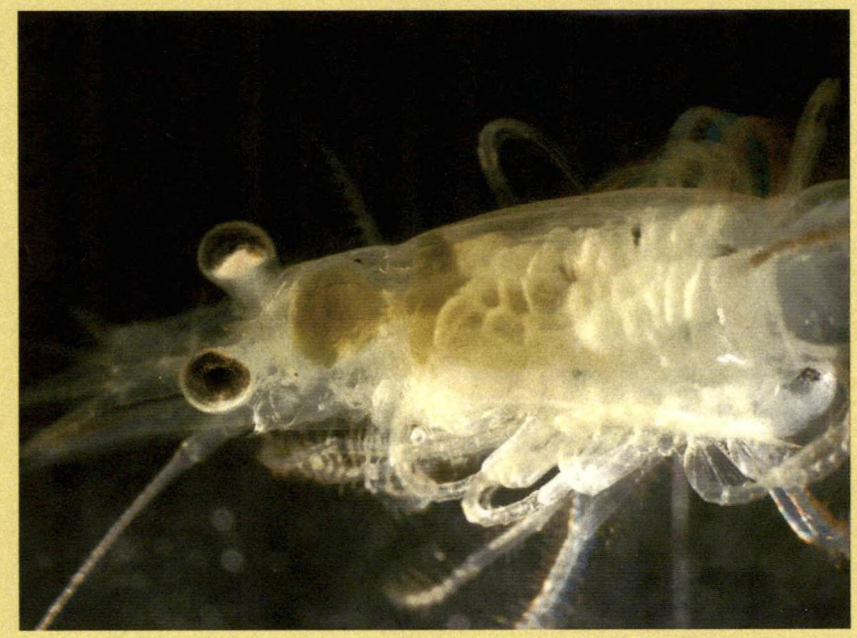
UNIVERSITEIT GENT
Faculteit Wetenschappen

Marine Biologie
Vakgroep Biologie
Krijgslaan 281-S8, B-9000 Gent, BELGIUM

Diet and growth of *Neomysis integer* (Leach, 1814) (Crustacea, Mysidacea)

Dieet en groei van *Neomysis integer* (Leach, 1814) (Crustacea, Mysidacea)



Nancy Fockedey

Promotor: Prof. Dr. Magda Vincx
Co-Promotor: Prof. Dr. Jan Mees

Academic Year 2004-2005

Rector: Prof. Dr. Andreas De Leenheer
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A thesis submitted in partial fulfilment of the requirements for the degree of
Doctor in Sciences (Biology)

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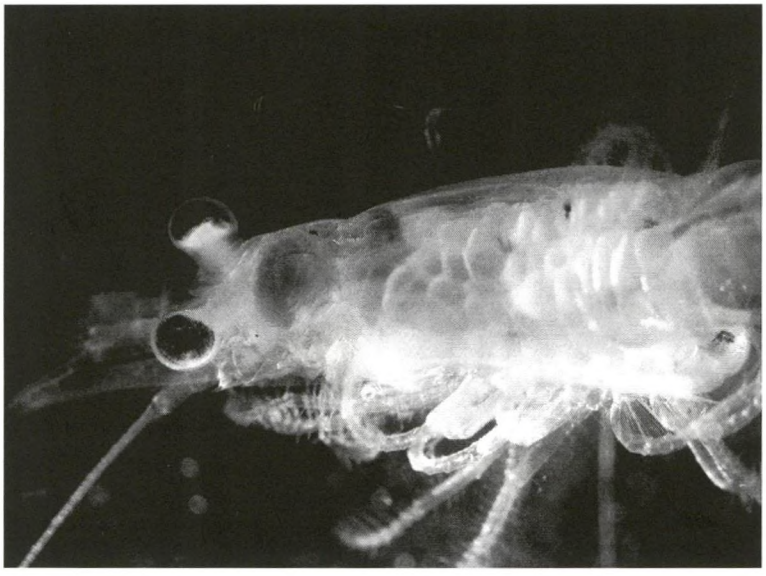

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Mijn carrière in de mariene biologie begon met het kiezen van een thesisonderwerp. Als studente in de biologie was die keuze gebaseerd op (1) een nogal onverklaarbare verbondenheid met de zee, vis en schepen (een 'gen' van mijn vader meegekregen?), en (2) de mogelijkheid om mee te kunnen op 'expeditie' op onderzoeksschepen. In drie weken tijd reden we van het zuiden van Frankrijk naar het noorden van Nederland om er evenveel estuaria te bemonsteren. We verbleven aan boord met Jacques Castel, overnachtten op het onderzoeksinstituut van Jean-Claude Sorbe, en ten huize van Karel Essink: iets wat als ik "groentje" toch wel indrukwekkend vond!

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Summary

Neomysis integer (Leach 1814) is a common mysid crustacean along the Atlantic coast of western Europe and the Baltic Sea. It is a hyperbenthic, euryhaline and eurythermic species, confined to the brackish environment within estuaries, and inland water bodies, which were once connected to the sea.

Neomysis integer is used as a model for studies on the physiology, behaviour and general ecology of brackish water crustaceans. A lot of information is available on the feeding and life history characteristics of *N. integer* living in partly enclosed systems like ponds, lakes and the Baltic Sea, but ecological information of *N. integer* living in the estuarine environment is much less documented.

In **Chapter 1** of this thesis, an extensive literature review on *Neomysis integer* is presented, focussing on its feeding, life history aspects, physiology, behaviour and energy budgets. All distribution records of the species are listed in an appendix. The aim is to make a summary of the currently available information on the species and to identify the gaps in our knowledge. The author's own contributions (published papers, submitted manuscripts and unpublished work) are highlighted in the text.

More quantitative information on the position of *Neomysis integer* in the heterotrophic food web of the brackish, turbid reaches of estuaries is needed. *N. integer* plays an important role in the diet of higher trophic levels, but a quantitative description of its own diet was missing. In **Chapter 2** of the thesis, a methodology is described for the quantitative diet analysis of mysids. The method brings together two techniques: (1) microscopic stomach content analysis and (2) a fullness index determination based on weight. After dissecting and preparing the stomach in a semi-permanent slide, food items are counted and measured using image analysis. Amorphous, unidentifiable detritus was characterised further by EDAX (Energy Dispersive Spectroscopy X-ray Microanalysis). The stomach fullness technique was adopted from fish feeding studies and this is the first time it is applied to small invertebrates like mysids.

This methodology is used to compare the diet of *Neomysis integer* collected from the maximum turbidity zone of the Schelde, Gironde and Elbe estuaries in spring. *N. integer* was found to be an omnivore which mainly utilizes mesozooplankton and detritus carbon pools. The quality of the diet did not differ between the sexes or between different developmental stages, although smaller individuals consumed fewer items. In all three estuaries, the (spring) animal fraction in the diet was dominated by calanoid copepods (3 – 10 *Eurytemora affinis* ind^{-1}) and supplemented with rotifers and cladocerans. Phytoplankton and benthic organisms, though present in the stomachs, were negligible. Macrophytal detritus and amorphous material, the latter unidentifiable under the light microscope, were very abundant food items too. The amorphous detritus was found to originate from the suspended sediment flocs which are characteristic of the estuarine turbidity zone and mainly consisted of clay minerals. The energetic value of these flocs for *N. integer* remains unclear.

There has been an increasing interest in using the brackish water mysid *Neomysis integer* as a toxicological test species for Western European estuarine systems. In this respect, more data on the growth, moulting and development in this species was needed. The influence of the prevailing environmental variables (*e.g.* temperature, salinity, food quality and quantity) on these processes, as well as their optimal range, have to be known in order to develop optimal laboratory cultures and to differentiate between chemically-induced variability and natural variability in toxicity testing. Furthermore, this knowledge is essential for ecological modelling and food web analyses. The following four chapters provide more insight in the growth of *N. integer* and the impact of salinity and temperature on the one hand (Chapter 3), and food quality on the other hand (Chapter 4 and 5) on the post-marsupial growth and moulting processes of the species. Chapter 6 deals with the impact of temperature and salinity on the intra-marsupial growth and embryonic development of *N. integer*.

In **Chapter 3** the individual post-marsupial growth (size, intermoult period, growth factor) of *Neomysis integer* was studied from first day neonates until adulthood under eight environmentally relevant temperature-salinity conditions. Three salinities (5, 15 and 30 psu) were tested at 15 and 20 °C, and two more extreme temperatures (8 and 25 °C) were tested at a salinity of 5 psu.

Survival and growth of *Neomysis integer* were studied within the whole range tested. Sexual maturation was only possible in the narrower range of 15 – 25 °C and 5 – 15 psu. The size-at-maturity of *N. integer* increased with decreasing temperature and increasing salinity. Salinity had a stronger effect on the time needed for maturation than temperature. Higher temperatures mainly resulted in shorter intermoult periods, but had less effect on the growth increment. Salinity effects on these growth parameters were less straightforward and dependent on the water temperature. A tool is provided to estimate the age, moult number, intermoult period, growth factor and growth rate from the standard body length of *N. integer* at all tested temperature-salinity combinations.

The von Bertalanffy growth model could be fitted to the individual and pooled data, except for the 8 °C experiment where growth was linear. Growth parameter estimates from pooled data were comparable with individually-based estimates, but generally underestimated the asymptotic length. Temperature was negatively correlated with the asymptotic length L_{inf} and positively correlated with the growth constant K. Experimentally-derived von Bertalanffy parameter estimates resulted in a higher growth performance index than field-based estimates for Schelde and Galgenweel populations of *Neomysis integer*.

Estuarine aggregates were an abundant dietary item in the stomachs of *Neomysis integer* in the turbid reaches of European estuaries. Growth experiments (**Chapter 4**) have given a decisive answer on their nutritive value for the mysid by monitoring the ability of *N. integer* to survive and grow when feeding on them. Because estuarine macro-aggregates fall apart when sampling in strongly bound microflocs, a roller table was used to regenerate the macro-aggregates from samples of natural estuarine water from the oligohaline part of the Schelde estuary. In a preceding experiment, the effect of tidal dynamics on the floc formation process, as well as on the floc size and shape, was examined.

The effect of continuous rotation in the roller tanks on the growth of *Neomysis integer* was negligible and the device turned out to be an adequate tool for performing feeding experiments with *N. integer* and fragile estuarine aggregates.

Survival, growth, intermoult period, growth factor and intermoult growth rate of subadult mysids (4 – 10 mm standard length) were monitored over a period of 4.5 weeks and compared to the growth performance of *N. integer* feeding *ad libitum* on *Artemia salina* nauplii. Also, the feeding rate of *N. integer* feeding on laboratory-generated macro-aggregates was estimated.

The estuarine aggregates were a valuable food source for *Neomysis integer* as the mysids showed good survival (80 %) and growth (0.08 ± 0.01 mm d^{-1}) on this dietary item, although growth was slower than on *Artemia salina* nauplii (0.11 ± 0.01 mm d^{-1}). A high feeding rate of subadult *N. integer* on the laboratory-made flocs (38 flocs ind^{-1} h^{-1}) may compensate for their low energetic value.

For *Neomysis integer* living in the maximum turbidity zone of estuaries, the estuarine aggregates may be an important additional food source, especially during periods when mesozooplankton prey (mainly calanoid copepods) are scarce. The rich bacterial and protozoan communities associated with the flocs and the incorporated amorphous organic matter – normally too small to be efficiently consumed by mysids – become part of their diet. This pathway thus constitutes a short-cut in the estuarine food chain.

The addendum to Chapter 4 (**Addendum 1**) reports on the results of a preliminary study to identify the composition of estuarine aggregates and to quantify the associated micro-organisms. In November 1997 and June 1998, the composition of estuarine macro-aggregates in the Schelde estuary was investigated at 4 sites situated in the estuarine turbidity maximum. The flocculation process was simulated *in vitro* using the roller table. Macro-aggregates were separated from the surrounding water by sedimentation and decantation. 47 – 90 % of the suspended particulate matter, 29 – 67 % of the particulate organic carbon, 6 – 57 % of the chlorophyll *a*, 1 – 39 % of the bacteria, 5 – 14 % of the heterotrophic nanoflagellates and 5 – 25 % of the ciliates in the water column were found to occur in association with the macro-aggregates. The fraction of total chlorophyll *a* associated with the macro-aggregates was, at all sites, lower in June compared to November. The fraction of total bacteria that was associated with the macro-aggregates was highest in the freshwater tidal reaches and tended to decrease in downstream direction. Concentrations of bacteria, heterotrophic nanoflagellates and ciliates in the macro-aggregates were generally one to two orders of magnitude higher than in the surrounding water. Despite high concentrations of micro-organisms in the macro-aggregates, living biomass contributed at most to 3.2 % of total organic carbon of the macro-aggregates.

Chapter 5 evaluates to what extent diets of *Artemia salina* (nauplii), *Eurytemora affinis* (copepodites and adults), laboratory-made estuarine flocs and macrophytal detritus (*Scirpus maritimus* and *Spartina anglica*), all administered *ad libitum*, influence the survival and somatic growth of subadult *Neomysis integer*. Growth was monitored in three alternative ways: (1) by the increase in standard length (SL), (2) by the intermoult period (IMP), and growth factor (GF), or (3) by the intermoult growth rate (GR).

Detritus originating from non-leached *Spartina anglica* was toxic to *Neomysis integer*, leading to morphologic aberrations and a high mortality. The growth of *N. integer* individuals was slower on a diet of *Scirpus maritimus* detritus than on a diet of animal food or laboratory-made flocs. *Artemia salina* nauplii were the highest quality food for *N. integer*: a relatively shorter IMP and higher GF and GR resulted in a significantly higher SL at the end of the experiment.

When fed with laboratory-made flocs, *N. integer* moulted as frequently as when fed with *Artemia*, but GR decreased over the course of the experiment. A *Eurytemora affinis* diet resulted in a significantly elongated IMP from the first moult onwards compared to mysids fed with *Artemia* or flocs. The mean associated growth rate was, however, comparable with the flocs treatment and significantly lower than fed *Artemia*.

In an addendum to Chapter 5 (**Addendum 2**) some experimental observations are reported on the gut passage time, egestion rate and faecal pellet production of *Neomysis integer* when feeding *ad libitum* on a variety of environmentally relevant food types and a reference diet of *Artemia salina* nauplii.

Gut passage times were calculated by measuring the voided faecal pellets. When feeding on *Artemia salina* nauplii, gut passage times were variable (from 4.1 to 12.9 h), but significantly longer than when feeding on the post-naupliar stages of the calanoid copepod *Eurytemora affinis* (2.6 h). Estuarine flocs passed through the intestine within 0.5 hour after ingestion, and *N. integer* produced daily up to twice its own body length in compact faecal material. The gut residence time of macrophytal detritus was 1.9 h and no difference was found between fresh and aged detritus.

The egestion rate of *Neomysis integer* feeding on estuarine flocs (0.163 ± 0.001 mm^3 h^{-1}) was significantly higher than in all other treatments (0.011 ± 0.001 mm^3 h^{-1}). The faecal pellet production rate, when feeding on flocs, amounted to 0.044 mgDW mgDW^{-1} h^{-1}.

Preliminary results on the C:N ratio of food and faecal pellets demonstrated a general enrichment in nitrogen in the faecal pellets, probably due to bacterial growth on the pellets, the peritrophic membrane and disintegrating cells of the mysid intestine. The faecal pellets produced by *Neomysis integer* are still potential sources of energy themselves. Scanning electron micrographs of faecal pellets give details about the peritrophic membrane and pellet content.

In **Chapter 6** a protocol is developed to examine the intra-marsupial development of *Neomysis integer in vitro* and a morphological description of the embryonic and larval developmental stages is presented. Daily survival percentage, percentage survival days, hatching success, total development time, duration of each developmental stage and the size increment of the embryos/larvae were evaluated as potential endpoints for ecotoxicological testing and their response to temperature and salinity was investigated.

The survival and hatching success are highly dependent on the salinity conditions, while the development time is strongly affected by temperature. High temperatures (21 °C) shorten the development time in comparison with low temperatures (11 °C) from 22 to 10 days, but have an opposite effect on survival. Optimal salinity for *in vitro* embryonic development of *Neomysis integer* is 14 – 17 psu. Living in lower or higher salinities thus implies suboptimal conditions for the juvenile recruitment to the population, unless the species can actively regulate the concentration of its marsupial fluid.

The developed *in vitro* technique may be used for testing the effect of both abiotic factors and (endocrine) disrupting toxicants on the intra-marsupial development of *Neomysis integer*. Survival, hatching success and development time appeared to be adequate endpoints, while size and growth increment of the embryos seemed unsuitable.

In **conclusion**, *Neomysis integer* living in the turbid reaches of estuaries is omnivorous, and mainly feeds on calanoid copepods, macrophytal detritus and estuarine sediment aggregates. The quality of all of these food items is sufficiently high to provide a good survival for the mysid, but growth rates are significantly higher when *N. integer* feeds on animal food in comparison to detrital diets. For *N. integer* living in the maximum turbidity zone of estuaries, the estuarine flocs and macrophytal detritus may be important additional food sources, especially in periods when mesozooplankton prey (mainly calanoid copepods) is scarce.

Under experimental conditions, the following conclusions on the effect of environmental variables (temperature and salinity) on the growth and development of *Neomysis integer* can be presented: the post-marsupial growth of *N. integer* is possible over a wide temperature and salinity range, but sexual maturation is only possible within the narrower range of 15 – 25 °C and 5 – 15 psu. Intra-marsupial survival (> 50 %) and development are confined to an even more restricted salinity range of 14 – 17 psu, unless female mysids can actively regulate the concentration of their marsupial fluid. The duration of the intra-marsupail development of *N. integer* is strongly affected by temperature, while survival and hatching success are dependent on the salinity conditions. Although survival is lower, the post-marsupial growth and development of *N. integer* are both accelerated at a higher temperature, mainly due to a more frequent moulting of the animals. The size-at-maturity decreased at a higher temperature. Salinity even had a stronger effect than temperature on the time needed to become mature.

The results of the experimental research presented in this thesis contribute to our basic knowledge of the ecology of the mysid *Neomysis integer*, a key species in the brackish water zone of temperate European estuaries. More specifically, this research contribute to the understanding and quantification of the species' feeding ecology and population dynamics, *i.e.* the impact of environmental variables (temperature, salinity and food quality) on processes like growth, moulting and pre- and post-marsupial development. These data are relevant for ecological modelling and the techniques developed and described for assessing the effects of environmental conditions on individual growth, moulting, and *in vitro* embryology are currently used in bioassays for the evaluation of the effects of toxic substances (mainly endocrine disrupting chemicals) in the estuarine ecosystem.

Samenvatting

De brakwateraasgarnaal *Neomysis integer* (Leach 1814) is een veel voorkomende soort langsheen de Atlantische kust van West Europa en in de Baltische Zee. Het is een hyperbenthische, euryhaliene en eurytherme soort, die specifiek aanwezig is in riviermondingen (estuaria) en brakwaterplassen en -kanalen.

Neomysis integer wordt vaak gebruikt als modelsoort in studies over de fysiologie, het gedrag en de algemene ecologie van brakwater crustaceeën. Er is dan ook grote hoeveelheid informatie beschikbaar over de voeding en de populatiedynamiek van *N. integer* populaties, maar deze beperkt zich vooral tot ingesloten brakwatermassa's (plassen, meren, kanalen en de Baltische Zee). Ecologische informatie van estuariene *N. integer* populaties ontbreekt tot nu toe nog grotendeels.

In **Hoofdstuk 1** wordt een uitvoerig literatuuroverzicht gegeven van de aasgarnaal *Neomysis integer*, met de nadruk op de voedingsecologie, de ontwikkelingsgeschiedenis, de fysiologie en biochemie, het gedrag en het energie budget van de soort. In een appendix van dit hoofdstuk werden alle waarnemingen van *N. integer* geïnventariseerd. Dit hoofdstuk wil een samenvatting geven van alle tot nu toe beschikbare informatie over *N. integer*, om zo de hiaten te kunnen aanduiden in onze kennis over de soort. De auteur haar eigen gegevens (gepubliceerd als artikel, ingediende manuscripten en ongepubliceerd werk) zijn opgenomen in de tekst in grijze kadertjes.

Er is nood aan kwantitatieve informatie over de rol van *Neomysis integer* in het heterotrofe voedselweb van het turbiede brakwater gebied van estuaria. *Neomysis integer* is er een belangrijk onderdeel in het dieet van de hogere trofische niveaus (vnl. van vissen en garnaal), maar een kwantitatieve beschrijving van het dieet van *N. integer* zelf ontbreekt tot nu toe. In **Hoofdstuk 2** van de thesis wordt een methodologie beschreven voor het uitvoeren van kwantitatieve maaganalyses van aasgarnalen. De methode bestaat erin om 2 technieken samen te gebruiken: (1) een microscopische analyse van de maaginhoud en (2) het bepalen van een maagvulling index, gebaseerd op gewicht. Na het uitprepareren van de maag en het monteren ervan in een semi-permanent preparaat, worden de aanwezige voedselitems geteld en gemeten met behulp van beeldanalyse technieken. Amorfe detritus, verder niet identificeerbaar onder de lichtmicroscoop, wordt verder gekarakteriseerd met behulp van EDAX (Energy Dispersive Spectroscopy X-ray Microanalysis). De techniek voor het bepalen van de maagvulling a.d.h.v. het gewicht, vaak gebruikt in de studie van de voedingsecologie van vissen, werd hier voor het eerst toegepast op kleine ongewervelden (Mysidacea).

De methodiek werd toegepast om het dieet te vergelijken van *Neomysis integer* individuen die in de lente bemonsterd werden in de maximum turbiditeitzone van de estuaria Schelde, Gironde en Elbe. *N. integer* voedt zich in deze gebieden voornamelijk met mesozooplankton en detritus. De kwaliteit van het opgenomen voedsel verschilt niet sterk tussen de verschillende geslachten en ontwikkelingstadia, maar kleinere aasgarnalen consumeren minder items.

In de 3 estuaria wordt het dieet gedomineerd door calanoide copepoden (3 – 10 *Eurytemora affinis* ind^{-1}) en aangevuld met rotiferen en cladoceren. Phytoplankton en benthische organismen zijn aanwezig in de maag in verwaarloosbare hoeveelheden, terwijl macrofyten detritus en amorf materiaal zeer abundant zijn. Het amorfe materiaal bleek, na een EDAX analyse, afkomstig van gesuspendeerde sediment vlokken, typisch voor de estuariene turbiditeitzone waar de soort leeft. Het is onzeker welke voedingswaarde deze vlokken hebben voor *N. integer*.

Er is een grote interesse om de brakwateraasgarnaal *Neomysis integer* te gebruiken als testorganisme voor toxicologisch onderzoek voor West-Europese estuariene systemen. In die zin is het belangrijk om de kennis uit te breiden over de groei, het vervellingsproces en de ontwikkeling van de soort. De invloed van omgevingsvariabelen (zoals temperatuur, saliniteit en voedsel kwantiteit en – kwaliteit) op deze processen, alsook hun optimale bereik, moeten gekend zijn zodat een optimale laboratorium cultuur kan opgezet worden en in toxiciteit-testen onderscheid kan gemaakt worden tussen chemisch geïnduceerde variabiliteit en natuurlijke variabiliteit. Verder is deze informatie ook nuttig bij het opstellen van ecologische modellen en voedselweb analyses. De volgende 4 hoofdstukken van de thesis geven meer inzicht in de groei van *N. integer* en de impact van enerzijds de saliniteit en temperatuur (Hoofdstuk 3) en anderzijds van de voedselkwaliteit (Hoofdstuk 4 en 5) op de post-marsupiale groei van de soort. Hoofdstuk 6 behandelt de impact van saliniteit en temperatuur op de intra-marsupiale groei en embryonale ontwikkeling van *N. integer*.

In **Hoofdstuk 3** werd de individuele post-marsupiale groei (lengte, 'intermoult period' en groeifactor) van *Neomysis integer* bestudeerd bij 8 relevante temperatuur- en saliniteitscombinaties. De overleving en groei werden gevolgd bij individuele aasgarnalen, vanaf de eerste dag na het vrijkomen uit het marsupium tot in het adulte stadium; dit bij 3 saliniteiten (5, 15 and 30 psu) bij 15 en 20 °C, en bij 2 meer extreme temperaturen (8 en 25 °C) bij een saliniteit van 5 psu.

De aasgarnalen overleven en groeien bij alle geteste temperatuur- en saliniteitscombinaties, maar de seksuele ontwikkeling van *Neomysis integer* was enkel mogelijk binnen het nauwe bereik van 15 – 25 °C en 5 – 15 psu. De 'size-at-maturity' van *N. integer* werd groter bij een hogere temperatuur en lagere saliniteit. Het zoutgehalte had een sterker effect dan temperatuur op de seksuele ontwikkelingstijd. Hogere temperaturen leidden voornamelijk tot een kortere 'intermoult period', en hadden een kleiner effect op de groeifactor. Het effect van saliniteit op deze twee groeiparameters was minder duidelijk en afhankelijk van de temperatuur. In het werk is een tabel voorzien die kan gebruikt worden als instrument voor het afleiden van de leeftijd, het aantal voorafgaande vervellingen, de 'intermoult period', de groeifactor en groeisnelheid a.d.h.v. de standaard lichaamslengte van de aasgarnaal bij alle geteste temperatuur- en saliniteitscombinaties.

De data werden gefit met het 'von Bertalanffy' groeimodel, uitgenomen bij 8 °C waar de groei lineair verliep. De groeiparameters, geschat door het fitten van het model op de gepoolde data, waren zeer vergelijkbaar met schattingen gebaseerd op het fitten van individuele data. Over het algemeen werd de asymptotische lengte overschat bij de gepoolde data. Temperatuur was negatief gecorreleerd met de asymptotische lengte L_{inf} en positief gecorreleerd met de groeiconstante K. De von Bertalanfy groeiparameters afkomstig van experimentele data resulteren in een hogere 'growth performance index' in vergelijking met schattingen gebaseerd op veldgegevens van *N. integer* populaties uit Schelde en Galgenweel.

Estuariene vlokken hadden een belangrijk aandeel in de maaginhoud van *Neomysis integer* afkomstig uit het turbiede brakwatergebied van Europese estuaria. Groei-experimenten (**Hoofdstuk 4**) kunnen uitsluitsel geven over de nutritieve waarde van deze vlokken voor de aasgarnaal. Estuariene macrovlokken vallen uit elkaar in kleinere, sterk gebonden microvlokken op het moment van staalname. Er werd gebruik gemaakt van een roltafel om de macrovlokken te regenereren uitgaande van water bemonsterd in de oligohaliene zone van het Schelde estuarium. In een voorafgaand experiment werd nagegaan welke het effect was van het moment van waterstaalname (tidale fase) op de vlokvorming (aantal en grootte van de vlokken). Het effect van continue rotatie op de groei van *Neomysis integer* was verwaarloosbaar. De roltafel bleek een nuttig instrument voor het uitvoeren van voedingsexperimenten waarbij de aasgarnalen zich voedden op de fragiele macrovlokken. De overleving, groei, 'intermoult period', groeifactor en groeisnelheid van subadulte aasgarnaal (4 – 10 mm standaard lengte) werden opgevolgd over een periode van 4.5 weken en vergeleken met de overleving en groei van subadulte *N. integer* die zich *ad libitum* voedden met *Artemia salina* nauplii. In een bijkomend experiment werd een schatting gemaakt van de voedingsnelheid van *N. integer* op de macrovlokken.

Estuariene macrovlokken bleken een goede voedselbron te zijn voor *Neomysis integer*. De aasgarnalen vertoonden een goede overleving (80 %) en groeiden 0.08 ± 0.01 mm d^{-1}, hoewel de groei trager was dan wanneer gevoed met *Artemia salina* nauplii (0.11 ± 0.01 mm d^{-1}). De hoge voedingsnelheid van subadulte *N. integer* op de gegenereerde macrovlokken (38 flocs ind^{-1} h^{-1}) duidt op een compensatie voor hun lage energetische waarde. De vlokken kunnen een belangrijke aanvulling zijn in het dieet van *Neomysis integer* in de maximum turbiditeitzone van estuaria, vooral in periodes wanneer mesozooplankton (vnl. calanoide copepoden) schaars is. De rijke gemeenschap van bacteriën en protozoa op de macrovlokken en het geïncorporeerde amorfe organische materiaal, normaal te klein om efficiënt geconsumeerd te worden door de aasgarnaal, gaan zo deel uitmaken van het dieet van *N. integer*, wat een 'short-cut' betekent in de estuariene voedselketen.

Het addendum van hoofdstuk 4 (**Addendum 1**) rapporteert over de studie waarin de samenstelling van de gegenereerde macrovlokken geïdentificeerd werd en de ermee geassocieerde micro-organismen gekwantificeerd werden. In november 1997 en juni 1998 werden de samenstelling van estuariene macrovlokken van het Schelde estuarium onderzocht van 4 plaatsen in en rond de maximum turbiditeitzone. Het vlokvormingsproces werd gesimuleerd door gebruik te maken van een roltafel. De macrovlokken werden gescheiden van het omgevende water door sedimentatie en decantatie. 47 – 90 % van het gesuspendeerde particulair materiaal, 29 – 67 % van de particulaire organische koolstof, 6 – 57 % van het chlorofyl *a*, 1 – 39 % van de bacteriën, 5 – 14 % van de heterotrofe nanoflagellaten en 5 – 25 % van de ciliaten aanwezig in de waterkolom waren geassocieerd met de macrovlokken. De fractie totale chlorofyl *a* geassocieerd met de vlokken was in alle stations hoger in juni in vergelijking met november. De fractie van de totale bacteriën geassocieerd met de vlokken was het hoogst in de zoetwater stations en daalde stroomafwaarts. De aantallen bacteriën, heterotrofe nanoflagellaten en ciliaten aanwezig in en op de vlokken was algemeen gezien één tot twee ordes hoger dan in het omgevende water. Niettegenstaande deze hoge concentraties aan micro-organismen, maakt de biomassa slechts max. 3.2 % uit van de totale organische koolstof in de macrovlokken.

Hoofdstuk 5 evalueert in welke mate een dieet van *Artemia salina* (nauplii), *Eurytemora affinis* (copepodieten en adulten), in het laboratorium gegenereerde estuariene vlokken en macrofyten detritus (*Scirpus maritimus* en *Spartina anglica*), alle toegediend *ad libitum*, de overleving en de somatische groei van subadulte *Neomysis integer* beïnvloeden.

De groei werd opgevolgd op drie alternatieve manieren: (1) m.b.v. de toename in standaard lengte (SL), (2) m.b.v. de 'intermoult period' (IMP) en de groeifactor (GF), en (3) m.b.v. in 'intermoult'-groeisnelheid (GR).

Detritus afkomstig van niet-uitgeloogde *Spartina anglica* bleek toxisch voor *Neomysis integer* en resulteerde in morfologische aberraties en een hoge mortaliteit. De groei van *N. integer* individuen was trager op een dieet van *Scirpus maritimus* detritus dan op een dierlijk dieet of op een dieet van estuariene macrovlokken. *Artemia salina* nauplii bleek het meest kwalitatieve voedsel voor *N. integer*, gezien een relatief korte IMP en een hoge GF en GR leidde tot een significant hogere SL aan het einde van het experiment. Indien de aasgarnaal gevoed werd met estuariene vlokken, vervelde *N. integer* even snel als wanneer gevoed met *Artemia*, maar de groeisnelheid daalde geleidelijk over het verloop van het experiment. Een dieet op *Eurytemora affinis* resulteerde al vanaf de eerste vervelling in een significant langere IMP in vergelijking met individuen gevoed met *Artemia* of vlokken. De gemiddelde groeisnelheid op copepoden was echter vergelijkbaar met deze op vlokken en significant lager dan wanneer gevoed met *Artemia*.

In een addendum van Hoofdstuk 5 (**Addendum 2**) worden enkele experimentele observaties gerapporteerd i.v.m. de 'gut passage time' (GPT) en 'egestion rate' (ER) van *Neomysis integer*, zijnde de tijd en snelheid waarmee de resten van het opgenomen voedsel uitgescheiden worden. Hierbij werden de aasgarnalen *ad libitum* gevoed op een variëteit aan diëten, relevant voor hun leefomgeving in de maximum turbiditeitzone in estuaria, en op een standaard dieet van *Artemia salina* nauplii.

De GPT werd geschat door het meten van de uitgescheiden fecale pellets. Wanneer de aasgarnaal gevoed werd met *Artemia salina* nauplii was de GPT zeer variabel (tussen 4.1 en 12.9 uur), maar significant langer dan wanneer gevoed met de latere stadia van de calanoide copepode *Eurytemora affinis* (2.6 uur). Estuariene vlokken passeerden in 0.5 uur doorheen het spijsverteringskanaal en *N. integer* produceerde dagelijks tot tweemaal zijn eigen lichaamslengte aan fecale pellets. De GPT op macrofyten detritus was 1.9 uur en er kon geen onderscheid gemaakt worden in de GPT tussen vers en verouderd detritus.

Neomysis integer die zich voedt op estuariene macrovlokken heeft een significant hogere ER (0.163 ± 0.001 mm^3 h^{-1}) dan bij alle andere diëten (0.011 ± 0.001 mm^3 h^{-1}). De productiesnelheid van fecale pellets, wanneer gevoed met estuariene vlokken, bedroeg tot 0.044 mgDW mgDW^{-1} h^{-1}.

Voorlopige resultaten van de C:N ratio van voedsel en fecale pellets toonden aan dat de pellets over het algemeen aangerijkt worden met stikstof, waarschijnlijk veroorzaakt door de bacteriële groei op de pellets, de afgescheiden peritrofe membraan en afgescheiden cellen uit het darmkanaal van de aasgarnaal. Gezien hun C en N inhoud, zijn de fecale pellets van *Neomysis integer* nog een potentiële voedselbron. Scanning elektronen microscopische opnames van de fecale pellets geven details over de peritrofe membraan en hun inhoud.

In **Hoofdstuk 6** wordt een protocol ontwikkeld voor het *in vitro* opvolgen van de embryogenese van *Neomysis integer*. Er is tevens een morfologische beschrijving van de embryologische ontwikkeling toegevoegd. De dagelijkse overleving, de 'percentage survival days', het 'hatching success', de totale ontwikkelingstijd en de duur van de deelstadia, evenals de lengtetoename van de embryo's werden geëvalueerd als mogelijke eindpunten voor verder gebruik in een ecotoxicologische test met de soort. Verder werd de respons van deze eindpunten op temperatuur en saliniteit bestudeerd.

De overleving en het 'hatching success' zijn sterk afhankelijk van de saliniteit, terwijl de ontwikkelingsduur bepaald wordt door de temperatuur. Hoge temperaturen (21 °C) verkorten de ontwikkelingstijd in vergelijking met lage temperaturen (11 °C) van 22 tot 10 dagen, maar hebben een negatief effect op de overleving van de embryo's.

De optimale saliniteit voor de *in vitro* embryo- ontwikkeling van *Neomysis integer* is 14 – 17 psu. Het leven van de soort in een lagere of hogere saliniteit moet dus suboptimaal zijn voor de rekrutering van juvenielen tot de populatie, tenzij de soort het zoutgehalte van het marsupiale vocht kan regelen.

De *in vitro* ontwikkelingstechniek blijkt zeer gebruiksvriendelijk en reproduceerbaar te zijn voor het testen van de effecten van zowel omgevingsvariabelen als van (endocriene) verstorende stoffen op de embryologie van *Neomysis integer*. Overleving, 'hatching success' en ontwikkelingsduur blijken goede eindpunten, terwijl het gebruik van de grootte en groei van de embryo's als eindpunt af te raden zijn.

Tot **besluit:** *Neomysis integer* is een omnivoor in de turbiede zone van estuaria en voedt er zich voornamelijk met calanoide copepoden, macrofyten detritus en estuariene sediment aggregaten. De kwaliteit van al deze voedselitems is voldoende hoog voor de overleving van *N. integer*, maar de groeisnelheid is hoger wanneer dierlijk voedsel toegediend wordt en lager bij een detritus dieet. De estuariene vlokken en macrofyten detritus kunnen echter een goede aanvulling zijn in het dieet van *N. integer* in de maximum turbiditeitzone van estuaria, zeker in periodes van mesozooplankton schaarste.

Onder experimentele omstandigheden, kunnen de volgende conclusies genomen worden betreffende de effecten van de omgevingsvariabelen temperatuur en saliniteit op de groei en ontwikkeling van *Neomysis integer*: De post-marsupiale groei en overleving van *N. integer* is mogelijk over een brede range van temperaturen en saliniteiten, maar de seksuele ontwikkeling is enkel mogelijk in het nauwere bereik van 15 – 25 °C en 5 – 15 psu. De embryologische overleving (>50 %) en ontwikkeling zijn slecht mogelijk in een nog smallere saliniteit range van 14 – 17 psu, tenzij de aasgarnalen het zoutgehalte van hun marsupiale vocht actief kunnen regelen. De duur van de embryonale ontwikkeling van *Neomysis integer* is sterk afhankelijk van de temperatuur, terwijl overleving en 'hatching success' afhankelijk zijn van de saliniteitcondities. Hoewel een hogere temperatuur resulteert in een lagere overleving van *Neomysis integer*, worden de postmarsupiale groei en ontwikkeling versneld, voornamelijk door een hogere vervellingsfrequentie. De 'size-at-maturity' daalt bij hogere temperaturen. Saliniteit heeft een grotere impact dan temperatuur op de ontwikkelingsduur van juveniel tot adult.

Het experimentele onderzoek dat in deze thesis voorgesteld wordt, draagt bij tot de kennis van de ecologie van de brakwateraasgarnaal *Neomysis integer*, een sleutelsoort in de turbiede brakwater zone van gematigde Europese estuaria. Meer specifiek, draagt het werk bij tot het begrijpen en kwantificeren van de voedingecologie van de soort en zijn populatiedynamica: nl. de effecten van omgevingsvariabelen (temperatuur, saliniteit en voedselkwaliteit) op processen zoals groei, vervelling en pre- en post-marsupiale ontwikkeling. Deze data zijn relevant voor ecologische modellering. De technieken, hier ontwikkeld en beschreven voor het bepalen van de effecten van omgevingsvariabelen op individuele groei, vervelling en *in vitro* embryologie, worden nu verder toegepast in bioassays voor de evaluatie van de effecten van toxische stoffen (vnl. endocriene verstoorders) in het estuariene ecosysteem.

Preface and outline

Neomysis integer is one of the most common mysids along the Atlantic coast of western Europe and the Baltic Sea. It is a hyperbenthic, euryhaline and eurythermic species, confined to the brackish environment within estuaries and inland water bodies (Tattersall and Tattersall, 1951), where it reaches high densities and biomasses and often plays a key role in the food web as an important prey for fish and epibenthic macrocrustaceans (*e.g.* Mauchline, 1980; Hostens and Mees, 1999).

The position of *Neomysis integer* in the heterotrophic food web of the brackish, turbid reaches of estuaries remains unclear and more quantitative information on this topic is invaluable for the development of accurate C-flux models. The description of detritus based food web patterns, including the quantification of transfer coefficients is a key-item in estuarine ecology. To date, few models have taken hyperbenthic mysids into account, because only limited quantitative information is available on the diet of the mysid species. Therefore, quantitative stomach analyses are important to elucidate the diet of *N. integer* in the estuarine habitat.

Estuarine organisms are exposed to fluctuating environmental factors such as temperature, salinity, and food quantity and quality. All of these factors may act either singly or in concert to modify the life history and distribution of the species. Survival of the estuarine organisms in such a dynamic environment requires both physiological and behavioural adaptations (McKenney and Celestial, 1995; Devreker *et al.*, 2004). Although some descriptions are available on the life history characteristics of *Neomysis integer* in the field (Mees *et al.*, 1994 and the references therein), an experimental approach can help to clarify the specific impact of temperature, salinity and food quality and quantity on population dynamics like post-marsupial growth, reproduction and intra-marsupial development of the species.

The specific aims of the thesis are:

1. To review all available literature on *Neomysis integer*, focussing on its distribution, feeding and life history aspects, behaviour, physiology and energy budget in order to identify current knowledge and indicate the gaps in the knowledge of the species (Chapter 1).

2. To describe the diet of *Neomysis integer* in the maximum turbidity zone of estuaries, including variations due to latitude, gender and developmental stage by means of quantitative stomach content analyses of field caught animals (Chapter 2).

3. To study the combined effect of temperature and salinity on the post-marsupial growth and development of *Neomysis integer* by means of individually-based laboratory growth experiments over its entire life span (Chapter 3).

4. To examine the effect of food quality on post-marsupial growth of *Neomysis integer* through individually-based laboratory growth experiments (Chapter 4 and 5).

5. To study the combined effect of temperature and salinity on the intra-marsupial development of *Neomysis integer* based on *in vitro* experiments (Chapter 6).

OUTLINE OF THE PHD THESIS

The aims of **Chapter 1** are to make a summary of the currently available information on *Neomysis integer* and to identify the gaps in our knowledge of the species. As *N. integer* is often used as a model to study the ecology of estuarine crustaceans, numerous investigations are encountered in literature. The older studies (< 1980) are often superficial or based on a limited number of observations; many data are often unpublished (as Ph.D. theses or reports) or published in local journals. Information extracted from this grey literature has been integrated in the review. In the last 10 years, more in-depth studies have been published concerning aspects of the feeding ecology, population dynamics, physiology, bioenergetics, behaviour, and ecotoxicological use of the species.

Although originally not planned to be so extensive, Chapter 1 finally includes an extensive literature review on *Neomysis integer* with focus on its distribution, feeding and life history aspects, physiology, behaviour, biochemical composition and energy budgets. All distribution records of the species are listed in an appendix. Some of the identified gaps in the knowledge of *N. integer* have reference to the work performed further in the thesis (Figure 1), but reading of the review is not necessary to understand the context of the following chapters. One should consider Chapter 1 as a reference work, to refer to when specific information on one of the topics is needed. The author's contributions to the knowledge of the species (results presented in this thesis and some unpublished work) are highlighted in the text. The chapter has a separate font and reference list because its contents differ from the remainder of the thesis.

In **Chapter 2** the diet of *Neomysis integer* living into brackish reaches of estuaries is described. A methodology is presented for the quantitative diet analysis of mysids by means of stomach fullness measurements and microscopic stomach content analyses. The diet of *N. integer* in the maximum turbidity zone of three western European estuaries (Elbe, Schelde and Gironde) was investigated in spring 1993. The quantitative technique allows for an objective comparison of latitudinal, sexual and ontogenic shifts in the diet. The chapter has been published as *Fockedey, N., Mees, J., 1999. Feeding of the hyperbenthic mysid Neomysis integer in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries. J. Mar. Syst., 22: 207-228.*

In **Chapter 3** post-marsupial growth, moulting and development of *Neomysis integer* is examined in detail over its whole life span under laboratory conditions and the effects of prevailing abiotic variables (temperature and salinity) and age on these processes were evaluated. Individual post-marsupial growth (size, intermoult period, growth factor) was studied from first day neonates until adulthood at eight environmentally relevant temperature-salinity conditions when feeding *ad libitum* on *Artemia salina* nauplii. Additional information was gathered on the timing of sexual differentiation and maturation of the mysids.

Generalized von Bertalanffy growth curves were fitted to the experimental data and compared with field derived estimates. This chapter is accepted for publication as: *Fockedey, N., Mees, J., Vangheluwe, M., Verslycke, T., Janssen, C. and Vincx, M. Temperature and salinity effect on post-marsupial growth of Neomysis integer (Crustacea: Mysidacea). J. Exp. Mar. Biol. Ecol. (in press).*

Both chapter 2 and chapter 3 delivered the basic knowledge to formulate the following research question: the impact of diet quality on the survival and growth performance of subadult *Neomysis integer* under laboratory conditions using environmentally relevant food items like calanoid copepods, estuarine flocs and macrophytal detritus (chapters 4 and 5).

Chapter 4 specifically deals with the methodological aspects of applying estuarine flocs as a food item in individual growth experiments with *Neomysis integer*. Because estuarine macroflocs fall apart upon sampling in strongly bound microflocs, a roller table (modified from Shanks and Edmondson, 1989) was used to regenerate the estuarine aggregates. We first tested the effect of tidal dynamics in the field on the process of floc formation in the laboratory. Subsequently an experiment was performed in which *N. integer* was reared on laboratory-made aggregates. The nutritional importance of the flocs to the mysid was assessed by measuring its survival and growth. Furthermore, the feeding rate of *N. integer* on laboratory-made flocs was estimated. This chapter is submitted for publication as: *Fockedey, N., Hermans, S., Mees, J., Vincx, M. Survival, growth and feeding rate of the mysid Neomysis integer (Crustacea, Mysidacea) on laboratory-made estuarine aggregates. Mar. Ecol. Prog. Ser.*

In an addendum to Chapter 4 (**Addendum 1**), the importance of estuarine flocs as a substrate for micro-organisms is examined at four stations along the estuarine gradient of the Schelde estuary. The estuarine flocculation process was simulated under laboratory conditions making use of a roller table (Shanks and Edmondson, 1989). Concentrations of suspended particulate matter, particulate organic carbon, chlorophyll *a*, bacteria, heterotrophic nanoflagellates and ciliates were quantified on the flocs (after sedimentation) in comparison to the surrounding water (after decantation). This addendum is published as a chapter of the Ph.D. thesis of dr. Koenraad Muylaert and a manuscript is being prepared for submission: *Muylaert, K., Fockedey, N., Mees, J., Vijverman, W., 1999. Association of microorganisms with estuarine flocs. In: Muylaert, K., 1999. Distribution and dynamics of protist communities in a freshwater tidal estuary. Ph.D. Thesis, Ghent University: 137-147.*

The aim of **Chapter 5** was to compare the survival and growth performance of subadult *Neomysis integer*, when feeding for several weeks on diets of *Artemia salina* nauplii, calanoid copepods (*Eurytemora affinis*), estuarine flocs or artificial detritus made from *Spartina anglica* and *Scirpus maritimus*. As all diets were administered *ad libitum*, the variation in growth could be fully attributed to the food quality. The impact on growth and moulting processes is evaluated in three alternative ways: (1) as increase in length, (2) by the intermoult period and growth factor at successive moults and (3) as the intermoult growth rate. This chapter is submitted for publication as: *Fockedey, N., De Pauw, N., Mees, J., Vincx, M. The effect of food quality on the growth of the brackish water mysid Neomysis integer. Estuar. Coast. Shelf Sci.*

In an addendum of Chapter 5 (**Addendum 2**) some experimental observations are presented on the gut passage time and the egestion rate of *Neomysis integer* when feeding on a diet of *Artemia salina* nauplii, *Eurytemora affinis*, laboratory-made macro-aggregates, and artificially-made (fresh and aged) macrophytal detritus from *Scirpus maritimus*. Additionally, the C:N ratio was determined for the food and the faecal pellets produced.

Scanning electron micrographs of faecal pellets produced on the different food types give details about the peritrophic membrane and the pellets content. This addendum is submitted for publication as: *Fockedey, N., Mees, J., Vincx, M. Some experimental observations on gut passage time, egestion rate and faecal pellet production of the brackish water mysid Neomysis integer (Mysidacea: Crustacea) feeding on different food items. J. Exp. Mar. Biol. Ecol.*

Data on the intra-marsupial development of *Neomysis integer* are available as well. One of the aims of **Chapter 6** was to develop and optimize a methodology to follow *in vitro* the intra-marsupial development of *N. integer*. It allows a detailed description of the embryonic and larval mortality, morphology and the duration of subsequent developmental stages. The chapter also presents the results of an experiment performed to determine the temperature and salinity effects on intra-marsupial development of *N. integer* on endpoints like survival, hatching success, duration of development and size of the embryonic and larval sub-stages. This chapter is submitted for publication as: *Fockedey, N., Ghekiere, A., Bruwiere, S., Janssen, C.R., Vincx, M. Effect of salinity and temperature on the intra-marsupial development of the brackish water mysid Neomysis integer (Crustacea: Mysidacea). Mar. Biol.*

In **Addendum 3** a review is added on the use of mysids (a.o. *Neomysis integer*) as an ecotoxicological test organisms to evaluate environmental endocrine disruption. It was written under the incentive of the Ph.D. of Tim Verslycke (2003), in which several authors have participated. It is published as: *Verslycke, T., Fockedey, N., McKenney, C.Jr., Roast, S.D., Jones, M., Mees, J., Janssen, C.R., 2004. Mysid crustaceans as potential test organisms for the evaluation of environmental endocrine disruption: a review. Environmental Toxicology and Chemistry, 23 (5): 1219-1234.*

A scheme is added (Figure 1) on how the different chapters of this Ph.D. thesis relate to each other. The diet analysis of field-caught *Neomysis integer* (Chapter 2) and the experiments on its post-marsupial growth (Chapter 3) were performed parallel in time. Both studies delivered the basic knowledge for the following research question on how food quality influences post-marsupial growth (Chapter 4 and 5). Although the intra-marsupial development comes earlier in the life-cycle of *N. integer*, we chose to deal with it only in chapter 6 because of the different techniques used in comparison with the common one used in chapters 3, 4 and 5.

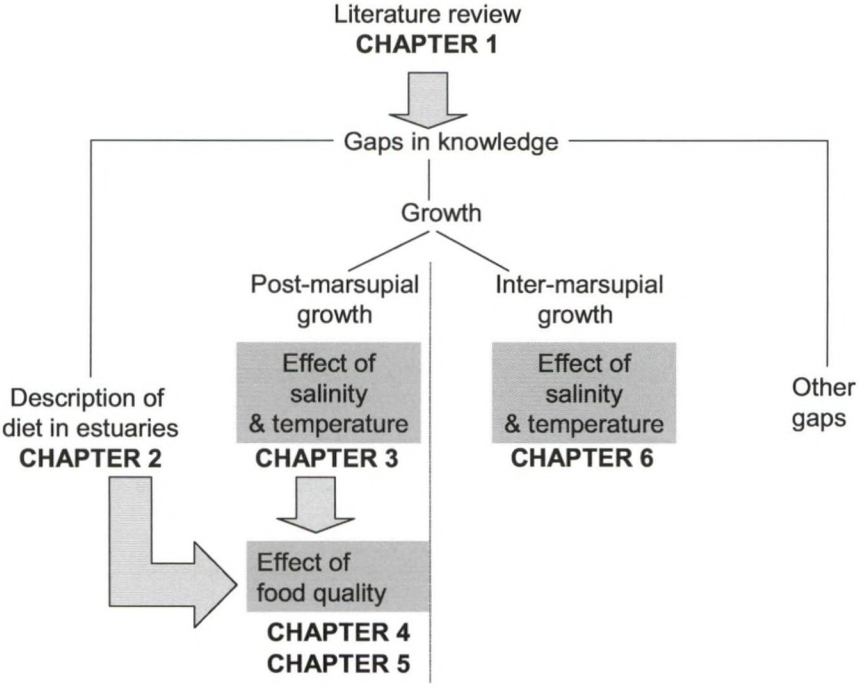

Figure 1: Scheme of the outline of the PhD thesis, indicating the relation between the different chapters. Arrows indicate the results that lead to new research questions.

Chapter 1

Neomysis integer: a review

ABSTRACT

The present chapter aims to be a literature review on the brackish water mysid *Neomysis integer*, with focus on its feeding ecology, life history aspects, behaviour, physiology, biochemical composition, bioenergetics and ecotoxicology. All records on the species, available from literature, are listed as an appendix. The review aims to identify the state-of-the-art and the gaps in our knowledge on the species.

Abundant information is available on the distribution patterns of *Neomysis integer* in enclosed brackish waters and estuaries, although on has to keep in mind that the swarming behaviour, vertical and horizontal migration, segregation of life stages and escape behaviour of *N. integer* (all not fully understood yet) can handicap the quantification of the density and biomass, and may hamper the calculation of the production and the study of the life history of the species.

There is a great need for the description of the feeding ecology of key species – like *Neomysis integer* – in estuarine environments for the development of accurate C-flux models and the description of detritus based food web patterns, including the quantification of transfer coefficients. Although *N. integer* is described as an important food item for many demersal and pelagic fish, larger epibenthic crustaceans and wading bird species, quantitative information is still lacking on its own diet, feeding rates, feeding patterns and selectivity (especially for populations living in estuarine conditions).

Numerous data are available on the life history of *Neomysis integer* over a wide geographical and habitat range, although southern populations (< 51°N) are more poorly known. Variations are observed between these populations in the number of cohorts, size-at-maturity, fecundity and growth rate. Growth and reproduction are affected by prevailing environmental conditions as generally observed in Crustacea. However, in the eurythermic and euryhaline *Neomysis integer*, typically living in the highly dynamic estuarine environment, this is not thoroughly studied yet. Details on how intra- and post-marsupial development, moulting processes and reproduction are affected by a wide range in salinity, temperature, food quantity and quality are still lacking.

The biochemical composition and the ecophysiology of *Neomysis integer* are well known and several methodologies to calculate the energy budget have been applied to the species.

There has been an increasing interest in using the brackish water mysid *Neomysis integer* as a toxicological test species for Western European estuarine systems. Mortality, respiration, swimming behaviour, testosterone metabolism and energy budgets are well established endpoints for bioassays with the species. However, more data on its growth, moulting and development are needed (at the individual- and population-level). The influence of prevailing environmental variables on these processes, as well as their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically-induced variability and natural variability in toxicity testing.

Because *Neomysis integer* is often used as a model species to study the ecology of brackish water crustaceans, and because the species is easy to sample qualitatively in shallow water and easy to keep in the laboratory, many studies and data are available concerning the species. The older studies (< 1980) are often superficial or based on a limited number of observations; many data are often unpublished (as Ph.D. theses or reports) or published in local journals. Information extracted from this *'grey'* literature has been integrated in the review. In the last 10 years, more in-depth studies have been published concerning aspects of the feeding ecology, population dynamics, physiology, bioenergetics, behaviour, and ecotoxicological use of the species, as well as on molecular work.

One should consider this chapter as a reference work, to refer to when specific information on one of the topics is needed. Reading of the review is **not** necessary to understand the context of the following chapters. Since its content differs from the remainder of the thesis, the chapter has a separate reference list and font. It has a separate table of contents to help to navigate through the text. The author's contributions (results presented in this thesis and some unpublished work) are highlighted in the text.

1 INTRODUCTION

1.1 Taxonomy

The order Mysidacea (Crustacea: Peracarida) includes species that inhabit most aquatic environments from the open sea to inland water bodies, and from strictly marine to freshwater conditions. At present 1065 species of mysids have been described, distributed over 170 genera (Deprez *et al.*, 2004; http://www.vliz.be/vmdcdata/nemys). The genus *Neomysis* (Czerniavsky, 1882) consists of 17 species, of which at least 10 are confined to brackish water conditions (Mauchline and Murano, 1977; www.iorbis.org). The only European representative is *Neomysis integer* (Leach, 1814).

Phylogenetic discussion: MYSIDA - MYSIDACEA

In the 80 – 90's of the 20th century the phylogenetic relationship of the constituent taxa of the Order Mysidacea were discussed in literature. Watling (1981; 1999) suggested that the Lophogastrida and the Mysidacea could not be considered as sister groups and that they both were rather related to the Eucarida than to the other orders of the Peracarida. The family Mysidae (to which Neomysis integer *belongs) was considered to be part of the order Mysida (e.g. http://tolweb.org/tree?group=Peracarida; Kobusch, 1998).*

However, based on a complex of 93 morphologic, anatomic and embryonic characters, Richter and Scholtz (2001) recently concluded that the taxa Mysida and Lophogastrida are indeed sister-groups within the monophyletic Order Mysidacea and that they are not separated from the other peracarid orders (Amphipoda, Mictacea, Spelaeogriphacea, Cumacea, Tanaidacea, Isopoda and Thermosbaenacea). The discussion continues ... In the present work we use the term Mysidacea.

1.2 External morphology

Mysidacea are shrimp-like animals that are characterized by a marsupium within which the entire larval development takes place (*cfr.* opossum shrimps). The brood pouch is composed of two, three or seven pairs of lamellae (oostegites) attached to the thoracic limbs. The uropods consist of lamellar endopods and exopods forming a tail fan with the telson. The endopods usually possess a statocyst. The telson is always wider at the base than at the apex. The abdominal legs never have chelae. The shield-like carapax covers the greater part of the cephalothorax, but is not attached to it in the last thoracal segments. For a more detailed definition of the order we refer to Tattersall and Tattersall (1951).

Neomysis integer (Leach, 1814) has a slender habitus and grows up to about 17 mm in length (Figures 1, 2). Its transparent body has occasional brown pigmentation. The large, stalked eyes are conspicuous. The well developed carapax, protecting the head and thorax, leaves the 7th and 8th thoracic somites uncovered and has a short rostrum. The two pairs of antennae are long and biramous. In adult males, the peduncle of the first antennae (antennule) bears a setose lobe (lobus masculinus) between the flagellae. The outer extension (exopod) of the second pair of antennae, takes the form of a flattened plate, known as the antennal scale. It is an important diagnostic characteristic for the species. The antennal scale of *N. integer* is very long and narrow, tapering to a point. It is bordered along its margins with setae and has a distinct distal suture.

The thoracopods are well developed and biramous, but lacking branchiae. The exopods are fringed and look feather-like. The adult female has a marsupium consisting of two pair of brood lamellae (oostegites) present on the 7th and 8th thoracopods. The lamellae are thin-walled, transparent concave plates fringed with strong, short setae that interlock ventrally to form a closed chamber below the thorax. Typical for the genus *Neomysis* are the two pairs of median finger-like processes extruding from the sterna of the last 2 thoracic somites into the marsupial cavity.

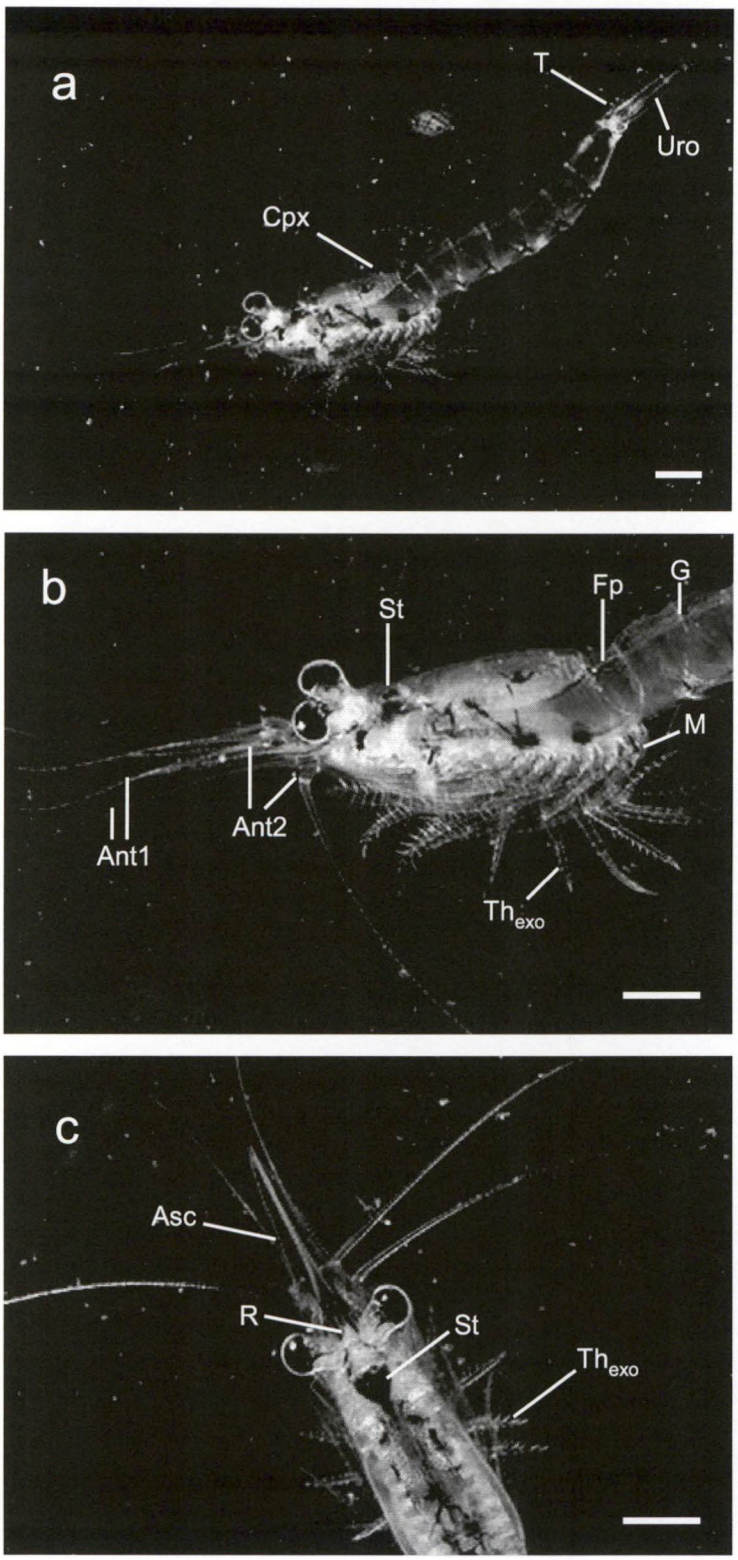

Figure 1: (a) habitus of the brackish water mysid *Neomysis integer*; (b) Cephalothorax in lateral view; (c) Cephalothorax in dorsal view. Ant1: 1st antennae (antennules), Ant2: 2nd antennae, Asc: Antennal scale, Cpx: Carapax, Th_{exo}: exopodite of thoracopod, Fp: faecal pellet, G: gut, M: (developing) marsupium, R: rostrum, St: Stomach, T: Telson, Uro: Uropods. Scale bar: 1 mm (Pictures: Offermans, R.)

The abdominal limbs (pleopods) are less developed, with the exception of 4th pair in adult males. They have an elongated 4th pleopod with a terminal pair of barbed setae. The last pair of pleopods is biramous and flattened, and forms the tail fan (uropods). The endopods of the uropod are as long as the telson, and have a statocyst at their basis. The two endopods are armed with a dense row of comb-like spines on their ventral surface near the inner margin. The exopods are one and a half times as long as the telson. They are not armed with spines but do have setae all round. The telson is long, triangular in outline with a narrow, uncleft apex (*cfr. integer*). Short spines border the lateral margins, while the apex has two pairs of spines of which the outer are twice as long as the inner.

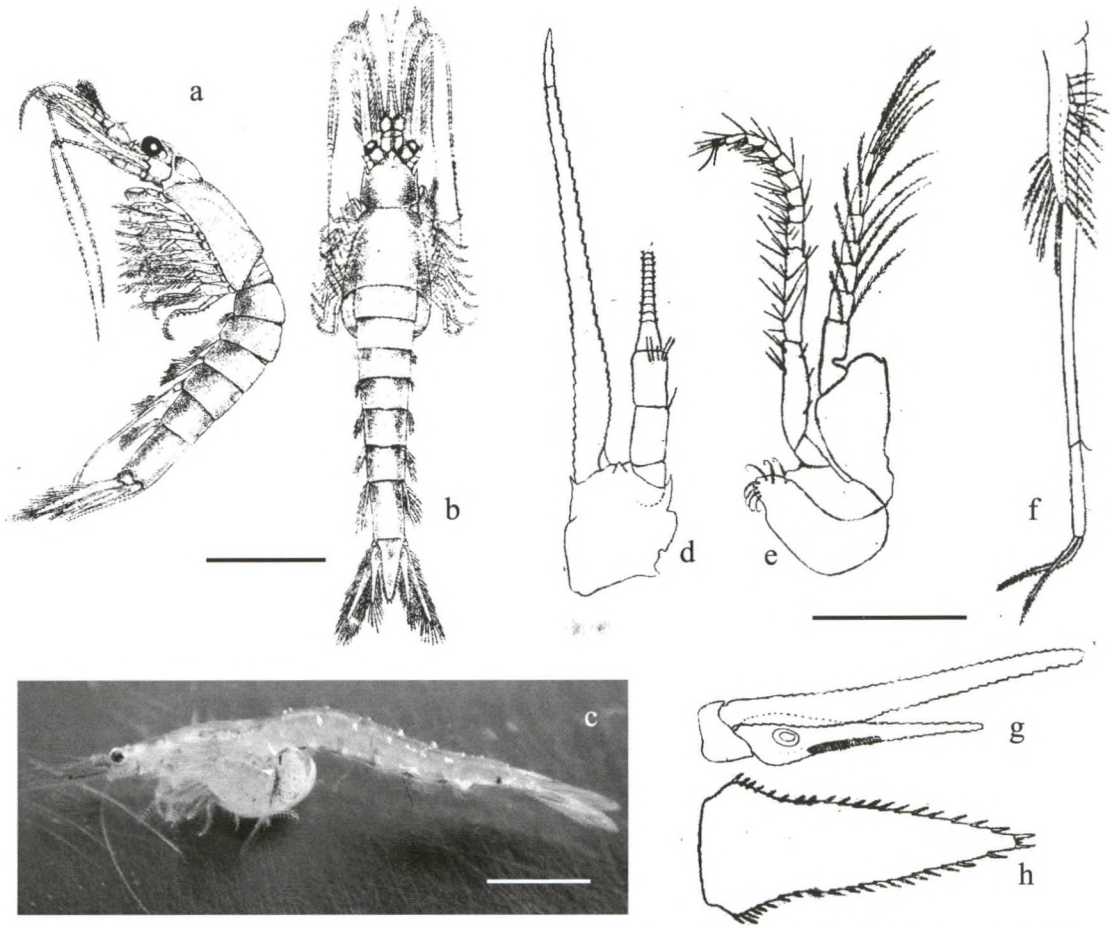

Figure 2: Habitus of adult male (a), female (b) and ovigerous female (c) *Neomysis integer* (scale bar 5 mm). Details on antennal scale (d) , 8th thoracic limb of male (e), 4th pleopod of male (f), uropod (g) and telson (h); scale bar 500 µm. (Drawings from: Tattersall and Tattersall, 1951).

The morphology of the feeding appendages and the intestine of *Neomysis integer* are treated in paragraphs 3.7 and 3.8. The description is adapted from Tattersall and Tattersall (1951), Mauchline (1980), Hayward and Ryland (1995), http://www.marlin.ac.uk/species/taxon_Neomysisinteger.htm and http://ip30.eti.uva.nl/bis/crustacea.php. The telson and antennal scale, important diagnostic characteristics for the species, are particularly susceptible to injury, causing atypical morphology which may lead to misidentification (Hayward and Ryland, 1995).

1.3 Geographical distribution and habitats

Neomysis integer is one of the most common mysids along the Atlantic coast of Western Europe. It is a hyperbenthic, euryhaline and eurythermic species, confined to the brackish environment within estuaries (Tattersall and Tattersall, 1951). It is also abundant in brackish (oligohaline to freshwater) inland water bodies, which were connected to the sea in recent geological history, *i.e.* brackish water ponds, lakes, ditches and canals (Tattersall and Tattersall, 1951; Bremer and Vijverberg, 1982). Occasionally, the species is reported for the open sea in British and Belgian continental waters (Kramp, 1910; Makings, 1977; Dewicke, 2002), but it is very uncommon there. *N. integer* is also reported as a resident species in the Belgian surf zone water (Lock *et al.*, 1999, Beyst, 2001; Beyst *et al.*, 2001), although with low densities (< 1 ind m^{-2}).

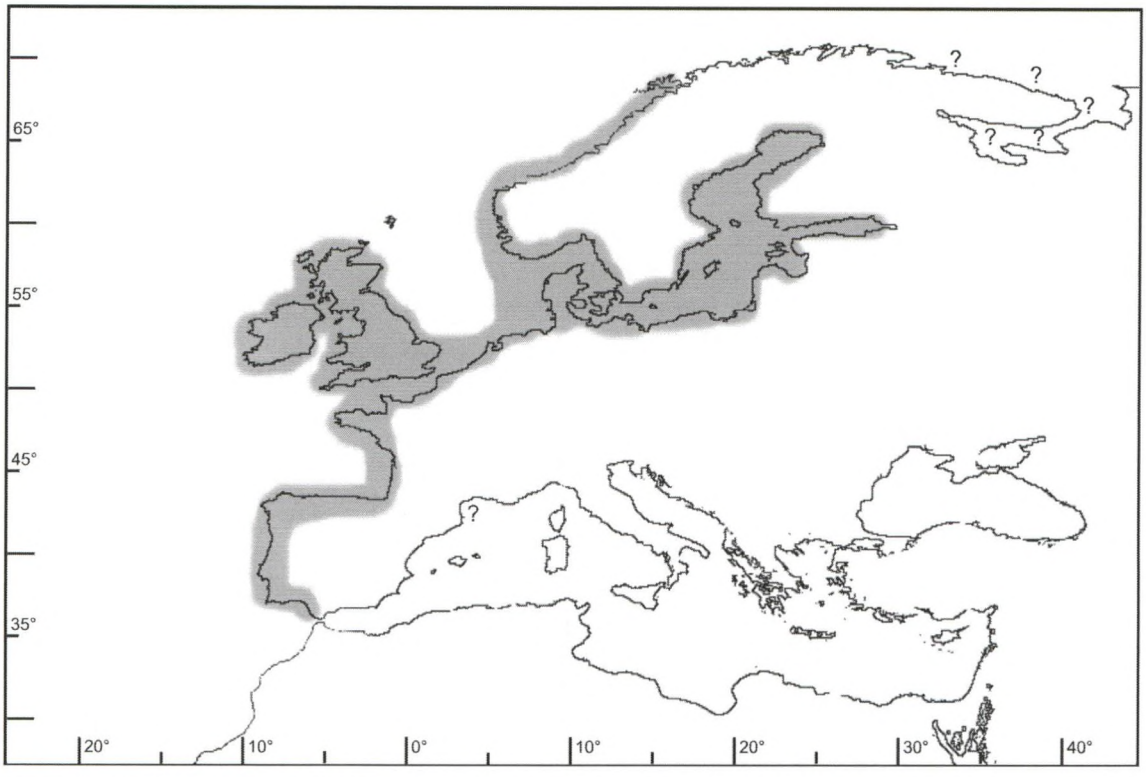

Figure 3: Distribution of *Neomysis integer* based on records in literature (Based on: Remerie, 2005).

Neomysis integer is commonly recorded along the Atlantic coastline and Britain between the longitudes 68°N (coast of Norway, Lofoten) and 36°N (South coast of Spain) and in the Baltic Sea (Figure 3, Appendix 1). The species is reported to occur more to the North (68 to 71°N), up till the Murman coasts of the Barents Sea and the White Sea (Wagner, 1885 referring to Jarzynsky), but recent observations do not confirm this (Väinölä, personal communication). Also, the two records of *N. integer* along the Mediterranean coastline in the canal d'Arlès and the mouth of the Rhone estuary (Bacescu, 1941) have not been confirmed since (Wittmann and Ariani, 2000).

1.4 Sampling

Mauchline (1980), Janssen (1985) and Mees and Jones (1997) summarize the different quantitative sampling techniques that can be applied to catch *Neomysis integer* in a variety of habitats. One should keep attention to the fact that the swarming behaviour, the vertical and horizontal migration, the often apparent segregation of the life stages and the escape behaviour of *N. integer* can handicap the exact estimation of their abundance and biomass, and hampers the calculation of the production and the study of the life history of the species (Mauchline, 1980; Arndt and Jansen, 1986; Thiel, 1992; Mees *et al.*, 1994).

2 Distribution patterns

2.1 Density, biomass and associated species in estuaries

Because of its sediment affinities (Hough and Naylor, 1992; David *et al.*, 2005), *Neomysis integer* can be considered as an element of the hyperbenthos (syn. suprabenthos), *i.e.* the fauna living in the lowest meter of the water column. Baseline studies on the spatial and temporal patterns in the estuarine hyperbenthic community, and on the contribution of *N. integer*, have been published for several NE Atlantic estuaries (Table 1).

Table 1: Available studies on the spatial and/or temporal distribution of *Neomysis integer* in the hyperbenthos of western European estuaries.

Latitude	Estuary	Source	Max density (biomass)
53°N	Elbe	Bernát et al., 1994; Köpke and Kausch, 1996	210 m^{-3} (bottom) 4-360 m^{-3} (surface)
53°N	Weser	Schuchardt et al., 1989; Haesloop, 1990	-
53°N	Jade Bay	Apel, 1992	-
53°N	Eems	Mees et al., 1995	22 m^{-2}
53°N	Conwy	Hough and Naylor, 1992	-
52°N	Shannon	O'Sullivan, 1984	-
51°N	Severn	Bamber and Henderson, 1994; Moore et al., 1979	-
51°N	Schelde	Subtidal: Mees and Hamerlynck, 1992; Mees et al., 1993; 1993b; 1994; 1995	160-190 m^{-2} (225mgADW m^{-2})
		Marshes: Cattrijsse et al., 1994; Hampel et al., 2003; 2003b	240 m^{-3} (840mgADW m^{-3})
50°N	Tamar	Milner, 1986 Moffat and Jones, 1992; Moffat, 1996	1400 m^{-3} 200 m^{-3}
49°N	Seine	Zouhiri et al., 1998; Mouny et al., 1996; 1998, 2000; Dauvin et al., 2000	667 – 2160 m^{-3} (200mgADW m^{-3})
45°N	Gironde	Sorbe, 1980; Mees et al., 1995; David et al., 2005	26 m^{-2}
43°N	Ria Deba	San Vicente, 1993; 1996	< 0.5 m^{-2}
43°N	Ria Urola	San Vicente, 1993; 1996	< 0.5 m^{-2}
43°N	Ria Oria	San Vicente, 1993; 1996	< 0.5 m^{-2}
43°N	Ria Uremea	San Vicente, 1993; 1996	< 0.5 m^{-2}
43°N	Ria Bidasoa	San Vicente, 1993; 1996	Absent
40°N	Ria de Aveiro	Cunha et al., 1999	-
37°N	Ria de Alvor	Rodriguez and Dauvin, 1987	-
36°N	Bay of Cádiz	Drake et al., 1997	-
36-37°N	Guadalquivir	Drake et al., 2002	9 m^{-3} (59mgWW m^{-3})

The hyperbenthic fauna in the upper reaches of European estuaries is dominated by the mysids *Neomysis integer* and *Mesopodopsis slabberi* (Sorbe, 1980; Mees *et al.*, 1993; 1993b; 1994; 1995; Moffat and Jones, 1992; 1993; Moffat, 1996; Köpcke and Kausch, 1996; Drake *et al.*, 2002; David *et al.*, 2005). In winter, the latter species is virtually absent in the estuary (*e.g.* Schelde, Tamar, Jade). Throughout the year *N. integer* occupies the upper, oligohaline zone (*e.g.* Moffat and Jones, 1992; Mees *et al.*, 1994; 1995), both in the subtidal and the adjacent marshes (Ralph, 1965; Mees *et al.*, 1993b). It is generally found in lower saline water than *M. slabberi* and recorded right up to the point at which the high-tide salinity is 0.1 psu (Percival, 1929; Sorbe, 1980; Zouhiri *et al.*, 1998).

Neomysis integer is typically associated with the zone of highest suspended matter concentrations (Bernát *et al.*, 1994; Mees *et al.*, 1995; Moffat, 1996; Köpcke and Kausch, 1996; Fockedey, unpublished).

The (daytime) vertical distribution of *Neomysis integer* in and around the maximum turbidity zone of the Elbe, Schelde and Gironde estuaries was studied by Fockedey (unpublished – EU project MATURE). By sampling a shallow system in two depth strata (near the surface and near the bottom) with a High Speed Plankton Sampler (mesh size 1 x 1 mm), the entire water column (generally < 10 m) was considered to be sampled and the relative abundance of *N. integer* in the two layers was compared.

In turbid waters, *i.e.* when suspended particulate matter concentrations were higher than 200 mg l^{-1}, the *Neomysis integer* population was evenly distributed over the water column, as was the case for the maximum turbidity zone of the Elbe and along the entire longitudinal transect of the Gironde. In the Schelde estuary, *N. integer* was predominantly present near the bottom during daytime, as turbidity is relatively low compared to the two other estuaries.

In many estuaries this zone of maximal turbidity at the fresh-water/brackish-water interface (maximum turbidity zone MTZ or estuarine turbidity maximum ETM) is associated with low dissolved oxygen levels (Costa and Elliott, 1991; Baeyens *et al.*, 1998; Mouny *et al.*, 2000; Roast *et al.*, 2002), especially in summer periods. These low dissolved oxygen levels cause problems for metazoan life. Concentrations lower than 40 % oxygen saturation level (*i.e.* 3.3 – 5.1 mg l^{-1} at 5 – 25 °C) are limiting for *N. integer* (Mees *et al.*, 1995), while concentrations lower than 7 mg l^{-1} produce a barrier for upstream migration of estuarine fish (Costa and Elliott, 1991). *N. integer* is found in the Schelde at a much higher salinity (10 – 15 psu) than in other, less organically polluted estuaries where the population maximum is found at around 3 – 5 psu. In the polluted Schelde, the maximal population densities are found near the limit of viable oxygen concentrations regardless of salinity (Mees *et al.*, 1993, 1993b, 1994; 1995), where no adverse oxygen conditions hamper their development.

In shallow estuarine water, *Neomysis integer* is often associated with the coexisting mysid *Praunus flexuosus* (Muus, 1967; Mauchline, 1971; Välipakka, 1992). *P. flexuosus* is more restricted to intertidal areas and occurs in much lower numbers in comparison with *N. integer*. Other typical associated hyperbenthic species in the subtidal are Gammarids (*Gammarus zaddachi, Gammarus salinus*), the isopod *Eurydice pulchra*, shrimp (*Crangon crangon, Palaemonetes varians, Palaemon longirostris*) and fish (*Pomatoschistus microps, P. minutes, Pleuronectes flesus* and larvae and juveniles of clupeids) (Moore *et al.*, 1979; Sorbe, 1980; Rudstam *et al.*, 1986). Typical associated zooplankton species are the calanoid copepods *Eurytemora affinis* and *Acartia* spp. (Moore *et al.*, 1979; Castel, 1993; Soetaert and Van Rijswijk, 1993; David *et al.*, 2005). The endobenthic fauna of the oligohaline zone of the estuary is impoverished (mainly *Corophium* sp., oligochaetes, and chironomids), due to the high turbidity clogging their feeding mechanisms, salinity stress and pollution, in addition to possible regular dredging of the upstream shipping channels (Pearson and Rosenberg, 1987; Ysebaert *et al.*, 1993; Mouny *et al.*, 1998).

Densities

Most authors (*e.g.* Moore *et al.*, 1979; Mees *et al.*, 1994) report distinct peaks of density (and biomass) of *Neomysis integer* in spring and summer, while densities generally drop in wintertime. Seasonal fluctuations in the densities can be partially explained by periods of active breeding when the population increases in size or by predation and increased winter mortality when the densities drop (Mauchline, 1971c; Gameson, 1982), although migration can also take part in explaining variable densities (Schuchardt *et al.*, 1989). The geographical position of the population within the estuary shows tidal variation due to advection within the tidal cycle and shows seasonal variation due to the freshwater discharge, with a more downstream position in winter and a more upstream position in summer and autumn (Sorbe, 1980; Schuchardt *et al.*, 1989; Hough and Naylor, 1992; Köpcke and Kausch, 1996; Mouny *et al.*, 1996).

In the subtidal of the **Schelde estuary**, the peak densities were 160 – 240 ind m^{-2} in spring and summer (Mees *et al.*, 1994; 1995; Mees and Jones, 1997). In the marshes of this estuary, maximal densities of 240 ind m^{-3} were recorded in early summer (Cattrijsse *et al.*, 1994; Hampel *et al.*, 2003; 2003b). In the bottom waters of the main channel in the **Elbe estuary**, the densities of *N. integer* were more or less constant during the day (210 ind m^{-3}), while at the surface densities fluctuated between 4 and 360 ind m^{-3}, with maximal values at the beginning of the ebb tide, especially at night (Bernát *et al.*, 1994; Köpcke and Kausch, 1996). Moffat and Jones (1992) and Moffat (1996) reported maximal *N. integer* densities of 200 ind m^{-3} in the **Tamar estuary** in late spring. Their value is considered to be an underestimate due to inefficient sampling of the larger immatures and adults with fine meshed plankton nets. In the same estuary, Milner (1986) reported a density of up to 1400 ind m^{-3} during summer. For the meso-oligohaline area of the **Seine estuary**, maximal densities of 667 and 2160 ind m^{-3} were reported for spring and autumn respectively (Zouhiri *et al.*, 1998; Dauvin *et al.*, 2000). In the **Gironde and Eems estuaries**, maximum densities were much lower in summer, respectively 26 and 22 ind m^{-2} (Mees *et al.*, 1995). In the **Guadalquivir**, mean density of *N. integer* was 9 ind m^{-3} in the surface water (Drake *et al.*, 2002). In the small **Rias Deba, Urola, Oria and Uremea** the estimated densities never exceeded 0.5 ind m^{-2} (San Vicente, 1993). See Table 1.

Neomysis integer is the most widespread mysid species in the **Baltic Sea** (Köhn, 1992). It is a typical species of very shallow waters (Välipakka, 1992) with densities of 100, 10 and 1 ind m^{-3} at respective depths of 0.5, 2 and 5 metres (Weslawski, 1981). In shallow Danish estuaries and lagoons the maximal densities of *N. integer* can amount to 3000 – 4000 ind m^{-2} (Muus, 1967). Rasmussen (1973) even reported 50000 ind m^{-2}. In open water, the species is far less abundant than the co-occurring *Mysis mixta* and *Mysis relicta* (Margonski and Maciejewska, 1999). Generally, the densities of *N. integer* are higher along the southern coasts of the Baltic Sea in comparison with the more northern areas (Thiel, 1992). In the northern Baltic region, with low densities of 24 ind m^{-2} (Hansson, 1990) *N. integer* contributes only 3 % of the total mysid biomass (Rudstam *et al.*, 1992). In the South, densities between 160 ind m^{-2} (Arndt and Jansen, 1986) to more than 600 ind m^{-2} (Thiel, 1992) were reported for shallow stations, while in open waters the densities reported by Margonski and Maciejewska (1999) were only 15 ind m^{-2}. In the middle reaches of the Baltic, along the Swedish coast of the Baltic proper, maximal densities of *N. integer* were 24 ind m^{-2} (Rudstam *et al.*, 1986) and in the Gulf of Riga (eastern Baltic, Latvia) maximal densities were 20 – 40 ind m^{-2} (Kotta and Simm, 1979).

Biomass

The standing stock available at a certain moment is usually estimated by calculating the available biomass per surface or volume unit (in dry weight or ash free dry weight) using the length frequency distributions and a length-dry weight regression (Table 2). These regressions do not show significant differences between males and females (Beattie and de Kruijf, 1978; Mees *et al.*, 1994). The relationship shows no variation with the time of the year (Beattie and de Kruijf, 1978; Mees *et al.*, 1994), although Aaser *et al.* (1995) constructed separate regressions for winter and summer. When formalin-preserved animals are used for the biomass determination, some authors (*e.g.* Bremer and Vijverberg, 1982) use a correction factor for the weight loss and add 10 % to the calculated dry weight (Beattie, 1982). Some studies directly weigh the total biomass available as wet weight. The (dry weight) standing stock is then calculated as 22 % of the wet weight for *Neomysis integer* (Maciejewska and Opalinski, 2002). The ash weight amounts approximately 10 % of the dry weight of *N. integer* (Astthorsson, 1980). The ash free dry weight thus is 90 % of the dry weight. Biomass can also be expressed as volume through the water-displacement method based on the principle that an immersed mass displaces its own mass of water (Margonski and Maciejewska, 1999).

In the **Schelde estuary**, the standing stock biomass of *Neomysis integer* peaked in late spring with 225 mgADW m^{-2} (Mees *et al.*, 1994). In the marshes along this estuary, maximal biomass values of 840 mgADW m^{-3} were measured in the same period (Cattrijsse *et al.*, 1994). In the **Seine estuary**, the biomass of the species peaked in September with 200 mgADW m^{-3} (Dauvin *et al.*, 2000). In the **Guadalquivir**, the mean biomass of *N. integer* only amounted to 58 mgWW m^{-3}, *i.e.* 13 mgDW m^{-3} (Drake *et al.*, 2002). In the southern **Baltic** the maximal biomass was estimated as 620 (Arndt and Jansen, 1986) and up to 9.5 g WW m^{-2} (*i.e.* 2100 mgDW m^{-2}) in the shallow areas (Thiel, 1992). See table 1.

Table 2: Length – weight regressions available for *Neomysis integer.*

lnADW = -5.539 + 2.267 lnSL	n=100; r^2=0.997	Mees et al., 1994
lnDW* = 1.432 + 2.853 lnSL	n=9, r^2=0.99, p<0.001	Irvine et al., 1995
Log DW = 2.7847 log TL – 2.5229	N=132; r=0.99, p<0.01	Astthorsson, 1980
DW = 0.00391 $TL^{2.48}$	n=15; r^2=0.998	de Kruijf, 1977
DW = 0.0041 $SL^{2.78}$	n=51; r^2=0.97, p<0.0001	Gorokhova and Hansson, 1999
DW = 0.00254 $TL^{2.7676}$	r=0.99	Jansen, 1985c; Arndt and Jansen, 1986
DW = 0.00638$TL^{2.435}$	n=100; r^2=0.98; p<0.0001	Aaser et al., 1995
DW = 0.00347 $TL^{2.7046}$ (winter)	n=50; r^2=0.98; p<0.0001	Aaser et al., 1995
DW = 0.00621$TL^{2.4873}$ (summer)	n=50; r^2=0.98; p<0.0001	Aaser et al., 1995
DW = 0.0016 $TL^{3.086}$	n=201; r^2=0.97	Winkler and Greve, 2004
log_{10}DW = -0.58 + 2.64 log_{10}TL	N=45; r=0.99	Summers, 1980
WW = 0.0022715 $TL^{3.46}$	r=0.996	Jansen, 1985c; Arndt and Jansen, 1986

With **DW**: dry weight (in mg); **DW***: dry weight (in µg); **WW**: wet weight (in mg); **ADW**: ash free dry weight (in mg); **SL**: Standard length, *i.e.* from the front of the eyes to the last abdominal segment (in mm); **TL**: Total length, *i.e.* from the front of the eyes to the end of the telson or the end of the uropods excluding the setae (in mm) TL= 1.165 SL - 0.080 (n = 112; r^2 = 0.997; p < 0.001; Mees et al., 1994); TL = 3.78 CL (Schuchardt et al., 1989); **CL**: Carapax length, from the basis of the eyed to the median end of the carapax, in lateral view (in mm); CL= 0.266 SL + 0.439 (n = 112; r^2 = 0.908; p < 0.001; Mees et al., 1994).

2.2 Brackish ponds and lakes

Neomysis integer is common in brackish lakes and ponds which were once connected to the sea until recent historical times. In those enclosed brackish water bodies, the *N. integer* population can undergo blooms and crashes (*e.g.* Barnes *et al.*, 1971; Parker and West, 1979; Fockedey and Remerie, unpublished). Its abundance and biomass can vary considerably between years and between lakes within the same district (Bremer and Vijverberg, 1982; Aaser *et al.*, 1995). In the Frisian brackish lake district, the peak densities reported for the Bergumermeer were 1000 ind m^{-2} in one year and 5800 ind m^{-2} in another year (Beattie and de Kruijf, 1978) or 6 ind m^{-2} in the Tjeukemeer in one year and 110 ind m^{-2} in another year (Vijverberg, unpublished). In the pond Galgenweel (Belgium) maximal densities of 1100 ind m^{-2} were recorded in the 90's (Soselisa, 1994; Fockedey, unpublished), but since 2002 densities dropped to < 1 ind m^{-2} (Verslycke *et al.*, 2000; Fockedey and Remerie, unpublished). The factors controlling these large population fluctuations remain to be determined. Annual variations in densities can be caused by adverse winter conditions (*e.g.* low oxygen concentrations due to ice cover). Another reason for a low standing stock in some years might be due to varying levels of predation by young and larval fish. Also, high summer temperatures, combined with the low salinity of these lakes, might cause a population crash due to physiological constraints of *N. integer*. In deeper lakes the colder, but often anoxic, hypolimnion is not a refuge from the warm surface layers (Parker and West, 1979). The decline of a *N. integer* population in the Belgian pond Galgenweel might be related to the invasion of the Pontocaspian invader *Hemimysis anomala* (Verslycke *et al.*, 2000).

Some lakes can support this very high density and biomass, while other lakes sustain a significantly lower order of densities. A high density of 1100 – 10400 ind m^{-2} was reported for *Neomysis integer* in Danish, Belgian and English brackish lakes (Irvine *et al.*, 1993; 1995; Jeppesen, *et al.*, 1994; Soselisa, 1994; Aaser *et al.*, 1995), while maximal densities of 6 ind m^{-2} are registered in the Slotermeer (Bremer and Vijverberg, 1982) or 0.5 ind m^{-2} in Lake Grevelingen (Platenkamp, 1983). The species is mainly abundant in those ponds or lakes with poorly developed submerged vegetation. According to Espeel (1982), wave action in these ponds is stronger due to the absence of macrophytes and more detritus is brought into suspension, providing a food resource for *N. integer*.

High densities of *Neomysis integer* in brackish lakes are often associated with a low predation pressure by fish. High densities of 3000 – 13000 *N. integer* ind m^{-3} are present in Danish brackish lakes with low fish biomass (Aaser *et al.*, 1995; Jeppesen, *et al.*, 1994) and a removal of 75 % of the fish stock in a Dutch Lake resulted in a major increase in *N. integer* density (Hosper and Meijer, 1993; Meijer *et al.*, 1994). When fish predation decreased due to a toxic bloom, the population size of *N. integer* substantially increased in Hickling Broad, a brackish lake in England (Irvine *et al.*, 1993). However, high densities of *N. integer* in brackish lakes may also reflect the hypertrophic character of the water mass (Aaser *et al.*, 1995), where the species even enhances eutrophication, both directly and indirectly (see later paragraph). In the brackish lake Hickling Broad, the *N. integer* population itself was suppressed by toxic algal blooms stimulated by the ingress of black-headed gull guano (Bales *et al.*, 1993).

The maximal biomass of *Neomysis integer* (in summer) in brackish lakes can be less than one mg (Bremer, 1980), several mg (Platenkamp, 1983), around 1g (Soselisa, 1994; Aaser *et al.*, 1995; Irvine *et al.*, 1995) or in the order of several grams DW m^{-2} (Beattie and de Kruijf, 1978).

In brackish lakes and pools, *Neomysis integer* is often associated with *Palaemonetes varians* (Norman, 1892; Kemp, 1910) and *Pleuronectes flesus* (Parker and West, 1979). In the more saline Lake Grevelingen, the species lives together with *Praunus flexuosus*, *Idotea chelipes* and *Gammarus locusta* (Platenkamp, 1983).

2.3 Swarming behaviour

Large line-formed swarms of *Neomysis integer* have been observed in shallow estuarine waters (Percival, 1929; Mauchline, 1971c; Arndt and Jansen, 1986; Walesby, 1973; Köpcke and Kausch, 1996). The shoals are one to two meters across by up to 10 m long and stay approximately at 10 cm under the water surface (Parker and West, 1979). At the turn of the tide at flood and some time afterwards during ebbing, the shallow water along the edge of the estuary and the edges of mud banks can be overcrowded with *N. integer* thus forming a thick 'mysid soup' (Percival, 1929). Köpcke and Kausch (1996) encountered far higher densities (1230 ind m^{-3}) in a shallow area of the upper Elbe estuary as compared to the subtidal (210 ind m^{-3}). Also, in the Baltic and in brackish water ponds the species has an extremely aggregated spatial distribution (*e.g.* Espeel, 1982; Arndt and Jansen, 1986; Rudstam *et al.*, 1986).

Although difficult to sample quantitatively (Mauchline, 1971c; Arndt and Jansen, 1986; Thiel, 1992), Roast *et al.* (2004) made an attempt to realistically estimate the maximal density of *Neomysis integer* swarms present over a subtidal flat at low tide by using a dip net and a Perspex cylindrical drop trap. They obtained densities of 11000 to 27000 ind m^{-2} or 36000 to 89000 ind m^{-3}. The volume taken by the swarms was extremely variable, ranging from 0.07 to 1.1 m^3 and containing from 6000 to 51000 individuals.

Next to creating a protection against predation (see later paragraph), increasing the reproductive success and helping in maintaining position (Clutter, 1969), living in a shoal may improve the filtration of suspended food by creating unidirectional currents (Zelickman, 1974). Individuals in large swarms had a decreased individual respiration rate and saved energy (Ritz *et al.*, 2001). For other aggregating crustaceans like Penaeids (Cortoni Valenti *et al.*, 1993), daphniids (Burns, 2000) and euphausiids (Haywood and Burns, 2003), it has been demonstrated that the cost of forming aggregations lead to a decreased growth rate and a lower asymptotic length (possibly by allelopathy). This is not yet demonstrated for *Neomysis integer*.

2.4 Segregation / Partitioning of the life stages

It has often been observed that juvenile and adult stages of *Neomysis integer* shoal separately (Tattersall and Tattersall, 1951; Kinne, 1955; Milner, 1986). Adults of *N. integer* are frequently found in dense swarms near the shore margins or in tidal marshes at certain phases of the tidal cycle in estuaries, while juveniles stay in deeper water (Välipakka, 1992). Sorbe (1980) described the reverse situation in the Gironde estuary with the juveniles occupying the shore, while the adults prefer the subtidal reaches.

Ralph (1965) noted that females utilize the marsh creeks, while newly released juveniles in the smallest size classes and males did not co-occur there. Cattrijsse *et al.* (1994) observed juveniles to be more present in the marsh than in samples from the subtidal of the Schelde estuary. Kinne (1955), Parker and West (1979) and Platenkamp (1983) did not find the early post-marsupial juveniles (2 – 4 mm total length) in bottom samples. This segregation of juveniles from the adults can help to prevent cannibalism (Välipakka, 1992). The swimming speed of adults and juveniles of a species can differ considerably, with the result that a mobile shoal or school becomes a self-sorting mechanism, separating age groups and so producing swarms of individuals of similar body size (Clutter, 1969). Differential swimming speed of juveniles and adults has not been investigated yet for *Neomysis integer*.

Some authors found the larger *Neomysis integer* situated more upstream along the subtidal, longitudinal estuarine gradient than the juveniles. In the Tamar estuary, there is some evidence that the mature and immature mysids move upstream to occupy the lower salinity zones during some periods of the year (spring and September), while juveniles are fairly evenly distributed along the salinity gradient (Moffat, 1996). Smaller juveniles were found in higher salinities than the larger size classes of juveniles (Milner, 1986; Moffat, 1996). In the Conwy, Gironde and Weser estuaries, males occurred more commonly down-estuary, and gravid females and juveniles more upstream (Sorbe, 1980; Schuchardt *et al.*, 1989; Hough and Naylor, 1992). Also, Astthorsson (1980) observed a geographical segregation between the sexes in the Ythan. The different life stages must be able to select particular physico-chemical regimes (mainly temperature, salinity and current speed) in order to achieve the partitioning seen in the population along the gradient. The occurrence of ovigerous females higher up the estuary may relate to the selection of optimal salinity for embryonic development (Hough and Naylor, 1992). Small sexual and/or ontogenic differences in the substrate preference might also be the cause of this segregation in brackish lakes (Beattie and de Kruijf, 1978).

2.5 Position maintenance in the estuary

In estuaries, *Neomysis integer* faces the problem of retaining its position against conditions of net seaward transport (Hough and Naylor, 1992; Roast *et al.*, 1998). In general, there are three main control mechanisms for the positioning of invertebrate populations in estuarine systems: (1) reproductive compensation of seaward losses, (2) behavioural adaptations like alterations in swimming activity at different tidal phases, and (3) use of hydrodynamic processes within the estuary (*e.g.* Siegfried *et al.*, 1979; Wooldridge and Erasmus, 1980; Wooldridge and Bailey, 1982; Moffat and Jones, 1993; Schlacher and Wooldridge, 1994).

Position maintenance has a high environmental relevance, as the mysids have to maintain in areas of optimum feeding conditions, lowest competition and lowest vulnerability to predation. However, due to the position maintenance, the animals experiences tidal and seasonal salinity and temperature fluctuations (Roast *et al.*, 1998). In the Looe estuary for example, *Neomysis integer* is exposed to daily temperature fluctuations of 5 to 15 °C and daily salinity fluctuations of 1 to 34 psu (Roast *et al.*, 1998). In the Schelde the daily and seasonal fluctuations are smaller (Mees *et al.*, 1993), while in other estuaries like for example Elbe, Weser, Conwy and Gironde the tidal variations are notable (Sorbe, 1980; Schuchardt *et al.*, 1989; Hough and Naylor, 1992; Köpcke and Kausch, 1996; Mouny *et al.*, 1996).

Neomysis integer maintains its position in estuaries by a combination of the three previously described factors: by a reproductive compensation through the production of relatively large numbers of juveniles per brood, by using hydrodynamic processes within the estuary (Moffat and Jones, 1993) and by an adapted swimming behaviour (Roast *et al.*, 1998). It migrates during the ebbing tide laterally out of the main channel towards the shallow areas with lower current velocity. By horizontal lateral movements prior to low water tide, *N. integer* can take advantage of the upstream transport provided by the subsequent flood tide and thereby compensates for the downstream drift (Köpcke and Kausch, 1996). The mysids stay predominantly close to the bottom during the peak ebbing tide. At the low tide and the beginning of the flood tide, *N. integer* undertakes vertical and lateral migration to reach the surface of the water in the channel of rapid current flow in order to be transported upstream (Köpcke and Kausch, 1996; Fockedey, unpublished).

In laboratory experiments, Hough and Naylor (1992) demonstrated a maximum swimming activity by *Neomysis integer* after the time of the expected high tide, which indicates that there is an endogenous ebb-phased circa-tidal rhythmicity. This result contrasts with the above described field data to prevent displacement of the animals out of the estuary. In general, mysids (also *N. integer*) exhibit positive rheotactic behaviour and swim forward into the current at low current velocities (Parker and West, 1979; Hough and Naylor, 1992; Roast *et al.*, 1998). At velocities greater than 12 cm s^{-1}, position maintenance of *N. integer* breaks down (Roast *et al.*, 1998) and animals are displaced by the current. In the East Looe river estuary (UK), *N. integer* is generally only present in areas where current velocities do not exceed 15 cm s^{-1} (Roast *et al.*, 1998; Lawrie *et al.*, 1999). Persistent swimming throughout the ebb tide under endogenous control, coupled with the rheotactic behaviour prevents the *N. integer* individuals occupying shallow zones of stranding in a pool in the intertidal. Aggregations of *N. integer* in imminent risk of stranding initially head into the current, but as the water level drops, and before a pool is completely cut off, the species swims with the current draining from the pool and enters the stream before finally re-orientating and swimming into the current (Hough and Naylor, 1992). In the inner part of Arcachon Bay near the mouth of the l'Eyre river, a substantial part (12 %) of the macrobenthic community of intertidal mudflats sampled during ebb consisted of (stranded) *N. integer* (Bachelet and Dauvin, 1993).

As swimming speed is an important factor in the position maintenance of the species, it is likely to be beneficial for the mysid to utilize any available shelter in order to conserve energy (Roast *et al.*, 1998b). The species aggregates in shallower water with low current velocity (Roast *et al.*, 1998; Lawrie *et al.*, 1999) and in the lee of rocks and macroalgal clumps where water flow rates were less than 10 cm s^{-1} (Hough and Naylor, 1992; Lawrie *et al.*, 1999). In experimental conditions, Roast *et al.* (1998b) observed *Neomysis integer* to enter the boundary layer where lower velocity flows are experienced. This corresponds with field studies, where *N. integer* is generally caught in greatest abundance in near-bottom samples (Hough and Naylor, 1992; Bernát *et al.*, 1994; Köpcke and Kausch, 1996; David *et al.*, 2005; see earlier remark of Fockedey, unpublished). Attachment to the substratum and even shallow burrowing into the sediment are also common means of position maintenance in moving waters (Roast *et al.*, 1998b).

A decreased swimming activity of *Neomysis integer* was demonstrated under conditions of hypoxia and pollution with heavy metals and pesticides (Roast *et al.*, 2000b; 2000c; 2001b; 2002; 2002b). *In situ* this alteration in the swimming activity may be reflected in an altered position maintenance of the population in the estuary (cfr. Schelde?).

2.6 Vertical and horizontal migration

Vertical migration

Diel and tidal vertical migration and distribution patterns of *Neomysis integer* in **estuaries** were described for populations in the Elbe (Bernát *et al.*, 1994; Köpcke and Kausch, 1996; Fockedey, unpublished – EU project MATURE), Severn (Moore *et al.*, 1979), Schelde (Mees, unpublished – EU Project JEEP; Fockedey, unpublished - EU project MATURE), Seine (Zouhiri *et al.*, 1998; Dauvin *et al.*, 2000) and Gironde (Fockedey, unpublished - EU project MATURE). A considerable amount of the *N. integer* population is distributed all over the water column in the turbid reaches of the estuary, especially at the beginning of the flooding tide (just after low water) when the current velocity is low (Köpcke and Kausch, 1996; Fockedey, unpublished). In the Elbe, the animals showed a pronounced day-night rhythm in their vertical distribution pattern (Bernát *et al.*, 1994; Köpcke and Kausch, 1996; Fockedey, unpublished). During daylight hours the mysids remained near the bottom, while during the night equally high numbers were found in bottom and surface waters. In the Seine estuary, the mysids were sampled near the bottom in high abundance only during the day at low tide. At high tide and during the night the densities in the benthic boundary layer were up to seven times lower, due to dispersal into the water column (Zouhiri *et al.*, 1998; Dauvin *et al.*, 2000). In the Westerschelde and Gironde estuaries, *N. integer* did not show a diurnal vertical migration pattern (Fockedey, unpublished).

Diel vertical migration of *Neomysis integer* in the **Baltic** is well documented (Jansson and Källander, 1968; Jansen, 1985; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; Debus *et al.*, 1992). In deeper stations of the Baltic *N. integer* stays near the sediment during daytime and migrates to the surface with the beginning of the evening twilight (Rudstam *et al.*, 1986; Debus *et al.*, 1992).

Also the diurnal vertical migration patterns of *Neomysis integer* in shallow **brackish lakes** are well described (Beattie and de Kruijf, 1978; Bremer and Vijverberg, 1982; Irvine *et al.*, 1993; Aaser *et al.*, 1995). From noon until sunset, virtually 100 % of the population is living near the bottom. At midnight the animals are more or less evenly distributed over the water column. At sunrise a substantial part of the population is still present in the water column. Densities of *N. integer* caught at the bottom (with bottom traps) during daytime can be 2 to 3-fold greater than at night, while daytime densities based on vertical hauls are 4-fold less than at night (Aaser *et al.*, 1993). There are no evident differences in the population structure over the water column at night, indicating that all stages and both sexes perform the vertical migration to the same extent (Beattie and de Kruijf, 1978). The vertical migration is more pronounced in summer than in late autumn and winter (Beattie and de Kruijf, 1978). Through their vertical migrations *N. integer* is considered to form a direct link between the benthos and the pelagos (Elliott *et al.*, 2002).

Horizontal migration and distribution patterns

The **longitudinal distribution** of the population of *Neomysis integer* along the estuary can change in response to seasonal changes in the position of the salinity gradient and the maximum turbidity zone, which in turn are influenced by the long-term seasonal cycle of freshwater run-off (Moore *et al.*, 1979; Sorbe, 1980; Eisma, 1986; Köpcke and Kausch, 1996; Allen *et al.*, 2003). In winter and spring, during high run-off, the *N. integer* population of the Elbe estuary was geographically situated more downstream than in summer. However, the salinity range where the species is present varied from 1.2 – 27.7 psu in winter to a more compressed salinity zone at 0.6 – 3.4 psu in summer (Köpcke and Kausch, 1996). In the Schelde, however, the salinity zones are relatively stable and the *N. integer* population remained in a narrow zone of 20 km close to the Dutch-Belgian border due to adverse oxygen conditions more upstream, regardless of salinity (Mees *et al.*, 1994). Hough and Naylor (1992) designated the middle part of the Conwy as a distribution centre for *N. integer*, from which the species spread upstream and downstream in the estuary as their numbers increased during summer. *N. integer* is also reported to perform active seasonal migration along the longitudinal axis of the estuary (Kühl and Mann, 1969; Schuchardt *et al.*, 1989).

In the estuarine environment, *Neomysis integer* actively migrates during certain periods of the **tidal** cycle from the subtidal channel towards the sheltered shores with low current velocities (Astthorsson, 1980; Hough and Naylor, 1992; Mees *et al.*, 1993b; Köpcke and Kausch, 1996; Speirs *et al.*, 2002; see former paragraphs). Speirs *et al.* (2002) could demonstrate that the high numbers of *N. integer* over the immersed intertidal mudflats were not the result of a passive diffusion process, nor the result of an attraction to the organically rich sediments as feeding grounds, nor a predator avoidance response, but were the result of an active migration to areas with a low current velocity.

Neomysis integer is known to make extensive use of **salt marshes** on the margins of estuaries (Ralph, 1965; Jansen, 1985; Mees *et al.*, 1993b; Cattrijsse *et al.*, 1994; Hampel *et al.*, 2003; 2003b). The non-vegetated subtidal channels of European marshes do not provide shelter to predation. On the contrary, migration into the intertidal marsh is associated with an increased predation pressure on the mysids by juvenile and adult fish and shrimp, using the marsh respectively as a nursery and feeding ground (Cattrijsse *et al.*, 1994; Dean *et al.*, 2005). In the Schelde estuary, *N. integer* follows the edge of the tidal cycle and makes maximal use of the salt marsh from the first hour to the last hour of the high water period (Cattrijsse *et al.*, 1994; Hampel *et al.*, 2003). Migration in and out of the marsh is maximal when current velocities are minimal, and is considered to be an – at least partly – active process (Cattrijsse *et al.*, 1994). Possibly, the marshes of the Schelde estuary serve as areas favoured by *N. integer* for reproductive purposes. Smaller individuals were more frequently found in the marsh area and peak abundance of juveniles occurred earlier in the season than in the subtidal channels of the estuary (Cattrijsse *et al.*, 1994; Mees *et al.*, 1993). Probably the large amount of detritus inside the marsh creeks attracts the mysids as well (Zagursky and Feller, 1985; Cattrijsse *et al.*, 1994).

Neomysis integer living in the coastal waters of the Baltic Sea performs **seasonal horizontal migrations** from the shallow littoral zone to deeper water when temperatures became too low (< 2 °C) or too high (> 20 °C) (Kinne, 1955; Muus, 1967; Jansen, 1979; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; Thiel, 1992; Välipakka, 1992; Jansen, 1993). Similar observations were made for *N. integer* inhabiting brackish lakes and ponds (Vorstman, 1951; Barnes *et al.*, 1977).

Part of the latter populations can make seasonal horizontal migrations towards almost freshwater environments in the connected ditches and channels. However, *N. integer* is dependent on higher saline waters to complete its reproductive cycle and is not present all year in these fresh water bodies (Haesloop and Scheffel, 1991).

Neomysis integer living in the Baltic performs additional **daily horizontal migrations** from the shallow shores towards the central part of the water body at night. In the daytime, *N. integer* forms large shoals (up to 600 ind m^{-2}) in the shallow nearshore regions of the Baltic Barther Bodden to reduce the danger of predation by visually predating fish. At that moment the organisms are restricted to feed on detritus. At night, when young and small fish do not feed, the swarms break up and *N. integer* migrates to the offshore region to search actively for zooplankton (Debus *et al.*, 1992).

In the stagnant water of brackish lakes and ponds, large variations in the horizontal distribution pattern of *Neomysis integer* are described. These result from the swarming behaviour and the **bottom substrate preferences** of the species (Beattie and de Kruijf, 1978; Bremer and Vijverberg, 1982). In the Slotermeer (The Netherlands), *N. integer* preferred sand substrates with a thin layer of fine mud on top over mudless sand or peat substrates (Bremer and Vijverberg, 1982). In Bergumermeer (The Netherlands), the densities of *N. integer* were on average 3 times higher on the hard sandy bottoms in comparison with the bottoms of soft mud or peat (Beattie and de Kruijf, 1978). In loch Furnace (Ireland), *N. integer* forms 1 metre wide and 1 metre deep band at a few decimetres from the water surface above steep bouldery shores (Parker and West, 1979). Where the bottom was weedy the shoals tend to be more concentrated, while in more exposed areas the shoals tend to be deeper. In shallow parts with stands of *Phragmites* the distribution of *N. integer* is patchier, being associated with bottom features like occasional stones or plants.

3 ROLE OF *Neomysis integer* IN THE FOOD WEB

In the brackish part of estuaries, the high standing stock of many functional units (hyperbenthos, but also epibenthos, mesozooplankton, and both demersal and pelagic fish) is explained by the import of large quantities of allochtonous organic matter (natural inputs and discharges from various effluents). The food web has been described as heterotrophic, *i.e.* respiratory processes exceed the *in situ* autotrophic production and the food web tends to be based on detritus (Hummel *et al.*, 1988; Hall and Raffaelli, 1991; Hamerlynck *et al.*, 1993; Heip *et al.*, 1995). This compares with the autotrophic food chain in the mouth of the estuary, where primary production lies at the basis of the food web (Hamerlynck *et al.*, 1993). Heterotrophic bacteria are not only responsible for the remineralisation of the nutrients (Goosen *et al.*, 1992); they simultaneously constitute the basis of the food web for higher trophic levels (Azam *et al.*, 1983; Sherr and Sherr, 1988; Billen *et al.*, 1990). Detritus and/or their associated bacteria are consumed, directly or indirectly, by the microzooplankton, the mesozooplankton and the hyperbenthos (Fenchel, 1988; Hamerlynck *et al.*, 1993). Fish and epibenthic macro-invertebrates can then feed at this 'secondary energy level'. There is a great need for the description of the feeding ecology of key species in estuarine environments.

3.1 Predators

Mysids are important food items for many demersal and pelagic fish, larger epibenthic crustaceans and wading bird species (Mauchline, 1980). In particular environments, where mysids are present in large numbers (e.g. coastal and estuarine waters), they form an important link in the food web. Often they replace copepod prey progressively in the diet of fish during ontogenic development from the postlarval to juvenile stages (Sorbe, 1981). In estuaries, the hyperbenthos, and mysids in particular, often dominate in the diet of 0-group individuals of commercially important fish and also sustain high densities of non-commercial demersal fish (e.g. gobies), which are an important prey for the larger size classes of the commercial species (Elliott *et al.*, 2002). Despite the differences in the species composition of the fish fauna between estuaries, similar feeding guilds can be distinguished over a wide geographical range. Each estuary has a dominant food web that relies on small detritivore epibenthic crustaceans; in some cases this can be mysids, while in others it relies on gammaridean amphipods or even isopods (McLusky and Elliott, 2004). A mysid feeding guild is described for several estuaries (Costa and Elliott, 1991; Mees and Jones, 1997; Hostens and Mees, 1999; Elliott *et al.*, 2002), although it is replaced by a gammaridean feeding guild in e.g. the Humber (Elliott, personal communication) and in Scottish Loch systems (Kislalioglu and Gibson, 1977), despite the presence of large densities of *Neomysis integer* (Mauchline, 1971; Budd, 2002). Next to these small epibenthic crustaceans, larger epibenthic crustaceans (like *Crangon crangon* or *Palaemonetes varians*) and mesozooplanktonic calanoid copepods (like *Eurytemora affinis*, *Acartia* spp.) also play an important role in the food web of the upper part of estuaries (Costa and Elliott, 1991; Maes *et al.*, 2003). Burke (1995) even suggested that the gradient in mysid densities in North-Carolina estuaries (*Neomysis americana*) can act as a guide for the migration of flounder to their nursery grounds.

The low-salinity reaches of **estuaries**, around the turbidity maximum, have proved their role as a nursery for larval and juvenile stages of marine and freshwater fish species and epibenthic crustaceans (Elliott and Hemingway, 2002). A large biomass of food must be available to sustain the populations of these rapidly growing individuals. In turbid waters, the number of sessile macrobenthic species generally decreases due to burial and/or clogging of their feeding apparatus; macrobenthic prey being largely ignored by fish predators in the upper reaches of the Schelde (Maes *et al.*, 2003). Hyperbenthic animals, like *Neomysis integer*, are present in high densities all over the year (Mees *et al.*, 1994) and certainly contribute to the nursery function of the area (Maes *et al.*, 2003; Hostens and Mees, 1999). Some examples of quantitative studies are available (Table 3) for the estuaries of the Schelde (Hostens and Mees, 1999; Maes *et al.*, 2003), Darss-Zingst (Thiel, 1996), Medway (Van den Broek, 1978), Tagus (Moreira *et al.*, 1992) and Loch Etive (Kislalioglu and Gibson, 1977). Qualitative studies are available for the upper Elbe (Thiel, 2000) and the Forth (Costa and Elliott, 1991).

In the mesohaline part of the **Schelde** estuary (The Netherlands), *Neomysis integer* is preyed upon by at least 15 fish species (Hostens and Mees, 1999). It is a dominant prey item in the stomachs of bib (*Trisopterus luscus*), sand goby (*Pomatoschistus minutus*), Lozano's goby (*P. lozanoi*) and herring (*Clupea harengus*). *N. integer* is also a numerically important food item for whiting (*Merlangius merlangus*), tub gurnard (*Trigla lucerna*), intertidally caught plaice and flounder (*Pleuronectes platessa* and *P. flesus*), sea bass (*Dicentrarchus labrax*), sea snail (*Liparis liparis*), pipefish (*Syngnathus rostellatus*) and hook-nose (*Agonus cataphractus*). For the common goby (*P. microps*), sprat (*Sprattus sprattus*), sole (*Solea solea*) and subtidally caught plaice (*P. platessa*) *N. integer* was only supplementary food. In contrast to the intertidal, the stomachs of subtidally caught flounder never contained *N. integer*. In the upper reaches of the Schelde estuary (power plant of Doel, Belgium) the diet of *Pomatoschistus minutus*, *Anguilla anguilla* and pike perch (*Stizostedion lucioperca*) was dominated all over the year by the mysid species (Maes *et al.*, 2003). *N. integer* and *Mesopodopsis slabberi* are important food supplements for young clupeids (*Clupea harengus* and *Sprattus sprattus*), sea bass (*Dicentrarchus labrax*) and *Pomatoschistus microps* in the Schelde estuary, especially in autumn (Maes *et al.*, 2003).

Next to fish, the very abundant caridean shrimp and prawn (*Crangon crangon*, *Palaemonetes varians* and *Palaemon longirostris*) are also important predators of the estuarine *Neomysis integer* population (Kemp, 1910; Marchand, 1981; Sorbe, 1983b; Mouny *et al.*, 1998; Hostens and Mees, 1999; Maes *et al.*, 2003; Hostens, 2003). They compete for the same resources (mainly *N. integer*) with the 0-group fish in the upper reaches of the estuary (Maes *et al.*, 2003).

In the southern **Baltic Sea** (Darss-Zingst estuary, Germany) *Neomysis integer* is dominant in the diet of pike-perch (*Stizostedion lucioperca*), perch (*Perca fluviatilis*), smelt (*Osmerus eperlanus*), pleuronectids and the sand goby (*Pomatoschistus minutus*), while it is an addition to the diet for the common goby (*Pomatoschistus microps*), roach, three-spined stickleback and herring (Arndt and Jansen, 1986; Thiel, 1996 and references therein).

In **inland water bodies** the typical predators of *Neomysis integer* are three-spined stickleback (*Gasterosteus aculeatus*), smelt (*Osmerus eperlanus*), perch (*Perca fluviatilis*), juvenile pike-perch (*Stizostedion lucioperca*), ruffle (*Gymnocephalus cernua*) and palaemonid shrimp (Bremer and Vijverberg, 1982; Irvine *et al.*, 1993; Søndergaard *et al.*, 2000). Occasionally eel (*Anguilla anguilla*) feeds on *N. integer* when its densities are high and chironomid larval densities are rather low (Bremer and Vijverberg, 1982).

Neomysis integer is also described to be an important prey for birds (Patterson, 1905; Cramp, 1977; 1983; Van De Vijver, 1983): a.o. avocet (*Recurvirostra avosetta*), common goldeneye (*Bucephala clangula)*, common and red-breasted merganser (*Mergus merganser* and *M. serrator)*, black-legged kittiwake (*Rissa tridactyla*), little tern (*Sterna albifrons*), tufted duck (*Aythya fuligula*), shoveler (*Anas clypeata*), green sandpiper (*Tringa ochropus*), common sandpiper (*Actitis hypoleucos*), eared grebe (*Podiceps nigricolli*), and non-specified wading birds. The low density of *N. integer* populations in several brackish inland waters was significantly related to the high density of foraging birds (Van De Vijver, 1983).

In laboratory feeding experiments, Winkler and Greve (2004) demonstrated the intraguild predation of the mysid *Praunus flexuosus* on juvenile (< 4 mm) *Neomysis integer*. However, *P. flexuosus* preferred calanoid copepods over *N. integer* in mixed prey conditions since they were easier to catch. In the Baltic the isopod *Saduria entomon* predates on *N. integer*, but laboratory and field experiments demonstrated that the large isopod caught more efficiently its own juveniles compared to the juvenile mysids (Leonardsson, 1991).

Estuaries are believed to provide non-limiting resources to the level of consumers (Barnes, 1974). As a result, estuarine fish often feed opportunistically on copepods, mysids and caridean shrimp (Hostens and Mees, 1999). The high productivity and standing stock of invertebrate prey may cause high niche overlap between the species since there is no need to partition the available food resources (Pianka, 1982).

Table 3: Some studies demonstrating the role of *Neomysis integer* as food for estuarine macro-invertebrates and fish. For the quantitative studies the degree of dominance (including a measure indicating the importance in the diet) is given.

Scientific name	Common name	Estuary	Country	Dominance in diet	Measure of importance	Scource
Crangon crangon	Brown shrimp	Schelde – subtidal	The Netherlands	D+	%O=50%	Hostens, 2003
		Schelde - march	The Netherlands	0 (<10mm SL)	-	Cattrijsse et al., 1997
Palaemon longirostris	Delta prawn	Seine	France	D+ (spring)	%N= 24-27%	Mouny et al., 1998
Pomatoschistus microps	Common goby	Darss-Zingst	Germany	S	RI_i = 6%	Thiel, 1996
		Schelde – subtidal	The Netherlands	S	%O=16%	Hostens and Mees, 1999
		Schelde – marsh	The Netherlands	D-	%N= 15%; %G= 39%	Hampel and Cattrijsse, 2004
		Schelde – power station	Belgium	S (esp. autumn)	%O=3%; %W=7%	Maes et al., 2003
		Seine	France	D+ (esp. 39-50 mm)	%N=57%	Mouny et al., 1998
		Ythan	UK, Scotland	S (mainly in summer)	%O=9%	Healey, 1972
		Tagus	Portugal	D+	no information	Moreira et al., 1992
Pomatoschistus minutus	Sand goby	Darss-Zingst	Germany	D	RI_i = 13%	Thiel, 1996
		Schelde	The Netherlands	D- (SL>30mm)	%O=59%	Hostens and Mees, 1999
		Schelde – power station	Belgium	D	%O=33%; %W=38%	Maes et al., 2003
		Tagus	Portugal	D+	no information	Moreira et al., 1992
Pomatoschistus lozanoi	Lozanoi's goby	Schelde – subtidal	The Netherlands	D+	%O=57%	Hostens and Mees, 1999
Dicentrarchus labrax	Sea bass	Schelde – subtidal	The Netherlands	D-	%O=22%	Hostens and Mees, 1999
		Schelde – salt marsh	The Netherlands	D	%A=40%; %G=70%	Cattrijsse, 1994
		Schelde – power station	Belgium	S	%O=14; %W=13%	Maes et al., 2003
		Tagus	Portugal	D+	no information	Moreira et al., 1992
Osmerus eperlanus	Smelt	Darss-Zingst	Germany	D	RI_i = 13%	Thiel, 1996
Pleuronectes flesus	Flounder	Medway – power station	UK	0	%O=0%	Van den Broek, 1978
		Schelde – subtidal	The Netherlands	0	%O=0%	Hostens and Mees, 1999
		Schelde – intertidal	The Netherlands	D-	%O=35%	Hostens and Mees, 1999
		Schelde – power station	Belgium	S	%O=21%; %W=38%	Maes et al., 2003
		Ythan	UK, Scotland	S	%N=<5%	Summers, 1980
Pleuronectes platessa	Plaice	Medway – power station	UK	0	%O=0%	Van den Broek, 1978
		Schelde – subtidal	The Netherlands	S	%O=4%	Hostens and Mees, 1999
		Schelde – intertidal	The Netherlands	D+	%O=21%	Hostens and Mees, 1999
Solea solea	Sole	Schelde	The Netherlands	S	%O=3%	Hostens and Mees, 1999
Clupea harengus	Herring	Darss-Zingst	Germany	S/D	RI_i = 9%	Thiel, 1996
		Schelde	The Netherlands	D-	%O=62%	Hostens and Mees, 1999
		Schelde – power station	Belgium	S (esp. autumn)	%O=24%; %W=25%	Maes et al., 2003
Sprattus sprattus	Sprat	Schelde	The Netherlands	S	%O=10%	Hostens and Mees, 1999
		Schelde – power station	Belgium	S (esp. autumn)	%O=17%; %W=2%	Maes et al., 2003

Table 3 (cont.)

Scientific name	Common name	Estuary	Country	Dominance in diet	Measure of importance	Scource
Anguilla anguilla	Eel	Schelde – subtidal	The Netherlands	0	%O=0%	Hostens and Mees, 1999
		Schelde – power station	Belgium	D	%O=57%; %W=68%	Maes et al., 2003
Gasterosteus aculeatus	Threespined stickleback	Darss-Zingst	Germany	S (not for O-group!)	RI_i = 3-4%	Thiel, 1996
Spinachia spinachia	Fifteen-spined stickleback	Loch Etive	W Scotland, UK	S	%N=12%	Kislalioglu and Gibson, 1977
Liparis liparis	Sea snail	Schelde	The Netherlands	D-	%O=31%	Hostens and Mees, 1999
Stizostedion lucioperca	Pike-perch	Schelde – power station	Belgium	D	%O=40%; %W=63%	Maes et al., 2003
Perca fluviatilis	Perch	Darss-Zingst	Germany	D	RI_i = 15-30%	Thiel, 1996
Rutilus rutilus	Roach	Darss-Zingst	Germany	S	RI_i = 1%	Thiel, 1996
Syngnathus rostellatus	Pipefish	Schelde	The Netherlands	D	%N=80%; %G=90%	Delbaere, unpublished
Syngnathus typhle	Pipefish (deep-nosed)	Tagus	Portugal	D+	no information	Moreira et al., 1992
Syngnathus abaster	Pipefish	Tagus	Portugal	D-	no information	Moreira et al., 1992
Syngnathus sp.	Pipefish	Tagus	Portugal	D-	no information	Moreira et al., 1992
Merlangius merlangus	Whiting	Medway – power station	UK	D- (< 80mm SL)	%O=21%; %A=12%	Van den Broek, 1978
		Schelde	The Netherlands	D-	%O=19	Hostens and Mees, 1999
Trisopterus luscus	Bib	Medway – power station	UK	D- *(< 100 mm SL)*	%O=22%; %A=19%	Van den Broek, 1978
		Schelde – subtidal	The Netherlands	D+ (SL: 50-130mm)	%O=57%	Hostens and Mees, 1999
Trigla lucerna	Tub gurnard	Schelde – subtidal	The Netherlands	D+	%O=33%	Hostens and Mees, 1999
Agonus cataphractus	Hook-nose	Schelde	The Netherlands	D-	%O=25%	Hostens and Mees, 1999
Ciliata mustela	Five-bearded rockling	Schelde	The Netherlands	S	%O=14%	Hostens and Mees, 1999

D+: Dominant food item (>50%); **D-**: Subdominant (10-50%); **S**: Supplementary food item; **0**: absent; **%W** = annual average of percentage biomass of the stomach content; **%A** = annual average of percentage abundance; **%N** = numerical abundance; **%O** = frequency of occurrence; **%G** = gravimetrical abundance; **RI_i** = relative importance index according to George and Hadley (1979)

E.g. while the feeding niches of *Pomatoschistus minutus* and *P. lozanoi* in the coastal zone are spatially segregated through interspecific competition, this is not the case in the brackish water zone, where mysids are highly abundant and food is unlikely to be a limiting factor. Here both species prefer to feed on *Neomysis integer* (Hostens and Mees, 1999). Although the resources meet the annual food demand of the dominant fish species in the Schelde estuary, Maes *et al.* (2003) found some degree of trophic segregation between the dominant members of the estuarine fish assemblage.

For some predators, like gadoids, *Neomysis integer* is only a preferred food item during a particular period in the life cycle. As the fish grow larger, an ontogenic shift from calanoid copepods to *N. integer* and amphipods has been described for bib (*Trisopterus luscus*) and whiting (*Merlangius merlangus*) (Van den Broek, 1978; Hostens and Mees, 1999). Later in their life they shift their diet to shrimp and small fish. Simultaneously the gadoids migrate out of the estuary to spend the rest of their lives in coastal areas and the open sea. Mysids are still encountered in stomach contents, but only the more marine species *Schistomysis spiritus* and *S. kervillei* (Hamerlynck and Hostens, 1993).

3.1.1 Predation pressure on *Neomysis integer*

A comparison between the annual consumption of the dominant fish species (610 mgADW m^{-3} y^{-1}) and the annual production of estuarine copepods (1600 mgADW m^{-3} y^{-1}; Escaravage and Soetaert, 1995) and *Neomysis integer* (300 mgADW m^{-2} y^{-1}; Mees *et al.*, 1994) in the Schelde **estuary,** suggest that food is not in short supply for the predators (Maes *et al.*, 2003). *N. integer* is consumed throughout the year by estuarine fish (Maes *et al.*, 2003). The impact of fish and macro-invertebrates on the local *N. integer* population is considerd to be rather low (1 % of the annual standing stock) and top-down control is unlikely (Hostens and Mees, 1999). Maes *et al.* (2003), on the other hand, found maximal consumption by fish on *N. integer* to coincide with minimal production and standing stock of the prey item (autumn-winter) in the upper Schelde estuary and concluded that predation sometimes may lead to a rapid depletion of the resources (a.o. *N. integer*), especially in years of high fish recruitment.

The impact of predation by young and small fish on the population of *Neomysis integer* in the Barther Bodden (southern **Baltic Sea**) is high and the mean annual consumption of *N. integer* mounts up to 9 gWW m^{-2}. This corresponds to 94 – 99 % of the total annual production of *N. integer* population (Thiel, 1992; 1996). In summer, *Clupea harengus* and *Osmerus eperlanus* consumed more than 5 times the local production of *N. integer* (Franek, 1988; 1989).

3.1.2 Predator avoidance mechanisms

For small aquatic invertebrates, predation is an important mortality pressure. The ability to detect the presence of predators and to adjust behaviour accordingly is a major advantage (Lindén *et al.*, 2003). The following abilities help *Neomysis integer* to diminishing the predation risk: vertical and horizontal migration patterns, swarming behaviour, well developed visual cues, mechanical and chemical predator detection, adapted feeding and swimming behaviour, and a direct escape response by tail flipping.

In brackish lakes and the Baltic, *Neomysis integer* performs diel vertical migrations (see before) to decrease the predation risk. They live in the dark, near bottom layers at daytime to avoid visual predators, and rise to the surface to feed at night time (Mauchline, 1980). In the daytime, *N. integer* is known to form large shoals in the shallow nearshore regions (Mauchline, 1971c) to reduce the danger of predation by visually predating fish. In the night time, when young and small fish do not feed, these swarms break up and *N. integer* migrate to the offshore region to search actively for zooplankton (Debus *et al.*, 1992).

The schooling behaviour of mysids is considered to be an effective anti-predator mechanism (Clutter, 1969; O'Brien and Ritz, 1988). If schools are attacked, the confusion effect serves to protect individual members and reduces the capture efficiency (Magurran, 1990). Information about approaching predators can be obtained through other school members, without each mysid needing to individually confirm the extent of the danger (Magurran, 1990).

In the littoral, mysids (like *Neomysis integer*) can use their good visual abilities (Fulton, 1982) in the well-lit shallow water to detect predators and derive an optimum escape direction. In darker or turbid waters, mechanical reception (Rademachers and Kils, 1996) and chemical reception of kairomones excluded by predators (Lindén *et al.*, 2003) are relatively more important. The eye function of *Neomysis integer* is studied by Hallberg *et al.* (1980) and Lindström (1992, 2000).

Another reaction described for *Neomysis integer* to a predator attack, is the direct escape response by tail flipping in which *N. integer* escapes at a high speed (796 mm s^{-1} or 80 body lengths s^{-1}) by flexion of the 3rd abdominal segment (Kaiser *et al.*, 1992b; Rademacher and Kils, 1996). Mysids like *N. integer* can be ranked with euphausiids and calanoid copepods as one of the fastest members of the zooplankton (Rademachers and Kils, 1996). The high-speed escape response by tail flipping seems to be very effective and only 25 % of the predator attacks were successful under laboratory condition (Rademachers and Kils, 1996). The tail flip itself seems to expose the telson to the bite of the predator, making it quite vulnerable for mechanical damage, even if the attack is not effective. The presence of *N. integer* individuals with an altered telson morphology (< 1 – 9 %; Holmquist, 1957; Chojnacki and Ciupinski, 1986; Mees *et al.*, 1995b) is considered to be a result of damage due to the unsuccessful predation attacks and subsequent regeneration of the damaged parts. The alternative theory (Norman, 1892; Holmquist, 1957; Chojnacki and Ciupinski, 1986), that the aberrant telson forms would result from mutations resulting from environmental pollution, was refuted by an amputation/regeneration experiment performed by Mees *et al.* (1995b), but cannot be ruled out completely. In contrast to the large escape reaction in the case of a fish predator attack, *N. integer* did not show any escape reaction at all in the case of *Praunus flexuosus* attacking (Winkler and Greve, 2004). Especially the juveniles of *N. integer* are strongly suppressed by this intraguild predation.

3.2 Diet of *Neomysis integer*

There is a great need for the description of the feeding ecology of key species (like *Neomysis integer*) in estuarine environments for the development of accurate C-flux models and the description of detritus based food web patterns, including the quantification of transfer coefficients. To date, few studies have taken hyperbenthos or mysids into account. Notable exceptions are Hall and Raffaelli (1991) and Soetaert and Herman (1995). Hyperbenthic mysids are thought to provide a significant link in the exchange of organic matter between the benthic and pelagic systems of estuaries, however, data on the contribution of *N. integer* to such food fluxes are limited (Roast *et al.*, 2000). The trophic position assigned to mysids and the hyperbenthos in general seems to be guessed rather than derived from field data.

Mysidacea are generally described as omnivores, feeding on detritus, algae and zooplankton (*e.g.* Mauchline, 1980). They can feed selectively on different zooplankton species and size groups (*e.g.* Cooper and Goldman, 1980; Murtaugh, 1981a; 1981b), and thus have the potential of structuring zooplankton communities (Fulton, 1982; Rudstam *et al.*, 1989). The phytoplankton (Kost and Knight, 1975; Siegfried and Kopache, 1980) and tychoplankton (Webb *et al.*, 1987; Wooldridge, 1989) are possibly also influenced through selective grazing by mysids. Mysid predation has even been reported as a possible control on meiofaunal densities (Siegfried and Kopache, 1980; Grossnickle, 1982; Johnston and Lasenby, 1982). Most mysids utilize organic detritus to a considerable extent and they can be responsible for the remineralisation of a substantial proportion of the non-refractory detritus (Kost and Knight, 1975; Jansen, 1985b).

3.2.1 Qualitative diet descriptions

Literature on the diet of the omnivorous *Neomysis integer* is scarce, and often only qualitative information is available. According to Lucas (1936) and Tattersall and Tattersall (1951) the species grazes on suspended organic detritus and/or planktonic diatoms. According to these authors the species only feeds on living copepods, dead mysids or amphipods when concentrations of other suspended food items are too low.

Later studies describe *N. integer* as an omnivore, consuming bottom detritus, organic matter in suspension, phytoplankton (a.o. diatoms), mesozooplankton (rotifers, calanoid and harpacticoid copepods), amphipods, carrion, fragments of leaves and of macroalgae, spores and seeds, insects and insect larvae and sand grains (Vorstman, 1951; Mauchline, 1971; 1980; Sorbe, 1980; Astthorsson, 1980; Jansen, 1985b). *N. integer* however prefers animal food (Kinne, 1955; Uitto *et al.*, 1995). In some locations phyto- and zoobenthos, like benthic diatoms, meiofaunal nematodes, harpacticoids and ostracods, amphipods, chironomids, and oligochaets, are present in stomach contents of *Neomysis integer* (Astthorsson, 1980; Bremer and Vijverberg, 1982; Haesloop, 1990; Speirs *et al.*, 2002; Vilas and Fockedey, unpublished).

In monospecific laboratory cultures, *Neomysis integer* feeds on dead or immobile individuals, just released juveniles, shed moults and faecal pellets (Vorstman, 1951; Molloy, 1958; Raymont and Krishnaswamy, 1960; Parker and West, 1979; Astthorsson, 1980; Sorbe, 1980; Kuhlman, 1982; Weisse and Rudstam, 1989; Roast *et al.*, 2000; Winkler, 2000; Verslycke and Janssen, 2002; Fockedey *et al.*, in press – Chapter 3). It can survive in the laboratory given a monospecific or mixed diet of *Artemia* nauplii, phytoplankton (*Nannochloris* spp.) and Cladocera like *Daphnia* spp. (Kuhlmann, 1984; Roast *et al.*, 1999), but it also effectively feeds on barnacle larvae and harpacticoid copepods (Mauchline, 1971) or oligochaetes (Haesloop, 1990). Astthorsson (1980) sustained the culture with a mixture of ground mussel tissue, *Enteromorpha*, detrital mud and *Artemia* nauplii. Raymont *et al.* (1964; 1966; 1968) kept *N. integer* for several weeks without additional food. They fed on the micro-organisms and detritus present in the water from which they were collected.

3.2.2 Feeding modes

Neomysis integer is a skilful swimmer and gathers small prey items, phytoplankton and suspended detrital material with the endopodites of the thoracopods, while simultaneously generating feeding currents with the exopodites of the thoracopods (Lucas, 1936; Tattersall and Tattersall, 1951; Astthorsson, 1980; Espeel, 1982). Furthermore, *N. integer* feeds raptorially on mesozooplankton and benthic invertebrates (Astthorsson, 1980; Mauchline, 1980; Bremer and Vijverberg, 1982). In brackish lakes the species grazes upon periphyton on the submerged plant (Irvine *et al.*, 1993; Bales *et al.*, 1993).

Neomysis integer feeds on the upper layer of the sediment substratum by stirring it up and feeding, both by filtering and raptorially, in the clouds of particles suspended (Raymont *et al.*, 1964; Parker and West, 1979; Astthorsson, 1980). The species is also able to collect aggregations of surface sediment prior to ingestion (Roast, 1997; Roast *et al.*, 2000; Roast *et al.*, 2004). While feeding according to this mode *N. integer* can actually contribute to the turbidity of its environment (and, more specifically, the maximum turbidity zone), either by de-stabilising the sediment surface by its feeding behaviour and so enhancing erosion rates or, by actively increasing the SPM due to sloppy feeding on sediment aggregates collected at the sediment surface (Roast *et al.*, 2004).

3.2.3 Quantitative diet descriptions

⇨ Stomach content analyses

Performing quantitative stomach content analyses of mysids is the most appropriate way to obtain information of the amount of food ingested in the field. This information is scarce for *Neomysis integer*, and is often missing details on the used methodology and results. Generally, the dietary items present in the gut are simply counted or recorded as present. Often only body parts of the larger zooplankton are found in the stomachs of *N. integer* as well as amorph detrital material, both hampering quantitative analyses. One study expresses the gut content of *N. integer* in terms of biomass (Bremer and Vijverberg, 1982). Biomass of the prey was derived from morphometric and length-wet weight regressions for zooplankton, and biomass of the ingested algal cells was derived from their bio-volume (assuming a specific weight of 1.0). The information available on quantitative stomach analyses of *N. integer* concerns populations from inland water bodies (Bremer and Vijverberg, 1982) and the Baltic Sea (Jansen, 1985b; Arndt and Jansen, 1986). Quantitative diet information is generally lacking for the maximum turbidity zone of estuaries.

Stomach content analyses were performed by Arndt and Jansen (1986) on *Neomysis integer* from the southern **Baltic Sea**. The diet composition was 53 % detritus, 37 % animal food, 6 % phytoplankton and 4 % sand grains. In **brackish lakes** *N. integer* feeds predominantly on zooplankton, plant detritus and algae (Bremer and Vijverberg, 1982; Irvine *et al.*, 1993). In terms of biomass, the diet consisted of more than 95 % detritus and animal food (Bremer and Vijverberg, 1982), mainly *Bosmina* sp., nauplii and copepodites of cyclopoids and calanoids. Rotifers and oligochaetes were taken occasionally. In numerical terms, algae (mainly benthic diatoms) were a very abundant food item, but their share in the biomass of the total gut filling was very small. *N. integer* feeds on filamentous blue-green algae (like *Oscillatoria*) and is capable of actively breaking down the longer algal filaments to smaller bits. Bremer and Vijverberg (1982) analysed only gut contents, *i.e.* faecal pellets; the content of the stomach itself was not included in their analysis.

In the Weser **estuary** *Neomysis integer* preferentially consumes animals prey (Haesloop, 1990). Next to zooplankton (mainly *Eurytemora affinis*), other prey were consumed too (*Corophium lacustre*, small gammarids, oligochaets and chironomids) as well as diatoms, macroalgae (*Enteromorpha* spp.) and macrophyte detritus. Sorbe (1980) described semi-quantitatively, and without giving further details, the diet of *N. integer* in the Gironde estuary. It consisted mainly of macrophytal detritus and benthic diatoms. Zooplankton was not considered important. In the intertidal areas of the Ythan, *N. integer* is a benthic feeder as sand grains, endobenthic harpacticoids (15 – 20 ind mysid^{-1}), nematodes and ostracods were the main dietary components (Astthorsson, 1980; Speirs *et al.*, 2002), while phytoplankton and other plant material (fragments, spores, and seeds) were eaten incidentally (Astthorsson, 1980).

⇨ Fullness index

Bremer and Vijverberg (1982) considered the alimentary track of *Neomysis integer* as a tube (without taking the spherical stomach into account) and determined its fullness by estimating the total amount of food (*i.e.* faecal pellets) present in the gut at a certain moment as a percentage of its total volume. Calculated in this way, the fullness index of *N. integer* in the brackish lake Slotermeer was used to monitor feeding intensity over a 24h cycle (see later paragraph). Astthorsson (1980) evaluated the quantity of food in the stomachs of *N. integer* on an arbitrary scale from 0 to 3. Throughout the year the majority of the mysids had a stomach fullness index of 3, reflecting that *N. integer* does not experience food shortage in the Ythan estuary. However, both techniques are subjective and do not deliver firm or detailed results.

A stomach fullness index, as the weight of the stomach relative to the weight of the animal, can give a better indication on the species' feeding periodicity and feeding intensity when studied at regular intervals over a certain time span. One can even calculate the feeding rate or ingestion rate from weight-based fullness index data by taking the gut passage time (*i.e.* gastric evacuation rate, turnover time) into account. An assumption is that animals only feed on one type of food and that they feed continuously. If the animals do not feed continuously, but in discrete meals, it is necessary to know the daily ration and the periods of feeding activity. Different calculation methods are available in fisheries research, but they have never been applied to animals as small as mysids (Eggers, 1977; Elliott and Persson, 1978). Fockedey and Mees (1999 – Chapter 2) applied the fullness index technique for the first time with *Neomysis integer* and used it to describe latitudinal, sexual and ontogenic variation in the diet, as well as seasonal and diurnal variation (Fockedey, unpublished – EU MATURE). By using the same technique, the diet of *N. integer* was compared between species (Remerie, 1999; Vilas and Fockedey, unpublished) and between populations of different habitats (Fockedey, unpublished; Soselisa, 1994; Fockedey, unpublished).

⇨ Ontogenic shifts and sexual variation

An ontogenic shift in the diet of *Neomysis integer* was demonstrated for a Dutch brackish lake (Bremer and Vijverberg, 1982), where the species fed intensively on zooplankton. *N. integer* showed no distinct size selective predation on Cladocera, but the larger cyclopoid copepods were only eaten by the larger mysids. Juvenile mysids (< 5 mm TL) fed more heavily on rotifers than the larger mysids. In an English brackish lake, Irvine *et al.* (1993) found that larger *N. integer* individuals had a higher number of *Eurytemora affinis* in their stomachs.

The size of the ingested *E. affinis* was not related to the size of the mysid. Juvenile *N. integer* of the Gironde estuary fed more on benthic diatoms, while adults had a relatively higher proportion of detritus in their stomachs (Sorbe, 1980). No ontogenic variation in the quality of the diet of *N. integer* could be demonstrated by Fockedey and Mees (1999 – Chapter 2), but smaller individuals consumed less items.

Sexual differentiation in the diet of *Neomysis integer* can be expected as well, as females probably need specific nutritional requirement for the development of the ovaries and the production of viable eggs (Kiørboe *et al.*, 1985). Fockedey and Mees (1999 – Chapter 2) could not demonstrate any sexual variation in the diet quality of *N. integer*, but they did not include gravid females in their study.

⇨ Temporal variation

The diet of *Neomysis integer* can vary over different time scales due to tidal, diurnal, monthly, seasonal and interannual variation. However, little information is available and is restricted to the study of the relative proportions of the diet constituents of *N. integer* over the course of the year. In the diet of *N. integer* from the southern Baltic Sea, detritus is more important in wintertime, while animal food is more important in summer (Arndt and Jansen, 1986). In an English pond, the number of higher plant remains was large in early spring in comparison to late spring and summer (Irvine *et al.*, 1993). In the Gironde estuary, the diet of *N. integer* shifts from a dominance of detritus in winter, to benthic diatoms in summer (Sorbe, 1980).

⇨ Feeding periodicity

The feeding periodicity and the variation in intensity of feeding can be demonstrated through the study of the variability in the gut fullness and from the frequency of occurrence of empty guts. In the brackish lake Slotermeer, *Neomysis integer*'s feeding activity reached a maximal intensity at sunset. It was still high during the first part of the night, but became low towards the morning and reached a minimum just after sunrise (Bremer and Vijverberg, 1982). *N. integer* is the only mysid from the Schelde estuary feeding at night and daytime. The other mysid species (*Schistomysis kervillei*, *Schistomysis spiritus*, *Praunus flexuosus* and *Gastrosaccus spinifer*) are typically night feeders (Remerie, 1999). More detailed patterns in the feeding intensity, coupled to tidal and diurnal rhythms, have not been studied yet for *N. integer*.

Fockedey and Mees (1999 – Chapter 2) developed a method to perform quantitative diet analyses of *Neomysis integer* individuals taken from the field. The method joins two techniques: (1) a microscopic stomach analysis and (2) a fullness index determination based on weight. After the dissection and preparing of the stomach in a semi-permanent slide, the counting and measurement of food items is further performed using image analysis. The technique includes an analysis of the amorph unidentifiable detritus by EDAX (Energy Dispersive Spectroscopy X-ray Microanalysis). The stomach fullness technique is adopted from fish feeding studies and here applied for the first time on small invertebrates like mysids.

Fockedey and Mees (1999 – Chapter 2) compared the diet of *Neomysis integer* collected from the maximum turbidity zone of the Schelde, Gironde and Elbe in spring. *N. integer* was found to be an omnivore which mainly utilizes the mesozooplankton and detritus carbon pools. In all three estuaries, the diet was dominated by calanoid copepod (up to 10 *Eurytemora affinis* ind^{-1}) and was supplemented with rotifers (esp. in Elbe) and cladocers. Phytoplankton, pollen and benthic organisms, though present in the stomachs, were negligible. Macrophytal detritus and amorphous material, originating from sediment macro-aggregates suspended in the water column or deposited on the sediment surface, were very abundant food items. Fullness indices in the Elbe and Schelde were comparable (< 1) and significantly lower than in the Gironde (1 – 4). A higher fullness index in the Gironde reflects the consumption of higher amounts of detritus in this estuary. No sexual, nor ontogenic shifts, could be demonstrated in the diet quality, nor in the prey size, but smaller mysids consumed less.

Additional measurements were made (Fockedey, unpublished) on the variation in the fullness index of *Neomysis integer* from the subtidal of the Schelde estuary over the course of a year. The lowest indices were measured in spring (< 1) as compared to summer (1.0 – 1.2) and winter (> 1.3). The higher stomach fullness in winter is related to a higher abundance of (macrophytal and unidentifiable) detritus in the stomachs of the mysids. The lower fullness indices in spring and summer were related to a more abundant feeding on higher quality food items like mesozooplankton (*Eurytemora affinis* in spring, *Acartia* spec. in summer).

The variation of the stomach fullness index of *Neomysis integer* was determined for several 24h cycles in the turbidity maximum of the Schelde, Gironde and Elbe (Fockedey, unpublished). Although variations in the fullness index are difficult to relate to diurnal and tidal cycles, the results indicate a continuous feeding over the day (fullness index between 0.8 and 1.4 in Elbe, 1.0 and 1.5 in Schelde, and between 1.8 and 2.9 in Gironde).

The stomach fullness of *Neomysis integer* caught in the surface layers of the maximum turbidity zone of the Schelde (at night) was significantly lower in comparison to the stomach fullness of individuals caught in the bottom layers (Fockedey, unpublished). No such pattern could be demonstrated for mysids living in the maximum turbidity zone of the Gironde estuary.

Vilas and Fockedey (unpublished) used the same techniques to compare the diet of *Neomysis integer* and *Rhopalophthalmus* sp. from the Guadalquivir estuary. *N. integer* was much more omnivorous than the predator *Rhopalophthalmus* sp. and mainly consumed calanoid copepods and detritus. The diet was supplemented relatively more with zoobenthic organisms, like nematodes and harpacticoids, as compared to the Schelde, Elbe and Gironde estuary.

The seasonal variation in the diet of *Neomysis integer* from the brackish pond Galgenweel was investigated as well (Soselisa, 1994; Fockedey, unpublished). The fullness indices of the mysids living in the pond were approximately 5 times as high (5.9 – 7.4) as compared to *N. integer* living in the mesohaline of the Schelde estuary (0.8 – 1.4). High values of the fullness index in winter, summer and autumn were associated with a high abundance of calanoid copepods (*Eurytemora affinis*) in the stomachs. The significantly lower fullness index in spring coincides with a dominance of Cladocera and pollen in the stomach contents of *N. integer*.

3.2.4 Isotope fractionation in the field

Food web structure can be described studying trophic interactions based on gut analysis. The interpretations however, can be biased by the lack of couplings to the microbial food web and direct errors in the diet analysis *e.g.* caused by differences in digestion rate between food types. Natural abundances of stable carbon and nitrogen isotopes can then be used to identify the sources of organic matter, the foraging location of organisms and trophic structures in aquatic food webs. Organisms tend to become enriched in heavy isotopes as compared to their food. Isotopic fractionation data are available from the northern Baltic Proper (Hansson *et al.*, 1997) and the low-salinity zone of the Schelde estuary (De Brabandere *et al.*, 2002 and references therein). Unfortunately, these studies did not include the mysid *Neomysis integer*, albeit the species is present in both systems.

The $\delta^{13}C$ and $\delta^{15}N$ values of subadult (summer) *Neomysis integer* in the Baltic Sea (South of Stockholm) ranged from -23.1 to -21.5 ‰ and 10.2 to 12.6 ‰, respectively (Rolff, 1998; Gorokhova, 1999; Gorokhova and Hansson, 1999). No variation could be demonstrated between sexes, but $\delta^{15}N$ values increased with body size. These ontogenic changes in the isotopic composition can be caused by either decreased growth efficiency with increasing size or by a shift in diet to food from a higher trophic level (Gorokhova and Hansson, 1999). Seasonal variations in $\delta^{13}C$ values might be due to the seasonally varying energy reserves, *i.e.* lipid content.

According to the $\delta^{13}C$ and $\delta^{15}N$ values reported by Rolff (1998) for zooplankton, it is suggested that *Neomysis integer* may be feeding on the largest mesozooplankton, namely older copepodites and adults (Gorokhova and Hansson, 1999). However the cyanobacteria ($\delta^{13}C$ = -22.9 ‰ and $\delta^{15}N$ = 4.5 ‰; Rolff, 1998) and microbially degraded material ($\delta^{15}N$ = 4.5 ‰) present in the water column cannot be ruled out as possible food sources (Gorokhova and Hansson, 1999).

The fractionation of heavy isotopes is a continuous process, whose influence on the isotopic composition of different tissues depends on their growth and turnover rate (Owens, 1987). The fractionation and the speed of incorporation of food in different body tissues (muscle, exuvia) and faeces was studied for *Neomysis integer* by Gorokhova and Hansson (1999). The muscle tissue did not get in balance with the diet isotopic composition, not even after 3 months. Exuvia of *N. integer* were enriched in $\delta^{13}C$ (+1.41 ‰) and depleted in $\delta^{15}N$ (-5.59 ‰) in comparison with muscle tissue, indicating that the exoskeleton is composed of nutrients that are more directly derived from the diet. The relative abundance of carbon isotopes in the excuvia of *N. integer* is in close balance with the carbon isotopic composition of the diet during the time of exoskeleton secretion, *i.e.* the previous intermoult period. Digestion leads to increased $\delta^{13}C$ (+1.4 ‰) and $\delta^{15}N$ (+3.4 ‰) values in the faeces compared to the food. The isotopic composition of each muscle tissue, exuvia or faecal pellets may form a basis for diet reconstruction of field caught mysids (Gorokhova and Hansson, 1999): the δ13C and $\delta^{15}N$ of the faeces mirror the diet over the last few hours, exuvia δ13C values represent nutrient metabolized 2 – 3 weeks ago and muscle tissue integrates the isotopic signal over a relatively long period (weeks to months).

To perform an isotope fractionation study with *Neomysis integer* from the low-salinity, turbid reaches of estuaries would be of great interest. It can confirm earlier conceptual estuarine food webs links based on the stomach analyses of the mysid species itself (Fockedey and Mees, 1999 – Chapter 2) and the higher trophic levels consuming it (Hostens and Mees, 1999; Maes *et al.*, 2003).

3.3 Feeding rate

Feeding rates (Table 4) can be measured directly in laboratory experiments or calculated from stomach content weights combined with gut evacuation time, given the animals feed continuously on one type of food. In the laboratory the ingestion rate (DW of food ingested per time unit) and the egestion rate (DW faecal pellet produced per time unit) can be measured and the assimilation efficiency calculated. While it is well established that the feeding rates of many crustaceans are influenced by various factors including temperature, salinity, weight, gender and food density, few of these factors have been investigated for mysids and specifically for *Neomysis integer* (Roast *et al.*, 2000; Winkler and Greve, 2004).

⇨ Phytoplankton

Experimenting with *Neomysis integer* in very dense diatom cultures, Lucas (1936) measured an average consumption rate of over 1.10^6 (max 6.10^6) *Nitzschia* cells mysid^{-1} h^{-1} (Table 4). The species is feeding much in excess of their actual requirements under these circumstances (Lucas, 1936). They survived optimally at an intermediate food density of 25 – 100 cells mm^{-3}. The filtration rate of *N. integer* on *Chlorella vulgaris* suspensions yielded a mean intake of 0.5 10^6 cells ind^{-1} h^{-1}, corresponding to a specific feeding rate of 108 µgDW mysid^{-1} d^{-1} (Arndt and Jansen, 1986).

Filter feeding on phytoplankton is often measured as the clearance rate (Gauld, 1951) and expressed as the volume filtered by the animals per hour. Astthorsson (1980) performed feeding experiments with *Neomysis integer* feeding on different species of cultured phytoplankton (Table 4). Highest clearance rates were obtained on the dinoflagellate *Prorocentrum micans* (26 µm) and the coccolithophore *Cricusphaera oblongata* (13 µm), respectively 6.6 and 6.7 ml mgDW h^{-1}. Far lower filtering rates were recorded on the diatom *Chaetoceros curnectum* (13 µm) and a further unidentified small chain diatom species (10 µm): 1.7 and 2.4 ml mgDW h^{-1}. No feeding was recorded on the small flagellate *Tetraselmis* sp. (8 µm). Energetic benefit, with a higher carbon intake than respired and excreted during the course of the feeding experiments, was only possible when feeding on *C. oblongata*.

Estimations of feeding rates of *Neomysis integer* on the *in situ* phytoplankton community of the brackish lake Hickling Broad indicated that phytoplankton cells were not an important component in the diet of the species in comparison with the calanoid *Eurytemora affinis* and epiphytic algae (Irvine *et al.*, 1993; 1995). Stomach analyses of *N. integer* in a variety of habitats confirmed the minor role of algae in terms of biomass in its diet (Bremer and Vijverberg, 1982; Irvine *et al.*, 1993; Fockedey and Mees, 1999 - Chapter 2).

⇨ **Zooplankton**

Laboratory experiments and field evidence revealed that *Neomysis integer* feeds extensively on the calanoid copepod ***Eurytemora affinis*** (*e.g.* Aaser *et al.*, 1995; Irvine *et al.*, 1993; Fockedey and Mees, 1999 – Chapter 2; Winkler and Greve, 2004). Experimentally derived predation rates (Table 4) of *N. integer* on a mixture of nauplii and copepodites averaged 23 nauplii and 17 copepodites mysid^{-1} h^{-1} (Irvine *et al.*, 1993). The maximal predation rate on a similar mixture was 52 nauplii and 8 copepodites mysid^{-1} h^{-1} (Aaser *et al.*, 1995). Winkler and Greve (2004) measured a predation rate of maximally 170 copepodites mysid^{-1} d^{-1} by adult *N. integer*, significantly decreasing with decreasing mysid size and lower temperature. Fockedey *et al.* (submitted a – Chapter 5) reports a feeding rate of only 9 copepodites mysid^{-1} d^{-1}. Also ***Daphnia* sp.** is readily taken by *N. integer* (Irvine *et al.*, 1993). The feeding rates did not differ when other prey items were present or not (± 15 daphnia mysid^{-1} h^{-1}). An egg-carrying *E. affinis* female was handled for approximately 2 minutes by *N. integer* (Viitasalo *et al.*, 1998), whereas small cladocerans (like *Bosmina*) could be swallowed in its entirety.

Often ***Artemia* nauplii** are used as animal food for *Neomysis integer* under laboratory culture conditions. The maximal feeding rate of *N. integer* on *Artemia* nauplii amounts to 600 – 800 nauplii d^{-1} for adults (Astthorsson, 1980) and 200 – 300 nauplii d^{-1} for subadults (De Pauw, 1998; Fockedey, unpublished). Feeding rate is lower at a smaller body size and each time when the animals are about to moult (Astthorsson, 1980). The feeding rate increases with the prey density according to a rectilinear model (Astthorsson, 1980). The feeding rate of adult *N. integer* rapidly increases at initial prey concentrations of less than 700 nauplii l^{-1}; while higher prey densities do not longer affect the feeding rate. At prey densities below the maximum feeding rate, *N. integer* appears to search for and find all available *Artemia* nauplii (Astthorsson, 1980). On the contrary adult mysids do not attempt to hunt at low concentrations of calanoid copepod prey (Irvine *et al.*, 1993).

Filter feeding on zooplankton can also be expressed as clearance rate in volume filtered by the animals per time unit (Cooper and Goldman, 1980). It provides an estimate of the proportion of total prey removed by the predator in a given time. Clearance rate values of *Neomysis integer* when feeding on *Eurytemora affinis* are 0.34 – 0.62 l mysid^{-1} h^{-1} for naupliar prey and 0.08 – 0.15 l mysid^{-1} h^{-1} for copepodites (Irvine et el., 1993; Aaser *et al.*, 1995).

Using length-weight regressions of zooplankton (Bottrell *et al.*, 1976; Burkell and Kendall, 1982) or assigned values for the discrete life stages (Lavens, 1978; Aaser *et al.*, 1995) the feeding rates can be expressed as weight-specific feeding rate. When feeding on a mixture of nauplii and copepodites of *Eurytemora affinis*, *N. integer* specific feeding rate varies according to the predator size, the prey density and the temperature between 18 – 137 µgDW mysid^{-1} d^{-1} of 20 – 350 % body C mysid^{-1} d^{-1} (Irvine *et al.*, 1993; Winkler and Greve, 2004). When feeding on daphniid prey, the specific feeding rate varied from 67 – 149 µgDW mysid^{-1} d^{-1} (Irvine *et al.*, 1993).

Laboratory studies on the predation rates of mysids on zooplankton can have quite some artefacts. A homogeneous spread of *Neomysis integer* individuals in an experimental set-up is difficult, since the species appears to be attracted to structures and surfaces (Irvine *et al.*, 1993). Prey like *Artemia*, cladocers and calanoids have the tendency to respond photopositively and to concentrate at the side of the feeding arena where the light intensity is greatest (Astthorsson, 1980; Bergström and Englund, 2004). To make the distribution random and prevent that *N. integer* is able to collect all prey from one concentrated spot, one can work in the dark or obscure the feeding containers (Astthorsson, 1980). The attack rate of *N. integer* on cladocerans is significantly higher in small containers in comparison with large feeding arenas due to a higher encounter rate (Bergström and Englund, 2004).

Table 4: Feeding rates reported for *Neomysis integer* on different food items

Food item	Concentration	Vol (L)	Time (h)	Conditions	Size mysids	Feeding rate	Scource
Nitzschia closterium (30-400 µm)	> 2000 cells ml^{-1} 1000-2000 cells ml^{-1}	? ?	? ?	- -	? ?	1.3 10^6 cells $mysid^{-1}$ h^{-1} 0.7 10^6 cells $mysid^{-1}$ h^{-1}	Lucas, 1936
Chlorella vulgaris (4µm)		?	?	-	?	0.5 10^6 cells.ind^{-1}.h^{-1} =108 µgDW.d^{-1}	Arndt and Jansen, 1986
Prorocentrum micans (26µm)	160 cells ml^{-1}	0.45	12	9°C (10 psu) – dark	4.3 – 7.2 mgDW	5.7 – 7.7 ml mgDW h^{-1} 3000-4300 cells $mysid^{-1}$ h^{-1} 3 -4 µgC $mysid^{-1}$ h^{-1}	Astthorsson, 1980
Cricusphaera oblongata (13µm)	12000 cells ml^{-1}	0.45	12	9°C (10 psu) – dark	4.3 – 7.2 mgDW	5.1-8.9 ml mgDW h^{-1} 160-190 10^3 cells $mysid^{-1}$ h^{-1} 25-30 µgC $mysid^{-1}$ h^{-1}	Astthorsson, 1980
Chaetoceros curnectum (13µm)	3000 cells ml^{-1}	0.45	12	9°C (10 psu) – dark	4.3 – 7.2 mgDW	1.6-1.8 ml mgDW h^{-1} 23-30 10^3 cells $mysid^{-1}$ h^{-1} 2-3 µgC $mysid^{-1}$ h^{-1}	Astthorsson, 1980
chain diatom (10µm)	8000 cells ml^{-1}	0.45	12	9°C (10 psu) – dark	4.3 – 7.2 mgDW	2.0-2.7 ml mgDW h^{-1} 37-70 10^3 cells $mysid^{-1}$ h^{-1} 2-3 µgC $mysid^{-1}$ h^{-1}	Astthorsson, 1980
Tetraselmis sp (8µm)	12700 cells ml^{-1}	0.45	12	9°C (10 psu) – dark	4.3 – 7.2 mgDW	0.6 ml mgDW h^{-1} 50 10^3 cells $mysid^{-1}$ h^{-1} 2 µgC $mysid^{-1}$ h^{-1}	Astthorsson, 1980
Eurytemora affinis	45 N + 39 COP l^{-1} 90 N + 78 COP l^{-1} 180 N + 156 COP l^{-1} ? ? ? ? 174N + 249 COP l^{-1}	4 4 4 6 6 6 6 4.4	1 1 2 5.5 5.5 5.5 5.5 1.2	0.5-2.6 mysids l^{-1}	8-9 mm SL 8-9 mm SL 8-9 mm SL 4 mm SL 6 mm SL 8 mm SL 10 mm SL 9 mm SL	20 N + 7 COP $mysid^{-1}$ h^{-1} 30 N + 11 COP $mysid^{-1}$ h^{-1} 34 N + 10 COP $mysid^{-1}$ h^{-1} 12 N + 11 COP $mysid^{-1}$ h^{-1} 9 N + 14 COP $mysid^{-1}$ h^{-1} 16 N + 17 COP $mysid^{-1}$ h^{-1} 55 N + 30 COP $mysid^{-1}$ h^{-1} 27 N + 44 COP $mysid^{-1}$ h^{-1}	Irvine et al., 1993
Eurytemora affinis	440 AD + COP l^{-1}	0.5 0.5 0.5 0.5 0.5	24 24 24 24 24	10°C – 20 psu – 3 mysids 10°C – 20 psu – 1 mysid 15°C – 20 psu – 5 mysids 15°C – 20 psu – 3 mysids 15°C – 20 psu – 1 mysid	Subadults 5-7 mm Adults 8-17 mm Juvenile 4-5 mm Subadult 5-7 mm Adult 8-17 mm	<5 COP $mysid^{-1}$ day^{-1} 50 COP $mysid^{-1}$ day^{-1} 20 COP $mysid^{-1}$ day^{-1} 40 COP $mysid^{-1}$ day^{-1} 170 COP $mysid^{-1}$ day^{-1}	Winkler and Greve, 2004
Eurytemora affinis	100 N + 100 COP l^{-1} 200 N + 100 COP l^{-1} 400 N + 100 COP l^{-1}	1 1 1	2 2 2	15°C mysid density 3 l^{-1}	?	15 N + 8 COP $mysid^{-1}$ h^{-1} 26 N + 5 COP $mysid^{-1}$ h^{-1} 52 N + 6 COP $mysid^{-1}$ h^{-1}	Aaser et al., 1995
Eurytemora affinis	143 COP l^{-1}	0.35	24	15°C – 5 psu – 1 mysid	Subadult 4 -8 mm SL	3-18 COP $mysid^{-1}$ day^{-1}	Fockedey et al., submitted a – Chapter 5

Table 4 (cont.)

Food item	Concentration	Vol (L)	Time (h)	Conditions	Size mysids	Feeding rate	Scource
Daphnia hyalina	60 Dh l^{-1}	3	1.75	0.5-2.6 mysids l^{-1}	Mysid 8-9 mm SL	14 Dh $mysid^{-1}$ h^{-1}	Irvine et al., 1993
Daphnia hyalina + Eurytemora affinis	60 Dh + 78 N + 86 COP l^{-1}	3	1.75	-	Mysid 8-9 mm SL	16 Dh+17N+10COP$mysid^{-1}h^{-1}$	
Daphnia magna+ Chydorus sphaericus	54 Dm + 165 CH l^{-1}	4.4	1.2	-	Mysid 9 mm SL	10 Dm+48 Ch $mysid^{-1}$ h^{-1}	
Daphnia magna+ Chydorus sphaericus Eurytemora affinis	29Dm + 88 CH + 85N + 121COP l^{-1}	4.4	1.2	-	Mysid 9 mm SL	9 Dm+ 31CH-13N+?COP $mysid^{-1}$ h^{-1}	
Artemia nauplii	50 N l^{-1}	2	3	9-16°C - ?psu (Finnish Baltic water)	18.2 mm TL =5.82mg DW	11.62 - 16.26 µg C h^{-1}	Lindén et al., 2003
Artemia nauplii	50 – 2400 N l^{-1}	0.5	24	9°C (10psu)	Adult	Max at 300-450 N $mysid^{-1}$ day^{-1}	Astthorsson, 1980
Artemia nauplii	2000 N l^{-1}	0.5	24	9°C (10psu) 16°C (10 psu)	1.5 - 7.4 mg DW 0.7 - 10.2 mg WD	90 – 480 N $mysid^{-1}$ day^{-1} 70 – 450 N $mysid^{-1}$ day^{-1}	Astthorsson, 1980
Artemia nauplii	2000 N l^{-1} 1000 N l^{-1} 500 N l^{-1}	0.5 1 2	24	9°C (10 psu)	1.5 - 7.4 mg DW 1.5 - 7.4 mg DW 1.5 - 7.4 mg DW	90 – 480 N $mysid^{-1}$ day^{-1} 40 – 380 N $mysid^{-1}$ day^{-1} 20 – 360 N $mysid^{-1}$ day^{-1}	Astthorsson, 1980
Artemia nauplii	5 N l^{-1} 13 N l^{-1} 25 N l^{-1} 50 N l^{-1} 100 N l^{-1} 150 N l^{-1} 200 N l^{-1} 400 N l^{-1}	5	6	9°C (10 psu)	4.5 mg DW	3.8 N $mysid^{-1}$ h^{-1} 10.2 N $mysid^{-1}$ h^{-1} 15.7 N $mysid^{-1}$ h^{-1} 11.5 N $mysid^{-1}$ h^{-1} 16.0 N $mysid^{-1}$ h^{-1} 22.3 N $mysid^{-1}$ h^{-1} 20.8 N $mysid^{-1}$ h^{-1} 17.7 N $mysid^{-1}$ h^{-1}	Astthorsson, 1980
Organic poor sediment	?	?	16	5°C – 10 psu – males 15°C – 10 psu – males 1 psu – 10°C – males 30 psu – 10°C – males	?	0.057 mg faeces mg^{-1} mysid DW h^{-1} 0.132 mg faeces mg^{-1} mysid DW h^{-1} 0.084 mg faeces mg^{-1} mysid DW h^{-1} 0.096 mg faeces mg^{-1} mysid DW h^{-1}	Roast et al., 2000
Estuarine flocs		0.85	24	5 subadults	Mysid 7 – 10 mm SL	38 flocs $mysid^{-1}$ h^{-1}	Fockedey et al., submitted c – Chapter 4
		0.85	24	8-10 small subadults 2-3 large subadults	Mysid 4 – 6 mm SL Mysid 9 – 11 mm SL	0.039 mgDW faecesmg^{-1} mysid DW h^{-1} 0.021 mgDW faeces mg^{-1} mysid DW h^{-1}	Fockedey et al., submitted d – Addendum 2

Ch : *Chydorus sphericus* ; **Dm**: *Daphnia magna*; **Dh** : *Daphnia hyalina* ; **N** : nauplii ; **COP** : copepodites and adults

Astthorsson (1980) though, report the container size not to have a significant impact on the maximal feeding rates of *N. integer* on *Artemia* nauplii. Gorokhova and Hansson (1997) report the effects of other experimental conditions like light regime, duration of the experiment and extent of starvation on the feeding rate estimate of mysids (*Mysis mixta*). Laboratory derived feeding rates must be considered as overestimates (Aaser *et al.*, 1995) due to the relative high prey density in comparison to the field, an aberrant prey and predator behaviour and due to the fact that *N. integer* in the field does not feed exclusively on zooplankton, but has an omnivorous diet.

⇨ **Phyto- and zoobenthos**

Neomysis integer is known to feed in some locations on phyto- and zoobenthos like benthic diatoms, meiofaunal nematodes, harpacticoids and ostracods, amphipods, chironomids, and oligochaets (Astthorsson, 1980; Bremer and Vijverberg, 1982; Haesloop, 1990; Speirs *et al.*, 2002; Vilas and Fockedey, unpublished). Although it can survive on harpacticoid copepods (Mauchline, 1971) or oligochaets (Haesloop, 1990) in aquaria, no further data are available on the feeding rates on these food itmes. Recently, Albertsson (2004) hypothesised a predator-interaction of *N. integer* on juveniles of the amphipod *Monoporeia affinis* but could not confirm this in mesocosm experiments. The mesocosm experiments however, could demonstrate a significant predation of *N. integer* on near-bottom zooplankton (mainly cyclopoids) in the Baltic (Albertsson, 2004).

⇨ **Detritus and sediments**

Qualitative stomach content analyses of *Neomysis integer* described the species to feed on fragments of leaves of higher plants and macroalgae, and on amorph suspended detritus. Ingestion rates of macrophytal detritus can be measured directly by weight difference of the food eaten over a certain time period (*e.g.* Nilsson, 1974). However, a significant decrease in food weight is difficult to measure and leads to a very variable feeding rate (Marchant and Hynes, 1981). The duration of the experiment have to be long enough and/or should contain many individuals. However, no experiments to determine ingestion, egestion and gut evacuation rates on macrophytal detritus or suspended particulate matter have been performed with *N. integer*, except for Fockedey *et al.* (submitted c – Chapter 4) and Fockedey *et al.* (submitted d – Addendum 2).

Neomysis integer also feeds on the sediment surface. It feeds raptorially on the whirled up particles (Parker and West, 1979) or directly ingests aggregations of surface sediment (Roast *et al.*, 2000). The egestion rate of *N. integer* on sieved, organically poor sediments was determined as a measure for the ingestion rate (Roast *et al.*, 2000). Egestion rate increased with increasing salinity and temperature.

3.4 Selectivity experiments

Selectivity of *Neomysis integer* for specific prey types or prey sizes can be determined in two ways: (1) via feeding experiments in artificial laboratory conditions, or (2) via the analysis of ingested food items. In laboratory experiments, nauplii and the smallest copepodites of *Eurytemora affinis* are most strongly selected (Irvine *et al.*, 1993; Aaser *et al.*, 1995). *N. integer* also readily feeds on *Daphnia hyalina* and again smaller prey individuals are preferred. Selectivity coefficients are significantly greater for *Daphnia* sp. than for copepodites of *E. affinis* (Irvine *et al.*, 1993). When administering a mixture of natural zooplankton, *N. integer* seemed to be largely missing the older *Eurytemora* copepodites as these are capable of more vigorous flight reactions (Arndt and Jansen, 1986). In the Elbe, *N. integer* was found to feed on elder developmental stages of *E. affinis* (Bernát *et al.*, 1994). Juvenile mysids preferred small rotifers and copepod nauplii, while larger *N. integer* favoured larger prey like Cladocera and calanoid copepodites over Rotifera (Arndt and Jansen, 1986). Larger *N. integer* individuals consumed a larger size of *E. affinis* (Fockedey and Mees, 1999 – Chapter 2).

In a Frisian brackish lake *N.integer* showed a strong negative selection for the filamentous blue-green alga *Oscillatoria* and a rejection of the filamentous green alga *Planctonema*, while it was preferably feeding on *Bosmina* sp., cyclopoids and detritus (Bremer and Vijverberg, 1982).

3.5 Structuring of zooplankton and phytoplankton populations

It is well known from fresh- and brackish water lakes, that high mysid densities may affect phytoplankton and zooplankton composition and abundance, especially in periods of low vertebrate predator abundance (Moss and Leah, 1982; Hanazato, 1990; Jeppesen *et al.*, 1994; Aaser *et al.*, 1995). This phenomenon has also been suggested for brackish coastal areas, e.g. the Baltic (Rudstam *et al.*, 1986; Hansson *et al.*, 1990). Information on the structuring impact of *N. integer* on the mesozooplankton (and phytoplankton) communities in dynamic estuarine systems is still lacking.

The high abundance of *Neomysis integer* in **brackish water ponds and lakes** is typically associated with a low abundance of pelagic Cladocera and copepods (Espeel, 1982; Van De Vijver, 1983; Aaser *et al.*, 1995), indicating the governing role of mysids in the structuring of zooplankton communities. In a Danish lake, Aaser *et al.* (1995) could demonstrate the negative impact of *N. integer* on *Eurytemora affinis*. By its heavy predation on the zooplankton community *N. integer* reduces the grazing pressure on the phytoplankton and enhances the eutrophication in nutrient-rich brackish lakes (Aaser *et al.*, 1995; Samuels and Mason, 1998). Moreover, *N. integer* also directly stimulates the phytoplankton growth (Aaser *et al.*, 1995), perhaps because nutrients consumed when feeding on the sediment surface are subsequently excreted to the water column and become available to the phytoplankton.

In laboratory experiments, *Neomysis integer* extensively feeds on nauplii and copepodites of *Eurytemora affinis* and Cladocera and thus has the potential to dramatically affect the population structure and density of its prey (Irvine *et al.*, 1993). However, in the field (Hickling Broad) no evidence was found of a link between the population dynamics of *N. integer* and *E. affinis* (Irvine *et al.*, 1995), except for a slight reduction in the total biomass and size-at-maturity of *E. affinis* in periods when larger mysids are relatively more abundant (cfr. these feeding more effectively on larger prey). The copepod reproduces so rapidly that its population is unlikely to be controlled by the mysid predation (Irvine *et al.*, 1993). Vice versa there was no indication that *Neomysis integer* was affected by (interannual) changes in the *Eurytemora affinis* population dynamics. The potential for omnivorous feeding in mysids implies that they may modify their diets in response to changes in the food quality and abundance. In periods of low zooplankton densities, the omnivorous *N. integer* can switch its diet from zooplankton to other food items, like epiphytic algae, (macrophyte) detritus and even organic matter in sediments. In this way, *N. integer* can keep its densities high and maintain a potentially high predation pressure on the remaining zooplankton (Irvine *et al.*, 1995).

Neomysis integer is an important regulator of zooplankton abundance and species composition in the **Baltic** as well (Jansen *et al.*, 1983; Jansen and Heerklos, 1983; Hansson *et al.*, 1990), except for the northern part of the Baltic Sea where the species contributes only 3 % of the total mysid biomass (Rudstam *et al.*, 1992), while another mysid species (*Mysis mixta*) and clupeid young-of-the-year larvae are the most dominantly zooplanktivores (Rudstam *et al.*, 1992). In coastal waters of the southern Baltic, *N. integer* consumption was estimated to amount to 2.7 – 20.1 % of the total annual zooplankton production in the Darss-Zingst estuary (Jansen, 1985b; Thiel, 1992). *N. integer* has a minor impact on the phytoplankton in the Baltic region (Jansen *et al.*, 1983; Jansen and Heerklos, 1983). Jansen (1985b) found zooplankton to constitute up to 37 % of the stomach content of *N. integer* in a bay of the southern Baltic Sea.

During periods when juveniles dominated the *N. integer* population, small Rotifera and copepod nauplii played a greater role in the food spectrum, while larger mysids selectively preferred larger prey like Cladocera and calanoid copepods (Arndt and Jansen, 1986). Only 8 % of the total annual rotifer production is consumed by *N. integer* in the southern Baltic. Still, *N. integer* juveniles are the main predators and select the larger rotifer species (like *Brachionus plicatilis*), the larger size classes and egg-bearing females (as demonstrated by feeding experiments by Arndt (1988).

A long-term dataset on the seasonal variation of *Neomysis integer* density in the Gironde **estuary** revealed a significantly positive correlation with copepod densities (*Eurytemora affinis* and *Acartia* spp.) and suggested predation by *N. integer* on copepods in the turbid reaches of the estuary with a possible food limitation (David *et al.*, 2005). No correlation between *N. integer* and *E. affinis* or chlorophyll *a* could be statistically demonstrated in the brackish regions of the Elbe estuary (Köpcke and Kausch, 1996).

3.6 Diet overlap with coexisting species

Larvae and young of zooplanktivorous estuarine fish (like herring and smelt) can heavily predate on zooplankton in some periods of the year, especially in years of high recruitment (Thiel, 1996), and then show a high degree of diet overlap with *Neomysis integer* (Kinne, 1955, Sanina, 1961, Maciejewska, 1992). The zooplanktivorous fish select their prey visually and prefer larger prey like larger cladocerans, calanoid copepods, and *N. integer* itself (Thiel, 1996). The mysid exhibits a more tactile behaviour (next to visual cues) in their prey selection and is able to prey on smaller organisms like larger rotifers and nauplii of copepods and cladocerans (Jansen, 1985b). *N. integer* and planktivorous fish respectively consume 20.1 % and 25.4 % of the total annual zooplankton production in the Baltic (Thiel, 1992). *N. integer* competes for food with the early fish developmental stages, but is at the same time a food component of the older stages of *e.g.* herring and sprat (Maciejewska and Opalinski, 2002).

Neomysis integer and *Praunus flexuosus* compete for the same food resource *Eurytemora affinis* (Winkler and Greve, 2004). In situations where not a lot of copepods are available, *Praunus flexuosus* will feed on the younger life stages of *N. integer* (Winkler and Greve, 2004), while *N. integer* will shift to a more detrital feeding mode under limited prey circumstances (*e.g.* Arndt and Jansen, 1986).

Remerie (1999) compared the stomach contents of *Neomysis integer* of the Schelde with other estuarine mysid species like *Gastrosaccus spinifer*, *Praunus flexuosus*, *Schistomysis kervillei* and *S. spiritus N. integer* was the only species feeding during day ánd night, while the others were mainly night feeders. For all of the species, stomachs contained large amounts of unidentifiable detritus, originating from estuarine micro-flocs typically formed in the estuary by flocculation of sediment, particular suspended and dissolved organic matter (Eisma, 1986).

3.7 Feeding appendages

The feeding appendages of mysids consist, in anterior to posterior order, of an unpaired labrum, and paired mandibles, paragnaths (labia), maxillules and maxillae (Mauchline, 1980). The mouth is situated ventrally and is enclosed by a chamber formed by the labrum, mandibles and paragnaths. The mandible has cutting, grinding and macerating regions and a forwardly directed mandibular palp. The morphology of all these mandibular features vary between species according to their feeding habits (Mauchline, 1980). The endopods of the thoracic legs function in filter feeding. The anterior thoracic endopods, together with the mouthparts form a "food basket" in which organic matter collected from the sediment surface is collected, mainly by the mandibular palp, and held in the basket prior to maceration by the mouthparts (Mauchline, 1980; Roast *et al.*, 2004).

Remerie (1999) and Maciejewska (1992) studied the morphology of the mandibles and thoracopods of *Neomysis integer*, since it can give information on their diet. The length and the distance between setae of the thoracopod endopods, identifying the surface and mesh size of the sieving device (Jerling and Wooldridge, 1994), was small in *N. integer* and indicate that filter feeding is of minor importance to the species. The structure of the mandibles, like the surface of the *pars molaris* (pm) and the length of the *pars incisivia* (pi), makes it possible to hypothesise on the degree of herbivory or carnivory of a species. For *N. integer* the pm/pi-ratio is low in comparison to other estuarine species and the presence of large, sharp incisive teeth on the *pars molaris* indicates a carnivorous or detritivorous feeding. When mysids are filter feeding they use the setae on the proximal endite of the maxilla for food collection (Cannon and Manton, 1927). Maxilla have feather-like setae with 2 rows of setules and a second type of more robust setae with short irregular setules overlaying the heather-like ones. The distance between setae and between setules on the maxilla of *N. integer* are respectively 4 – 5 and 2 – 3 µm apart (Astthorsson, 1980) and should be able to retain particles as small as 2 – 3 µm.

3.8 Gut morphology, chitinases and cellulases

The alimentary canal of crustaceans is typically devisable in three regions (Molloy, 1958; Brunet *et al.*, 1994): foregut, midgut and hindgut. In Mysidacea the foregut is more or less distinctly divided into four regions (Kobusch, 1998): the oesophagus, the cardiac and pyloric chambers of the stomach and the funnel. The midgut consists of a comparatively long straight intestine bearing a number of blind-ending caeca at its junction with the foregut.

Gelderd (1909), Molloy (1958), Haffer (1965), Mauchline (1980) and Kobusch (1998) all described the histological and morphologic structure of the gut of *Neomysis integer* (Figure 4a, b). Its oesophagus is short and muscular, lined with chitin and has spines that tend to point upwards towards the stomach. The stomach is divided into a large anterior 'cardiac' region and a smaller posterior 'pyloric' region. Both are armed internally with strong spines and setae and posses folds to assist the passage of food through the stomach. In general, the omnivorous *N. integer* retains a large mass of food in the cardiac chamber of the stomach, where it is continuously being worked up by the various cardiac folds, spines and teeth (Molloy, 1958).

Neomysis integer has a single, large dorsal diverticulum (Figure 4b) that arises as an extension of the midgut in the posterior dorsal area of the pyloric region of the stomach. It continuously secretes the peritrophic membranes into the intestine, within which the faecal material is bound (Molloy, 1958). The digestive glands (or hepatopancreas), consisting of two groups of five blind-ending caeca, open with a common duct into the posterior ventral area of the pyloric region of the stomach (Figure 4c, d). Part of the food passes from the stomach into the lumina of the digestive glands. The walls of the digestive glands posses circular muscles that power the movement of food and digestive enzymes within the lumina. The finely particulate food is digested and absorbed there. The caeca can reach up to the abdomen and is coloured yellowish green to brown in freshly caught specimens (Molloy, 1958). The intestine emerges from the stomach in the 3rd or 4th thoracic segment and extends posteriorly (as midgut) to the posterior end of the fifth abdominal segment where it becomes the hind gut, wider, lined with chitin and more muscular. Studies of a number of crustacean species revealed that cells of the digestive epithelium are cyclically shed (Brunet *et al.*, 1994) and replaced as is supposed to happen in *N. integer* as well (Bradshaw *et al.*, 1989). The hindgut is short, extending only from the 6th abdominal segment to the anus, which is situated ventrally at the base of the telson. The volume of the gut of *N. integer* is approximately 7.5 mm^3 for a 10 mm long (SL) mysid (Irvine *et al.*, 1993).

Cellulases and chitinases have been identified in whole specimen extracts of *Neomysis integer*, probably present in the alimentary canal (Molloy, 1958). So it can be assumed the species is capable of digesting exoskeleton remains of ingested zooplankton, their own moults and faeces (with chitinous peritrophic membrane) and the (refractile) macrophyte detritus. Also it is assumed that some kind of inhibitor must be present as well to prevent digestion of the chitinous lining of the hindgut and the chitinous peritrophic membrane surrounding the faeces (Molloy, 1958).

In other detritivore crustaceans, cellulolytic bacteria are described to mobilize previously unavailable material in the faeces (Hargrave, 1976). Starved *Neomysis integer* individuals are known to re-ingest their faeces and may increase the efficiency of nutrient extraction due to both processes (enzymes and bacterial breakdown). Its own digestive system can be complemented by the 'external rumen' of bacterial action through coprophagy (Parker and West, 1979). The cellulolytic bacteria are not yet specified in the mysid *N. integer* though, although Armitage *et al.* (1981) mention the presence of general gut micro-organisms in the species. Other authors (*e.g.* Hargrave, 1970) suggest that the main energy value of the macrophyte detritus for crustaceans lies in the associated bacteria, fungi and protozoans present on the detritus, while the detritus itself is egested unchanged.

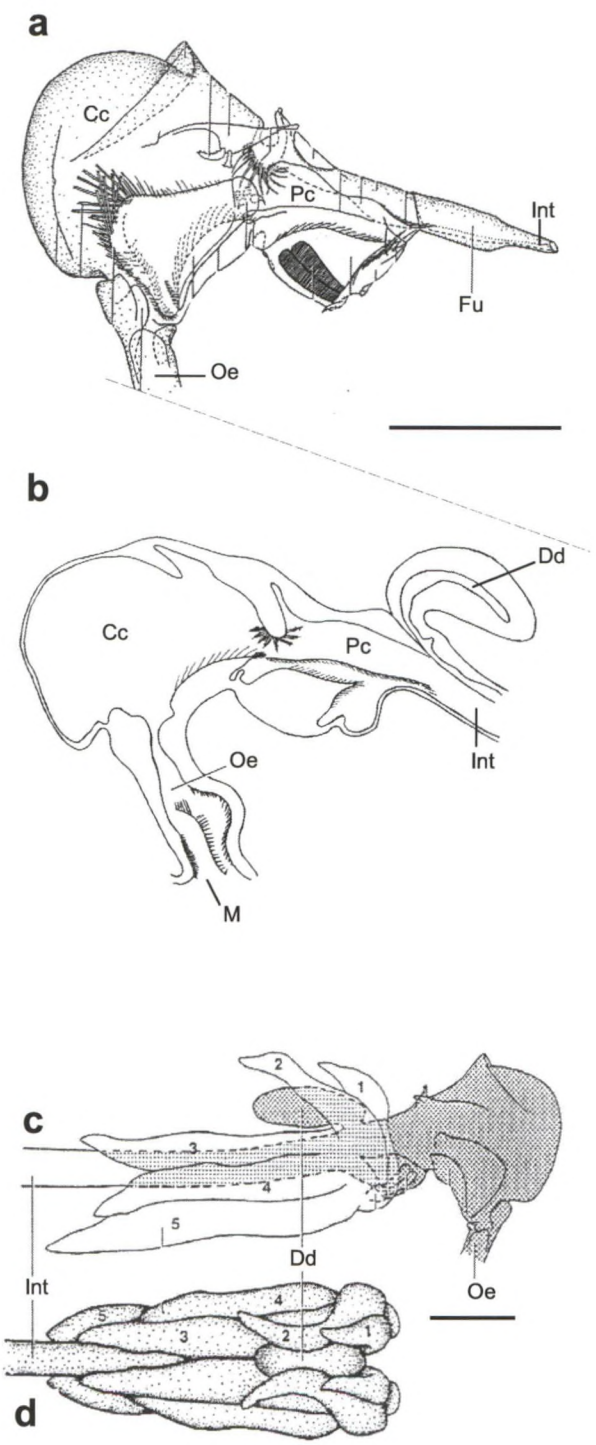

Figure 4: (a) Foregut of *Neomysis integer* in lateral view, with dorsal diverticulum omitted (Kobusch, 1998). (b) Schematic view of a longitudinal section through the foregut (Molloy, 1958; Mauchline, 1980). (c) Midgut glands in relation to the foregut in lateral view (Kobusch, 1998) and (d) in dorsal view with the foregut removed usch, 1998). Cc: Cardiac chamber (foregut); Dd: Dorsal diverticulum (midgut); Fu: Funnel (foregut); Int: Intestine (midgut); M: mouth; OE: oesophagus (foregut); Pc: Pyloric chamber (foregut); 1-5: five tubes of the digestive glands on each side. Scale bar: 500µm

3.9 Gut passage time

Molloy (1958) noted that carmine particles require 30 – 40 minutes, occasionally as much as 90 minutes, passing along the complete length of the alimentary track of *Neomysis integer*. The food stays in the stomach and anterior part of the midgut for most of the time. The intestine of *N. integer* starts anti-peristaltic movements when faecal material passes into the abdominal part of the intestine (Molloy, 1958). The rhythmic pumping of the hindgut muscles is associated with gulping in of water through the anus, and subsequent defecation. These gulping movements appear to initiate a forward wave of contractions along the intestine (anti-peristalsis).

Direct observations of the passage of food through the gut of *Neomysis integer* were performed by Ferguson (1973). Animal food (crab hepatopancreas) had a considerably longer time in the gut than the (natural and laboratory-made) detrital food sources; the first faecal pellets were produced after respectively 177 – 188 and 35 – 56 minutes and gut clearance took 4 to 6 times as long as on natural detritus. There was little difference in the digestion rate between juvenile and adults feeding on the same food source. *N. integer* produced more faecal pellets on the detrital diet as compared to the animal diet, although the time allowed for ingestion was similar (Ferguson, 1973). The higher the assimilation efficiency of a certain food source, the longer the gut passage time (Ferguson, 1973). The higher the ash content of the food, the shorter the gut passage time (Ferguson, 1973). Fockedey *et al.* (submitted d – Addendum 2) determined the gut passage time on environmentally relevant food sources for estuarine *N. integer* to be between 0.5h (estuarine aggregates) and 2.6h (*Eurytemora affinis*).

3.10 Faecal pellets and coprophagy

Neomysis integer produces faecal material surrounded by a chitinous peritrophic membrane. The pellets break apart into smaller pieces after egestion. One fifth of the produced faecal pellet weight disintegrates and disperses into the surrounding water in which the animal is living (Bradshaw *et al.*, 1989; Maciejewska and Opalinski, 2002).

Neomysis integer feeds on its own faeces when there is a shortage of food (Molloy, 1958; Parker and West, 1979; Weisse and Rudstam, 1989; Roast *et al.*, 2000). The faecal pellets of *N. integer* are potential sources of energy; especially when derived from organically rich food sources, the faecal pellets have a high carbon content (40 – 60 %; Ferguson, 1973). A more efficient assimilation of a specific food item does not automatically implicate organically poorer faecal pellets (Ferguson, 1973). An assimilation efficiency of more than 75 % resulted mostly in faeces containing > 35 – 66 % organic matter. When *N. integer* is offered its own faecal pellets as food, the assimilation efficiency is 10 – 25 %. Although this is not particularly high, it indicates that some nourishment can be derived from the faecal pellets. The faecal pellets produced by *N. integer* on a variety of food environmentally relevant sources are studied in detail (scanning electron micrographs and C:N analysis) by Fockedey *et al.* (submitted d – Addendum 2).

3.11 Starvation

Juvenile and subadult *Neomysis integer* survived no longer than 7 weeks when starved at 9 °C (Gorokhova and Hanssen, 1999). Morris *et al.* (1977) and Armitage *et al.* (1978) starved *N. integer* by feeding it with kaolin clay and obtained a mortality of 60 % after 6 to 8 days. Survival to starvation is dependent on salinity and temperature conditions and size of the mysid (Vlasblom and Elgershuizen, 1977; Winkler, 2000).

Starvation of *Neomysis integer* during 5 weeks resulted in a 36 % mortality, a dry weight decrease of 18.6 % and 7.6 % reduction of body carbon (Gorokhova and Hanssen, 1999; Gorokhova, 2002). Starvation reduces the protein content of *N. integer* (-41 %) and thus reduces the dry weight (Armitage *et al.*, 1977; 1978). Wet weight does not alter much during starvation, because of the increase in water content (Armitage *et al.*, 1978). The level of total free amino acids was reduced (28 – 29 %) in starved animals (Armitage *et al.*, 1977; 1978). Starvation did not change the isotopic composition of *N. integer*, as might be expected since progressively lighter isotopes are expected to be catabolized first (Gorokhova and Hanssen, 1999).

A prolongation of the starvation period from 6 to 30 hours significantly reduced the ammonia excretion rate of *Neomysis integer* (Weisse and Rudstam, 1989), but did not affect the oxygen consumption or dissolved inorganic phosphorus excretion rate. Starvation reduces the carbohydrate reserves of *N. integer* (Raymont and Conover, 1961), but does not reduce significantly the total lipid content (Linford, 1965). More recently, starvation was demonstrated to cause an increased atomic O:N ratio, indicator for the reserve substrate being catabolized (Weisse and Rudstam, 1989). The actual amount of glycogen is small, and allows the mysid to live for only a few hours (Raymont *et al.*, 1966; 1968). The (low) amount of lipid reserves of *N. integer* (12 – 15 % of DW) is enough to maintain respiration rates and activity levels for at least 48 h (Weisse and Rudstam, 1989). When starved for a longer period, the species can deaminate body protein (Raymont *et al.*, 1964; 1967; 1968; Verslycke and Janssen, 2002).

When starved, mysids are known to increase the retention time of the food present in their stomach (Murtaugh, 1984). Molloy (1958) starved *Neomysis integer* in order to clear the gut, but 5 – 7 days after being deprived of food, the alimentary track still contained food items. When food is limited, *N. integer* is feeding coprophagously on its own faeces (Weisse and Rudstam, 1989; Roast *et al.*, 2000). They flex their body and bring the faecal thread close to the mouthparts where it is seized, drawn out and eaten (Parker and West, 1979). They also feed on dead *N. integer* individuals, though living ones, other than lost larvae and just released juveniles, are not attacked when there is shortage of food (Raymont and Krishnaswamy, 1960; Parker and West, 1979; Fockedey, unpublished). Usually only the thoracic region of the dead *N. integer* is eaten. It is thus difficult to ensure complete starvation conditions experimentally for *N. integer*. Armitage *et al.* (1977, 1978) solve this by administering kaolin clay, since the mysids keep on feeding and hence do not retrieve the remaining food in the stomach.

3.12 Feeding ecology and changing temperature and salinity

Generally, the ingestion rate increases with increasing temperature, as anticipated from the general effect of temperature on most rates of physiological processes including the feeding responses of aquatic invertebrates. Increased temperature increases the predation rate of *Neomysis integer* (Astthorsson, 1980; Winkler and Greve, 2004) and the egestion rate on organically poor sediments (Roast *et al.*, 2000). Temperature has a strong effect on the excretion rates (ammonia and DIP) of *N. integer* (Weisse and Rudstam, 1989). The feeding rate of *N. integer* feeding on organically poor sediments increased also with increasing salinity (Roast *et al.*, 2000), probably because more energy is spent in osmoregulation.

Roast *et al.* (2000) used the response of the egestion rate (as a measure of the ingestion rate) to changes in salinity and temperature under laboratory conditions, to calculate the tidal and seasonal changes in the feeding rate of *Neomysis integer* in the East Looe River estuary (with extreme tidal fluctuations in the salinity and temperature). They concluded that the feeding rates of *N. integer* are generally low during the tidal cycle, except for a short period of 2 hours around high tide. Seasonal temperature changes cause seasonal changes in the feeding rate of *N. integer*, with increased rates in the warmer summer months, *i.e.* the main reproductive period (Roast *et al.*, 2000).

Two other aspects of the feeding ecology affected by the prevailing temperature and salinity are: (1) the survival to starvation (see above; Vlasblom and Elgershuizen, 1977) and (2) the assimilation efficiency of *Neomysis integer* increases with increasing temperature when feeding on animal food (Ferguson, 1973), though not when feeding on organically poor sediments (Roast *et al.*, 2000). Salinity has little or no impact on the assimilation efficiency of *N. integer* feeding on animal food or sediments (Ferguson, 1973; Roast *et al.*, 2000).

3.13 Survival, growth and reproduction on different diets

Survival, growth, development and reproduction rates can be used as indicators of food quantity and food quality. Feeding rate alone cannot explain the differences observed in growth, development, egg production or mortality (Koski *et al.*, 1998) and thus experiments are needed to elucidate the degree to which specific food items are ingested, digested and – especially – assimilated. Somatic tissue and eggs have a different chemical composition, which demand a different nutritional composition of the food. Probably specific lipids and fatty acids are more critical for gonad development and egg production, while proteins are more important for somatic growth (Kiørboe *et al.*, 1985).

Until now, few studies have examined the survival and growth rates of mysids in relation to food quality (Lehtiniemi *et al.*, 2002) and little information is available on the impact of food quality on their reproduction (Domingues *et al.*, 2002). Some studies are available on the impact of food quality on the feeding activity, ingestion and assimilation efficiency (Ferguson, 1973; Astthorsson, 1980). Filter feeding experiments with *Neomysis integer* on 5 species of cultured algae (Astthorsson, 1980) suggested that only when feeding on one species (*Cricussphaera oblongata*), the mysid was able to meet all its metabolic requirements. On the other phytoplankton *N. integer* experienced some kind of shortage.

For *Neomysis integer*, only Ferguson (1973) performed experiments for comparing growth efficiencies of selected size classes of mysids feeding them an animal diet (crab hepatopancreas) and two detrital diets (artificial detritus of aged algal culture and natural detritus). The smallest animals exhibited the highest growth efficiencies. When feeding artificial detritus, the growth efficiency was significantly smaller than on the animal food. On natural detritus, collected from the surface of the marsh creek beds, the animals lost weight due to the poor quality of the natural detritus sample. Fockedey *et al.* (submitted a – Chapter 5) evaluated survival, growth and moulting of subadult *Neomysis integer* when fed *Eurytemora affinis*, estuarine aggregates and macrophytal detritus in order to evaluate the nutritive value of these environmentally relevant food sources.

4 LIFE HISTORY

Reliable estimates of secondary production and well documented life history data are necessary for key species in order to understand the functioning of estuarine ecosystems. As life history characteristics can vary considerably from one habitat to another, local knowledge on the species' biology is essential for further use in ecosystem modelling, energy flow studies, experimental and toxicological work. For *Neomysis integer*, quite some data are available on its life history over a wide geographical and habitat range (Table 5), and quite some variations exists between populations. Life history of southern populations < 51°N are poorly known, with the exception of the Gironde population (Sorbe, 1980). Studies on the Guadalquivir population, at the southern most border of the species distribution area are ongoing and not published yet (Vilas, personal communication).

4.1 Seasonal dynamics of *Neomysis integer* populations in the estuary

Most studies on the population dynamics of *Neomysis integer* are exclusively based on field data (*e.g.* Mees *et al.*, 1994 and the references herein). Generally, length frequency distributions are obtained through regular sampling (once or twice per month) of the population for at least one year. As a result of the asynchronous moulting between individual mysids, the lengths within a cohort are presumed to be normally distributed. Cohorts can then be segregated by modal progression analysis, but this is often complicated due to the occurrence of overlapping generations and prolonged reproductive periods (*e.g.* Astthorsson and Ralph, 1984; Mauchline, 1985; Irvine *et al.*, 1995). In order to detect and separate cohorts in a more objective way, length frequency distributions can be analyzed with the Bhattacharya method (Bhattacharya, 1967 implemented in Pauly and Caddy, 1985). It splits composite length-frequency distributions into separate normal distributions. The means of the normal distribution for all sampling dates are then plotted over time to trace the modal length progression (growth curve) of the cohorts. This technique was used for *N. integer* populations in the Schelde, Galgenweel, Gironde, Tamar and Guadalquivir (Mees *et al.*, 1994; Fockedey, unpublished; Mees *et al.*, unpublished; Villas, personal communication; Moffat, 1996). For each of the cohorts identified, density, biomass and production can be estimated, and growth curves can be fitted. The growth rate (mm d^{-1}) of each cohort can then be calculated as the increase in the mean length during each sampling interval (Omori and Ikeda, 1984).

Based on the development of the secondary sexual characteristics, the mysid individuals within the population can easily be staged and sexed (Mauchline, 1971) into the following classes: (1) juveniles, (2) immature males, (3) mature males, (4) immature females, (5) adult females and (6) gravid females. Adult males are distinguished by their well developed 4th pleopods which reach beyond the posterior edge of the last abdominal segment. They are further characterised by a well-developed and setose lobus masculinus between the flagellae of the antennal peduncle. Adult females all have a well developed marsupium between the thoracic legs. Juveniles lack secondary sexual characteristics. A further distinction between adult and subadult (immature) males and females is often more subjective. For subadult males the following criteria were used by Mees *et al.* (1994), Mees *et al.* (unpublished) and Fockedey (unpublished): the 4th pleopod stop short of reaching the end of the last abdominal segment and/or the lobus masculinus is present but it is much smaller than in adult males and it is not yet setose. The latter criterion seemed to be the most reliable when distinguishing immature males from juveniles. Females were categorised as adults when their marsupial were large enough to be seen from the lateral side. In contrast, the developing Oostegites in subadult females are only visible between the thoracopods when the ventral side of the animal is carefully examined. Adult females are further divided into females without embryos or larvae (fully developed but empty marsupia) and 'gravid' or ovigerous females (embryos or larvae present in the marsupium). When gravid females are present one can make larval counts of females with complete broods.

Information on the population structure at subsequent moments within the year, like the relative number of adults and gravid females or the appearance of high amounts of small juveniles, helps to identify the breeding season and distinguish the subsequent cohorts within the length-frequency distributions.

Some authors distinguish juveniles from subadult stages at a fixed length: 6 mm TL (Platenkamp, 1983), 6 mm TL (Borghouts, 1978), 8 mm TL (Kinne, 1955) and 9 – 10 mm TL (Beattie and de Kruijf, 1978; Bremer and Vijverberg, 1982). However, this is an inadmissible simplification since the transition from juvenile to subadult occurs at a different size, dependent on the season, sex of the animal and the latitude it is living at (Mauchline, 1971; Schuchardt *et al.*, 1989; Mees *et al.*, unpublished).

4.1.1 Annual production

Annual production can be estimated from the length-frequency data and a length-weight regression (Table 2). Three commonly used methods, each with their own strengths and weaknesses are: (1) the growth summation method (Winberg, 1971; Crisp, 1984), (2) the removal summation method (Crisp, 1984) and (3) the size frequency method (Hynes and Coleman, 1968; Menzie, 1980). Furthermore, production of mysids can independently be estimated using the estimated mortality rate. This low effort method only requires the length-frequency distributions and an estimate of the mean annual biomass (Brey, 1986). The total mortality of a population equals the P/B ration, if the individual growth can be described by a von Bertalanffy function (Allen, 1971). The first method is applied most in production estimates of *Neomysis integer* (Bremer and Vijverberg, 1982; Arndt and Jansen, 1986; Mees *et al.*, 1994; Irvine *et al.*, 1995). The different methods were compared with each other in a study of the population dynamics of *N. integer* in the Schelde estuary (Mees *et al.*, 1994). The growth summation and removal summation method yielded comparable production estimates. The size-frequency method only gave similar results when applied to the 3 cohorts and to both sexes separately. Although the identification of cohorts is not a prerequisite to obtain a production value with this method, it is advisable in order to avoid an overestimation (up to 40 %). The production estimate based on the mortality rate of the different cohorts resulted in values comparable for the overwintering generation, but overestimated the spring and summer cohort production with 24 %.

The annual production of *Neomysis integer* in the Schelde **estuary** was 322 – 449 mgADW $m^{-2} y^{-1}$ (Mees *et al.*, 1994). Despite the long life span of the winter generation it generated only a quarter of the annual production, while the spring generation accounted for almost half of it.

Several annual production estimates of *Neomysis integer* are available for the **Baltic** region. In the southern Baltic (Darss-Zingst estuary and adjacent bays), the annual production was estimated as 3.0 – 4.7 gWW $m^{-2} y^{-1}$ (Arndt, 1985) or 437 – 876 mgDW $m^{-2} y^{-1}$ (Arndt and Jansen, 1986); but Thiel (1992) obtained a higher production of 9 gWW $m^{-2} y^{-1}$ for the same region. The annual production in the shallow littoral water (< 1.5 m) is one order of magnitude higher as compared to deeper water of the Darss-Zingst Bodden: 400 – 800 vs 30 – 60 mgDW $m^{-2} y^{-1}$ (Arndt and Jansen, 1986).

The annual production of *Neomysis integer* in **brackish lakes** is variable. For Lake Ferring (Denmark) it amounted to 2.2 gDW $m^{-2} y^{-1}$ (Aaser *et al.*, 1995), and daily production peaked in July and August when respectively 35 and 29 mgDW $m^{-2} d^{-1}$ were recorded. In Hickling Broad (England) the daily production ranged from 2 to 73 mgDW $m^{-2} d^{-1}$ (Irvin *et al.*, 1995), yielding a total annual production (including intra-marsupial production) of 5.8 gDW $m^{-2} y^{-1}$. Soselisa (1994) reports an annual production of 5.6 gADW $m^{-2} y^{-1}$ in the Belgian brackish pond Galgenweel. Bremer and Vijverberg (1982) and Bremer (1980) calculated a far lower production of *N. integer* in the Dutch lakes Slotermeer and Tjeukemeer as 10 mgDW $m^{-2} y^{-1}$ and 0.03 mgDW $m^{-2} y^{-1}$, respectively.

Most estimates of the production of *Neomysis integer* must be considered to be underestimates, since net efficiency, mesh selection or weight-loss due to formalin conservation are generally not taken into account. In none of the former studies the loss through moulting or the intra-marsupial production were considered. One exception on the latter is Irvine *et al.* (1995), who calculated the intra-marsupial production rate using the growth increment summation method as being always lower than 10 % of the total production.

Table 5: Available life history studies on *Neomysis integer*

Lat	Long	Name location	#cohorts	Longelivity cohorts	Breeding season	**Summer (Aug-Sept)** TL gravid females (in mm)	Brood size (# embryo ind^{-1})	Source
58°N	17°E	Baltic – Himmerfjärden (S)	2	no information	no information	12-13	no information	Rudstam et al., 1986
57° N	2° W	Estuary – Ythan (Scot)	2	S: May to Aug/Sept W: Jul/Sept to May+	Mar-Sept + winterstop	13-17 (16°C/ ? psu)	15-32	Astthorsson, 1980; Astthorsson and Ralph, 1984
56°N	5°W	Estuary/Loch – Etive (Scot)	3†	Sp: Mar/May to Jul S: Jun/Jul to Oct/… W: Sept/… to Jun/Jul+	All year round Low in winter	9-15	14-32	Mauchline, 1971
56°N	8°E	Lake – Lake Ferring (D)	2	S: May/Jun to Aug/Sept W: Jul/Aug to May+	May-Oct + winterstop	no information (3-6 psu)	no information	Aaser et al., 1995
54°N	18-19°E	Baltic – Gdansk Bay, Vislinkiy Bay (P)	2	S: 5-6 months W: 11 months	May-Nov	13	17	Wiktor, 1961; Tehn, 1992
54°N	9-10°E	Kiel Canal, at entrance Eider-Ring Canal (G)	2/3	Sp: May-Jun to Jul/Aug S: Jul to Sep or Jun+ W: Sep to June+	Apr-Sept + winterstop	10-13 (16°C / 7-14 psu)	9-22	Kinne, 1955
54°N	12-13°E	Baltic – Darss-Zingster Bodden (G)	2	S: May/Jun to Aug/Sept W: Aug/Sept to May/Jun+	May-Nov + winterstop	10-17 (15-18°C/ 0.5-15psu)	8 – 30	Jansen et al., 1980; Arndt and Janssen, 1986; Jansen, 1986
53°N	9°W	Estuary/Loch – Furnace (IR)	3†	Sp: Apr/May to Aug S: ? to … W: Sept/… to May+	All year round Low in winter	10-14 (0-2psu)	10	Parker and West, 1979
53°N	6°E	Lake – Bergumermeer (NL)	2	Sp: May/Jun to Sept W: Sept to May+	May-Sept + winterstop	no information	no information	Beattie and de Kruijf, 1978; de Kruijff, 1977
53°N	8°E	Estuary – Weser (G)	3	Sp: May/Jun to Jul/Aug S: Jul/Aug to Nov W: Sept/Oct to May+	Apr-Oct + winterstop	10-15 (10°C)	18-20	Schuchardt et al., 1989; Haesloop, 1990
52°N	1°E	Lake – Hickling Broad (Eng)	2	S: Apr/May to Jul (or Jul+?) W: Jul to May+	Feb/Mar-Oct + winterstop	7-8 (?°C/?psu)	no information	Irvine et al., 1993 Irvine et al., 1995
52°N	4°E	Lake - Rowing course (NL)	---	Sp: Jul – die in August! W: imigration in Jun/Jul	No survival	Died! (>21°C / 0.7psu)	No offspring	Vorstman, 1951
52°N	5°E	Lake – Slotermeer (NL)	3	Sp: Jun to Jul S: Jul to Sept W: Aug/Oct to Jul+	May-Oct + winterstop	10-13 (20°C / 0.4psu)	13-22	Bremer, 1980; Bremer and Vijverberg, 1982

Table 5 (cont.)

Lat	Long	Name location	#cohorts	Longelivity cohorts	Breeding season	Summer (Aug-Sept) TL gravid females (in mm)	Brood size (# embryo ind^{-1})	Source
52°N	5°E	Lake -Tjeukemeer (NL)	3	Cfr. Slotermeer, but with 1-2 weeks delay	no information	No information (0.1-0.3psu)	No information	Bremer, 1980
52°N	5°E	Lake – Barnegat (NL)	3	Sp: Apr/May to Aug S: Jul to Aug W: Aug to Jul+	Mar-Sept + winterstop	7-13 (?°C / 2.5psu)	± 6	Vorstman, 1951
51°N	3°E	Lake – Den Inkel (NL)	3	Sp: May to Jun/Jul S: Jun to Aug/Sept W: Aug to May+	May-Aug + winterstop	7-9 (17°C / 16psu)	± 5*	Borghouts, 1978
51°N	3°E	Lake –Veerse meer (NL)	3	Sp: May to Jun/Jul S: Jun to Sept W: Sept to May+	Apr-Sept + winterstop	10-15 (17°C / 13-23psu)	8-42*	Borghouts, 1978
51°N	3-4°E	Grevelingen (NL)	3	Sp: May to Jun S: Jun/Jul to ? W: ? to May/Jun+	Apr-? + winterstop	no information (13psu)	no information	Platenkamp, 1983
51°N	4°E	Estuary – Schelde (NL/B)	3	Sp: Apr/May to Sept S: Jun to Nov W: Aug/Sept to Jun+	Apr-Oct + winterstop	10-13 (19°C/23psu)	14-22*	Mees et al., 1994
51°N	4°E	Galgenweel (B)	3	Sp: Mar to Jul S: June to Sept W: Aug to May+	Mar-Sept	7-12 (18°C/4psu)	5-25*	Soselisa, 1994; Fockedey, unpublished
51°N	2°E	Lake - Stuivekenskerke (B)	2/3?	Sp: May to Jul S: Jul to ? W: ? to May+	Apr-Sept + winterstop	no information	no information	Espeel, 1979; 1982
51°N	1°W	River Test (UK)	---	Sp: March to ? no further information	All year round Low in winter	no information (15-21°C/2-27psu)	no information	Raymont et al., 1966
45°N	0°W	Estuary – Gironde (F)	>3†	Sp: Feb to May S†: May to Aug/Dec W: Aug to May+	Feb-Nov + winterstop	7-8 (?°C / ? psu)	no information	Sorbe, 1980; Mees et al., unpublished

TL: Total length; **Sp**: Spring generation; **S**: summer generation; **W**: winter generation; **+**: following year; **?**: no information on salinity and/or temperature conditions; ***** derived from fecundity-size regression given in the paper; **†** no distinct generation, but rather modal age groups due to intensive breeding in certain periods of the year against a backgroup of continous breeding; **...**: juveniles appear through winter alt a very low rate

4.1.2 P/B ratio: biomass-specific production

The P/B coefficient can be of more general significance than the production value itself (Greze, 1978). The P/B decreases with size and body mass within and between populations. Within a population the young animals show low body mass and relatively high growth (*i.e.* production) and have a comparatively high P/B, whereas the large and older animals have a reduced growth and a high biomass and resulting in a comparatively low P/B ratio. P/B ratio decreases with each trophic level by one order of magnitude, as the transfer efficiency among trophic levels amounts to 10%. P/B is mostly measured on an annual scale or daily scales.

The **estuarine** *Neomysis integer* population from the Schelde has an annual P/B ratio of 6, while the average cohort P/B was 3 (Mees *et al.*, 1994). The daily P/B coefficients of *N. integer* in the southern **Baltic** are 0.011 to 0.013 (Arndt and Jansen, 1986). In **brackish lakes**, the daily P/B ratio for *N. integer* varies between 0.001 and 0.050 in Frisian lakes (Bremer and Vijverberg, 1982), while in a lake in Jutland values between 0.001 and 1.0 were recorded (Aaser *et al.*, 1995). Peak values of the daily P/B coefficient coincide with periods when the recruitment of juveniles into the population is high (Bremer and Vijverberg, 1982). In brackish lakes, the annual P/B ratio is 3.3 to 14.8 (Bremer and Vijverberg, 1982; Irvine *et al.*, 1995; Soselisa, 1994; Fockedey, unpublished). The lower and upper limits of the daily P/B coefficients of *N. integer* based of **laboratory experiments** are 0.009 to 0.051 (Kuhlmann, 1982) and are comparable to the daily P/B ratios measured in the field.

4.2 Number of cohorts or generations per year

In the most northern populations (> 53°N) generally 2 generations per year are reported, while in the more southern studies (< 53°N) 3 generations or more are described per year. In the Baltic region of Darss-Zingst (Arndt and Jansen, 1986), the Swedish coast (Rudstam *et al.*, 1986), Gdansk bay (Wiktor, 1961) and the Kiel Canal (Kinne, 1955), only 2 generations are reported. In some brackish lakes, like Lake Ferring (Aaser *et al.*, 1992), Bergumermeer (Beattie and de Kruijf, 1978), Hickling Broad (Irvine *et al.*, 1995) the spring or summer generation is not developed or it is difficult to distinguish them from each other (Borghouts, 1978; Parker and West, 1979). In the Ythan estuary (Astthorsson, 1980; Astthorsson and Ralph, 1984) only 2 generations are described. Sorbe (1980) originally categorized *N. integer* in the Gironde estuary as a bivoltine species as well, although the author expected an additional spring generation to be present between February and May. A later study of Mees *et al.* (unpublished) concluded that even more than 3, highly overlapping generations are present in the Gironde estuary.

The creation and maturation of the specific cohorts differs between the different populations (Table 5). Along the west coast of Ireland and Scotland, in lakes Hickling Broad, Barnegat, Galgenweel and in the estuaries of the Schelde and Gironde, the **spring generation appears** in the population in April –May. In other locations the juveniles of the spring generation are released in May and/or June (Baltic populations, east coast of Scotland; lake Ferring, Dutch lakes Grevelingen, Bergumermeer, Slotermeer, Tjeukemeer, lake Veere, Den Inkel, the clay pits of Stuyvekenskerke and the Weser estuary).

The moment when this **spring generation reaches maturity and releases brood** is not linked to the moment they appear in the population but probably to environmental variables (like temperature, salinity, food conditions, ...). In Slotermeer and Tjeukemeer e.g., the spring generation only appears in the course of June but rapidly reaches maturity to release a **summer generation** in the course of July (Bremer and Vijverberg, 1980; Bremer, 1980). This phenomenon is also observed in the other Dutch lakes (Borghouts, 1978; Platenkamp, 1983), with the exception of the oligohaline Barnegat (Vorstman, 1951). Here the summer generation is considered as the latest hatching product of the overwintering generation before they die. In other populations with a 'late' spring generation, like for example in Lake Ferring (Aaser *et al.*, 1995) or the Barther Bodden (Arndt and Jansen, 1986), the **summer generation** is completely **lacking** due to the delayed maturation of the spring generation in late summer (August/September). Those populations with an early spring generation all produce a summer generation, with the exception of Hickling Broad (Irvine *et al.*, 1993; 1995).

The **appearance** of the individuals that will **overwinter** as large juveniles or subadults generally occurs in late summer (August/September) in most populations with 3 annual generations per year. In the populations of the Weser and Slotermeer this period is prolonged up to October. In loch-like estuaries along the Irish and Scottish west coast breeding continues in winter, although at a very low rate.

In populations with 2 generations a year, the juveniles of the overwintering generation generally appears in July. In the Darss-Zingster Bodden and the Bergumermeer, overwintering juveniles appear in late August/September. The **overwintering generation disappears** from the population generally within the same month the spring generation appears or 1 month later at the most. However in loch Etive, Barnegat, Schelde and Gironde, the overwintering adults only die 3 to 5 months later.

4.3 Maximal longevity / life span

Most epipelagic species of the littoral zone have relatively short lives of 2 – 9 months (Mauchline, 1972). *Neomysis integer* is estimated to be tri- or bivoltine (see Table 5). The winter generation has a longer life span than the spring and summer generations. The latter reach maturity within 1 – 2 months after having left the brood pouch and probably live for maximally 4 months (Mauchline, 1971). The winter generation has a retarded growth and maturation due to the low winter temperature, and starts reproduction only in spring of the following year. They are generally present for 9 – 11 months in the population (Table 5). Mauchline (1971b) reports for the Clyde Sea that remnants of the spring generation (born in June/July) contribute to the winter generation that reproduces in March/May and their longevity is thus maximally 12 – 13 months. Some survivors of the summer generation of the previous year probably are still present in the population of Hickling Broad the next summer (Irvine *et al.*, 1995) and longevity is estimated to be one year. Tattersall and Tattersall (1951) estimate a maximal longevity of 18 months.

Along the west coast of Scotland and Ireland, females of the overwintering generation die first, the males later (Mauchline, 1971; Parker and West, 1979), while in the Grevelingenmeer, Lake Veere, pond Den Inkel and the Schelde estuary females outlive the males (Borghouts, 1978; Platenkamp, 1983; Mees *et al.*, 1994). In the Bergumermeer the males and females died simultaneously (Beattie and de Kruijf, 1978). No difference in the mortality between the sexes is observed for spring and summer cohorts (Mees *et al.*, 1994).

4.4 Size-at-maturity

The animals from the spring and summer generations reach a smaller length at maturity in comparison with the overwintering generation (*e.g.* Mees *et al.*, 1994). In Frisian lakes for example, animals of the summer generation reach at most 15 mm TL, while adults of the overwintering generation reach a maximum length of 19 mm TL (Bremer and Vijverberg, 1982).

Marked differences are apparent in the growth patterns of the 2 sexes within each cohort. Adult females generally have a larger length than males. Adult females of (late summer) *Neomysis integer* (Baltic proper) are approximately 30 % larger than males (Weisse and Rudstam, 1989). Dry weight of adult males from the Baltic ranged from 2.7 to 3.2 mg (overall mean 2.9 mg) and for females between 4.0 and 4.8 mg (overall mean 4.2 mg) (Weisse and Rudstam, 1989). Mees *et al.* (1994) found adult males and females of the overwintering generation – respectively 10 and 14 mm SL (*i.e.* 12 and 16 mm TL) – to be larger than the spring/summer generation at respectively 9.5 and 10 mm SL (*i.e.* 11 and 12 mm TL).

Comparing the population dynamics of *Neomysis integer* in 2 lakes in SW Netherlands, Borghouts (1978) found the size at maturity to be significantly smaller in the more densely populated lake (Den Inkel). Size at maturity of the spring generation was respectively 15 – 19 mm TL and 10 – 13 mm TL in Lake Veere and Den Inkel; in summer respectively 10 – 15 mm TL and 7 – 9 mm TL.

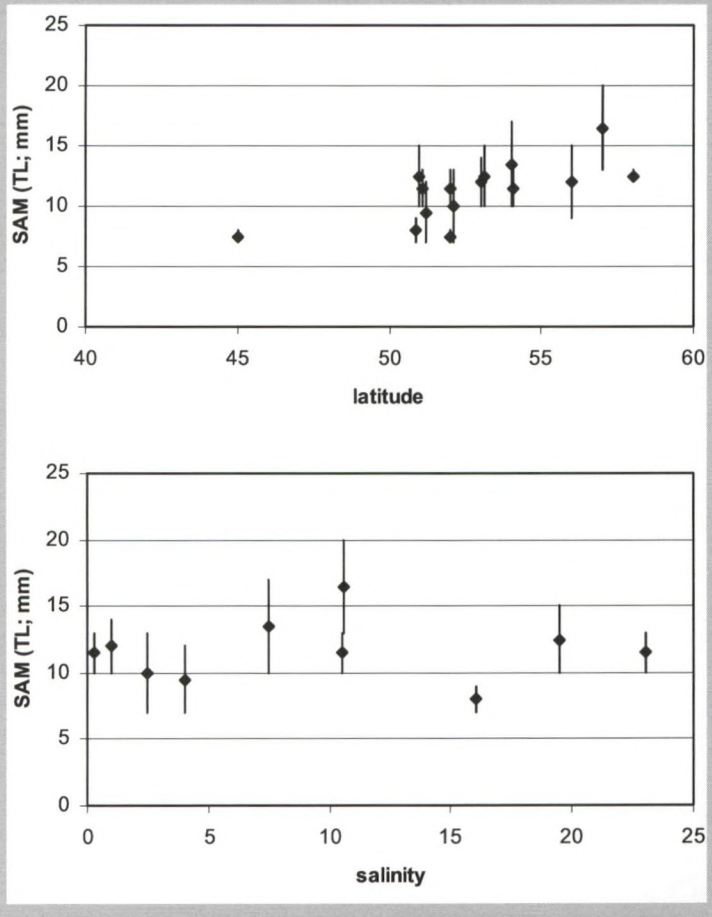

Figure 5: Effect of latitude and salinity on the size-at-maturity (SAM) of all available information in literature

Parker and West (1979) report the *Neomysis integer* of Loch Furnace to be smaller (10 – 14 mm TL) than elsewhere in Europe. Comparing all studies available up till 2005, this postulation no longer holds. The reported size-at-maturity in August/September is smallest (<10 mm TL) in the lakes Hickling broad, Den Inkel and the Gironde estuary. Largest size at maturity (>15 mm TL) are observed in the Ythan and the Darss-Zingster estuaries. Late summer size-at-maturity (August-September) is significantly related to latitude (Spearman rank = 0.66, p = 0.01), but no effect of salinity could be demonstrated (Figure 5).

The comparison of the published data resulted to difficult, mainly because of differences in the measuring and staging techniques used by the different authors. In order to overcome this problem, Mees *et al.* (unpublished) measured and staged *N. integer* collected in spring and summer from populations of the estuaries Guadalquivir, Gironde, Adour, Schelde, Eems, Elbe, Shannon and Lay (Figure 6). A clear logitudinal effect could be observed in the size-at-maturity of *N. integer*.

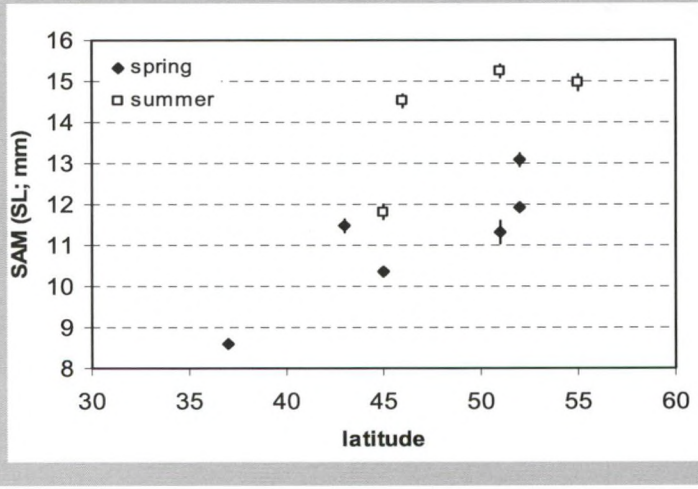

Figure 6: Effect of latitude on the size-at-maturity (SAM) of *Neomysis integer* from Guadalquivir, Gironde, Adour, Schelde, Eems, Elbe, Shannon and Lay (according to Mees *et al.*, unpublished).

4.5 Fecundity

The fecundity of a *Neomysis integer* population, *i.e.* the number of young recruiting to the population, is determined by (1) the prevailing temperatures which governs the breeding season, (2) size-at-maturity and associated brood size, (3) the number of successive broods, (4) intra-marsupial development rate and (5) intra-marsupial mortally. Also, (7) the size of the pool of large mysids that successfully reaches maturity, especially after the winter, and the time to reach maturity also have an important role in the population's fecundity (Irvine et al, 1995).

4.5.1 Breeding season

In the most western populations of *Neomysis integer* of the Scottish and Irish Lochs and the estuaries Severn, Tamar, Test and Conwy breeding is continuous over the year (Raymont *et al.*, 1966; Mauchline, 1971; Parker and West, 1979; Moore *et al.*, 1979; Hough and Naylor 1992; Moffat, 1996). However, periods of intensive breeding result in the production of distinct spring (March-May), summer (June-July) and overwintering (August-September) generations (Mauchline, 1971; Parker and West, 1979). There is always a proportion of the population breeding, but the intensity of breeding in winter (October-February) is low with gravid females representing less than 1 – 2 % of the population (Mauchline, 1971; Moore *et al.*, 1979). Moffat (1996) also mentions continuous breeding in the Tamar estuary, since juveniles are present all year round in the samples.

Other authors (see Table 5) report no reproductive activity during the cold winter period when no gravid females are found, but the breeding season varies substantially between the different populations. The species is described to breed for 5 – 7 months between April/May and September/October in most of the populations. *E.g. Neomysis integer* breeds for 7 months, respectively between April and October in the Schelde estuary (Mees et al., 1994) and between March and September in the pond Galgenweel (Soselisa, 1994; Fockedey, unpublished). The breeding season starts earlier in February/March in the *Neomysis integer* populations of Hickling Broad, Barnegat and Gironde and gets on to November in the populations of Darss-Zingster Bodden and Gironde. An extremely short breeding season of 4 months is recorded in Den Inkel, while extreme long (but intensive) breeding is observed in Hickling Broad (9 months) and the Gironde (10 months).

During the period of most intensive breeding (spring), the relative abundance of gravid females into the population can increase to 17 – 30 % of the total population or more than 80 % of the total number of adults females (Kinne, 1955; Mauchline, 1971; Moore *et al.*, 1979). In the Weser (Schuchardt *et al.*, 1989) and Bergumermeer (Beattie and de Kruijf, 1978) only 40 – 50% of the adult females were gravid. Schuchardt *et al.* (1989) ascribe this relative low value of the proportion of gravid females in the breeding season to (1) an inaccurate separation of the subadult and adult females during the analysis while distinguishing stages; or (2) to high relative numbers of females with the brood recently hatched, or (3) to a relative long period of sexual inactivity in between subsequent broods, although a bad conservation can also be the cause of this low number (Mees, personal communication).

The first breeding in spring is quite synchronous. Given the multiple broods of one female, the synchronicity diminishes over the course of summer and it becomes more and more difficult to distinguish the breeding efforts in subsequent cohorts (Schuchardt *et al.*, 1989). Some authors give the advice to consider the whole period of development of the population as one period of continuous reproduction with 2 or 3 periods of intensive breeding which can be followed for some time as pulses in the length frequency distribution of the population (Mauchline, 1971; Beattie and de Kruijf, 1978; Bremer and Vijverberg, 1982; Schuchardt *et al.*, 1989).

Table 6: Length-specific brood size regressions

Location	Lat	Salinity (psu)	Relation brood size (F) and length		Time period	Source
Estuary – Ythan (Scot, UK)	57°N	10.5	$F_I = 6.0209\ TL - 45.0079$	n=198; r=0.61	Stage I, winter generation	Astthorsson, 1980
			$F_{III} = 5.2774\ TL - 42.1283$	n=86, r=0.36	Stage III, winter generation	
			$F_I = 1.2470\ TL + 12.4118$	n=251; r=0.36	Stage I, summer generation	
			$F_{III} = 2.6434\ TL - 1.2122$	n=297, r=0.38	Stage III, summer generation	
Fjord – Loch Etive (Scot, UK)	56°N	>18	$Log\ F = 1.0385\ log\ (TL^3)-2.0887$	N=750; r=0.739; p<0.01	All year	Mauchline, 1973
Estuary – Kiel Canal (G)	54°N	18-33	$F = 0.0082\ TL^{3.19}$	No information	All year	Kinne, 1955
Baltic – Darss-Zingst (G)	54°N	0.5-15	$F=TL^{1.397298}$	$R^2 = 0.95$	Winter	Arndt and Jansen, 1986
			$F=0.056998\ TL^{2.26274}$	$R^2=0.92$	Summer	
Lake – Bergumermeer (NL)	53°N	0.2-0.4	$F = 0.328\ TL^{1.62}$	No information	All year	de Kruijf (1977)
Lake – Hickling Broad (Eng, UK)	52°N	2.7-3.6	$lnF = -2.41 + 2.28\ lnSL$	n=10; r^2=0.96; p<0.001	1988	Irvine et al., 1995
			$lnF = -4.11 + 2.75\ lnSL$	n=9; r^2=0.99; p<0.001	1989	
Lake – Slotermeer (NL)	52°N	0.2-0.4	$F = 0.0522\ TL^{2.34}$	n=260, r=0.77	All year	Bremer and Vijverberg, 1982
Lake – Tjeukemeer (NL)	52°N	0.2-0.4	$F = 0.00706\ TL^{3.25}$	No information	All year	Vijverberg, unpublished*
Lake – Den Inkel (NL)	51°N	15-16	$F = 0.957(TL-7)^{1.7512}+3.3$	No information	All year	Borghouts, 1978
Lake – Veerse meer (NL)	51°N	11-24	$F = 0.957(TL-7)^{1.7512}+3.3$	No information	All year	Borghouts, 1978
Estuary – Schelde (NL/B)	51°N	15	$F = 0.0365\ SL^{2.656}$	n=420, r=0.866; p<0.001	All year	Mees et al., 1994
		15	$lnF=-3.720+2.828\ lnSL$	n=167, r=0.655; p<0.01	Winter	
		16	$lnF=-2.307+2.223\ lnSL$	n=149, r=0.562; p<0.01	Spring	
		16	$lnF=-0.974+1.673\ lnSL$	n=104, r=0.615; p<0.01	Summer	
Lake – Galgenweel (B)	51°N	4	$F = 0.008\ SL^{3.242}$	N=1070; r=0.852; p<0.001	All year	Soselisa, 1994; Fockedey, unpublished

*Cited in Bremer and Vijverberg, 1982; **F**: Brood size (in number of embryo per marsupium); **TL**: Total length of the female (in mm), as distance between the base of the eyestalk and the posterior end of the uropods excluding the setae (Mauchline, 1971) = distance between the front of the eye till the end of the telson (Kinne, 1955) = 1.05x (distance between the front edge of the eye and the end of the uropods excluding the setae (Vorstman, 1951)); **SL**: Standard length of the female (in mm); **B**: Belgium; **Eng, UK**: England; **G**: Germany; **NL**: The Netherlands; **Scot, UK**: Scotland

4.5.2 Brood size

The number of embryos/larvae in the marsupium depends on body size, the size of the individual eggs, and the season of the year. Brood size also differs between populations living at different latitudes (Mauchline, 1980; Mees et al., unpublished). The maximum brood size reported for *Neomysis integer* is 98 embryos per female in the Ythan estuary (Astthorsson, 1980) and in the Schelde estuary (Mees *et al.*, 1994).

For *Neomysis integer* brood size is demonstrated to be highly depended on female **body size** (Table 6). Bremer and Vijverberg (1982) report a little variation of this relationship over the course of the year, but other authors (Astthorsson, 1980; Arndt and Jansen, 1986; Mees *et al.*, 1994) observed a significantly different size-fecundity relationship between the overwintering generation and the spring- and summer generations.

Seasonal variation in size at maturity, and consequently in fecundity, between different cohorts has been reported. The late summer and autumn breeding animals usually have a smaller size at maturity and related fecundity as compared to those breeding in spring and early summer (Vorstman, 1951; Kinne, 1955; Borghouts, 1978; Parker and West, 1979; Astthorsson, 1980; Bremer and Vijverberg, 1982; Arndt and Jansen, 1986; Mees *et al.*, 1994). Females of the winter generation generally produce more eggs than the spring and/or summer females of the same body size (Mauchline, 1971; Arndt and Jansen, 1986; Mees *et al.*, 1994). For example, the brood size of *Neomysis integer* from the overwintering generation is 50 (40 – 65 ind^{-1}) eggs per female in the Darss-Zingst estuary of the southern Baltic (Arndt and Jansen, 1986); females of the summer generation generally produce far less eggs (9 – 28 ind^{-1}). In Loch Etive breeding continues during winter at a rate of 12 – 27 ind^{-1}, while brood size in spring, early summer and late summer was 29 – 46, 14 – 57 and 14 – 32 ind^{-1}, respectively (Mauchline, 1971). Also, size-specific brood size can vary between successive years (Irvine *et al.*, 1995).

Bremer and Vijverberg (1982) and Schuchardt *et al.* (1989) demonstrated a lower brood size in almost freshwater populations (0.2 psu) in comparison to populations living in more saline water (10 – 18 psu).

Comparing the size-specific brood size of *Neomysis integer* living in the Schelde estuary (15 psu) and a population living in a nearby brackish pond (5 psu), Fockedey and Mees (unpublished) can make a similar conclusion (Figure 7).

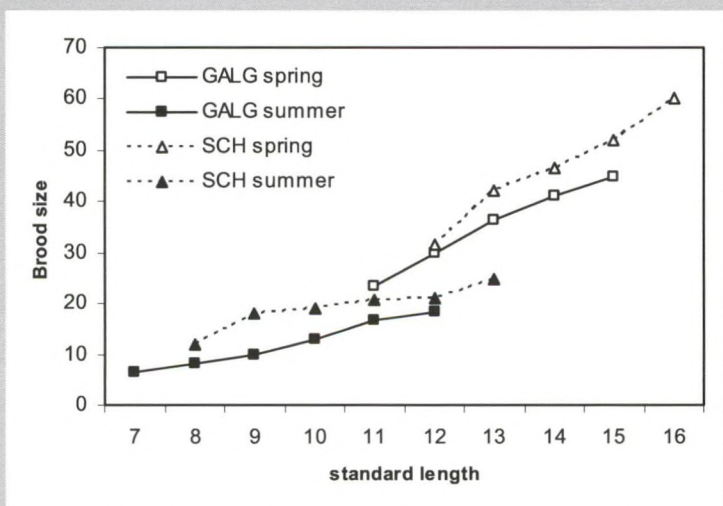

Figure 7: Size-specific brood size of *Neomysis integer* in Galgenweel (GALG) and Schelde (SCH) in the spring and summer generation.

Mees *et al.* (unpublished) report the Schelde population of *Neomysis integer*, living at a higher **salinity** (15 psu) than other estuarine populations (< 5 psu), to be characterised by the largest brood size.

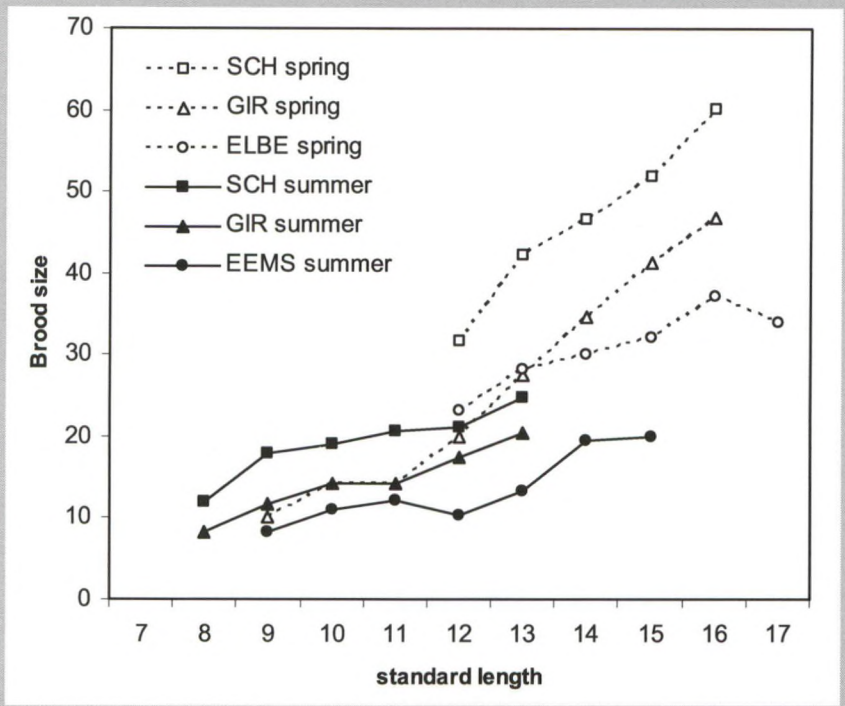

Figure 8: Effect of latitude on the brood size of the *spring and summer generation* in the Elbe (ELBE), Eems (EEMS), Schelde (SCH) and Gironde (GIR) estuaries.

It remains unclear why most estuarine populations live at a low salinity that finally results in a relatively smaller brood size. Possible explanations may include a trade-off for suboptimal brood sizes with competitive advantages of living in low-salinity waters which in estuaries coincide with higher turbidity, lower predation pressure, a higher food availability, less competition with other mysids or benthic filter feeders (Mees *et al.*, 1994).

4.5.3 Number of subsequent broods per cohort

Whether a higher brood size also results in a higher fecundity is difficult to say based on field data. Smaller brood sizes may be compensated by the production of several subsequent broods per female in the spring and summer generations. However, without laboratory observations, it is difficult to decide whether females breed more than once (Vorstman, 1951). Tattersall and Tattersall (1951) coin the possibility of 3 or 4 subsequent broods in the spring individuals. Astthorsson (1980) assumes that *Neomysis integer* in the Ythan produces 2 – 3 subsequent broods. Extrapolating laboratory derived development times to the field observations, Kinne (1955) concludes that the winter generation of *N. integer* in the Kiel Canal produces 3 broods and the summer generation 4 – 6 broods. Mees *et al.* (1994) report unpublished culture experiments (Janssen, unpublished) where *N. integer* produce 5 consecutive broods at 20 °C.

In the laboratory (Fockedey, unpublished; Table 7) *Neomysis integer* produced 4 successive broods at 15 °C, independent of salinity (5 or 15 psu). At 20 °C, up to 5 broods were produced per female. The time between successive brood is 0 – 4 days and 0 – 7 days at 15 and 20 °C.

Table 7: Unpublished laboratory experiments on the *in vivo* intra-marsupial development of *Neomysis integer*.

		15°C – 5 psu		15°C – 15 psu		20°C – 5 psu	
length experiment (days)		61		61		55	
# females brought into experiment		38		32		43	
50% mortality (days)		16		33		21	
Mauchline (1980)	Wittmann (1981)	days	%	days	%	days	%
Egg	Embryonic phase	5.3	32	5.1	32	3.2	29
Larva	Nauplioid phase	6.5	40	6.7	42	4.2	38
Eyed larva	Post-nauplioid phase	4.6	28	4.3	26	3.7	34
	Incubation time (days)	16.5		16.1		11.1	
	# successive broods	max. 4		max. 4		max. 5	
time between successive broods (days)		0 – 2		0 - 4		0 – 7	
# juveniles liberated per brood		24 / 13 / 6 / ?		22 / 11 / 7 / ?		7 / 7 / 6 / ? / ?	

4.5.4 Mortality during intra-marsupial development / hatching success

The number of early embryos in the marsupium always outnumbers the number of later larval stages (Astthorsson, 1980; Mauchline, 1980). Generally, 10 % of the young are lost in a premature condition (Mauchline, 1973; Wittmann, 1981). Mortality or loss of eggs initially laid in the marsupium was estimated to be 27 % for the winter generation; for the summer generation the marsupial mortality was dependent on the body size of the female and was 25, 17 and 3 % for gravid females with a total length of 12, 14 and 16 mm, respectively (Astthorsson, 1980). By comparing the abundance of embryos/larvae in brood carrying females and the subsequent abundance of neonates (< 3 mm) in the field, Irvine *et al.* (1995) suggest a high mortality (> 50 %), especially in the early embryo stages.

Little data were available on the hatching success of embryos of *Neomysis integer*. Vlasblom and Elgershuizen (1977) tested survival of embryos at a relative narrow range of salinities at 15 °C. **Hatching success** was optimal at a salinity between 11 and 18 psu. The optimal salinity for hatching is strongly related to the salinity the animals are adapted to and hatching percentage amounted to 60 – 80 % for animals adapted to low salinity (7 psu) and 40 – 60 % for animals adapted to higher salinity (23 psu). The early embryos had a smaller salinity tolerance than the later larvae (Vlasblom and Elgershuizen, 1977).

More in-depth information on the intra-marsupial development of *Neomysis integer* and the impact of environmental variables on mortality, hatching success, and the duration of the developmental stages is available in Fockedey *et al.*, submitted b – Chapter 6). Information on the impact of biotic factors (size female, brood size, egg size) is still lacking.

4.5.5 Morphology of the developing embryos

Mauchline (1980) divides the intra-marsupial development into three easily distinguishable stages. The early embryo, at first egg-like but later with rudiments of antennae and abdomen developing, are named 'stage I' or 'eggs'. The latter name however is a confusing term, since eggs are immediately fertilized after being deposited from the oviduct into the marsupium. Better is to use the term egg-shaped embryo or (sub)-spherical embryo. The stage I embryos are still surrounded by the egg membrane. Stage II larvae or 'eyeless larvae' are hatched from the egg membrane and develop the antennae and the thoracic appendages, while the eyes become pigmented. Stage II terminates in a moult. Stage III or 'eyed larvae' have stalked eyes and moult to a free living juvenile when released from the marsupium. Most authors follow this division (*e.g.* Wittmann, 1984).

Before, de Kruijf (1977) described 6 distinct developmental stages of the embryo in the brood chamber of *Neomysis integer* and can be distinguished as follows: Stage I is a simple egg-like structure, spherical in shape. The next stage (II) contained a rudimentary abdomen with a clear distinction between the rounded anterior and the pointed posterior of the larva. Development into stage III comprised a further extension of the abdomen, a rudimentary telson and two thoracic appendages. In stage IV the body was further extended and had the clear beginning of abdominal segmentation. In stage V there was a first projection of the head with development of the eyes, further segmentation of the abdomen, and first development of pleopods. Stage VI had distinct eye projections and development of the pleopods and uropods. The body contained an obvious carapax and elongated abdomen. The morphological stages described by de Kruijf (1977) were used by Irvine et al. (1995) in the intra-marsupial staging of *N. integer*, but Fockedey *et al.* (submitted b – Chapter 6) considered them to be impractical and based on subjective criteria. Wittmann (1981) even used a third set of criteria to stage the embryos and larvae of mysids in general and Fockedey *et al.* (submitted b – Chapter 6) compares the different terminologies used.

Detailed descriptions of the histology of successive larval stages of *Neomysis integer* are given by Wagner (1896) and Needham (1937). External morphological descriptions of the intra-marsupial development are available by Kinne (1955) and de Kruijf (1977). A description of the embryonic and larval stages of *N. integer*, together with pictures illustrating the development, is given in Fockedey *et al* (submitted b – Chapter 6).

4.5.6 Duration of the intra-marsupial development

Neomysis integer, like all other mysid species, carries its embryos in a marsupium within which the entire larval development takes place from oviposition to the release of free swimming juveniles (Wittmann, 1984). No correlation could be found between the duration of the intra-marsupial development and the prevailing salinities (0.4 – 16 psu) at 15 °C (Vlasblom and Elgershuizen, 1977). Individuals adapted to a higher salinity of 23 psu are able to develop their embryos, but over a longer time span (*i.e.* 17 – 22 days) than the population adapted to lower salinity of 7 psu (15 – 18 days). Irvine *et al.* (1995) report an intra-marsupial development time of 42 days at 6 °C and 5.8 days at 20 °C measured *in vivo* in laboratory experiments. Indirectly estimated development times are obtained from field observation in Hickling Broad (Irvine *et al.*, 1995): 56 days at 7 – 16.5 °C, 29 days at 16.5 – 18.0 °C and 13 – 14 days at 19.5 – 20 °C.

Generally, most breeding females are found with eyeless larvae in their marsupium, indicating that this is the longest larval stage in mysids intra-marsupial development (Mauchline, 1973; 1980; Parker and West, 1979). Astthorsson (1980) found on average almost equal proportions of gravid females with spherical larvae, eyeless larvae and eyed larvae in a 2 year study of the *Neomysis integer* population from the Ythan.

Following the intra-marsupial development in detail *in vivo*, *i.e.* through the semi-transparent oostegites, is difficult (Fockedey, unpublished; see table 7) or needs anaesthetization (Irvine *et al.*, 1995). Information on the dependence of the intra-marsupial development time with temperature or/and salinity is reported in Fockedey *et al.* (submitted b – Chapter 6).

4.5.7 Size, growth and intra-marsupial production of the embryos

Embryo size of *Neomysis integer* from Hickling Broad generally falls within the range of 0.48 – 2.1 mm (Irvine *et al.*, 1995). The diameter of the egg-shaped embryo of *N. integer* from Loch Etive equals 0.5 – 0.6 mm (Mauchline, 1972). The length of the eyeless larvae is 1.3 – 1.5 mm (Mauchline, 1972). The length of the eyed larvae is 1.9 – 2.1 mm (Mauchline, 1972) and the total length of freshly emerged juvenile is ± 2.0 mm, *i.e.* 1.6 – 2.4 mm (Vorstman, 1951; Kinne, 1955; Mauchline, 1971; 1972; Astthorsson, 1980; Aaser *et al.*, 1995; Moffat, 1996).

The biomass of embryos of *Neomysis integer* was estimated from their volumes as 11.3 µg DW for the early spherical embryos, 16.0 µg DW for ellipsoid embryos and 13 – 14.4 µg for the later larval stages (Irvine *et al.*, 1995). The size of the (earliest) embryos in *Neomysis integer* can vary seasonally (Mauchline, 1973; Irvine *et al.*, 1995), with winter embryos being larger than in spring or summer. The smaller embryo appeared to contain fewer and smaller oil globules (Mauchline, 1973). Although no more details are given, Irvine *et al.* (1995) estimated the intra-marsupial production rate of *Neomysis integer* at all times to be less than 10 % of the total production.

4.5.8 Instantaneous birth rate

The instantaneous birth rate b' of *Neomysis integer* can be estimated from the equation:

$$b' = \ln((E/N) + 1)/D$$

with E the egg density, N the total density of all life stages and D the development time at the appropriate temperature (Paloheimo, 1974). Since the mortality during the first embryonic stages is high (> 50 %) instantaneous birth rate b' was calculated by Irvine *et al.* (1995) based on the egg ratio E and development time of the latest larval stages only. In the reproductive periods (June to October) the instantaneous birth rate of *N. integer* in a brackish lake (Hickling broad) was measured as 0.05 – 0.15, *i.e.* between 5 and 15 births per 100 individuals (Irvine *et al.*, 1995). Generally, mysids have a low instantaneous birth rate and thus a low intrinsic rate of natural increase (Fager and Clutter, 1968).

4.5.9 Instantaneous rate of population change

Instantaneous rate of population change r' for successive population estimates (N_{t+1}, N_t) is calculated from

$$r' = (\ln N_{t+1} - \ln N_t)/t$$

with t the sampling interval. Generally r' is highest during periods of high reproduction and/or immigration into the population. Irvine *et al.* (1995) report the instantaneous rate of population change r' of *Neomysis integer* in a brackish lake (Hickling Broad) to be +0.06 to -0.10. Positive measurements occurred when the *N. integer* population was highly reproductive. A positive peak in r', measured before the reproductive period (during early spring), reflect the influx of adults into the population through immigration.

4.6 Mortality

Under certain conditions (a.o. that individual growth is described by a von Bertalanffy growth model) the total mortality of a population is equal to the P/B ratio of the population (Allen, 1971). Mortality rate (Z) can also be obtained from a length converted catch curve (Mees et al., 1994) and amounted from 3.15 to 4.41 over the year. Mortality rate was not different between the sexes for the summer cohorts of *Neomysis integer* in the Schelde (Mees et al., 1994).

Instantaneous dead rate d' is estimated as the difference between the instantaneous birth rate and the instantaneous rate of population change. Instantaneous dead rate of a *Neomysis integer* population in Hickling Broad amounts between 0.02 and 0.15 (Irvine *et al.*, 1995), *i.e.* 2 to 15 animals per 100 animals die daily. In wintertime dead rate is minimal. A negative dead rate in early spring indicates the immigration of individuals into the water mass from outside.

4.7 Sex-ratio

A shifted sex ratio (further always expressed males:females) is a well described phenomenon in mysid species (Mauchline, 1980). Differences in the sex-ratio over time can be explained by a differential mortality of one of the sexes, while spatial differences in the sex-ratio may be linked to sexual differential habitat preferences as described for other estuarine crustaceans (like *Palaemon longirostris*, Sorbe, 1983b; Schuchardt *et al.*, 1987). In most *Neomysis integer* populations the sex ratio is close to 1:1 (Mauchline, 1971). The sex ratio in Frisian lakes, ranged between 1:1.5 and 1:0.7; with a mean close to 1:1 (Bremer, 1980; Bremer and Vijverberg, 1982).

In loch Furnace (Parker and West, 1979), the sex ratio was strongly biased towards the females, being greatest in April (1:9). Only in June and August when the breeding females of the spring and summer generation were dying off, males outnumbered the females. Females are always more abundant than males in the Severn estuary (1:2 to 1:6) (Moore *et al.*, 1979). In the Kiel canal sex-ratio is 1:1.9 (Kinne, 1955). In the lower reaches of the Weser estuary significantly more females than males were caught (Schuchardt *et al.*, 1989). This shifted sex-ratio is different for the different generations, respectively 1:6 in the overwintering generation, and 1:2 – 1:4 in summer. In a study in the Tamer estuary (Moffat and Jones, 1992), the proportions of males, females and juveniles were not related to temperature. The sex-ratio ranged between 1:1 and 1:2 for most of the year, except for October when females outnumbered males with a ration of 1:9.

In the Weser and Gironde estuaries, a shifted sex-ratio was observed along the longitudinal axis of the estuary (Sorbe, 1980; Schuchardt *et al.*, 1989). More females (1:5) were caught in the most upstream reaches while in the mesohaline region an almost equilibrated ration was found (1:0.9). Along the longitudinal transect of the Ythan the overall sex-ratio equals 1.0 (Astthorsson, 1980). No information is available on this segregation of sexes along the salinity gradient for other estuaries.

4.8 Field-based growth curves

Growth curves of all cohorts present over the year have been derived from field data for several estuarine *Neomysis integer* populations (Mauchline, 1977; Astthorsson, 1980; Astthorsson and Ralph, 1984; Mauchline, 1985; Mees *et al.*, 1994), as well as in the Baltic (Arndt and Jansen, 1986) and for brackish lakes (Aaser *et al.*, 1995). Bremer and Vijverberg (1982) and Beattie and de Kruijf (1978) only derived a growth curve of the first summer generation of *N. integer* from Slotermeer and Bergumermeer.

Growth curves can be fitted to a growth model. The generalized von Bertalanffy growth function (Gayanilo *et al.*, 1989) is originally designed for describing fish growth but is recently applied to mysids growth with good results (Mees et al., 1994; Soselisa, 1994; Fockedey, unpublished). To describe the growth stop during the period of coldest temperatures of the winter generation, a seasonally oscillating version of the latter growth model can be used. Mees *et al.* (1994) demonstrated that a good fitting of the von Bertalanffy growth model in *Neomysis integer* was only possible if the differences in growth between the sexes were taken into account. *N. integer* grows almost linearly up to sexual maturity. From then on the growth rate slows down (Astthorsson, 1980).

To date, the field-estimated growth parameters have rarely been validated with laboratory observations. Only Astthorsson (1980), compared the field derived growth curve for *Neomysis integer* from the Ythan estuary with a growth curve derived from laboratory growth experiments at 9 and 16°C, however based on very few observations. Fockedey *et al.* (in press – Chapter 3) compared laboratory-based growth parameters estimates of *N. integer* with those from field studies in the Schelde estuary and the brackish pond Galgenweel.

4.9 Laboratory-based growth curve

Very little detailed information is available on the growth curves and growth rate of *Neomysis integer* cultured under constant laboratory conditions. Bremer and Vijverberg (1982) refer to a study performed by Schrotenboer (1980) who estimated growth and development rate of *N. integer* by means of laboratory culture at 15 °C and 17.5 °C over 110 days. The growth curve at 15 °C was linear and the one at 17.5 °C was curvilinear.

Kuhlmann (1984) described the growth of juvenile *Neomysis integer* (between 3 and 10 mm) to be linear. Astthorsson (1980) constructed average growth curves for *N. integer* based on laboratory length-specific measurements of the intermoult period and growth factor and an initial juvenile length of 2.4 mm TL. Irvine *et al.* (1995) describe post-marsupial growth under constant laboratory conditions (20 °C) as a linear regression: $SL = 2.8 + 0.105$ days ($n = 52$; $r^2 = 0.92$; $p < 0.001$).

Field observations of the same population (successive peaks in the length-frequency distributions) also suggested a linear growth rate. Fockedey *et al.* (in press – Chapter 3) described growth of *N. integer* under 8 salinity/temperature combination. At all treatments, except the coldest (8 °C) where growth was linear, the growth curves were best fitted with a von Bertalanffy growth model.

4.10 Growth rate

Field- and laboratory-based growth rates of *Neomysis integer* from several investigations are presented in Table 8. However, Astthorsson and Ralph (1984) questioned the validity of growth rates calculated from field data, based on the grounds that it is often difficult to follow the cohorts. On the other hand, growth rates calculated from laboratory experiments may be influenced by the experimental conditions. Arndt and Jansen (1986) and Bremer and Vijverberg (1982) found higher growth rates in a natural population in comparison with the available laboratory growth rates. When comparing the data from Table 8 this statement cannot be confirmed as maximal laboratory growth rates are all higher than those recorded in the field.

The growth rate of *Neomysis integer* kept in laboratory cultures at 20 °C and feeding on copepods and Cladocera was 1 mm per 9.5 days, *i.e.* 0.11 mmTL d^{-1} (Irvine *et al.*, 1995). The mean growth rate of *N. integer* maintained on a diet of *Artemia* nauplii was 0.06 mmTL d^{-1} at 9 °C and 0.09 mmTL d^{-1} at 16 °C (Astthorsson, 1980; Astthorsson and Ralph, 1984). Growth rate of the same population in the field (Ythan) was estimated from the time spent within each 1 mm size class and varied between 0.03 and 0.16 mm d^{-1} in the growth season from May to September, and from 0.03 – 0.13 mm d^{-1} in wintertime, *i.e.* from November to April (Astthorsson, 1980). Other field derived maximal growth rates amount to 0.13 mm d^{-1} in the Schelde (Mees *et al.*, 1994) and 0.16 mm d^{-1} in Slotermeer (Bremer and Vijverberg, 1982). Juveniles, just released from the marsupium, grew between 0.142 and 0.281 mm d^{-1} in laboratory experiments at different temperature/salinity combinations (Kuhlmann, 1984).

The growth rate is related to the length of the mysid and differs between and within the cohorts. In the Schelde (Mees *et al.*, 1994) *Neomysis integer* grows at a rate of 3 – 4 mm $month^{-1}$ in autumn, and ceases completely during wintertime. In spring the mysids regained their fast growth rate that they maintained during summer. In these cohorts the smaller juveniles and subadults grew fastest (3 – 4 mm $month^{-1}$, *i.e.* 0.10 – 0.13 mm d^{-1}), while the larger mysids grew slower (1 – 2 mm $month^{-1}$; *i.e.* 0.03 – 0.06 mm d^{-1}). Aaser *et al.* (1995) formulated semi-log transformed linear relationships between the cohorts-specific growth rate and the (total) length of *N. integer*.

The growth rate of *Neomysis integer* is related to environmental temperature (Kinne, 1955; Astthorsson and Ralph, 1984), although it cannot be seen as the only determining factor. At the same temperature of for example 12 °C, the growth rate of *N. integer* accelerates in spring, but decreases in autumn (Schuchardt *et al.*, 1989). Probably the length of the daylight period also plays an important role (Schuchardt *et al.*, 1989). In the Bergumermeer, *N. integer* living near the warm water discharge of a power plant grows equally fast in spring and summer as in other parts of the lake. However, growth can continue longer into autumn in the warmer water sites (Beattie and de Kruijf, 1978).

Individual production or growth in terms of dry weight was 0.02 mgDW d^{-1} and 0.03 mgDW d^{-1}, respectively at 9 and 16 °C respectively (Astthorsson, 1980). Maciejewska and Opalinski (2002) measured the average body wet weight increase over an 18 days period at 14 °C feeding *ad libitum* on frozen *Daphnia*: 0.057 mgWW ind^{-1} d^{-1} or 0.01 mgWW $mgWW^{-1}$ d^{-1} for the smaller animals (initial length ± 8mm), and 0.0002 mgWW ind^{-1} d^{-1} or 0.0003 mgWW $mgWW^{-1}$ d^{-1} for the larger animals (initial length ± 15 mm). Production rates of *Neomysis integer*, calculated from field based growth curves, amounts 0.611 mgWW $mgWW^{-1}$ d^{-1} (Beattie and de Kruijf, 1978) in Bergumermeer, and 0.283 mgWW $mgWW^{-1}$ d^{-1} in Slotermeer, two Frisian brackish lakes (Bremer and Vijverberg, 1982). In the laboratory experiments at 15 °C performed by Schrotenboer (1978), *N. integer* individuals grew 0.277 mgWW $mgWW^{-1}$ d^{-1}.

Table 8: Growth rates available for *Neomysis integer* in literature (Adapted from Winkler and Greve, 2002)

Field	Temperature Salinity	Period	Stage	Growth rate (mm TL d^{-1})	
Hickling Broad	20 – 22 °C	Jun/Aug		0.18	Irvine et al., 1995
	?	Sept/Oct		0.08-0.11	
	?	Nov/Apr		0.04	
	?	Apr/May		0.14	
Schelde estuary	17 °C	Autumn		0.10-0.13	Mees et al., 1994
	9 °C	Winter		0	
	18 °C	Spring/Summer	Juvenile	0.10-0.13	
	18 °C	Spring/Summer	Adult	0.03-0.06	
Slotermeer	15 – 20 °C	May-Oct	2-14 mm TL	0.13-0.16	Bremer and Vijverberg, 1982
Ythan estuary	?	Summer	Juvenile	0.13-0.16	Astthorsson, 1980; Astthorsson and Ralph, 1984
	?	Summer	Adult	0.03-0.06	
	?	Winter	Juvenile	0.10-0.13	
	?	Winter	Adult	0.03-0.06	
Lake Ferring	?	Spring/Summer		3.14×0.567^{TL} ($n=5$; $r^2=0.82$; $p<0.03$)	Aaser et al., 1995
		Winter cohort (< winterstop)		2.41×0.538^{TL} ($n=7$; $r^2=0.97$; $p<0.001$)	
		Winter cohort (> winterstop)		0.024 mm d^{-1} ($n=5$; $p<0.0001$)	

Laboratory	Temperature Salinity	Food	Stage	Growth rate (mm TL d^{-1})	
Laboratory	14 °C – 13 psu	Green algae	Juvenile	0.142	Kuhlmann, 1984
	14 °C – 17 psu	*Daphnia* sp.		0.161	
	14 °C – 24 psu	*Artemia* sp.		0.166	
	17 °C – 13 psu			0.229	
	17 °C – 17 psu			0.233	
	17 °C – 20 psu			0.232	
	17 °C – 24 psu			0.189	
	19 °C – 13 psu			0.231	
	19 °C – 17 psu			0.274	
	19 °C – 20 psu			0.224	
	21 °C – 17 psu			0.281	
	21 °C – 20 psu			0.271	
	21 °C – 24 psu			0.186	
	24 °C – 17 psu			0.204	
	24 °C – 20 psu			0.194	
	24 °C – 24 psu			0.193	
Laboratory	20 °C	Copepoda Cladocera	?	0.11	Irvine et al., 1995
Laboratory	9 °C	*Artemia*	3-13 mm	0.02-0.09	Astthorsson, 1980; Astthorsson and Ralph, 1984
	16 °C	*Artemia*	3-12 mm	0.03-0.15	
Laboratory	10 °C	*Artemia*	3-7 mm	0.05 – 0.09	Winkler and Greve, 2002
	15 °C	*Artemia*	3-7 mm	0.12 – 0.19	
	10 °C	*Artemia*	8-12 mm	0.03-0.08	
	15 °C	*Artemia*	8-12 mm	0.03-0.09	

4.11 Moulting

Growth of mysids can be described in terms of intermoult periods (IMP) and growth factors (GF) (Mauchline, 1976; 1977; 1977b). The IMP is the period between two successive moults and the GF is the percentage increase in length at each moult. Generally the intermoult period increases when the animals become larger, while the growth factor decreases steadily at successive moults. Although mysids grow in discrete steps when moulting, the length increase at a moult can also be expressed as the mean length increase within the intermoult period or intermoult growth rate (GR) (Winkler and Greve, 2002).

Detailed information on the growth and moulting of *Neomysis integer* is scarce. Schrotenboer (1980), Astthorsson (1980), Astthorsson and Ralph (1984), Kuhlmann (1984), Irvine *et al.* (1995), Gorokhova (2002) and Winkler and Greve (2002) all performed growth experiments with *N. integer*, but only at very few temperature-salinity combinations or at limited periods within the life cycle.

According to Mauchline (1976; 1977; 1977b) the intermoult period is related in an exponential function to body length and moult number. Astthorsson (1980) and Gorokhova (2002) confirm this log-linear relationship for *Neomysis integer*. The relationship differed between the two experimental temperatures 16 °C and 9 °C (Astthorsson, 1980): the intermoult period ranged from approximately 4 and 8 days in juveniles to 12 and 16 days in adult *N. integer* at 16 and 9°C, respectively. The intermoult period is inversely related to temperature (Astthorsson, 1980). At higher temperatures the moulting occurs more frequently, resulting in a higher growth rate (Astthorsson and Ralph, 1984). However Winkler and Greve (2002) and Fockedey *et al.* (in press – Chapter 3), basing their conclusions on a more extended dataset, could not confirm the exponential relation between IMP and body length or moult number. No differences in IMP were found between the sexes (Gorokhova, 2002; Fockedey *et al.*, in press – Chapter 3).

Mauchline (1976; 1977; 1977b) demonstrated that the growth factor in mysids is related in an exponential function to mysid length and to moult number. The log-linear relationship was confirmed for *Neomysis integer* (Astthorsson, 1980; Astthorsson and Ralph, 1984; Gorokhova, 2002). Temperature (9 and 16°C) did not have an effect on the growth factor in the experiments of Astthorsson (1980) and Astthorsson and Ralph (1984), but was affected by temperature (8°C – 25°C) in the experiments of Fockedey *et al.* (in press – Chapter 3). The growth factor ranged from 14 % in juveniles to 3 % in adult *N. integer* (Astthorsson, 1980). However Winkler and Greve (2002) and Fockedey *et al.* (in press – Chapter 3), basing their conclusions on a more extended dataset at more temperature/salinity combinations, could not confirm the exponential relationship between GF and body length or moult number.

The moulting process can be grouped into 3 major sub-periods (Gorokhova, 2002): the postmoult, intermoult (not to confuse with the IMP (Mauchline, 1976; 1977a; 1977b) and premoult periods. Each period constitutes 34 %, 22 % and 44 % of the intermoult period in *Neomysis integer* (Gorokhova, 2002). Development of the new exoskeleton occurs during the premoult stage and involves the enzymatic digestion of the existing post-ecdysial layer and re-absorption of material from this layer into the new cuticle. At the time of moulting (ecdysis), this pre-ecdysial layer is fully developed. Another portion of the exoskeleton is formed after ecdysis, and the synthesis of this post-ecdysial layer is associated with the incorporation of assimilated nutrients into newly formed matter. Thus the old, shed cuticle contains material synthesized during one moult cycle before the last one (Gorokhova and Hansson, 1997).

Moulting in *Neomysis integer* always occurs at night (Fockedey, personal observation). The removal of exuvia proceeds fast (< 1 min) in three steps: (1) carapace and thoracopods, (2) abdomen with abdominal appendages, and (3) first antennae. Freshly moulted individuals lie on the bottom for a while (5 – 7 min), then swim and behave normally. The formation of the statolyth at the base of the endopod is completed within 3h after ecdysis (Gorokhova, 2002). In experiments, *Neomysis integer* is often seen to eat its own cast moults, even if enough other food is available. Possibly, the mysid can acquire important minerals and organic matter in this way (Astthorsson, 1980).

4.12 Intermoult growth

Mauchline (1973), Childress and Price (1978), Cuzin-Roudy *et al.* (1981) and Hartnoll (1982) report a limited growth increment during the intermoult period of mysids due to the stretching of the abdominal joints. For *Neomysis integer* the increase in length within a moult cycle amounts to 5 – 15 % (Mauchline, 1973). This phenomenon was described for field caught gravid females, which do not moult as long as they carry embryos and larvae in the marsupium. However, females moult in between successive broods and then increase (minimally) in length (Fockedey, unpublished). This was not taken into account by (Mauchline, 1973).

4.13 Response of post-marsupial growth to temperature, salinity, food quality and quantity

Juveniles just released from the marsupium and fed a mixture of green algae, *Daphnia* sp. and *Artemia* nauplii, grew maximally (0.25 mm d^{-1}) at 19 – 21 °C and 16 – 20 psu (Kuhlmann, 1984). However, a limited salinity and temperature range was selected in this study (between 14 – 24 °C and 13 – 24 psu) and the lower limits were far from reached.

Only a limited number of replicates were used per treatment (between 2 and 9 individuals) and no information on the mortality, intermoult period and growth factor was reported in the paper. Effects of temperature and salinity on the growth parameters IMP and GF were described in a previous paragraph 4.11 (Astthorsson, 1980; Astthorsson and Ralph, 1984; Winkler and Greve, 2002; Gorokhova, 2002; Fockedey *et al.*, in press – Chapter 3).

The growth over the whole life span in terms of IMP, GF and GR; and the sexual development of *Neomysis integer* is studied over an environmentally relevant salinity and temperature range by Fockedey *et al.* (in press – Chapter 3). The effect of food quality on these growth parameters is dealt with in Fockedey *et al.* (submitted c – Chapter 4) and in Fockedey *et al.* (submitted a – Chapter 5).

The effect of food quantity on the growth and moulting was studied as well (De Pauw, 1998; Gorokhova, 2002; Fockedey, unpublished). The duration of the IMP is extended in condition of suboptimal food quantity. The animals mainly prolonged their late postmoult and early premoult stages at a suboptimal feeding regime (Gorokhova, 2002). Starvation holds up the moulting process in the intermoult or premoult stage. Ecdysis was observed in some individuals only and was restricted to the first week (Gorokhova, 2002). Temperature affects the duration of the total intermoult period, but does not influence the relative duration of the substages of the intermoult period, as long as food supply is high (Gorokhova, 2002).

4.14 Effect of genotype on life history characteristics

For euphausiids (Haywood and Burns, 2003) it is demonstrated that the rate of development is different between siblings growing under exactly identical experimental conditions. For *Neomysis integer* this variation in growth between siblings resulted to be very small (Fockedey *et al.*, in press – Chapter 3) and thus laboratory-based studies on growth, development, physiological responses and ecotoxicological bio-assays can be performed with a relatively small number of replicates.

Genetically different populations of a species may differ in their growth and the response of their physiological processes towards salinity (Lee, 1999) and temperature (Wittmann, 1984). The phylogeographic patterns of *Neomysis integer* were examined through its distribution range by Remerie *et al.* (submitted a; submitted b), using mitochondrial cytochrome oxidase I sequencing. The *N. integer* population of the Schelde estuary was genetically homogenous along the longitudinal estuarine axis, but a large heterogeneity was observed between the populations from 11 estuaries. The responses of the distinct *N. integer* populations to temperature, salinity and other environmental conditions may be population-dependent and need further study.

5 BIOCHEMICAL COMPOSITION

5.1 Dry weight and ash content

The dry weight of *Neomysis integer* is 22 % of the wet weight for most of the year in Southampton waters (Raymont *et al.*, 1964). A slight reduction in the percentage dry weight was found in December and January (20 – 21 %) (Raymont *et al.*, 1966). Raymont and Krishnaswamy (1960) reported the dry weight as 19.0 – 19.9 % of the wet weight for the same population. The dry weight of *N. integer* from a Baltic population was 22 % of the wet weight (Maciejewska and Opalinski, 2002). In the Schelde estuary, the dry weight of *N. integer* is measured as around 14 – 17 % of the wet weight (Verslycke, 2003). See also Table 2 for more conversions.

Some authors use a correction factor when calculating the dry weight out of the wet weight in formaldehyde-preserved animals and add another 10 % to the calculated dry weight (Beattie, 1982). The length reduction of *N. integer* after a 100 days conservation in a 4 % formaldehyde-seawater solution is4 %, corresponding to a wet weight reduction of 17.4 %. The major shrinkage occurs in the first day after conservation (Kuhlmann *et al.*, 1982).

The wet weight of individual *Neomysis integer* is significantly dependent on the season, since size varies between cohorts (Verslycke *et al.*, 2004). Adult animals collected in spring (± 28 mg) weighed significantly more than adults collected in summer (± 21 mg) and winter (± 14 mg), these data ar ein agreement with the population biology (Mees *et al.*, 1994). The wet weight of *N. integer* is dependent on the location along the estuarine gradient (Verslycke *et al.*, 2004). Animals collected at the most upstream locations of the Schelde estuary have a significantly lower wet weight.

The ash content of *Neomysis integer* from the Ythan was 10.8 % of the dry weight, without sexual differentiation (Astthorsson, 1980). Total ash content of ovigerous females of Southampton Water amounts to 7.9 % of the dry weight (Raymont *et al.*, 1964). Ash and chitin together amounted to 14 % of the dry weight (Raymont *et al.*, 1966). See also Table 2 for more conversions.

The weight of a moult shed is related to the weight of *Neomysis integer* according to the factor 0.1057 (Astthorsson, 1980). For a juvenile *N. integer* the moult is approximately 8 % of the dry weight of the animal that produced it, while for mature individuals this figure is 10 % (Astthorsson, 1980). The ash content of shed moults equals 51.4 % of the DW (Astthorsson, 1980).

The dry weight and ash content of the eggs (*remark: probably meant stage I embryos?*) of *Neomysis integer* are on average 0.04 mg DW and 8.1 % (Astthorsson, 1980). Irvine *et al.* (1995) consider the dry weight of the embryos to be 10 % of their wet weight.

5.2 Chitin content

Chitin content is 7.1 % of the dry weight of ovigerous *Neomysis integer* from Southampton waters (Raymont *et al.*, 1964). The exoskeleton of *N. integer* contains large amounts of protein. Moulting of *N. integer* is temperature-, salinity- and age-dependent (Fockedey *et al.*, in press – Chapter 3). Moulting can thus create large variation in the protein content of *N. integer* (Verslycke and Janssen, 2002).

5.3 Caloric content

The caloric content of 10 – 15 mm sized *Neomysis integer* is 4.95 ± 0.10 cal $mgDW^{-1}$ (Summers , 1980). Astthorsson (1980) obtained a somewhat smaller mean caloric conversion factor of 4.68 cal mg DW^{-1}. The mean annual caloric value of adult females (4.75 cal $mgDW^{-1}$) was significantly larger than that of adult males (4.62 cal $mgDW^{-1}$), probably related to the (energetically rich) egg production in the ovaries (Astthorsson, 1980). Maciejewska and Opalinski (2002) used the conversion factors of 1.03 cal $mgWW^{-1}$ (4.33 J) as the body energy equivalent for *N. integer*. Kaiser *et al.* (1992) determined the energy content of a *N. integer* individual (body length 14.5 mm) to be 0.15 KJ. In terms of ash free dry weight (ADW) the mean annual caloric values is 5.17 and 5.32 cal $mgADW^{-1}$, respectively for adult males and females (Astthorsson, 1980). The caloric value of *N. integer* shows little seasonal variation (Astthorsson, 1980).

The mean caloric content of *N. integer* is 19.89 J $mgDW^{-1}$ (12.91 J $mgDW^{-1}$ for juveniles and 22.65 J $mgDW^{-1}$ for adults) (Arndt and Jansen, 1986). Shed moults have a mean caloric content of 1.28 cal $mgDW^{-1}$ or 2.63 cal $mgADW^{-1}$ (Astthorsson, 1980). The caloric content of stage I embryos is 7.4 cal $mgDW^{-1}$ or 0.30 cal per embryo (Astthorsson, 1980).

5.4 C, N and P content; C:N ratio

Total nitrogen content of ovigerous female *Neomysis integer* from Southampton is 11.4 % of the dry weight (Raymont *et al.*, 1964) and does not vary significantly over the year (Raymont *et al.*, 1966). The non-protein nitrogen is equivalent to 22 % of the total nitrogen, but varies with environmental salinity (Raymont *et al.*, 1966).

Total organic carbon ranges between 22 and 35 % (mean 30 %) of the dry weight in ovigerous females over the course of a year (Raymont *et al.*, 1964; 1966). Parsons *et al.* (1977) used a general conversion factor of 40 %. The organic matter as the summation of the protein, lipid and carbohydrate content, is highly constant over the year (Raymont *et al.*, 1966). The conversion of organic carbon to total organic matter in *Neomysis integer* is approximately 1:3 (Raymont *et al.*, 1966).

Subadult *Neomysis integer* from the Baltic (mean DW 1.67 mg) contained 42 % carbon and 11 % nitrogen (Gorokhova and Hansson, 1999). No variations in the C:N ratio (3.65 – 3.95, mean 3.78) could be detected between the sexes or between animals from the summer generation of subsequent years (Gorokhova and Hansson, 1999).

The total phosphorous content of *Neomysis integer* is determined by Tundisi and Krishnaswamy (1967). Values were 53.5 µg $mgWW^{-1}$ for mature females, 41.0 µg $mgWW^{-1}$ for adult males and 128.6 µg $mgWW^{-1}$ for juvenile *N. integer*.

5.5 Protein and amino acids content

The total protein content of ovigerous females of *Neomysis integer* is 70 µg $mgWW^{-1}$, *i.e.* 70 % of the dry weight, 7 % of the wet weight, 80 and 90 % of the total organic matter excluding chitin. Proteins make up the main part of the total N content of the animals and are quantitatively the most important energy fraction of ± 1700 mJ $mgWW^{-1}$ (Raymont *et al.*, 1964; 1966; Srinivasagam *et al.*, 1971; Verslycke and Janssen, 2002; Verslycke *et al.*, 2004). Throughout the year there are no significant changes in total protein levels (Raymont *et al.*, 1966; Verslycke et al; 2004). The protein of *N. integer* has been found to contain 13 % nitrogen (Raymont *et al.*, 1968). Therefore the total N values can be multiplied by a factor 7.7 to obtain an estimate of the protein content (Raymont *et al.*, 1966). The protein in *N. integer* showed a wide but not unusual range of amino acids: in descending order glutamate, aspartate, leucine, serine, glycine, alanine and lysine (Raymont *et al.*, 1968). The major yolk protein in eggs of *N. integer* is vitellin and it is essential to the nutritional needs of the embryo (Ghekiere *et al.*, 2004).

Non-protein N (including free amino acids) is 20 % of the total N, but the proportion varies with the environmental salinity from 14 % to 23 % (Raymont *et al.*, 1966; 1968). Free amino acids constitute up to 80 % of the non-protein N (Raymont *et al.*, 1968). The non-protein amino acids mainly consist of glycine, alanine and glutamate (Raymont *et al.*, 1968; Srinivasagam *et al.*, 1971). They are more prominent in individuals living at higher salinities and are responsible for the osmoregulatory mechanism of the species (Raymont *et al.*, 1968).

The most dominant amino acids in *Neomysis integer* are aspartate, glutamate and lysine (together accounting for 33 %), while alanine, serine, valine, glycine and praline represent a further 31 % (Armitage *et al.*, 1978). Glycine, taurine, alanine, proline, aspartate, glutamate, threonine, arginine, tyrosine, lysine, phenyl-alanine, isoleucine, leucine, valine, serine, cysteic acid and ornithine appeared to be non-essential to *N. integer* as they could be synthesised by the species or/and its digestive micro-organisms in the gut (Armitage *et al.*, 1981). The first 4 or 5 amino acids of this series are involved in the osmoregulation of the species (Armitage and Morris, 1982; Moffat, 1996).

The very high frequency of the incorporation of glycine and taurine underlines their importance to *N. integer* (Armitage *et al.*, 1981). Methionine and histidine are not synthesised by *N. integer*.

Since lipid and carbohydrate reserves in *Neomysis integer* are small (see following paragraphs), and only used as short-term energy sources, the species can use its own body proteins during nutritional stress (Armitage *et al.*, 1978). Starvation of 6 days (on kaolin clay) significantly decreased the protein content of *N. integer* (Morris *et al.*, 1977). When fed on a starch diet, the total protein and most amino acid concentrations were also drastically reduced (Armitage *et al.*, 1977). When fed pure protein (albumin), the mysids total protein was reduced as well (-39 %), probably because some nutritive requirements (*e.g.* cofactors or vitamins) were not met by the diet (Armitage *et al.*, 1978).

Neomysis integer takes up more ^{14}C from the food into their free and protein amino acids from a starch based diet (100 % glucose, aspartate or glutamate) than from a protein based diet (70 % egg yolk + 20 % cod liver oil + 10 % starch) (Armitage *et al.*, 1981). Probably, in the absence of proteins the metabolism of tissue protein of *N. integer* is increased and incorporation of food ^{14}C is more rapidly transferred into the tissues, while in protein-fed animals nitrogen and amino acids are supplied in the diet and catabolism of tissue protein is not necessary (Armitage *et al.*, 1981). In comparison with another mysid species (*Gnathophausia* sp.), *N. integer* metabolises diet proteins at a high rate and relies to a great extent on protein as a metabolic substrate (Armitage *et al.*, 1981). The differential uptake of ^{14}C from the food illustrates the higher uptake efficiency across the intestine membrane when fed starch based diets in comparison to a protein-based diet (Armitage *et al.*, 1981).

5.6 Lipid content

The lipid content of ovigerous *Neomysis integer* is 13 % of the dry weight (Raymont *et al.*, 1964). The lipid content of adults is on average 37 µg $mgWW^{-1}$ or 4 % of the WW and represents an energy equivalent of 1600 mJ $mgWW^{-1}$ (Verslycke and Janssen, 2002; Verslycke *et al.*, 2004). Mysids are capable of storing lipids in the R and B-cells of the digestive glands (Molloy, 1958; Raymont *et al.*, 1968; Brunet *et al.*, 1994). In comparison to other mysids (Adare and Lasenby, 1994; Azeiteiro et al., 2001; Richoux et al., 2004), the lipid content of *N. integer* is relatively small (Raymont *et al.*, 1968; Morris and Sargent, 1973) and lipids are not a very important energy reserve for *N. integer* (Raymont *et al.*, 1964). The problem of food shortage in *N. integer* may not be so acute for an estuarine omnivore exploiting a wide range of feeding methods and habits and always has a choice of dietary sources.

The total lipid content varies moderately over the year – between 8.5 and 15.6 % of the organic matter excluding chitin – and shows significant decreases in winter time in Southampton waters (Raymont *et al.*, 1966; Morris, 1971), while in the Schelde estuary *Neomysis integer* has lower lipid contents in summer and winter as compared to spring (Verslycke *et al.*, 2004). These patterns can be associated with local conditions of breeding, food supply and metabolic rates. Along the estuarine gradient of the Schelde estuary, the average lipid concentration increases from downstream to upstream locations (Verslycke *et al.*, 2004).

The eggs of *Neomysis integer* contain large quantities of lipid material (Linford, 1965). Females have a higher lipid content than the males or juveniles, resulting from a larger triglyceride fraction (Morris, 1973). The lipid metabolism of ovigerous females is assumed to be different from males and is probably slower in winter when the demand for lipid for gamete production comes to halt (Linford, 1965; Verslycke and Janssen, 2002; Verslycke *et al.*, 2004). It has been demonstrated that adult mysids have a lower O:N ratio than juveniles, as adults rely relatively more on protein substrates, which results in a higher relative lipid content of the adults than juveniles (McKenney, 1998).

When held at a high temperature, high salinity and low dissolved oxygen concentration, lipid content of *Neomysis integer* decreases (Verslycke and Janssen, 2002). Morris (1971) found significant changes in the lipids fatty acid composition of *N. integer* in a series of experiments to assess the effects of temperature and salinity.

A decreasing temperature leads to a build-up of the long-chain polyunsaturated acids at the expense of short-chain low unsaturated acids. Salinity does not have an effect on the fatty acid composition.

Starvation for 96h did not show any statistically significant decrease in the total lipid level of *Neomysis integer* (Linford, 1965), although a starvation period of 6 days (on kaolin) did decrease lipid levels significantly (Morris *et al.*, 1977). A diet of carbohydrate (starch) is not adequate for the maintenance of a normal lipid composition in *N. integer* on the long term, causing a reduction in the total lipid, triglyceride, and long-chain polyunsaturated fatty acids (Morris *et al.*, 1977). A diet of detritus and phytoplankton, taken from the mysids natural marsh habitat, caused a significant total lipid loss of 25 % after 7 days and of 45 % after 12 days (Morris, 1971). Incorporation of dietary ^{14}C into the lipid fraction of *N. integer* was highest on a diet of glucose and glutamate (Armitage *et al.*, 1981).

The lipids of *Neomysis integer* (Linford, 1963; Raymont *et al.*, 1968; Morris, 1971) contain 3 – 18 % non-saponifiable material, 50 % phospholipids, 30 % triglycerides, hydrocarbon, diglycerides and traces of monoglycerides, and low levels (< 10 %) of sterols. The dominant lipid class in *N. integer* is thus fatty acids (86 %), the next abundant class is sterols. *N. integer* lacks sterol esters, wax esters and fatty alcohols (Morris, 1971; Bradshaw *et al.*, 1989; 1990). The fatty acid:sterol ratio in *N. integer* equals 1.8 – 2.7 (Bradshaw *et al.*, 1989; 1990). Triglycerides are thought to function primarily as an energy store, being metabolized fairly rapidly, whereas phospholipids are considered more as a structural lipid, its metabolism being slower (Morris, 1971; Morris *et al.*, 1977). *N. integer* was found to be capable of converting dietary starch or short-chain saturated fatty acids into long-chain polyunsaturated fatty acids and incorporating them mainly in the triglyceride and phospholipid fractions (Morris *et al.*, 1973; 1977). The species' requirements for triglycerides are easily met by biosynthesis from a range of diets within 3 – 4 days. To obtain all needed phospholipids, *N. integer* needs more time to biosysnthesize them from diets not containing polyunsaturated acids (Morris *et al.*, 1973). When starved, the lipid metabolism was maintained for over 1 week, but the long-chain polyunsaturated fatty acids gradually declined (Morris *et al.*, 1977).

The **fatty acid composition** is relatively constant over the year, and is dominated (51 %) by poly-unsaturated fatty acids or PUFAs (Morris, 1971; Bradshaw *et al.*, 1989). The fatty acid content is 3.2 $\mu g\ mgWW^{-1}$ (Bradshaw *et al.*, 1989). In summer and autumn, these PUFAs increase in concentration at the expense of short-chain low unsaturated acids (Morris, 1971). An increase in short-chain fatty acids in early summer can be related to the feeding of *Neomysis integer* on the phytoplankton bloom containing high amounts of these fatty acids (Morris, 1971).

The fatty acid composition of *Neomysis integer* differs between sexes and between developmental stages. Mono- and long chain PUFAs are present in higher concentrations in adult females, mainly due by a high concentration in the triglyceride fraction (Morris, 1973). Probably these differences can be related to the egg production or embryo formation in females, with the high levels of triglycerides in ovigerous females being used as an energy source for the eggs (Morris et al, 1981). Saturated fatty acids remain at constant levels, irrespective of sex or age, and can be explained by their presence as stable fractions within glycerides and phospholipids (Morris, 1973).

The **sterols** of *Neomysis integer* are dominated by cholesterol (85 – 90 %). The fairly simple sterol composition is affected to some degree by the sex and the level of maturity (Morris *et al.*, 1981), with juveniles having a different composition in comparison to adult females. This may be the result of the juveniles having a very different diet from the adults or of the juveniles having less capacity for the bioconversion of their other dietary sterols to cholesterol (Morris *et al.*, 1981). Environmental temperature does not have any effect on the sterol composition of *N. integer*, while subtle changes are observed as salinity changes, especially lower than 10 psu (Morris *et al.*, 1981; 1982).

When food is passing through the gut, the amount and composition of the dietary lipids alters. *N. integer* decreases the fatty acids and increases the sterols during herbivory (Bradshaw *et al.*, 1990). *N. integer* also significantly contributes lipids to its faecal pellets, mainly cholesterol and fatty acids when gut epithelium cells are added to the faecal material.

The enteric microbes also contribute to the fatty acids of the faeces of *N. integer*. All the contributed lipids are modified and/or reabsorbed by digestive processes while still in the gut system (Bradshaw *et al.*, 1989).

5.7 Carbohydrate content

The amount of carbohydrates in *Neomysis integer* is very low: 0.20 – 0.42 % of the wet weight (Raymont and Krishnaswamy, 1960; Verslycke and Janssen, 2002) or 1.06 – 1.30 % of the dry weight (Raymont and Krishnaswamy, 1960). Using another technique, Raymont *et al.* (1964) obtained a value of 2.4 % of the dry weight for ovigerous *N. integer*.

Over the year the carbohydrate levels are between 2 and 3 % of the total organic matter excluding chitin (Raymont *et al.*, 1966) or 1 – 2 % of the total energy reserve (Verslycke *et al.*, 2004). In the Schelde estuary, adult *Neomysis integer* contains on average 2µg $mgWW^{-1}$ sugars (Verslycke *et al.*, 2004). Sugar content is significantly lower in winter than in spring and summer (Raymont *et al.*, 1966; Verslycke *et al.*, 2004). This can be explained by a higher energy demand and/or reflects the lower food availability or quality in winter (Verslycke *et al.*, 2004).

The carbohydrate content in fed *Neomysis integer* tended to be somewhat higher than in starved animals, indicating that carbohydrates are stored during feeding (Raymont and Krishnaswamy, 1960). Mysids (*N. integer*) are capable of storing glycogen in the R and B-cells of the digestive glands (Molloy, 1958; Raymont *et al.*, 1968; Brunet *et al.*, 1994). However, the generally low carbohydrate content of *N. integer* implicates that glycogen, making up 25 – 30 % of the total carbohydrates (Raymont *et al.*, 1968), cannot be a significant energy storage material (Raymont *et al.*, 1964). The problem of food shortage in *N. integer* may not be so acute for an estuarine population and may explain the lack of energy reserves (Raymont *et al.*, 1964). Sugars are probably only used as a short-term fast energy source (Raymont *et al.*, 1968) and preferred over lipid and protein as the fuel for metabolic processes (Morris, 1999).

6 ENERGY BUDGETS

There is a general lack of information on the quantities of energy consumed by *Neomysis integer* in the field and the role of the species in the trophic web. However, it is often difficult to directly determine the food ration of such small organisms like *N. integer*, especially since they are omnivorous. Indirect studies which measure the respiration, production and egestion are often the only way to estimate the daily food ration or energy requirement of mysids (*e.g.* Rudstam, 1989; Thiel, 1996; Maciejewska and Opalinski, 2002). Bioenergetic models like 'Scope for Growth' (Roast *et al.*, 1999b) or 'Cellular Energy Allocation' (Verslycke *et al.*, 2003; 2004) have recently been adapted for use with *N. integer* and are relatively rapid, alternative measurements of the energy status (fitness) of a *N. integer* population allowing the evaluation of the potential for growth and reproduction or the quality of the environment the population is living in (*e.g.* food availability and quality, pollution).

Despite the role of *Neomysis integer* in the estuarine food web, it is surprising that very little information is available on the parameters which affect their food requirements and how the ingested food is partitioned to meet the animal's requirements (Astthorsson, 1980). Energy is acquired through feeding and food absorption, and lost through respiration, excretion and moulting, with the surplus energy available for somatic growth and reproduction. *N. integer* has little energy reserves and depends mainly on the daily food intake to meet its energy requirements (Raymont *et al.*, 1964; 1966; Linford, 1965; Verslycke *et al.*, 2004b). This stresses the need for information on daily food consumption, daily energy requirements and assimilation efficiencies on different dietary items.

6.1 Energy intake – daily ration

Few studies on *Neomysis integer* give data on the energy intake. Often this is the parameter estimated from energy budgets given all energy expenditure values (*e.g.* Maciejewska and Opalinski, 2002). Generally, mysids need daily approximately 10 % of their total body carbon content to meet their basic metabolic requirements (Froneman, 2000).

Arndt and Jansen (1986) calculated the specific ingestion rate of *Neomysis integer* feeding on *Chlorella vulgaris* to be 108 µgDW d^{-1}. The daily energy intake of a juvenile *N. integer* was estimated as 13 % of the body weight at 10 °C and as 28 % of the body weight at 20 °C (Arndt and Jansen, 1986). Specific consumption rate (daily ration) was 7 – 13 % for juvenile *N. integer* feeding on *Artemia* nauplii (Kuhlmann, 1982) at 10 – 19 °C.

Astthorsson (1980) estimated energy intake over the complete life span of *Neomysis integer*, using a laboratory-derived growth curve, and a relationship between mysid size and daily food consumption, the latter based on laboratory experiments with *N. integer* feeding on *Artemia* nauplii. Maximum feeding rate was 300 – 450 *Artemia* nauplii for adult *N. integer*. In relation to the DW of the mysid, the intake of *Artemia* increased according to a log-log relation and was highest at higher temperatures (Astthorsson, 1980). The average daily food consumption of an immature *N. integer* (1mg DW) equals 70 and 129 *Artemia* d^{-1}, at 9 °C and 16 °C respectively. This corresponds to 0.10 and 0.18 mg $DW_{Artemia}$ d^{-1} or 10 and 18 % of the mysid's body DW. Adult mysids (6 mg DW) consumed 265 and 378 Artemia d^{-1}, at 9 °C and 16 °C respectively. This corresponds to 0.37 and 0.53 mg $DW_{Artemia}$ d^{-1} or 6 and 9 % of the mysid's body DW. Throughout its life from juvenile (2.4 mm TL) to a mature 15 mm sized animal (after 277 days), *N. integer* consumed 305 cal at 9 °C and 370 cal at 16 °C.

6.2 Specific dynamic action

The energetic cost to find and process food is defined as the specific dynamic action. If *Neomysis integer* is offered *Artemia* nauplii in a concentration beneath its maximal feeding rate, it is capable of capturing all available prey (Astthorsson, 1980). It is not stopping its feeding activities to minimise the energy loss by the increased searching effort, as was suggested for some copepod species (Frost, 1975). When feeding on calanoid copepodites (*Eurytemora affinis*) in low concentration, *N. integer* makes no attempt to hunt (Irvine *et al.*, 1993). Increased respiration rates, attributable to the dynamic action, usually occur in the first hours after feeding (Kiørboe *et al.*, 1985).

Results from experiments with other crustaceans give values for the specific dynamic action within the range of 17 – 20 % of the assimilated food (Kiørboe *et al.*, 1985; Lampert, 1986). Rudstam (1989) assumes a specific dynamic action of 17 % for *Mysis mixta*. No measurements have been done with other mysids or *N. integer*.

6.3 Assimilation efficiency

The assimilation efficiency or absorption efficiency is the proportion of the organic matter that is absorbed from the ingested food through the gut membrane; thus excluding the amount of organic matter that is egested in the faecal pellets (Conover, 1966). The assimilation efficiency can be measured quantitatively in the laboratory, provided the exact amount of food offered, the amount of food remaining uneaten and the weight of the faecal pellets produced can be estimated (Ferguson, 1973). Often this is not possible and/or very time consuming. The simpler ratio-method can be used as well, were only a sample of the food and a sample of the faeces are needed, and their respective DW and ADW are measured. The latter technique assumes (1) that the faeces contain organic matter derived entirely from the food; (2) that all unassimilated food can be collected and (3) all fraction of the food must be ingested in the same proportion as contained in the food, *i.e.* no preferential selection of certain food items may occur. Both assumptions are contested by Johannes and Satomi (1967), but Ferguson (1973) found both methods to be in good agreement for animal food (bivalve mantle).

The assimilation efficiency of *Neomysis integer* feeding on dead animal food (bivalve mantle, crab hepatopancreas and dead mysids) is 68 – 92 % (Ferguson, 1973), and 57 – 65 % when feeding on *Artemia* nauplii (Astthorsson, 1980). When feeding on monospecific phytoplankton cultures, the assimilation efficiency is 73 – 90 % for dinoflagellates and diatoms, except for *Skeletonema* (58 – 66 %) (Ferguson, 1973). Assimilation efficiency on natural detritus is 9 – 10 % and on laboratory-made detritus (aged algal cultures) 42 – 46 % (Ferguson, 1973). A low absorption efficiency of 35 % was measured in *N. integer* when feeding on sediments with a low organic content (Roast *et al.*, 2000) in comparison with an absorption efficiency of 70 – 90 % and 60 – 90 % when feeding on zooplankton and phytoplankton respectively (Astthorsson, 1980).

Juveniles had a lower assimilation efficiency than adults on animal food and on a diatom diet of *Coscinodiscus* or *Skeletonema* cells (Ferguson, 1973). When feeding on detritus no such trend was visible. The assimilation efficiencies of juvenile *Neomysis integer* on natural phytoplankton assemblages or green alga *Chlorella vulgaris* suspensions however, were significantly higher (respectively 70 and 90 %) than those of adults (respectively 62 and 80 %) (Arndt and Jansen, 1986).

The assimilation efficiency decreased when animals released their young or when they are moulting (Ferguson, 1973). The higher the ash content of the food, the lower the assimilation efficiency (Ferguson, 1973). Assimilation efficiency of *Neomysis integer* increases with increasing temperature between 5 and 25 °C when feeding on animal food (Ferguson, 1973); but is not affected by temperature when feeding on organically poor sediments (Roast *et al.*, 2000). Salinity has little or no impact on the assimilation efficiency of *N. integer* feeding on animal food or sediments; at least in the first 24 hours (Ferguson, 1973; Roast *et al.*, 2000).

Assimilation of carbohydrates by *Neomysis integer* is 75 – 80 %, with the highest assimilation efficiency on diets with the lowest carbohydrate content (Ferguson, 1973). *N. integer* assimilates the protein from animal food with an efficiency of 80 – 90 %, from diatom food with an efficiency of 75 % (Ferguson, 1973). Highest assimilation efficiency of carbohydrates and proteins is observed when *N. integer* is feeding on dead members of its own species, probably because identical or closely resembling proteins and carbohydrates are more easily absorbed (Ferguson, 1973).

6.4 Egestion: faecal pellet production

Maciejewska and Opalinski (2002) determined the daily faeces egestion of *Neomysis integer*, when feeding on frozen zooplankton (at 14 °C), as 0.169 mgDW ind^{-1} d^{-1} or 0.01 mgDW $mgWW^{-1}$ d^{-1}. During the course of the experiment (24 h) however, 22 % of the initial weight of the faecal material is leached into the water and a correction factor was applied. Egestion rate of *N. integer*, when feeding on organically poor sediments, was 0.017 – 0.049 mg faeces mg^{-1} dry weight mysid h^{-1} (Roast *et al.*, 2000). The egestion rate increased with increasing temperature and salinity (Roast *et al.*, 2000). Egestion rates were not affected by gender (Roast *et al.*, 2000). Egestion rate ranged between 0.022 – 0.044 mg faeces mg^{-1} dry weight mysid h^{-1} when feeding on laboratory-made estuarine aggregates (Fockedey et al., submitted d – Addendum 2). Faecal pellet production, and thus feeding activity, slows down when the mysids are releasing their young from the marsupium or when they are moulting (Ferguson, 1973).

The caloric value of *Neomysis integer* faeces (when feeding on frozen zooplankton) was 3.66 cal $mgDW^{-1}$ faeces or 15.37 J $mgDW^{-1}$ faeces (Maciejewska and Opalinski, 2002). The caloric value of faeces when feeding on other dietary items is not reported in literature. The organic content of the faeces is dependent on the organic content of the food (Ferguson, 1973). When feeding on diets with 45 – 65 % organic matter the organic content of faecal pellets decreased little, while feeding on organically rich food diminished the relative organic matter content from 40 up to 70%. When feeding on detrital food sources (< 45 %), the organic content of the faecal pellets contained relatively more carbon than the food (enrichment of organic matter). This is probably due to the preferential selection of the organic fraction of the food supplied (Ferguson, 1973).

6.5 Egestion: excretion of soluble excretory product (N, P)

The excretion of soluble nitrogen and phosphorous by animals is an important pathway for the remineralisation of nutrients in aquatic ecosystems. The measurement of N excretion can give indication of the level of protein turnover (Raymont *et al.*, 1968). Also a link between osmoregulation through free amino acids and the nitrogen excretion has been demonstrated (Raymont *et al.*, 1968). Nitrogen excretion is of significance in the disposal of excess free amino acids and is mainly done in the form of ammonia (Raymont *et al.*, 1968).

Not much information is available on the excretion of soluble products by *Neomysis integer*. The weight-specific mean ammonia excretion is highly temperature dependent and amounts to 5 – 10, 5 – 15, 10 – 25 and 15 – 35 µg NH^{4+} gDW^{-1} h^{-1}, respectively at 6, 10, 15 and 20 °C (Laughlin and Lindén, 1983). Weisse and Rudstam (1989) measured the ammonium excretion of *N. integer* to be 3 – 11 µmol NH^{4+} gDW^{-1} h^{-1} between 6 and 16 °C. At a constant salinity (0.4 – 35 psu at 15 °C), *N. integer* excretes 1.0 ± 0.5 µg NH_3 $mgDW^{-1}$ h^{-1} (Raymont *et al.*, 1968). A reduction in the environmental salinity leads to a rapid but temporary rise in ammonia secretion up to 6 times the basal excretion rate. A transfer from low to high salinity caused little to no effect on the excretion rate (Raymont *et al.*, 1968). Weight-specific mean dissolved inorganic phosphorus (DIP) amounted 0.2 –1.0 µmol PO_4^{3-} gDW^{-1} h^{-1} (between 6 and 16 °C) and was correlated to temperature (Weisse and Rudstam, 1989). Sex and dry weight of the mysids has no effect on the excretion rates of N and P (Laughlin and Linden, 1983; Weisse and Rudstam, 1989). Caloric conversions for ammonia-N and urea-N are respectively 0.00594 and 0.00551 cal $µgN^{-1}$ (Elliott, 1976).

Laboratory rearing conditions tend to increase the ammonia excretion compared to field-collected mysids (Laughlin and Lindén, 1983), probably due to differences in the dietary composition (N-rich food in excess) and feeding frequency in the experimental setup in comparison with the wild mysids.

Although some authors (Clutter and Theilacker, 1971; Lasenby and Langford, 1972; Roast *et al.*, 1999) considered the soluble organic excretion in mysids to be insignificant (< 5 %), Ferguson (1973) indirectly estimated that *Neomysis integer* excreted 15 % of the ingested energy in this form. By assuming that the excretion is a fixed percentage of the energy ingested, this value was used by Astthorsson (1980) to calculate the energy loss by excretion of soluble excretory products as 45 cal at 9 °C and 55 cal at 16 °C.

6.6 Growth

The energy used for growth can be derived from the growth curve. The length can be expressed in terms of weight and subsequently in terms of calories. Astthorsson (1980) converted laboratory growth curves (in length) into energy content curves (in cal). Freshly released juveniles represent 0.19 cal, while mature adults contain 29.22 cal. For data on the caloric conversions factors of *Neomysis integer* see higher.

The gross growth efficiency, *i.e.* the percentage of the ingested food which is used for growth, is between 8 and 10 % for the whole life time of *Neomysis integer* (Astthorsson, 1980), whereas the net growth efficiency, *i.e.* the percentage of the assimilated food used for growth, equals 12 – 17 % taken over its whole life span (Astthorsson, 1980). The gross growth efficiency decreases from 20 – 25 % in juveniles to 5 – 8 % in adults and significantly larger for *N. integer* living at 9°C than at 16°C and feeding on *Artemia* (Astthorsson, 1980). Ferguson (1973) report gross growth efficiencies of *N. integer* on a diet of crab hepatopancreas as 28 %, 19 % and 12 % for animals with a total length of respectively 8 – 10, 10 – 12 and 12 – 14 mm. Net growth efficiencies for the same size classes are respectively, 43 %, 27 % and 17 %. Gross and net growth efficiencies on laboratory-made detritus (aged algal culture) are considerably lower. For animals with a total length of 8 – 10 and 10 – 12 mm, the gross and net growth efficiencies are very similar as 8 and 19 %; larger animals had the lowest gross (3 %) and net (8 %) growth efficiency. All experiments of Ferguson (1973) were performed at 15 °C. Growth conversion efficiencies reported from growth experiments with well fed *N. integer* juveniles on *Artemia* nauplii are 34 % at 10 °C and 38 % at 19 °C (Kuhlmann, 1982).

6.7 Moulting

Organic matter, *i.e.* energy, is lost during moulting. The dry weight of a moult of *Neomysis integer* is linearly related to the dry weight of the animal that sheds the moult. Moults of juveniles and adult *N. integer* respectively weigh about 8 to 10 % of the dry weight of the individuals that produced them (Astthorsson, 1980). The caloric value of moults of *N. integer* is 1.28 cal $mgDW^{-1}$ (Astthorsson, 1980). Over a complete life span, *N. integer* moults on average 23 times (at 9 and 16 °C) and the total energy loss amounts to 6 cal (Astthorsson, 1980).

6.8 Reproduction

The amount of energy used by male mysids for sperm production is probably negligible (Clutter and Theilacker, 1971; Astthorsson, 1980). The energy used by females for egg production can be estimated using field derived data on the length-dependent brood size (Table 6) and knowledge on the number of successive broods produced by one female. The caloric content of 1 egg is estimated to be 0.30 cal (Astthorsson, 1980). In age-dependent energy budgets one need to allocate the total number of energy spend in brood production over the time that females invest energy in the development of the ovaries.

6.9 Respiration

Weight-specific oxygen consumption rates can be used to estimate the daily oxygen consumption of differently sized *Neomysis integer*. Different methods are used (Winkler bottles, electrodes, Gilson respirometers, CO_2 production, pH changes) and respiration is reported in a variety of units (µg-mg, µl, mm^{-3} per mg-gWW, mg-gDW) over a range of time scales (h-day). A summary is given in Table 9. Nothing is known about the respiration of embryos and larvae during intra-marsupial development.

Using data of the laboratory growth curves of *Neomysis integer*, Astthorsson (1980) calculated the overall energy expenditure over the complete life cycle. At 9 °C it takes *N. integer* 277 days to grow from a 2.4 mm juvenile to a 15 mm adult and during this period the total energy expenditure on metabolism (measured as respiration) is estimated to be 95 cal. At 16 °C it takes 166 days to grow to an adult and metabolic cost is 127 cal.

Table 9: Weight-specific respiration rates for *Neomysis integer* available in literature.

Temp	Sal	Method	measurement	Source
10°C	8 psu	Winkler method	3.5-11.1 μgO_2 mgWW day^{-1} 15.9 and 50.4 μgO_2 mgDW day^{-1}	Raymont et al., 1966
18°C	?	CO2 production, pH change	0.10 to 0.62 μlCO_2 $mgWW^{-1}$ h^{-1}	Raymont and Krishnaswamy, 1968
5°C 15°C	0.3-18.7 psu	Respirometer (Gilson)	1.4-1.7 μlO_2 mgDW h^{-1} 1.9-2.1 μlO_2 mgDW h^{-1}	Vlasblom and Elgerhuizen, 1977
6°C 10°C 15°C 20°C	7 psu	Oxygen electrode	0.8-1.0 mgO_2 gDW^{-1} h^{-1} 1.0-1.3 mgO_2 gDW^{-1} h^{-1} 1.3-1.6 mgO_2 gDW^{-1} h^{-1} 1.6-2.8 mgO_2 gDW^{-1} h^{-1}	Laughlin and Lindén, 1983
5-15°C	?	Winkler apparatus	1-3 μlO_2 $mgDW^{-1}$ h^{-1}	Weisse and Rudstam, 1989
5-15°C	1-30 psu	Oxygen electrode	0.14-0.41 μlO_2 $mgWW^{-1}$ h^{-1}	Roast et al., 1999
5°C 10°C 20°C	2-24 psu 2-24 psu 10-15psu <10 – >15	Flow through-Respirometer	0.7-1.8 μlO_2 $mgDW^{-1}$ h^{-1} 1.2-1.8 μlO_2 $mgDW^{-1}$ h^{-1} ± 1.7 μlO_2 $mgDW^{-1}$ h^{-1} 3-4 μlO_2 $mgDW^{-1}$ h^{-1}	Arndt and Jansen, 1986
14°C	2 psu	Oxygen electrode	0.889 mm^3O_2 $mgWW^{-1}$ h^{-1}	Maciejeska and Opalinski, 2002

With **WW**: wet weight; **DW**: dry weight = 19 - 22% WW; density of Oxygen gas = 1.429 kg/m^3)

Several oxycaloric coefficients are available. The conversion factor of 4.8 cal ml^{-1} O_2 suggested by Schmidt-Nielsen (1979) implies a metabolisation of equal mixtures of carbohydrate, fat and protein, as is the case when *Neomysis integer* is offered animal food (Ferguson, 1973). Maciejewska and Opalinski (2002) used an oxycaloric coefficient of 0.0047 cal mm^{-3} O_2. The oxygen consumed is transformed to energetic equivalents using oxyenthalpic equivalents for an average lipid, protein and sugar mixture as 484 kJ mol^{-1} O_2 (Verslycke and Janssen, 2002). Roast *et al.* (1999b) used a heat equivalent of oxygen uptake of 0.02008 J μl^{-1} O_2. Also 13600 J g^{-1} O_2 can be used as oxycaloric conversion factor (Elliott and Davidson, 1975).

6.10 Energy budgets

A preliminary energy budget for 12 – 14 mm sized *Neomysis integer* has been constructed by Ferguson (1973). The quantity of food ingested is allocated to growth, faecal pellets, respiration and excretion. The quantity of food channelled into to latter two functions was found by difference. The allocation percentage to growth, *i.e.* net growth efficiency, is low (16 %) for 12 – 14 mm *N. integer* in comparison with other species (Ferguson, 1973). The growth efficiency was highest (27 %) with animal food (crab hepatopancreas) and lowest (7.5 %) with artificial detritus (Ferguson, 1973). Respiration and excretion are also higher with animal food. When considering the amount of faecal pellets produced however, a much lower percentage of animal food is being egested (23 – 37 %) compared with detrital food (63 – 66 %). Although a much greater quantity of detritus diet (max. 7.2 mgDW) was ingested per animal compared to the animal diet (max. 2.6 mgDW), the latter supported a higher growth efficiency. Thus, *N. integer* can feed far more economically on an animal diet than on de detrital diet.

A simple energetic budget was used to calculate the daily energy requirements of *Neomysis integer* in the Baltic (Maciejewska and Opalinski, 2002) from measurements on production, respiration and egestion. Daily food ration (in mg WW of microzooplankton ind^{-1} d^{-1}) of *N. integer* as a function of mysid size (WW, in mg) is expressed as: $C = 0.66\ WW^{0.61}$ (r^2=0.9992; n=10). For example, the daily ration for an average individual (20mg WW) is 2.86 cal of food daily, *i.e.* 4.22 mg of zooplankton (WW). All supporting experiments were performed at 14 °C and animals were allowed to feed on natural zooplankton assemblage and/or *ad libitum* frozen *Daphnia*.

A more complicated energy budget (Parsons *et al.*, 1977) was used to calculate the energy relations for the entire life span of *Neomysis integer* (Astthorsson, 1980). The energy ingested equals the energy loss due to metabolism, excretion, moulting, growth and gonad production. The energy expenditure in the gonad production is different between the two sexes and energy budget for males and females were calculated separately. In general, 14 % of the assimilated food is used for growth, 3 % is lost by moulting, 50 % was used for respiration and 24 % was presumed to be excreted in a soluble form. In adult females 10 % of the assimilated food is used for egg production. No differences were found for animals kept at winter or summer temperatures (9 and 16 °C).

6.11 Cellular Energy Allocation

Cellular Energy Allocation (CEA) is a relatively new methodology (De Coen and Janssen, 1997) to assess the individual-based energy budget and is used to assess effects of abiotic variables like salinity, temperature and oxygen (Verslycke and Janssen, 2002; Verslycke *et al.*, 2004) and exposure to pollutants (Verslycke *et al.*, 2003; 2004b; 2004c) on the metabolic processes of *Neomysis integer*. The ratio of the total available energy reserves used (decrease in total sugars, lipid and protein content) and the average energy consumption (measured as cellular respiration rate from electron transport activity) indicate the energy available in excess of that required for maintenance and reflects the energy available for growth and reproduction (Verslycke and Janssen, 2002; Verslycke *et al.*, 2004). CEA is predictive of long-term effects on growth and other long-term population effects in the field (De Coen and Janssen, 1997).

Sugar, lipid and protein content is transformed into energetic equivalents using their respective energy of combustion, respectively 17500 mJ mg^{-1} glycogen, 24000 mJ mg^{-1} protein and 39500 mJ mg^{-1} lipid. The oxygen consumed as measured by electron transport activity (ETA) is transformed to energetic equivalents using oxyenthalpic equivalents for an average lipid, protein and sugar mixture as 484 kJ mol^{-1} O_2.

The CEA can be influenced by a large array of factors like diet, reproductive status, sex, age, location, season, hence careful interpretation of the data is required (Verslycke *et al.*, 2004). When starved (4 days) at a higher temperature, energy reserves and CEA of *Neomysis integer* decreased although not significantly (Verslycke and Janssen, 2002). The energetic metabolic processes of *N. integer* were relatively unaffected in the laboratory by short-time changes in salinity, temperature and dissolved oxygen (Verslycke and Janssen, 2002). *N. integer* from the Schelde estuary, on the other hand, expressed a clear seasonal response in their CEA with spring and summer values significantly higher than in winter (Verslycke *et al.*, 2004). Also, important spatial differences were observed in the CEA along the estuarine gradient, with smaller CEA in the (most polluted) upstream stations (Verslycke *et al.*, 2004).

The latter was not confirmed however by more recent CEA measurements (Ghekiere, personal communication). The average total energy content of adult *N. integer* of the Schelde estuary is on average 3200 mJ $mgWW^{-1}$ and was significantly affected by the location along the estuarine gradient, but not by season (Verslycke *et al.*, 2004). Protein was the most important fraction (70 µg $mgWW^{-1}$), followed by lipids (37 µg $mgWW^{-1}$) and sugars (2 µg $mgWW^{-1}$), making proteins and lipids the most quantitative energy sources for the species (Verslycke *et al.*, 2004). The individual fractions were differentially affected by season and location along the estuarine gradient. Energy consumption of *N. integer* in the Schelde was on average 31 mJ $mgWW^{-1}$ h^{-1} (Verslycke *et al.*, 2004) and was not affected by season but increased towards the more upstream sampling locations. CEA values in the *Artemia* fed *N. integer* from laboratory cultures (± 66) was significantly lower than in the field (± 135), since energy content was comparable (3200 – 3300 mJ $mgWW^{-1}$) but energy expenditure was significantly higher in the laboratory (54 mJ $mgWW^{-1}$ h^{-1}) than in the field (31 mJ $mgWW^{-1}$ h^{-1}) (Verslycke and Janssen, 2002; Verslycke *et al.*, 2004).

6.12 Scope for growth

Scope for growth (SFG) is a technique where several physiological responses are integrated into a single bioassay originally designed for bivalves (Widdows and Salkeld, 1993). It has been adopted for *Neomysis integer* by Roast *et al.* (1999b). It provides a rapid, instantaneous measurement of the energy status of an organism, as does the Cellular Energy Allocation technique (Verslycke *et al.*, 2004b). However, SFG integrates respiration, feeding and excretion rates, rather than respiration rate and biochemical composition in CEA. The SFG method is used in *N. integer* for the evaluation of the toxic effect of the pesticide chlorpyrifos on the metabolic processes (Roast *et al.*, 1999b), not yet for the impact of abiotic (*e.g.* salinity, temperature, dissolved oxygen) and biotic variables (*e.g.* gender, age, size, developmental state, food quality and quantity).

7 PHYSIOLOGY

The upper region of the estuarine environment is characterized by strong fluctuating conditions of salinity and temperature. Both are considered dominant 'ecological abiotic master factors', which may act either singly or in concert to modify the population dynamics (survival, development and growth rates), the distribution and physiology and metabolism of estuarine organisms (Kinne, 1970; 1971; McKenney and Celestial, 1995). As a typical estuarine species, the brackish water mysid *Neomysis integer* experiences strong tidal, diel and seasonal changes in temperature and salinity (Moffat and Jones, 1992; Roast *et al.*, 1999).

7.1 Salinity tolerance

Neomysis integer is euryhaline and tolerates a wide salinity range of 1 to 40 psu (Mauchline, 1971; Vlasblom and Elgershuizen, 1977; Arndt and Jansen, 1986; Roast *et al.*, 2001), although survival is low in salinities greater than 35 psu (Ralph, 1965). Kuhlmann (1984) reported however, the upper salinity tolerance limit of adult *N. integer* to range between 25 – 30 psu at 10 °C. No particular preference was shown by *N. integer* within the salinity range from 2 to 12 psu (Arndt and Jansen, 1986). The species possesses considerable powers of adaptation to waters of low salinity (Tattersall and Tattersall, 1951), but is unable to maintain itself in absolute fresh water (Stammer; in Segerstrale, 1945) when summer temperatures become higher than 23 – 24 °C (Merker, 1928). *N. integer* is highly tolerant to large, acute salinity fluctuations between 1 and 30 psu (Moffat and Jones, 1992; Roast *et al.*, 1998). It can attain osmotic balance within 2 hours of exposure to a change in salinity (Moffat, 1996).

In the field *Neomysis integer* can be found at salinities between 0.1 and 38 psu, although it is rare in waters of more than 20 psu (Tattersall and Tattersall, 1951; Vlasblom and Elgershuizen, 1977). In the Schelde estuary, *N. integer* was recorded at salinities ranging from 8 to 25 psu with a maximal abundance at around 15 psu (Mees *et al.*, 1994). Ongoing studies suggest that the population is shifting towards the more oligohaline zone of the estuary (Verslycke *et al.*, 2004; Fockedey, personal observation) as a consequence of improved oxygen conditions in the upstream reaches in winter and early spring (*e.g.* De Brabandere *et al.*, 2002; http://waterbase.nl). In other, more oxygenated Western European estuaries such as the Guadalquivir (Spain), Gironde (France), Tamar (UK), Elbe (Germany) and Ems (The Netherlands) the abundance peak is typically found around 5 psu (Mees et al, 1995; Moffat, 1996; Drake *et al.*, 2002; Fockedey, personal observation).

7.2 Temperature tolerance

Neomysis integer is an eurythermic species that occurs in brackish waters along the Western European coast at longitudes between 36°N and 63°N and in the Baltic Sea (Deprez *et al.*, 2004; http://intramar.ugent.be/NeMys). Within the range Elbe – Guadalquivir, the summer water temperature of the brackish estuarine zone varies from 26 °C in the North to 29 °C in the South, while winter water temperatures range from 0 °C in the North to 10 °C in the South (Drake *et al.*, 2002, Zimmermann, 1997).

Neomysis integer can sustain temperatures from 0 to 33 °C under laboratory conditions (Merker, 1928; Mauchline, 1980), although its optimal resistance tend towards the lower temperature ranges (Arndt and Jansen, 1986). The tolerance of mysids to ambient temperatures may vary **among populations** of the same species (Mauchline, 1980). *N. integer* from the Baltic Sea dies within 2.5 weeks at 20 °C, probably because this population rarely experiences temperatures above 15 °C, and never for periods exceeding a few days at a time (Laughlin and Lindén, 1983). No particular preference was shown by *N. integer* from the Baltic within the temperature range from 4 to 18 °C (Arndt and Jansen, 1986). The tolerance of mysids to ambient temperatures may vary **between generations** within the same population. Kuhlmann (1984) reports a distinct difference in the upper tolerance limit between the winter and summer generations of adult *N. integer* from Kiel Canal as respectively 10 to 12 °C and 20 to 25 °C (at 10 psu). Furthermore, temperature tolerance limits vary **ontogenically**. Juveniles seem to have a different range of temperature tolerance than the adults (Kinne, 1955).

In the coastal water of the western Baltic Sea, the shoals are leaving the shallow littoral zone when water becomes too cold (< 2 °C), or too warm (> 20 °C) (Välipakka, 1992). The fact that it is easy to keep *Neomysis integer* at 0 °C for long periods shows that the species has a good resistance to cooling, whereas its frost resistance to freezing at -10 °C is relatively poor with all animals dying within 8 minutes (Arndt and Jansen, 1986). However, Jansen (1979) described some individuals of *N. integer* to be present in the shallow zone of the Darss-Zingst Peninsula when ice is present in winter time. Espeel (1979) mentions a 'lethargic' behaviour of *N. integer* in very cold winter conditions (< 1 °C) in a brackish lake, with the animals lying at the bottom without any movement.

Temperature is generally thought to overshadow salinity in its effects on growth and reproduction in crustaceans. Still, temperature can influence the salinity tolerance of a species (Vlasblom and Elgershuizen, 1977; Arndt and Jansen, 1986) and interaction effects of both on the survival and growth of a species can change with age (Kinne, 1955; McKenney, 1994; McKenney and Celestial, 1995). *Neomysis integer* is better able to tolerate both low and high salinities at the low temperature range, *i.e.* thermophobic behaviour (Arndt and Jansen, 1986). In freshwater *N. integer* dies rapidly when the temperature is 23 – 24 °C, while in water of a higher salinity they can support temperatures up to 33 °C (Merker, 1928).

The effect of temperature on metabolic rates is commonly expressed as the Q_{10} value. Temperature has a significant effect on the respiration rate of *Neomysis integer*, with a Q_{10} of 1.5 to 3.3 (Clutter and Theilacker, 1971; Astthorsson, 1980; Laughlin and Lindén, 1983; Weisse and Rudstam, 1989; Roast *et al.*, 1999). Temperature also has a significant effect on the ammonia and DIP excretion rate of *N. integer*, with a Q_{10} of respectively 1.4 – 2.9 and 2.6 – 4.9 (Weisse and Rudstam, 1989).

Short term temperature changes of 5 °C (from 15 to 20°C) did not appear to cause an acute temperature chock in *Neomysis integer* (Laughlin and Lindén, 1983). During diel vertical migration *N. integer* can encounter temperatures from 18 °C at the surface to 5 °C at the bottom (in a 30 m deep Baltic station). Since metabolic rates, like respiration and excretion, are strongly affected by temperature, these rates can vary with a factor 2 – 3 during a 24h cycle (Weisse and Rudstam, 1989).

7.3 Oxygen tolerance

The abundance of *Neomysis integer* is affected by the temperature in relation to the oxygen content of the water (Jorgensen, 1929). The scarcity of the species in the field is particularly well marked when oxygen content drops below 40 %, *i.e.* 3.3 – 5.1 mg l^{-1} at 5 – 25 °C (Jorgensen, 1929; Mees *et al.*, 1993; 1995; Margonski and Maciejewska, 1999). Also in (organically) polluted waters the oxygen is more quickly depleted and has its impact on the relative abundance of *N. integer*. In enclosed waters where a permanent halocline is present under which an anaerobic stagnant water mass (hypolimnion), the *N. integer* populations are confined to the oxygenated surface layers (*e.g.* Parker and West, 1979). However, *N. integer* can sustain low oxygen-tensions under laboratory conditions. An oxygen saturation decreasing to 20 % had no influence on the survival and behaviour of adult *N. integer* under laboratory conditions at 18 °C and 18 psu (Kuhlmann, 1984). From 20 % on, their behaviour became abnormal (not further described) and the lethal threshold of oxygen tolerance was at 13 % O_2 saturation. In the complete absence of oxygen, *N. integer* can survive for 20 minutes (Arndt and Jansen, 1986).

7.4 Ammonium tolerance

The toxic threshold of adult *Neomysis integer* to increased ammonia concentration (at 16 °C and 16.5 psu) lies at 0.2 mg NH3 l^{-1}; the 96h LC_{50} is measured at 1.7 mg l^{-1} (Kuhlmann, 1984). This value can be considered as high in comparison with other crustaceans (*Daphnia*), freshwater fish or marine fish larvae (Kuhlmann, 1984).

7.5 Osmoregulation

The isosmotic point of *Neomysis integer*'s blood is 16 psu at 15 °C (Southampton waters, Ralph, 1965) to 19 psu at 5 °C (Loch Etive - Scotland, McLusky and Heard, 1971). Moffat (1996) described the isosmotic point of *N. integer* to be higher than 20 psu at 15 °C for a population in the Looe estuary. It is not unusual for the blood concentration at a given salinity to vary between different populations of the same species (Moffat, 1996).

Neomysis integer is an extremely efficient hyper-hypo-osmoregulator. It is capable of maintaining the concentration, composition and volume of its body fluids such that the metabolic functioning of the cells can continue normally. It can maintain its blood concentration hyperosmotic to the medium when living in diluted sea water environments in the range 0.5 to 20 psu and hyposmotic at salinities between 20 and 40.6 psu (Ralph, 1965; McLusky and Heard, 1971; Moffat, 1996). In fact, the blood concentration itself varies between 14.5 and 24 psu (McLusky and Heard, 1971).

Neomysis integer can rapidly respond to acute changes in salinity and attains osmotic balance within 2 hours of experiencing a change in salinity (Moffat, 1996). This osmoregulatory ability is correlated with changes in (mainly non-essential) free amino acid concentrations. Glutamine, glycine, taurine and alanine were identified as important osmo-effectors in *N. integer* (Moffat, 1996), while Armitage and Morris (1982) report glycine, alanine and proline to be involved in the osmoregulation of this species. Armitage and Morris (1982) report major changes in the free amino acid concentrations within the first 4 hours after an abrupt salinity change. In the field, the adjustment of free amino acid concentration in *N. integer* to salinity change occurs at the changing of the tide at either high or low water (Armitage and Morris, 1982).

Alternating high and low salinity leads to a net decrease in protein in *Neomysis integer* (Austin, 1965), suggesting that the proteins, relatively rich in glycine, alanine and glutamine (Raymont *et al.*, 1968), are the direct, irreversible source for the free amino acids needed for osmoregulation. The proteins are broken down and the free amino acids excreted when no longer required. The discovery of several intracellular transaminases in *N. integer* provides another possible mechanism (by extensive amino acid conversion) for the mobilisation of those large quantities of free amino acids needed in function of osmoregulation (Raymont *et al.*, 1967; Raymont *et al.*, 1968).

Subtle changes in the structural membrane sterols of *Neomysis integer* do appear when environmental salinity changes, especially when salinity drops below 10 psu (Morris *et al.*, 1982). This may be the result of the animal requiring to modify its membrane structure (*i.e.* orientation of the lipid layers) in order to cope with the changing external salinity. These changes occur (in the laboratory) in less than 48 hours. In the field such changes happen regularly during the tidal cycle and are part of *N. integer*'s mechanisms to cope with it sliving in fluctuating environmental salinity (Morris *et al.*, 1982).

The isosmotic point of *Neomysis integer* is a little lower in comparison with the coexisting species *Praunus flexuosus* (25 psu). *N. integer* is a better osmoregulator and thus better adapted to live in more oligohaline waters than *P. flexuosus*, since the range of salinities experienced by the body tissues of *N. integer* is less than in *P. flexuosus* over the same range of media (McLusky and Heard, 1971). The isosmotic point of the other coexisting species *Mesopodopis slabberi* is not know in literature.

7.6 Respiration

Mysids oxygen consumption is dependent on several abiotic (*e.g.* temperature, salinity, season) and biotic (like generation, gender, weight, age and reproductive status) factors. Each factor may have a solitary effect or several factors may act in concert to modify the oxygen consumption (Roast *et al.*, 1999). The respiratory physiology of *Neomysis integer* is considered to be adapted for inhabiting a highly (acutely and longer term) variable environment (Roast *et al.*, 1999). The specialized respiratory physiology, taken with the broad salinity tolerance and an efficient osmoregulatory mechanism explains the presence of *N. integer* in the upper regions of European estuaries.

The impact of salinity, temperature and adaptation salinity on the respiration rate were studied by Vlasblom and Elgershuizen (1977), but no firm conclusions could be made. More recently, also Verslycke and Janssen (2002) measured oxygen consumption by electron transport activity (ETS) at varying salinity, temperature and dissolved oxygen combinations in the laboratory, but the small variations in respiration could not be explained by a single abiotic factor within the ranges tested, suggesting that biotic factors such as weight, age, and gender probably have a larger influence on the respiration rates.

7.6.1 Temperature effect

Several studies (Astthorsson, 1980; Laughlin and Lindén, 1983; Weisse and Rudstam, 1989; Roast *et al.*, 1999) proved that the weight-specific respiration rate of *Neomysis integer* is highly temperature dependent. The temperature dependency of the respiration of *N. integer* is moderate though (Q_{10} between 1.5 and 3.3) in comaprsion to other species, indicating a significant degree of temperature compensation.

The oxygen consumption of *Neomysis integer* was affected more by temperature changes at high than at low salinity (Roast *et al.*, 1999), as demonstrated in higher Q_{10} values at higher salinity. A temperature of 20 °C cannot be considered as optimal for Baltic *N. integer* (Arndt and Jansen, 1986), as is reflected in the high O_2 consumption in comparison with 5 and 15 °C; and especially the low (2 psu) and high (22 psu) salinities have a striking effect on the respiration rate. It can explain why mysids migrate from the littoral zone to deeper (and cooler) water in the summer.

In general there was little difference between male and female oxygen consumption in response to temperature changes (Roast *et al.*, 1999), with males increasing their respiration to a somewhat higher extent than females. Temperature has a significant effect on the O_2 consumption of *Neomysis integer* from the same generation, but respiration is also significantly influenced between seasons or generations by other factors than temperature (Astthorsson, 1980).

7.6.2 Salinity effect

The respiration of *Neomysis integer* is susceptible to changes in salinity as well, but the effect of salinity on physiological processes is less predictable. After all, temperature has a stronger effect on the oxygen consumption than salinity (Roast *et al.*, 1999). In experiments performed by Arndt and Jansen (1986), the salinity had no effect on the respiration at the low temperatures of 5 and 10 °C. At 20 °C respiration is generally higher, but is minimal at the intermediate salinities of 10 – 15 psu and highest at the low (2 psu) and high (24 psu) salinity treatments. In contrast to the former study, Vlasblom and Elgershuizen (1977) found the respiration of *N. integer* to be affected by salinity at 5 and 15°C. At any salinity tested (0.3 – 18 psu, except 2 psu) the respiration was always lower than at the adaptation salinity (12.8 psu). A change in salinity cause greater changes in the oxygen consumption at low (5 °C) than at high (15 °C) temperatures (Roast *et al.*, 1999). Salinity effects were not modified by the mysid gender (Roast *et al.*, 1999).

7.6.3 Body size effect

The respiration of *Neomysis integer* is strongly related to the body size, body surface or body weight (Astthorsson, 1980; Arndt and Jansen, 1986) and are therefore experessed as weight-specific respiration rates (Table 9). The oxygen consumption (R) increases with increasing body weight (DW) according to the following regression $R = 3.21\ DW^{0.6}$ (Arndt and Jansen, 1986).

7.6.4 Gender effect

At most temperature/salinity combinations, male *Neomysis integer* consumed significantly more oxygen than females (Roast *et al.*, 1999). Gender differences in respiration rates have been reported, but may also result from the reproductive status of the test organisms (Raymont *et al.*, 1966; Simmons and Knight, 1975) or from morphological differences between male and female mysids affecting weight-specific oxygen consumption rates (Smith and Hargreaves, 1985).

7.6.5 Season effect

According to Raymont *et al.* (1966) and Astthorsson (1980), the respiration rates of *Neomysis integer* are significantly affected by season. The weight-specific oxygen consumption, measured in the laboratory at 10 °C with animals from the field, showed to have a peak in March and in August, and a marked decline in September/October while respiration stays low during winter months. The respiration of similar sized animals at a specific temperature was found to be higher in summer than in winter (Astthorsson, 1980).

In contrast to the former studies, Verslycke *et al.* (2004) and Roast *et al.* (1999) found no seasonal effect (mainly temperature) in the respiration of *Neomysis integer* from the Schelde and East Looe River estuary, probably because temperature changes occur gradually and the mysids can acclimate to the changing conditions.

7.6.6 Other effects

In the Schelde the respiratory energy consumption of *Neomysis integer* increased upstream (Verslycke *et al.*, 2004) and can be explained by stress caused by decreased salinity and dissolved oxygen or increased pollution (Verslycke *et al.*, 2004).

Laboratory rearing conditions showed to increase oxygen consumption compared to field-collected mysids (Laughlin and Lindén, 1983), probably due to the dietary differences and feeding frequency between the two situations.

7.6.7 Field validation

Laboratory measurements on the effect of salinity and temperature on respiration rate of *Neomysis integer* were related to the field conditions the species is living in (East Looe River estuary) by Roast *et al.* (1999), and where high tidal fluctuations are experienced in temperature (10 °C) and salinity (33 psu); in order to evaluate the adaptive nature of temperature and salinity responses in *N. integer*. *N. integer* disposes of relative good temperature compensation (to the moderate temperature changes experienced in the field over a tidal cycle). The salinity response measured in the laboratory (higher respiration at lower salinity) cannot be seen to have an adaptive nature, since living at the low salinity reaches implies high metabolic costs due to high oxygen consumption (Kinne, 1971; Roast *et al.*, 1999). After all, combining the salinity and temperature responses together, the respiration is high at high water in comparison with low water (Roast *et al.*, 1999). It would be worthwhile to repeat this exercise in estuaries with less extreme tidal fluctuations in salinity and temperature.

On a seasonal basis the oxygen consumption of *Neomysis integer* is predicted to be higher in summer, when temperatures are higher (Roast *et al.*, 1999). At lower winter temperatures the effect of changing (tidal) salinity conditions are more distinct than in summer (Roast *et al.*, 1999). On the other hand, the seasonal temperature increase occurs gradually over a longer period of time and a gradual adaptation might occur, causing a minimal effect on the respiration of *N. integer* (Roast *et al.*, 1999). Also, Raymont *et al.* (1966) and Astthorsson (1980) report the respiration rates of *N. integer* to be significantly affected by season in itself. Predictions based on measurements of one sampling occasion like the one of Roast *et al.* (1999) would be invalid to extrapolate to other seasons in such a case.

8 ECOTOXICOLOGY

Mysids are sensitive to chemical contaminants at environmentally relevant concentrations and have been used in regulatory toxicity testing for more than 20 years (*e.g.* Nimmo and Hamaker, 1982; Gaudy *et al.*, 1991; Harmon and Langdon, 1996; Brandt *et al.*, 1993; Roast *et al.*, 1998; Veslycke *et al.*, 2004 and references herein). The U.S. Environmental Protection Agency and the American Society for Testing and Materials both have adopted the subtropical *Americamysis bahia* (formerly *Mysidopsis bahia*) as a key testing species for coastal and estuarine monitoring, and standard guides for conducting life--cycle toxicity tests with this species have been developed (USEPA, 2002). Although a relatively large amount of published toxicity data is available for *Americamysis* species, relatively limited data are available on the sensitivity of other mysid species to toxicants (Roast *et al.*, 1999c). However, the available evidence suggests that mysids are generally more sensitive to toxic substances than many other test species (Morton *et al.*, 1997; Roast *et al.*, 1998b; Verslycke *et al.*, 2003b).

Ideally, chemical risk assessment should be assessed by standardized endpoints that cover the molecular, individual and population level. For mysids, this implies that, in addition to evaluating mortality, acute, chronic and multigenerational bio-assays have to be developed for testing chemical effects on growth and moulting, reproduction, biochemical composition, metabolism, physiological processes, behaviour and morphologic aberrations (as reviewed by Verslycke *et al.*, 2004). Also, field studies and caging experiments with mysids have been published (Clark et al., 1986; Rand and Clark, 2000; Fossi et al., 2001; Verslycke, 2003). Because of their ecological importance, wide geographic distribution, year-round availability in the field, ease of transportation, ability to be cultured in the laboratory, and sensitivity to contaminants, mysids are appropriate toxicity test organisms. Clearly, field validation of the biomarkers is a strong research need for the future.

Recently, there has been increasing interest in using the brackish water mysid *Neomysis integer* (Leach, 1840) as a toxicological test species for estuarine systems (Emson and Crane, 1994; Roast *et al.*, 1998b; Verslycke *et al.*, 2004 – Addendum 3). It is an alternative to *Americamysis bahia* for use in European water quality testing (Roast *et al.*, 1998a; Verslycke *et al.*, 2004). Due to its relatively high temperature requirements, the subtropical species *A. bahia* can not be used in temperate regions and its low tolerance for low salinities of 0.1 to 5 psu makes it an inappropriate test species to use in the turbid upper reaches of estuaries or oligohaline inland water bodies (Roast *et al.*, 2000a; 2001).

Next of being applied in acute toxicity tests (von Oertzen *et al.*, 1988; Emson and Crane, 1994; Wildgust and Jones, 1998; Roast *et al.*, 1999c; 2001; Verslycke *et al.*, 2003b), *Neomysis integer* have been used successfully in various sublethal tests for evualuating swimming ability (Roast et al., 2000b; 2000c; 2001b; 2002; 2002b), energy budget (Roast et al., 1999b; Verslycke et al., 2003; 2004b; 2004c), testosterone metabolism (Verslycke et al., 2002; 2003c; 2004c), respiration (Laughlin and Linden, 1983; von Oertzen *et al.*, 1988), ammonia-excretion (Laughlin and Linden, 1983), growth and moulting (Ghekiere et al., submitted), vitellogenesis (Ghekiere et al., 2004), and intra-marsupial development (Ghekiere et al., in preparation).

9 CONCLUSIONS

The present work aims to gather all literature available on the brackish water mysid *Neomysis integer*, with focus on its distribution, feeding ecology, life history aspect, behaviour, physiology, biochemical composition, bioenergetics and ecotoxicology. It aims to identify gaps in our knowledge of the species.

Because *N. integer* is often used as a model species to study the ecology of brackish water crustaceans, and because the species is easy to sample (qualitatively) in shallow water and keep in the laboratory, many studies and data are available concerning the species. The older studies (< 1980) are often superficial or based on a limited number of observations; while their data are often unpublished (as Ph.D. theses or reports) or published in local journals. Information extracted from this *'grey'* literature has been integrated in the review. In the last 10 years, quite some in-depth studies have been published concerning aspects of the feeding ecology, population dynamics, physiology, bioenergetics, behaviour, and ecotoxicological use of the species. One should consider this chapter as a reference work, to grab to when specific information of one of the topics is needed. The author's contributions (results presented in this thesis and some unpublished work) are highlighted in the text.

Abundant information is available on the distribution patterns of *Neomysis integer* in enclosed brackish waters and estuaries, although on has to keep in mind that the swarming behaviour, vertical and horizontal migration, segregation of life stages and escape behaviour of *N. integer* (all not fully understood yet) can handicap the quantification of the density and biomass, and may hamper the calculation of the production and the study of the life history of the species.

There is a great need for the description of the feeding ecology of key species – like *Neomysis integer* – in estuarine environments for the development of accurate C-flux models and the description of detritus based food web patterns, including the quantification of transfer coefficients. Although *N. integer* is described as an important food item for many demersal and pelagic fish, larger epibenthic crustaceans and wading bird species, quantitative information is still lacking on its own diet, feeding rates, feeding patterns and selectivity (especially for populations living in estuarine conditions).

Numerous data are available on the life history of *Neomysis integer* over a wide geographical and habitat range, although southern populations (< 51°N) are more poorly known. Variations are observed between these populations in the number of cohorts, size-at-maturity, fecundity and growth rate. Growth and reproduction are affected by prevailing environmental conditions as generally observed in Crustacea. However, in the eurythermic and euryhaline *Neomysis integer*, typically living in the highly dynamic estuarine environment, this is not thoroughly studied yet. Details on how intra- and post-marsupial development, moulting processes and reproduction are affected by a wide range in salinity, temperature, food quantity and quality are still lacking.

The biochemical composition and the ecophysiology of *Neomysis integer* are well known and several methodologies to calculate the energy budget have been applied to the species.

There has been an increasing interest in using the brackish water mysid *Neomysis integer* as a toxicological test species for Western European estuarine systems. Mortality, respiration, swimming behaviour, testosterone metabolism and energy budgets are well established endpoints for bioassays with the species. However, more data on its growth, moulting, development and reproductive processes are needed (at the individual- and population-level). The influence of prevailing environmental variables on these processes, as well as their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically-induced variability and natural variability in toxicity testing.

Still, some literature published on the species *Neomysis integer* is not dealt with in the present literature review as its falls out of the scope of the present Ph.D and cover fields like environmental contamination, biomanipulation of brackish lakes, use of the species for drug detection, molecular, phylogenetic and phylogeographic work on *N. integer*, detailed morphologic descriptions of the cuticle, statocyst, sensory systems and the intra-marsupial development, visual sensitivity of the species, presence of intersexuality, culturing of the species, and use as food for other trophic levels. All references are grouped in Table 10 with an indication of the topics not treated in this review (*).

Table 10: All literature on *Neomysis integer* (* falling out of the scope of the present overview)

Taxonomy	Mauchline and Murano, 1977; Deprez *et al.*, 2004; http://www.vliz.be/vmdcdata/nemys; www.iorbis.org
Morphology – External (general)	Tattersall and Tattersall, 1951; Mauchline, 1980; Hayward and Ryland, 1995; http://ip30.eti.uva.nl/bis/crustacea.php; http://www.marlin.ac.uk/species/taxon_Neomysisinteger.htm
Morphology – Feeding appendages and gut structure	Gelderd, 1909; Lucas, 1936; Molloy, 1958; Tattersall and Tattersall, 1951; Haffer, 1965; Astthorsson, 1980; Mauchline, 1980; Armitage *et al.*, 1981; Espeel, 1982; Bradshaw *et al.*, 1989; Maciejewska, 1992; Brunet *et al.*, 1994; Kobusch, 1998; Remerie, 1999; Roast *et al.*, 2004
* **Morphology** – Cuticle	Putz and Buchholtz, 1991
* **Morphology** – Statocyst	Lowenstam and McConell, 1968; Espeel, 1984; 1985; 1986; 1987; Wittmann et al., 1993
* **Morphology** – Olfactory system	Guse, 1979; Guse, 1983; Hallberg et al., 1992; Johansson and Hallberg, 1992; 1992b
* **Morphology** – Sensilla	Guse, 1978; 1980; 1980b
* **Morphology** – Intra-marsupial development	Rathke, 1839; Wagner, 1896; 1998; Needham, 1937; Kinne, 1955; de Kruijf, 1977; Mauchline, 1980; Scholtz, 1984; Scholtz et al., 1993; Fockedey *et al.*, submitted b – Chapter 6
Morphology – morphometric regressions	Schuchardt et al., 1989; Mees et al., 1994
Morphology – length/weight regressions	de Kruijf, 1977; Beattie and de Kruijf, 1978; Astthorsson, 1980; Summers, 1980; Beattie, 1982; Bremer and Vijverberg, 1982; Jansen, 1985c; 1985c; Arndt and Jansen, 1986; Mees *et al.*, 1994; Aaser *et al.*, 1995; Irvine et al., 1995; Gorokhova and Hansson, 1999; Maciejewska and Opalinski, 2002; Winkler and Greve, 2004
Density & Biomass – Estuaries	Percival, 1929; Moore et al., 1979; Sorbe, 1980; O'Sullivan, 1984; Milner, 1986; Rodriguez and Dauvin, 1987; Schuchardt et al., 1989; Haesloop, 1990; Apel, 1992; Hough and Naylor, 1992; Mees and Hamerlynck, 1992; Moffat and Jones, 1992; Mees et al., 1993; 1993b; San Vicente, 1993; Bamber and Henderson, 1994; Bernát et al., 1994; Cattrijsse et al., 1994; Mees et al., 1994; 1995; Köpke and Kausch, 1996; Moffat, 1996; Mouny et al., 1996; San Vicente, 1996; Drake et al., 1997; Mouny et al., 1998; Zouhiri et al., 1998; Cunha et al., 1999; Dauvin et al., 2000; Mouny et al., 2000; Drake et al., 2002; Hampel et al., 2003; 2003b; David *et al.*, 2005; Fockedey, unpublished – EU MATURE
Density & Biomass – Baltic Sea	Muus, 1967; Rasmussen, 1973; Kotta and Simm, 1979; Weslawski, 1981; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; Hansson, 1990; Köhn, 1992; Rudstam *et al.*, 1992; Thiel, 1992; Välipakka, 1992; Margonski and Maciejewska, 1999

Density & Biomass – Ponds and lakes	Barnes *et al.*, 1971; Beattie and de Kruijf, 1978; Parker and West, 1979; Bremer, 1980; Bremer and Vijverberg, 1982; Espeel, 1982; Platenkamp, 1983; Bales *et al.*, 1993; Hosper and Meijer, 1993; Irvine *et al.*, 1993; Jeppesen, *et al.*, 1994; Meijer *et al.*, 1994; Soselisa, 1994; Aaser *et al.*, 1995; Irvine *et al.*, 1995; Verslycke *et al.*, 2000
Diet of *Neomysis integer* – description	Lucas, 1936; Tattersall and Tattersall, 1951; Vorstman, 1951; Kinne, 1955; Mauchline, 1971; 1980; Bremer and Vijverberg, 1982; Sorbe, 1980; Astthorsson, 1980; Jansen, 1985b; Haesloop, 1990; Uitto *et al.*, 1995; Speirs *et al.*, 2002; Vilas and Fockedey, unpublished
Diet of *Neomysis integer* – quantitative stomach analysis and fullness index	Astthorsson, 1980; Sorbe, 1980; Bremer and Vijverberg, 1982; Jansen, 1985b; Arndt and Jansen, 1986; Haesloop, 1990; Irvine *et al.*, 1993; Soselisa, 1994; Remerie, 1999; Speirs *et al.*, 2002; Fockedey and Mees, 1999; Fockedey, unpublished – EU MATURE; Fockedey, unpublished; Vilas and Fockedey, unpublished
Diet of *Neomysis integer* – isotope fractionation	Rolff, 1998; Gorokhova, 1999; Gorokhova and Hansson, 1999
Diet of *Neomysis integer* – Feeding rate (ingestion, egestion, gut evacuation rate, assimilation efficiency)	Lucas, 1936; Molloy, 1958; Ferguson, 1973; Astthorsson, 1980; Kuhlmann, 1982; Arndt and Jansen, 1986; Irvine *et al.*, 1993; 1995; Aaser *et al.*, 1995; De Pauw, 1998; Viitasalo *et al.*, 1998; Roast *et al.*, 2000; Maciejewska and Opalinski, 2002; Winkler and Greve, 2004; Bergström and Englund, 2004; Albertsson, 2004; Fockedey *et al.* submitted a – Chapter 5; Fockedey *et al.*, submitted c – Chapter 4; Fockedey *et al.*, submitted d – Addendum 2; Fockedey, unpublished
Diet of *Neomysis integer* – Selectivity and structuring effects on prey populations	Espeel, 1982; Moss and Leah, 1982; Jansen *et al.*, 1983; Jansen and Heerklos, 1983; Van De Vijver, 1983; Jansen, 1985b; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; 1992; Hansson *et al.*, 1990; Thiel, 1992; Irvine *et al.*, 1993; 1995; Bernát *et al.*, 1994; Jeppesen *et al.*, 1994; Aaser *et al.*, 1995; Fockedey and Mees, 1999 – Chapter 2
Diet of *Neomysis integer* – Feeding modes	Lucas, 1936; Tattersall and Tattersall, 1951; Raymont *et al.*, 1964; Parker and West, 1979; Astthorsson, 1980; Mauchline, 1980; Bremer and Vijverberg, 1982; Espeel, 1982; Irvine *et al.*, 1993; Bales *et al.*, 1993; Roast, 1997; Roast *et al.*, 2000; Roast *et al.*, 2004
Diet of *Neomysis integer* – diet overlap with other species	Kinne, 1955, Sanina, 1961, Jansen, 1985b; Maciejewska, 1992; Thiel, 1992; 1996; Remerie, 1999; Maciejewska and Opalinski, 2002; Winkler and Greve, 2004; Vilas and Fockedey, unpublished
Diet of *Neomysis integer* – Starvation	Raymont and Krishnaswamy, 1960; Raymont and Conover, 1961; Linford, 1965; Raymont *et al.*, 1964; 1967; 1966; 1968; Morris *et al.*, 1977; Vlasblom and Elgershuizen, 1977; Armitage *et al.*, 1977; 1978; Parker and West, 1979; Murtaugh, 1984; Weisse and Rudstam, 1989; Gorokhova and Hanssen, 1999; Winkler, 2000; Gorokhova, 2002; Verslycke and Janssen, 2002
Diet of *Neomysis integer* – Faecal pellets and coprophagy	Molloy, 1958; Ferguson, 1973; Parker and West, 1979; Bradshaw *et al.*, 1989; Weisse and Rudstam, 1989; Roast *et al.*, 2000; Maciejewska and Opalinski, 2002; Fockedey *et al.*, submitted d – Addendum 2

Predation on *Neomysis integer*	Patterson, 1905; Kemp, 1910; Redeke, 1941; Healey, 1972; Cramp, 1977; 1983; Kislalioglu and Gibson, 1977; Morawski, 1978; Van den Broek, 1978; Mauchline, 1980; Summers, 1980; Timola, 1980; Marchand, 1981; Sorbe, 1981; 1983b; Bremer and Vijverberg, 1982; Van De Vijver, 1983; Heeste, 1984; Van Densen, 1985; 1988; Arndt and Jansen, 1986; Janssen and Spannhof, 1987; Franek, 1988; 1989; Debus, 1989; Kostrzewska Szlakowska and Szlakowsk, 1990; Costa and Elliott, 1991; Leonardsson, 1991; Assis et al., 1992; Costa et al., 1992; Moreira *et al.*, 1992; Aarnio and Bonsdorff, 1993; Houthuijzen et al., 1993; Irvine *et al.*, 1993; Cattrijsse, 1994; Cattrijsse *et al.*, 1994; Holker and Hammer, 1994; Thiel, 1996 and references herein; Van Densen et al., 1996; Lazauskiene et al., 1996; Cattrijsse et al., 1997; Larsson and Berglund, 1998; Mouny *et al.*, 1998; Hostens and Mees, 1999; Thyrel et al., 1999; Søndergaard *et al.*, 2000; Thiel, 2000; Temming and Herrmann, 2001; Hostens, 2003; Maes *et al.*, 2003; Granqvist and Mattila, 2004; Hampel and Cattrijsse, 2004; Winkler and Greve, 2004; Dean *et al.*, 2005
Behaviour – Swarming	Percival, 1929; Mauchline, 1971c; Arndt and Jansen, 1986; Walesby, 1973; Parker and West, 1979; Espeel, 1982; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; Thiel, 1992; Köpcke and Kausch, 1996; Roast *et al.*, 2004
Behaviour – Segregation of life stages	Tattersall and Tattersall, 1951; Kinne, 1955; Ralph, 1965; Beattie and de Kruijf, 1978; Parker and West, 1979; Astthorsson, 1980; Sorbe, 1980; Platenkamp, 1983; Milner, 1986; Schuchardt *et al.*, 1989; Hough and Naylor, 1992; Välipakka, 1992; Cattrijsse *et al.*, 1994; Moffat, 1996
Behaviour – Position maintenance	Hough and Naylor, 1992; Moffat and Jones, 1993; Köpcke and Kausch, 1996; Roast *et al.*, 1998; 1998b; 2000b; 2000c; 2001b; 2002; 2002b
Behaviour – Swimming	Parker and West, 1979; Hough and Naylor, 1992; Roast *et al.*, 1998; 1998b; Lawrie *et al.*, 1999; Roast *et al.*, 2000b; 2000c; 2001b; 2002; 2002b
Behaviour – Predator avoidance	Mauchline, 1971c; Debus *et al.*, 1992; Kaiser *et al.*, 1992b; Rademachers and Kils, 1996; Lindén *et al.*, 2003
Behaviour – Vertical migration	Jansson and Källander, 1968; Beattie and de Kruijf, 1978; Moore *et al.*, 1979; Bremer and Vijverberg, 1982; Jansen, 1985; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; Debus *et al.*, 1992; Irvine *et al.*, 1993; Bernát *et al.*, 1994; Aaser *et al.*, 1995; Köpcke and Kausch, 1996; Zouhiri *et al.*, 1998; Dauvin *et al.*, 2000; Fockedey, unpublished – EU project MATURE; Mees, unpublished – EU Project JEEP
Behaviour – Horizontal migration	Vorstman, 1951; Kinne, 1955; Ralph, 1965; Muus, 1967; Kühl and Mann, 1969; Barnes *et al.*, 1977; Beattie and de Kruijff, 1978; Jansen, 1979; Moore *et al.*, 1979; Astthorsson, 1980; Sorbe, 1980; Bremer and Vijverberg, 1982; Jansen, 1985; Arndt and Jansen, 1986; Rudstam *et al.*, 1986; Schuchardt *et al.*, 1989; Hough and Naylor, 1992; Thiel, 1992; Välipakka, 1992; Jansen, 1993; Mees *et al.*, 1993b; 1994; Cattrijsse *et al.*, 1994; Köpcke and Kausch, 1996; Speirs *et al.*, 2002; Allen *et al.*, 2003; Hampel *et al.*, 2003; 2003b

Life history – field data (number of cohorts, life span, size-at-maturity, sex-ratio)	Vorstman, 1951; Tattersall and Tattersall, 1951; Kinne, 1955; Wiktor, 1961; Raymont et al., 1966; Mauchline, 1971; 1971b; de Kruijff, 1977; Beattie and de Kruijf, 1978; Borghouts, 1978; Espeel, 1979; 1982; Parker and West, 1979; Astthorsson, 1980; Bremer, 1980; Jansen et al., 1980; Sorbe, 1980; Bremer and Vijverberg, 1982; Platenkamp, 1983; Astthorsson and Ralph, 1984; Arndt and Janssen, 1986; Jansen, 1986; Rudstam et al., 1986; Schuchardt et al., 1989; Weisse and Rudstam, 1989; Haesloop, 1990; Moffat and Jones, 1992; Tehn, 1992; Irvine et al., 1993; 1995; Mees et al., 1994; Soselisa, 1994; Aaser et al., 1995; Fockedey, unpublished; Mees et al., unpublished
Life history – annual production	Bremer, 1980; Bremer and Vijverberg, 1982; Kuhlmann, 1982; Arndt, 1985; Arndt and Jansen, 1986; Thiel, 1992; Mees *et al.*, 1994; Soselisa, 1994; Aaser *et al.*, 1995; Irvine *et al.*, 1995; Fockedey, unpublished
Life history – fecundity (breeding season, brood size, number of broods, intra-marsupial mortality, hatching success, development time, size and growth, birth rate)	Vorstman, 1951; Tattersall and Tattersall, 1951; Kinne, 1955; Raymont *et al.*, 1966; Mauchline, 1971; 1972; Vlasblom and Elgershuizen, 1977; Beattie and de Kruijf, 1978; Borghouts, 1978; Parker and West, 1979; Moore *et al.*, 1979; Astthorsson, 1980; Bremer and Vijverberg, 1982; Arndt and Jansen, 1986; Schuchardt *et al.*, 1989; Haesloop and Scheffel, 1991; Hough and Naylor 1992; Mees et al., 1994; Soselisa, 1994; Irvine *et al.*, 1995; Moffat, 1996; Fockedey *et al.*, submitted b – Chapter 6; Fockedey and Mees, unpublished; Fockedey, unpublished; Janssen, unpublished; Mees *et al.*, unpublished
Life history – mortality	Mees et al., 1994; Irvine *et al.*, 1995
Life history – growth (field and laboratory-based growth curves, growth rate, moulting,)	Kinne, 1955; Mauchline, 11973; 976; 1977; 1977b; 1985; Beattie and de Kruijf, 1978; Astthorsson, 1980; Schrotenboer, 1980; Bremer and Vijverberg, 1982; Astthorsson and Ralph, 1984; Kuhlmann, 1984; Arndt and Jansen, 1986; Schuchardt *et al.*, 1989; Mees *et al.*, 1994; Soselisa, 1994; Irvine *et al.*, 1995; Aaser *et al.*, 1995; Gorokhova and Hansson, 1997; De Pauw, 1998; Gorokhova, 2002; Maciejewska and Opalinski, 2002; Winkler and Greve, 2002; Fockedey *et al.*, in press – Chapter 3; Fockedey *et al.*, submitted a – Chapter 5; Fockedey *et al.*, submitted c – Chapter 4; Fockedey, unpublished
Biochemical composition – dry weight, ash content, Chitin content	Raymont and Krishnaswamy, 1960; Raymont *et al.*, 1964; 1966; de Kruijf, 1977; Astthorsson, 1980; Summers, 1980; Jansen, 1985c; Arndt and Jansen, 1986; Mees *et al.*, 1994; Irvine *et al.*, 1995; Aaser *et al.*, 1995; Gorokhova and Hansson, 1997; Maciejewska and Opalinski, 2002; Verslycke, 2003; Winkler and Greve, 2004
Biochemical composition – caloric content	Summers , 1980; Astthorsson, 1980; Arndt and Jansen, 1986; Kaiser *et al.*, 1992; Maciejewska and Opalinski, 2002
Biochemical composition – C, N and P content	Raymont *et al.*, 1964; 1966; Tundisi and Krishnaswamy, 1967; Gorokhova and Hansson, 1999
Biochemical composition – proteins, amino acids	Raymont *et al.*, 1964; 1966; 1968; Srinivasagam *et al.*, 1971; Morris *et al.*, 1977; Armitage *et al.*, 1977; 1978; 1981; Armitage and Morris, 1982; Moffat, 1996; Verslycke and Janssen, 2002; Verslycke *et al.*, 2004; Ghekiere *et al.*, 2004

Biochemical composition – lipids	Molloy, 1958; Raymont *et al.*, 1964; 1966; 1968; Linford, 1963; 1965; Morris, 1971; 1973; Morris and Sargent, 1973; Morris *et al.*, 1973; 1977; 1981; 1982; Armitage *et al.*, 1981; Bradshaw et al., 1989; 1990; Brunet *et al.*, 1994; Verslycke and Janssen, 2002; Verslycke *et al.*, 2004
Biochemical composition – carbohydrates	Molloy, 1958; Raymont and Krishnaswamy, 1960; Raymont *et al.*, 1964; 1966; 1968; Brunet *et al.*, 1994; Morris, 1999; Verslycke and Janssen, 2002; Verslycke *et al.*, 2004
Energy budgets	Astthorsson, 1980; Rudstam, 1989; Thiel, 1996; Roast *et al.*, 1999b; Maciejewska and Opalinski, 2002; Verslycke and Janssen, 2002; Verslycke *et al.*, 2003; 2004; 2004b; 2004c
Physiology – Tolerance salinity	Merker, 1928; Stammer; in Segerstrale, 1945; Tattersall and Tattersall, 1951; Ralph, 1965; Mauchline, 1971; Vlasblom and Elgershuizen, 1977; Kuhlmann, 1984; Arndt and Jansen, 1986; Moffat and Jones, 1992; Mees *et al.*, 1994; 1995; Moffat, 1996; Roast *et al.*, 1998; 2001; Drake *et al.*, 2002; Verslycke *et al.*, 2004
Physiology – Tolerance temperature	Merker, 1928; Kinne, 1955; Vlasblom and Elgershuizen, 1977; Espeel, 1979; Jansen, 1979; Mauchline, 1980; Laughlin and Lindén, 1983; Kuhlmann, 1984; Arndt and Jansen, 1986; Välipakka, 1992
Physiology – Tolerance oxygen	Jorgensen, 1929; Parker and West, 1979; Kuhlmann, 1984; Arndt and Jansen, 1986; Mees *et al.*, 1993; 1993b, 1994; 1995; Margonski and Maciejewska, 1999; Roast *et al.*, 2000
Physiology – Tolerance ammonium	Kuhlmann, 1984
Physiology – Osmoregulation	Austin, 1965; Ralph, 1965; Raymont *et al.*, 1967; 1968; McLusky and Heard, 1971; Armitage and Morris, 1982; Morris *et al.*, 1982; Moffat, 1996
Physiology – Respiration	Raymont et al., 1966; Raymont and Krishnaswamy, 1968; Simmons and Knight, 1975; Vlasblom and Elgerhuizen, 1977; Astthorsson, 1980; Laughlin and Lindén, 1983; Smith and Hargreaves, 1985; Arndt and Jansen, 1986; Weisse and Rudstam, 1989; Roast et al., 1999; Maciejeska and Opalinski, 2002; Verslycke and Janssen , 2002; Verslycke *et al.*, 2004
Physiology – Excretion	Raymont *et al.*, 1968; Ferguson, 1973; Laughlin and Lindén, 1983; Weisse and Rudstam, 1989
Physiology – Visual sensitivity	Halberg, 1977; Kurnaty, 1979; Hallberg et al., 1980; Lindström, 1992; 2000
* **Culturing protocol**	Mauchline, 1971; Astthorsson, 1980; Kuhlmann, 1984; Arndt and Jansen, 1986; Haesloop, 1990; Roast *et al.*, 1999; Verslycke et al., 2003b
Ecotoxicology – General	Roast et al., 1998b; Verslycke et al., 1994 – addendum 3
Ecotoxicology – Metals	von Oertzen et al., 1988; Emson and Crane, 1994; Wildgust and Jones, 1998; Roast et al., 1999c; 2000b; 2001; 2002; 2002b; Verslycke et al., 2003b

Ecotoxicology – Oil pollution	Laughlin and Lindén, 1982; 1983; Lindén et al., 1982
Ecotoxicology – Pesticides	Zmudzinski, 1975; Zandmane and Stasulane, 1987; Zandmane, 1988; Zandmane and Zelcans, 1988; Davies et al., 1997; Roast et al., 2000c
Ecotoxicology – Endocrine disruption	Verslycke, 2003; Verslycke et al., 2003; 2003c; 2004c; 2004d; 2005
* **Environmental contamination**	Beattie and de Kruijf, 1978; Vobach and Felt, 1981; Foekema et al., 1998; Loizeau et al., 2001; 2001b
* **Biomanipulation in lakes**	Dietrich and Hesse, 1990; Hosper and Meijer, 1993; Meijer et al., 1994; Jeppesen et al., 1994; Moss et al., 1996
* **Detection anabolic drugs**	De Wasch et al., 2002; Verslycke et al., 2002; Van Hoof, 2004; Poelmans et al., 2005
* **Phylogeny & Phylogeography**	Kobusch, 1998; Remerie, 2005; Remerie et al., 2004
* **Molecular work**	Suomalainen, 1954; Salemaa, 1986; Nunn et al., 1996; Remerie, 2005; Remerie et al., 2004; Remerie et al., submitted a; submitted b
* **Parasitism**	Tatterssal and Tattersall, 1951; Gibson, 1972; Astthorsson, 1980; Espeel, 1984; Koie, 1993; Marcogliese, 1996
* **Intersexuality**	Hough et al., 1992; Mees et al., 1995b

Appendix

Records of *Neomysis integer*

approx. Latitude	approx. Longitude	Location name	Sytem	Source
71°03'N – 63°50'N	32°09'E – 44°04'E	? White Sea and Murman Seas	?	Wagner, 1885 (Jarzynsky); Zimmer, 1909
68°29-10'N	14°12E – 15°28'E	Lofoten, Norway	Fjord – Loch	Holmquist, 1957
67°14'N – 64°58'N	11°50'E – 15°43'E	Helgeland coast, Norway	Fjord – Loch	Holmquist, 1957
63°26'N – 58°00'N	4°37'E – 10°23'E	Norwegian coast, Christiania to Trondheim	Fjord - Loch	Wagner, 1885 (Sars); Zimmer, 1909
60°34'N – 53°40'N	9°28'E – 30°16'E	Baltic Sea (Estonian coastal waters, Gdansk Bay, Isefjord, Mecklenburg Bay, Vistula lagoon, Bay of Riga, Gulf of Bottnia, Finnish coastal area, Baltic proper, Swedish coast, Darss/Zingst Peninsula, coast Poland, Vislinsky Bay, Danish estuaries and lagoons)	Baltic	Wagner, 1885 (Lindström, Lilljeborg, Cajander, Kröyer); Blegvad, 1922; Dahl, 1944; Segerstrale, 1945; Wiktor, 1961; Järvekülg, 1965; Muus, 1967; Rasmussen, 1973; Kotta and Simms, 1979; Kotta 1979; 1980; 1984; Wiktor et al., 1980; Lumme et al., 1980; Salemaa, 1981; Laughlin and Lindén, 1983; Jansen, 1985; Arndt and Jansen, 1986; Chojnacki and Ciupinski, 1986; Rudstam et al., 1986; Arndt, 1989; Weisse and Rudstam, 1989; Rudstam and Hansson, 1990; Tehn, 1991; 1991b; 1992; Salemaa et al., 1990; Kohn, 1992; Välipakka, 1992; Petryashov, 1992; Pentti, 1992; Witek et al., 1993; Witek, 1995; Razinkovas, 1993; 1996; Thiel, 1996*; Margonski and Maciejewska, 1999; Lindström, 2000; Maciejewska and Opalinski, 2002 and references herein; Lindén et al., 2003; Bergström and Englund, 2004
54°24'N – 53°54'N	9°09'E – 10°13'E	Nordostseekanal/ Kiel canal, Germany	Brackish canal	Kinne, 1955; Schütz, 1969; Kuhlmann, 1984
60°51'N - 59°51'N	2°07'W - 0°47'W	Shetland Islands, Scotland, UK	Brackish pond	Scott and Duthie, 1895
59°23'N – 58°43'N	3°26'W – 2°22' W	The Orkneys, Scotland, UK	Fjord - Loch	Nicol, 1939
58°26'N	3°05'W	Loch Wester, Wick, Scotland, UK	Fjord - Loch	Scott, 1891
58°21-19'N	6°30-35'W	Loch Arnol, Loch Bravas and inland water, Island of Lewis, Outer Hebrides, Scotland, UK	Fjord - Loch	Elton, 1937; Tattersall and Tattersall, 1951*
57°43-40'N	3°16'W	Loch of Spynie, Scotland, UK	Fjord - Loch	Gordon, 1852
57°41-30'N	7°31-06'W	North Ulst, Scotland, UK	Fjord - Loch	Nicol, 1936
57°39-37'N	3°38-34'W	Bay of Findhorn, Scotland, UK	Estuary	Gordon, 1852
57°21-18'N	2°00W	Ythan, England, UK	Estuary – subtidal	Astthorsson, 1980; Astthorsson and Ralph, 1984

approx. Latitude	approx. Longitude	Location name	Sytem	Source
57°03'N - 56°56'N	7°23-32'W	Several lochs, Island Barra, (e.g. Loch St. Clair), Outer Hebrides, Scotland, UK	Fjord - Loch	Scott, 1894; Crichton and Young, 1936
56°42'N	2°28'W	North Esk, Scotland, UK	Estuary - subtidal	Tattersall and Tattersall, 1951*
56°34-32'N	8°07-10'E	Lake district (o.a.Lake Ferring), Denmark	Brackish lake	Aaser et al., 1995; Jeppesen et al., 1994; Søndergaard et al., 2000
56°27'N	3°04'W	Quarry near Tay, Scotland, UK	Brackish pond	Tattersall and Tattersall, 1951
56°34-11'N	5°03-37'W	Loch Craignish, Feochan, Etive, Dunstaffnage Bay, Crenan, Shuna, Linnhen, Eil, Torridon, Ardmaddy Bay, Scotland, UK	Fjord - Loch	Mauchline, 1971; McLusky and Heard, 1971; Kaiser et al., 1992
56°16'N- 55°24'N	5°28'W- 4°28'W-	Firth of Clyde, at head of lochs Striven, Riddon, Fyne, Gilp and Ranza; Bubh loch, Southannan and Hunterston sands and Kames Bay, Scotland, UK	Fjord - Loch	Mauchline, 1971; 1971b
56°00'N	2°51'W	Aberlady, Scotland, UK	Estuary – marsh	Nicol, 1936
55°23'N- 51°25'N	10°26'W - 6°05W	Ireland (Shannon, Lee, Dodder, Cork, Dublin Bay, Galway, Mayo, Wicklow, Youghal; Loch Leam, Loch Foyle, Loch Furnace)	Estuary Brackish lake/pond Fjord - Loch	Rankin, 1907; Kemp, 1910; Tattersall, 1912; Thompson, 1828; Thompson, 1845; Thompson, 1847; Kinahan, 1857; Melville, 1857; MacDonnald and MacMillan, 1951; Murray, 1977; Parker, 1978; Parker, 1979; Parker and West, 1979; O'Sullivan, 1984
55°21-20'N	1°36-34'W	Coquet, England, UK	Estuary	Jorgensen, 1924
54°59-57'N	1°36-25'W	Tyne, England, UK	Estuary	Jorgensen, 1929
54°18-15'N	9°54-59'E	Flemhuder See, Germany	Brackish lake	Rademacher and Kils, 1996
53°44-30'N	0°58-04'W	Humber, England, UK	Estuary – subtidal	Budd, 2002
53°44-43'N	2°51-50'W	Ponds on Hutton Marsh, England, UK	Estuary - marsh	Tattersall, 1919
53°26-17'N	3°12'W - 2°55'W	Pools in Wirral Peninsula, England, UK	Brackish pond	Tattersall, 1919; Standen, 1922; Tattersall and Tattersall, 1951
53°18'N	3°44'W	Landlocked pool at Colway Bay, Wales, UK	Brackish pond	Walker, 1895
53°55-32'N	8°48'W- 10°00'W	Elbe, Germany	Estuary – subtidal	Kraepelin, 1886; Dahl, 1891; Kraefft, 1908; Schlienz, 1924; Caspers, 1951; Kühl, 1964; 1972; 1973; Fiedler, 1991; Bernat et al., 1994; Mees et al., 1995b; Köpcke and Kausch, 1996; Winkler., 2000
53°33-05'N	8°45-34'E	Weser, Germany + tributaries and marsh ditches	Estuary – subtidal and intertidal	Klie, 1914; Schlienz, 1922; 1924; Schräder, 1941; Friedrich, 1960; Kühl and Mann, 1969; Michaelis, 1973; Teufert, 1980; Vobach and Feldt, 1981; Grotjan and Michaelis, 1985; Haesloop, 1990; Haesloop and Scheffel, 1991; Kolbe and Michaelis, 2001

approx. Latitude	approx. Longitude	Location name	Sytem	Source
53°40-28'N	8°05-12'	Jade estuary, Germany	Estuary - subtidal	Apel, 1992
53°33-05'N	8°45-34'E	Bremen, Germany Port and gravel pits (e.g. Hegemannsee) connected to the Weser, last course of rivers Lesum and Ochtum, Wümme (Borgfeld). Canals and waterways connected to the Ochtum (e.g. Krimpelfleet, Hucht-inger fleet, Arsten-Habenhauser-fleet,) and Kleine Wumme (e.g. Machinenefleet, HemmstraSSen-graben)	Brackish inland waters and ponds	Haesloop, 1990; Haesloop and Scheffel, 1991
53°02'N - 52°51'N	5°25-53'E	Brackish lakes in Friesland, The Netherlands (Tjeukemeer, Slotermeer)	Brackish lake	Otto, 1934; Vijverberg, unpublished; Bremer and Vijverberg, 1982
53°05'N - 52°31'N	5°02-39'E	IJselmeer (Zuiderzee) en Barnegat, The Netherlands	Brackish lake/pond	De Vos, 1941; Vorstman, 1951; Macan, 1974;
52°45-35'N	1°33-42'E	Hickling and Cockshoot Broad, England, UK	Brackish lake	Moss, 1991; Bales et al., 1993; Irvine et al., 1993; 1995; Moss et al., 1996
52°29-13'N	7°35'-11°44E	Weser-Elbe canal, Germany	Brackish canal	Munkemüller and Herhaus, 1978
52°26-12'N	6°50'E-7°24'E	Ems – Dollard, The Netherlands & Germany	Estuary – subtidal	Mees et al., 1995; 1995b
52°54-45'N	1°44-24E	Norfolk Broads, England, UK	Brackish pond	Gurney, 1904
?51°57-33'N	? 0°36E – 1°18'E	Lagoon in Essex, England, UK	Brackish lake	Howes, 1939
51°57-52'N	4°05-15'E	Brielse Maas, The Netherlands	Brackish lake	Leentvaar, 1955
51°48-31'N	3°37'E-4°24'E	Brackish lakes and ditches in Zeeland, The Netherlands (Grevelingen, Veere, Den Ingel, Ouwerkerk)	Brackish lake/pond	Tesch, 1911; Vlasblom and Elgershuizen, 1977; Borghouts, 1978; Platenkamp, 1983
51°36-13'N	3°06'W-2°37'W	Severn, England, UK	Estuary – subtidal	Moore et al., 1979; Bamber and Henderson, 1994
51°33'N	0°32'E	Thames, England, UK	Estuary - marsh	Lambert, 1930; Budd, 2002
51°18-14'N	3°48-51'W	Conwy estuary, Wales, UK	Estuary – subtidal	Hough and Naylor, 1992
51°25-21'N	0°28-23'E	Medway, England, UK	Estuary - subtidal	Van den Broek, 1978*
51°26-13'N	4°00-23E	Schelde, brackish part, The Netherlands & Belgium	Estuary – subtidal	Tesch, 1910; Mees and Hamerlynck, 1992; Mees et al., 1993; 1993b; 1994; 1995; 1995b; Hostens and Mees, 1999; Fockedey and Mees, 1999; Maes et al., 2003*; Hostens, 2003*; Verslycke, 2003; Verslycke et al., 2004; 2005
51°25-20'N	4°03-12'E	Schelde, salt marshes Saeftinghe, Waarde and Sieperda, The Netherlands	Estuary – salt marsh	Cattrijsse, 1994; Cattrijsse et al., 1994; 1997*; Hampel et al., 2003; 2003b; Hampel and Cattrijsse, 2004; Hampel, 2003

approx. Latitude	approx. Longitude	Location name	Sytem	Source
51°20-13'N	3°32'E-4°23'E	Brackish ponds, left bank Schelde estuary, East Flanders/Antwerpen Belgium & The Netherlands: Hollandersgat (St-Margriete), Boerenkreek (St-Jan-in-Eremo), Kleine Geul en Rode Geul (Assenede), St-Elooiskreek (Wachtebeke), Groot Gat (Doel), Galgenweel, Kanaaldok, Het Grote Gat, Plas van Steenland, Plas aan Boutweg, Burchtse Weel and Blokkersdijk, Braakman, Schuddebeurs	Brackish pond	Dumont and Gysels, 1971; Vlasblom and Elgershuizen, 1977; Van de Vijver, 1983; Soselisa, 1994; Mees et al., 1995b; Verslycke and Janssen, 2002; Verslycke, 2003; Verslycke et al., 2002; 2003; 2003b; 2004b; 2004c; Ghekiere et al., 2004; Fockedey et al., in press
51°30-06'N	2°25'E - 3°22'E	Continental shelf, Belgium	Coastal sea	Kramp, 1910; Dewicke, 2002; Tattersall and Tattersall, 1951(Oostende?)
51°22-05'N	2°32'E - 3°22'E	Sandy beaches, Belgium	Sandy beach	Van Beneden, 1861; Lock et al., 1999, Beyst, 2001; Beyst et al., 2001
51°12-14'N	2°56-57'E	Spuikom, Oostende, Belgium	Brackish lake	Polk, P., 1963
51°05'N	2°48'E	Clay pits, Stuyvekenskerke, Belgium	Brackish pond	Espeel, 1979; Espeel, 1982
51°00'N – 50°22'N	1°33'E – 2°05'E	Boulonnais, France	?	Wagner, 1885 (Giard)
50°55-53'N	1°23'W	River Itchen, Northam Bridge, England, UK	Estuary – intertidal	Raymont et al., 1964
50°53'N	1°24'W	River Test, Tottem Marsh, Redbridge, England, UK	Estuary – salt marsh	Ralph, 1965; Ferguson, 1973; Armitage, 1979; Raymont et al., 1964; 1966; 1968; Morris, 1971; Morris et al., 1981; 1982b; Armitage et al., 1981
50°48'N	1°19'W	Calshot, Hampshire	Estuary - marsh	Barnes and Jones, 1972
50°48'N	1°05'W	Artificial pond near Porthsmouth, England, UK	Brackish pond	Morris et al., 1977; 1981; 1982
50°30-26'N	4°11-12'W	Tamar, England, UK	Estuary – subtidal	Percival 1929; Tattersall, 1938; Milner, 1986; Bradshaw et al., 1989; 1990; Moffat and Jones, 1992; 1993; Moffat, 1996
50°25-24'N	4°17-18'W	Lynher, England, UK	Estuary – subtidal	Percival 1929
50°23-21'N	4°27-31'W	East Looe, England, UK	Estuary - intertidal	Roast et al., 1998; 1999, 1999b, 2000; Moffat, 1996
50°21'N	4°07'W	Plym, Chelson Meaddows landfill, England, UK	Estuary	Molloy, 1958
50°20-18'N	4°05-01'W	Yealm	Estuary	Molloy, 1958
50°09'N	5°04'W	Swanpool, Falmouth, England, UK	Brackisch pond	Barnes et al., 1971; 1977

approx. Latitude	approx. Longitude	Location name	Sytem	Source
49°28-25'N	0°07-29'E	Seine, France	Estuary - subtidal	Wagner, 1885 (Bonnier, de Kerville); Zouhiri et al., 1998; Mouny et al., 1996; 1998, 2000; Dauvin et al., 2000
48°41-38'N	1°29-21'W	Mont Saint Michel Bay, France	Estuary - marsh	Laffaille et al., 2001*
47°52'N	3°55W	Concarneau, Brittany, France	Estuary	Wagner, 1885 (Bonnier)
47°22-16'N	2°10'W - 1°30'W	Loire, France	Estuary	Marchand, 1981*
45°15-03'N	0°38-44'W	Gironde, France	Estuary - subtidal	Sorbe, 1980; Castel, 1993; Mees et al., 1995; 1995b; David et al., 2005
44°40'N	1°01'W	Arcachon Bay – L'Eyre, France	Estuary - intertidal	Bachelet and Dauvin, 1993
43°21-17'N	3°05'W – 1°55'W	Rias Guipuzcoa (Deba, Urola, Oria, Uremea), Spain	Estuary - subtidal	San Vicente, 1993; 1996
39°06'N-38°41'N	9°09'W-8°42'W	Tagus, Portugal	Estuary - subtidal	Salgado et al., 2004*
37°09-07'N	8°37-34'W	Ria de Alvor, Portugal	Estuary - subtidal	Rodrigues and Dauvin, 1987
36°34-27'N	6°10-12'W	Bay of Cádiz, tidal channels, Spain	Estuary - subtidal	Drake et al., 1997
37°28'N-36°46'N	6°21-00'W	Guadalquivir, Spain	Estuary - subtidal	Drake et al., 2002
43°20-41'N	4°37-40'E	? Bouche-du-Rhone and canal d'Arles, Mediterranean, France	Estuary	Bacescu, 1941

*: recorded from fish and decapod stomach contents.
Historical records not included (location not specified): Friedrichs, 1904; Redeke et al., 1923; Redeke, 1932; 1933; 1935; 1948; Dorgelo, 1928; Makings, 1977.

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Chapter 2

Feeding of the hyperbenthic mysid *Neomysis integer* in the maximum turbidity zone of the Elbe, Westerschelde and Gironde estuaries

ABSTRACT

The diet of the mysid *Neomysis integer* in the maximum turbidity zone (MTZ) of three European estuaries (Elbe, Westerschelde and Gironde) was investigated in spring 1993. The quality and quantity of the diet were assessed through measurement of the stomach fullness and microscopical analysis of the stomach content combined with image analyses. *N. integer* was found to be an omnivore that mainly utilizes mesozooplankton and detritus carbon pools. The quality of the diet did not differ between the sexes nor between different developmental stages, although smaller individuals consumed fewer items. In all three estuaries, the diet was dominated by Copepoda Calanoida (5 – 10 *Eurytemora affinis* ind^{-1} for adults; 2 – 5 ind^{-1} and 2 – 3 ind^{-1} for subadults and juveniles, respectively) and was supplemented with Rotifera and Cladocera. Phytoplankton and benthic organisms, though present in the stomachs, were negligible. Macrophytal detritus and amorphous material, the latter unidentifiable under the light microscope, were very abundant food items. The amorphous detritus was found to originate from the suspended sediment flocs that are characteristic for the MTZ and mainly consist of clay minerals. The energetic value of the flocs for *N. integer* remains unclear.

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INTRODUCTION

Recently, an increased research interest has focused on the description of the food web at the freshwater-seawater interface of estuaries. Since these systems receive large quantities of allochtonous organic matter (natural inputs and discharges from various effluents), their food webs have been described as heterotrophic, *i.e.* respiratory processes exceed the *in situ* autotrophic production (*e.g.* Heip *et al.*, 1995). Moreover, in the oligohaline zone at the freshwater-seawater interface, sediment and organic matter are accumulated by flocculation and sedimentation of suspended matter (*e.g.* Eisma, 1986; Wolanski, 1995). These phenomena result in a zone of increased turbidity (maximum turbidity zone or MTZ).

The suspended particulate matter (SPM) in the brackish zone of estuaries consists mainly of detritus and inorganic sediment particles next to living matter (bacteria, fungi, phytoplankton, and zooplankton). The amount of seston present in the water column of an estuary depends on (1) local primary and secondary production in the estuary proper and in adjacent intertidal areas, (2) the import of marine and fluvial materials and (3) the amount of sediment resuspended *in situ* (McLusky, 1989; Ketchum, 1983). In the Westerschelde estuary (SW-Netherlands), for example, 1.5 million tons SPM enter the brackish zone per year, 25.10^4 tons of which are detritus and organic matter (Wollast, 1976). Soetaert and Herman (1995b) calculated that 20 % of the imported organic particles degrade or flocculate and precipitate in the MTZ. SPM concentrations in the water column at the MTZ of the Westerschelde vary between 0.06 and 0.4 g l^{-1} (Soetaert and Van Rijswijk, 1993). In the MTZ of the Elbe (NW-Germany) SPM concentrations vary between 0.1 and 0.2 g l^{-1} (Brockmann, 1992), while in the Gironde (SW-France) concentrations higher than 1 g l^{-1} at the surface and 10 g l^{-1} near the bottom are regularly recorded (Jouanneau and Latouche, 1981).

The biogeochemical cycling in heterotrophic food webs has been shown to behave according to distinctly specific patterns (Smith *et al.*, 1989) and the food webs tend to be based on detritus (Hummel *et al.*, 1988; Hall and Raffaelli, 1991; Hamerlynck *et al.*, 1993). Heterotrophic bacteria are not only responsible for the remineralisation of the nutrients (Goosen *et al.*, 1992), they simultaneously constitute the basis of the food web for higher trophic levels (Azam *et al.*, 1983; Sherr and Sherr, 1988; Billen *et al.*, 1990). Detritus and/or its associated bacteria are consumed, directly or indirectly, by the microzooplankton, the mesozooplankton and the hyperbenthos (Fenchel, 1988; Hamerlynck *et al.*, 1993). Fish and epibenthic macro-invertebrates can then feed at this 'secondary energy level'.

There is a great need for the description of the feeding ecology of key species in estuarine environments for the development of accurate C-flux models and the description of detritus based food web patterns, including the quantification of transfer coefficients. To date, few studies have taken medium sized hyperbenthic animals into account. Notable exceptions are Hall and Raffaelli (1991) and Soetaert and Herman (1995b). Still, the trophic position assigned to the hyperbenthos seems to be somewhat guessed rather than derived from field data. The structure of the hyperbenthic community of the freshwater-seawater interface has been described for quite a few Western European estuaries (Mees and Jones, 1997), but studies on the functional impact of the hyperbenthos on suspended particles in the MTZ are lacking.

In the MTZ of West-European estuaries the hyperbenthic community is dominated, both in terms of density and biomass, by the brackish water mysid *Neomysis integer* (Mees *et al.*, 1993a; 1993b; 1995). This species probably has a key function in the energy transfer to higher trophic levels in the ecosystem (Mees *et al.*, 1994).

Therefore, *N. integer* was chosen as a model to assess the impact of the feeding of the hyperbenthic community on particles in the MTZ's of the Elbe, the Westerschelde and the Gironde.

Mysidacea are generally described as omnivores, feeding on detritus, algae and zooplankton (*e.g.* Mauchline, 1980). They can feed selectively on different zooplankton species and size groups (*e.g.* Cooper and Goldman, 1980; Murtaugh, 1981a), and thus have the potential of structuring zooplankton communities (Fulton, 1982; Rudstam *et al.*, 1989). The phytoplankton (Kost and Knight, 1975; Siegfried and Kopache, 1980) and tychoplankton (Webb *et al.*, 1987; Wooldridge, 1989) are possibly also influenced through selective grazing by mysids. Mysid predation has even been reported as a possible control on meiofaunal densities (Siegfried and Kopache, 1980; Grossnickle, 1982; Johnston and Lasenby, 1982). Most mysids utilize organic detritus to a considerable extent and they can be responsible for the remineralisation of a substantial proportion of the non-refractory detritus (Kost and Knight, 1975; Jansen, 1985).

Literature about the diet of *Neomysis integer* is scarce, and only qualitative information is available. According to Lucas (1936) and Tattersall and Tattersall (1951) the species is an efficient filter feeder, grazing on organic detritus and/or planktonic diatoms. According to these authors it only feeds on zooplankton when concentrations of other suspended food items are too low. More recent studies describe *N. integer* as an omnivore consuming detritus, algae, diatoms, rotifers, copepods, amphipods, and other crustaceans, carrion, fragments of leaves and of macroalgae, spores and seeds, terrigenous materials and insect larvae (Kinne, 1955; Mauchline, 1971; 1980; Jansen, 1985). Chitinases and cellulases have been found in the gut of *N. integer* (Molloy, 1958; Zagursky and Feller, 1985), so it can be assumed that they are capable of digesting exoskeletons and macrophyte detritus. Still, the growth efficiency of *N. integer* has been shown to be highest (27 %) with animal food (dead mysids) and lowest (7.5 %) with detritus (Ferguson, 1973; Zagursky and Feller, 1985).

This paper describes a methodology for quantitative and qualitative diet analyses of mysids by means of stomach fullness measurements and microscopical stomach analyses. These techniques are applied for a comparison of the diet of *Neomysis integer* in the MTZ of 3 West-European estuaries. Sexual and ontogenic shifts in the diet are also investigated.

MATERIAL AND METHODS

Samples

The *Neomysis integer* populations of the maximum turbidity zones of the Elbe (NW-Germany), Westerschelde (SW-Netherlands and Belgium) and Gironde (SW-France) estuaries were sampled in spring 1993. All samples were collected in a one month period. In each estuary, a station in the MTZ was sampled during daytime with a hyperbenthic sledge in the main estuarine channel (for a description of the sampling gear and the sampling strategy see Hamerlynck and Mees, 1991). In the Elbe a station near Brunsbüttel (53° 52' 30" N – 09° 09' 55" E) was sampled on April 22, 1993. In the Westerschelde the sampling point was located near Bath (51° 23' 40" N – 04° 12' 00" E; May 6, 1993). Since upstream of Bath dissolved oxygen concentrations are too low for hyperbenthic life (*e.g.* Mees *et al.*, 1995), this station – a few kilometres downstream of the MTZ – was chosen because it was characterized by highest mean *N. integer* densities in previous studies (Mees *et al.*, 1993b; 1994). In the Gironde a station near Pauillac (45° 14' 15" N – 00° 44' 50" W) was sampled on May 23, 1993. The salinity at the time of sampling was 4.84, 11.60 and 1.20 psu in Elbe, Westerschelde and Gironde respectively.

Catches were immediately fixed in a 7 % neutral formaldehyde solution. In the laboratory, the samples were rinsed over a 1 mm sieve. Adults, subadults and juveniles of *Neomysis integer* were picked out for quantitative and qualitative diet analyses. Sexes and developmental stages were identified according to Mees *et al.* (1994). No gravid females were used for the diet analysis. Individuals of *N. integer* were rinsed in distilled water to remove salts, formaldehyde crystals and other impurities. Additionally, the standard length (distance from the basis of the eyestalk to the last abdominal segment) was measured for around 100 individuals per stage and sex.

In order to obtain valuable information on the diet of a species it is advisable to combine several (objective) methods of stomach analysis: at least one method measuring the amounts of the different food items (here named qualitative analysis) and one measuring the bulk of the food material present (quantitative analysis). Ideally, the latter must be linked with the size of the individual (Hyslop, 1980).

Qualitative diet analyses

Information on the diet composition of *Neomysis integer* was obtained by light microscopic analysis of the stomach contents, in combination with image-analysis techniques. To obtain semi-permanent microscopic slides of the stomach contents, each mysid was first dehydrated (Seinhorst, 1959). A gradual dehydration series from a formaldehyde solution to glycerin causes no risk of abrupt shrinkage of the stomach or intestine: no ingested particles are pushed from the stomach to the intestine nor does digested material return from the intestine into the stomach. The carapax was then removed and the gut was cut just after the round stomach. The stomach (oesophagus included) was dissected out and pulled open in a drop of glycerine on a microscopic slide. Analysis of the slides was performed by light microscope (magnification x 250) connected to an Image Analyzer (Leica Quantimet 500+). Per estuary, 15 adult males, 15 adult females, 15 subadult males, 15 subadult females and 30 juveniles were processed.

The identification and processing of the different prey categories present in the stomach of *Neomysis integer* were made according to the following procedure:

The chitinous body of adult and copepodite stages of calanoid copepods were usually found to be fragmented (Figure 1b), depending on the degree of digestion. Mandibles (Figure 1a) were found to be the most persistent parts. The number of ingested copepods and copepodites was estimated by counting the mandibles and dividing this figure by 2. Uneven counts were rounded off upwards. The width of the mandible's cutting edge was measured with the image analyzer to investigate possible size selectivity of the different ontogenic stages. The calanoids were identified to genus or species level based on other recognisable parts: the caudal rami (Figure 1b), the antennae and the fifth pleopods.

Rotifera (Figure 1c, f), Cladocera (Figure 1d), Harpacticoida (Figure 1e) and nauplii of Copepoda (Calanoida and Harpacticoida) were usually found intact. Most specimens present could be identified to genus level and counted. Nauplii were noted as such. Halacaridae (Figure 1g) and insect larvae were found occasionally, but were not used in further analyses.

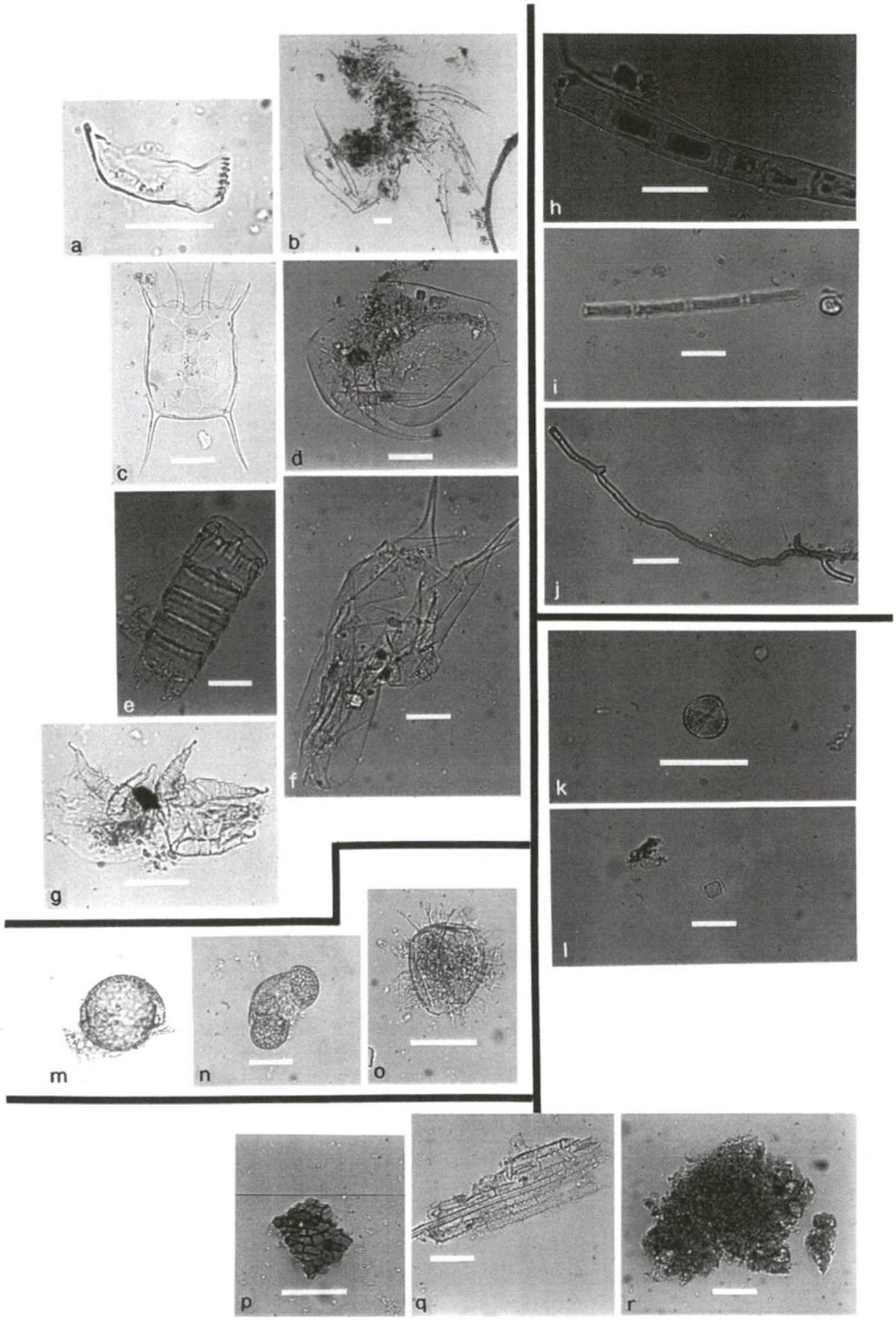

Figure 1: The dominant prey items found in the stomachs of *Neomysis integer*. Zooplankton: (a) mandible of *Eurytemora affinis*; (b) part of the calanoid copepod *Eurytemora affinis*; in the bottom left corner the caudal rami are recognisable; (c) *Keratella species*; (d) *Bosmina species;* (e) caudal part of a harpacticoid copepod; (f) *Brachionus species*; (g) Halacaridea species. Algae: (h) Filamental Chlorophyta; (i) filamental centricate diatom; (j) the intertidal benthic *Vaucheria species*; centricate diatom in frontal (k) and lateral (l) view. Pollen: (m and o) round forms and (n) pollen of gymnosperms. Detritus: (p and q) macrophytal and (r) 'unidentifiable' detritus particles. Scale bar: 50 µm

Phytoplankton cells were usually found intact and were counted as such. Based on size and shape, a distinction between different types was made: solitary phytoplankton cells (Figure 1k, l) and colonial cells or filamentous algae (Figure 1h, i, j) were counted separately. Only a minority of the specimens found could be identified to genus level. Still, a distinction could be made between species originating from intertidal areas, freshwater or brackish water in most cases (Muylaert and Sabbe, 1999).

Pollen were common in the stomachs of *Neomysis integer*. They were counted and divided into round forms (Figure 1m, o) and pollen of gymnosperms (Figure 1n). The round forms could not all be identified beyond doubt, and possibly resting stages or cysts of zooplankton are included in the counts.

Large particles with a plant cell structure were denoted as 'macrophytal detritus' (Figure 1p, q). Particles with no regular cell structure were classified as 'unidentifiable detritus' (Figure 1r). All the detritus particles present in the stomach were counted by means of an image analyzer and the surface areas and maximal lengths of the particles were measured.

The numerical abundance of each dietary item present in the stomachs was tested for differences between estuaries, ontogenic stages and sexes by means of Kruskal-Wallis tests and subsequent multiple comparisons (Conover, 1980). For macrophytal and unidentifiable detritus, the surface areas and length-frequency distributions of the particles were compared. The frequency of occurrence of the food items present in the stomachs was calculated as the proportion of stomachs containing a certain prey item (Hyslop, 1980). Also, possible size selectivity of the different ontogenic stages of *Neomysis integer* on Calanoida was tested with a Kruskal-Wallis test and subsequent multiple comparisons. No attempt was made to determine the relative importance of the various food items to the total energy intake of the mysid population.

Further characterisation of the 'unidentifiable detritus' was done by EDAX analysis, using a JEOL JSM-6400 scanning electron microscope with a Voyager II 2100/2110 microanalysis system (Noran Instruments). The stomachs of 10 adult animals per estuary were dissected out. The content was rinsed out in a drop of distilled water, placed on specimen mounts and dried in an oven (40 °C) for 30 minutes. The mounted samples were subsequently coated with carbon. The elemental composition of the detritus flocs was determined, recalculated for the eight most abundant elements (excluding C and O), and compared between estuaries.

Quantitative diet analyses

The stomach of each mysid was carefully dissected out after removing the carapax. The stomach (and its content) and the mysid were dried separately in small aluminium weighing pans for 4 days at 60 °C, after which the dry weight of both was determined with a microbalance to the nearest 1 µg. For the comparison of the three estuaries, 20 adult females and 20 adult males from each estuary were processed. For the ontogenic diet comparison 5 times 3 subadult and 5 times 5 juvenile individuals were pooled.

Additionally, the empty stomachs of 30 adults per estuary were weighed after carefully emptying the dissected stomach. A linear regression analysis was done on the dry weight of the mysids and the dry weights of the corresponding empty stomachs. The dry weight of the stomach content itself was then calculated as follows:

$$DW_{content} = DW_{stomach} - DW_{empty}$$

where: $DW_{empty} = a + b*DW_{mysid}$

with $DW_{content}$ the dry weight of the stomach content, $DW_{stomach}$ the dry weight of the stomach with its content, DW_{empty} the dry weight of the empty stomach derived by a regression from DW_{mysid} (the dry weight of the mysid without its stomach).

A fullness index (FI) was calculated with these data. This relative measure is frequently used in fisheries research for the comparison of stomach contents of fish taken from different size classes (e.g. Hyslop, 1980). The amount of food present in the stomach of *Neomysis integer* at a given time *t* is then expressed as the fullness index FI_t:

$$FI_t = \frac{DW_{content}}{DW_{mysid}} x100$$

Latitudinal, ontogenic and sexual differences in FI's were assessed with Kruskal-Wallis tests and subsequent multiple comparisons (Conover, 1980).

RESULTS

Neomysis integer was found with densities of 36.0, 9.4 and 10.8 ind m^{-2} and biomasses of 184.6, 39.0 and 21.5 mg ash free dry weight m^{-2} in the MTZ stations of the Elbe, Westerschelde and Gironde, respectively. The absolute and relative density and biomass of all sexes and stages of *N. integer* present in the MTZ in the three estuaries (spring 1993) are shown in table 1. The standard length of the mysids differed significantly between estuaries, ontogenic stages and sexes (ANOVA and contrast analysis).

Qualitative diet analysis: Comparison between estuaries

In the three estuaries, the diet of all ontogenic stages of *Neomysis integer* was composed of zooplankton, phytoplankton and detritus (Figure 2; Table 2). In the Westerschelde and the Gironde the diet of *N. integer* was numerically dominated by adult and copepodite stages of the calanoid copepod *Eurytemora affinis* (Figure 1a, b) with respectively 10.27 and 8.00 calanoids consumed per adult, 4.20 and 5.72 per subadult and 2.20 and 2.90 per juvenile mysid. In the Elbe rotifers were the most abundant animal prey items for the three ontogenic stages. Here, only 5.07 calanoid copepods were consumed per adult, 2.43 per subadult, and 1.77 per juvenile mysid.

Table 1: Absolute and relative density (in N/m^2 and %) and biomass (in mg ash free dry weight/m^2 and %) of *Neomysis integer* in the MTZ of Elbe, Westerschelde, and Gironde in spring 1993.

	Elbe		Westerschelde		Gironde	
	N/m^2 (%)	Mg/m^2 (%)	N/m^2 (%)	Mg/m^2 (%)	N/m^2 (%)	Mg/m^2 (%)
Adult female	9.4 (26.1)	70.5 (38.2)	2.0 (21.3)	16.9 (43.3)	0.7 (6.5)	2.3 (10.7)
Adult female (Gravid)	5.0 (13.9)	41.7 (22.6)	0.9 (9.6)	7.8 (20.0)	0.3 (2.8)	0.9 (4.2)
Adult male	3.6 (10.0)	13.6 (7.4)	1.6 (17.0)	7.7 (19.7)	0.1 (0.9)	0.2 (0.9)
Subadult female	12.7 (35.3)	44.6 (24.2)	1.8 (19.1)	3.0 (7.7)	6.3 (58.3)	13.1 (60.9)
Subadult male	5.1 (14.2)	13.8 (7.5)	1.9 (20.2)	2.9 (7.4)	1.8 (16.7)	3.2 (14.9)
Juvenile	0.2 (0.6)	0.4 (0.2)	1.2 (12.8)	0.7 (1.8)	1.6 (14.8)	1.8 (8.4)
Total	36.0	184.6	9.4	39.0	10.8	21.5

Adult and copepodite stages of harpacticoids (Figure 1e) were rare in the stomachs. They were only encountered in 7 % of the adult individuals of the Westerschelde and 3 – 7 % of the juveniles and adults in the Gironde. In the Elbe harpacticoids occurred in 20 – 33 % of the stomachs, though always in low numbers. Nauplii (of calanoids and harpacticoids) were present in low numbers in the stomachs from the Elbe (0.03 – 0.23 ind^{-1}) and the Gironde (0.10 – 0.15 ind^{-1}); they were not consumed in the Westerschelde. Their frequency of occurrence was low (3 – 20 % in adults and subadults of the Elbe and 10 – 15 % in the Gironde), except for the juveniles in the Elbe (70 %). Cladocera of the genus *Bosmina* (Figure 1d) were encountered in 33 – 76 % of the stomachs of Elbe and Gironde, but rarely in those of the Westerschelde (max. 7 %). Rotifers of the genera *Keratella* (Figure 1c) and *Brachionus* (Figure 1f) were the most abundant prey items for *N. integer* in the MTZ of the Elbe (16.10 – 22.17 ind^{-1}). In the Gironde and the Westerschelde they were consumed in lesser numbers (1.38 – 1.93 ind^{-1} and 0.17 – 0.40 ind^{-1} respectively). The frequency of occurrence of the rotifers was 100 % in the Elbe, 67 – 83 % in the Gironde and 17 – 27 % in the Westerschelde.

Other zooplankton prey were very rarely encountered and were excluded from further analyses. Halacaridae (Figure 1g) were infrequently encountered in the Westerschelde and the Gironde (a total of 6 observations) and one larval Homoptera (Insecta) was found in the Gironde.

For each prey item, the overall latitudinal effects were significant. The average numbers per stomach were tested for significant differences between the three estuaries for the three ontogenic stages (table 3a) by multiple comparisons. Except for copepod nauplii and harpacticoids in all ontogenic stages and calanoids in juveniles, most of the differences were highly significant.

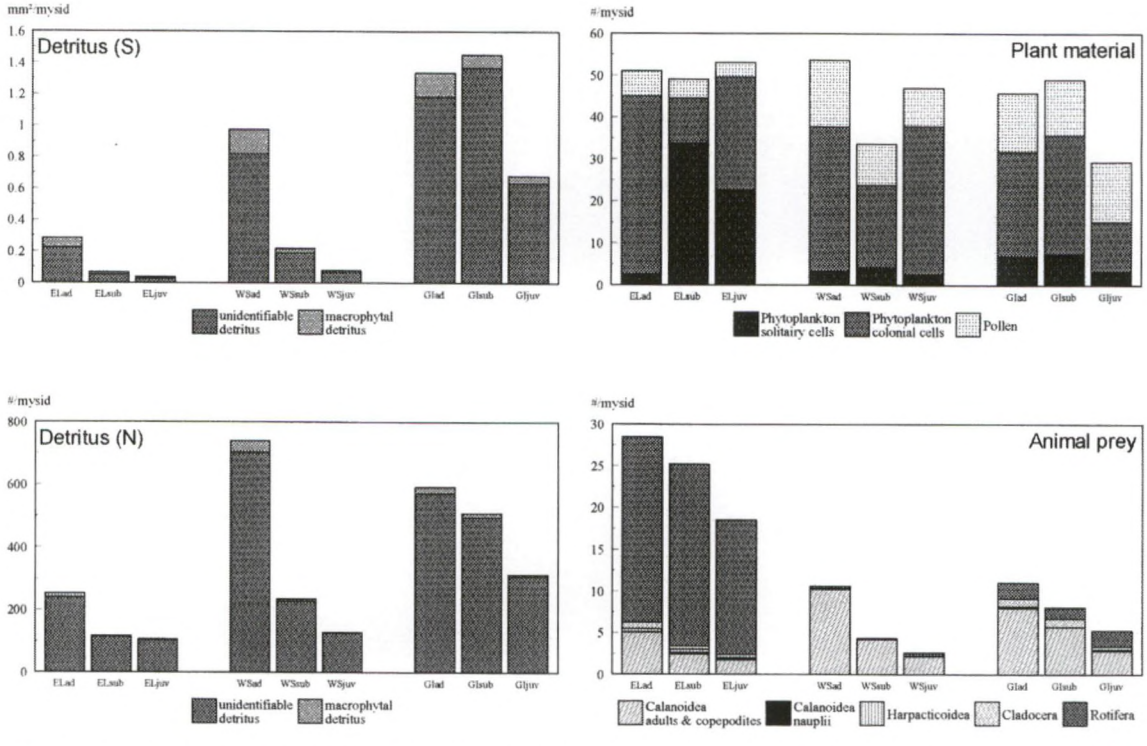

Figure 2: Absolute composition of the diet of adult, subadult and juvenile *Neomysis integer* in the Elbe, Westerschelde and Gironde. The surface of the detritus particles in mm² per mysid (upper left corner); plant material, animal prey and detrital particles in numbers per mysid. See table 2 for the explanation of the abbreviations.

Table 2: Mean and standard error of different dietary items per adult, subadult and juvenile *Neomysis integer* in the Elbe, Westerschelde and Gironde. Second line: frequency of occurence (%) and, between brackets, the number of individuals analyzed.

	Elbe adult (ELad)	**Elbe** subadult (ELsub)	**Elbe** juvenile (ELjuv)	**Westerschelde** adult (WSad)	**Westerschelde** subadult (WSsub)	**Westerschelde** juvenile (WSjuv)	**Gironde** adult (Glad)	**Gironde** subadult (GIsub)	**Gironde** juvenile (GIjuv)
Standard length (mm)	12.53 ± 0.27 (90)	9.70 ± 0.13 (89)	7.27 ± 0.20 (22)	13.44 ± 0.26 (90)	7.16 ± 0.12 (89)	4.69 ± 0.10 (45)	9.42 ± 0.09 (90)	7.95 ± 0.11 (75)	6.26 ± 0.10 (45)
Unidentifiable detritus Surface (mm^2)	0.224 ± 0.038 100% (15)	0.052 ± 0.009 100% (15)	0.030 ± 0.007 100% (15)	0.821 ± 0.125 100% (15)	0.192 ± 0.026 100% (15)	0.079 ± 0.013 100% (15)	1.184 ± 0.126 100% (15)	1.369 ± 0.149 100% (14)	0.634 ± 0.052 100% (15)
Unidentifiable detritus Number	241.53 ± 54.83 100% (15)	113.47 ± 13.37 100% (15)	103.87 ± 11.62 100% (15)	704.13 ± 140.98 100% (15)	229.93 ± 23.13 100% (15)	155.20 ± 13.06 100% (15)	572.20 ± 61.50 100% (15)	496.36 ± 34.57 100% (14)	308.13 ± 32.88 100% (15)
Macrophyte detritus Surface (mm^2)	0.061 ± 0.020 93% (15)	0.016 ± 0.006 77% (30)	0.007 ± 0.003 60% (30)	0.152 ± 0.048 100% (15)	0.027 ± 0.006 93% (30)	0.013 ± 0.003 73% (30)	0.152 ± 0.035 100% (15)	0.084 ± 0.014 100% (14)	0.047 ± 0.011 93% (15)
Macrophyte detritus Number	12.57 ± 1.89 97% (30)	3.43 ± 1.02 77% (30)	1.60 ± 0.41 60% (30)	36.50 ± 7.04 100% (30)	6.40 ± 1.12 93% (30)	3.23 ± 0.77 73% (30)	20.30 ± 2.05 100% (27)	12.62 ± 1.99 100% (29)	6.57 ± 0.91 93% (30)
Calanoidea Adult & copepodite	5.07 ± 0.62 100% (30)	2.43 ± 0.32 97% (30)	1.77 ± 0.18 93% (30)	10.27 ± 0.68 100% (30)	4.20 ± 0.32 100% (30)	2.20 ± 0.21 100% (30)	8.00 ± 0.73 100% (27)	5.72 ± 0.31 100% (29)	2.90 ± 0.32 97% (30)
Harpacticoidea Adult & copepodite	0.40 ± 0.12 33% (30)	0.40 ± 0.11 33% (30)	0.20 ± 0.07 20% (30)	0.07 ± 0.05 7% (30)	0.00 ± 0.00 0% (30)	0.00 ± 0.00 0% (30)	0.07 ± 0.05 7% (27)	0.00 ± 0.00 0% (29)	0.03 ± 0.03 3% (30)
Calanoidea & Harpacticoidea Mandible width (µm)	33.46 ± 0.93 (206)	35.82 ± 1.71 (85)	36.94 ± 2.04 (53)	43.47 ± 0.66 (407)	39.38 ± 0.84 (204)	38.04 ± 1.34 (84)	36.79 ± 0.58 (320)	35.08 ± 0.68 (271)	32.45 ± 0.90 (137)
Calanoidea & Harpacticoidea Nauplii	0.03 ± 0.03 3% (30)	0.23 ± 0.09 20% (30)	0.07 ± 0.05 70% (30)	0.00 ± 0.00 0% (30)	0.00 ± 0.00 0% (30)	0.00 ± 0.00 0% (30)	0.15 ± 0.07 15% (27)	0.00 ± 0.00 0% (29)	0.10 ± 0.06 10% (30)
Cladocera	0.80 ± 0.11 70% (30)	0.37 ± 0.10 33% (30)	0.40 ± 0.09 40% (30)	0.07 ± 0.05 7% (30)	0.00 ± 0.00 0% (30)	0.03 ± 0.03 3% (30)	0.96 ± 0.16 70% (27)	1.03 ± 0.14 76% (29)	0.40 ± 0.12 33% (30)
Rotifera	22.17 ± 2.50 100% (30)	21.83 ± 2.05 100% (30)	16.10 ± 1.43 100% (30)	0.27 ± 0.10 23% (30)	0.17 ± 0.07 17% (30)	0.40 ± 0.14 27% (30)	1.93 ± 0.38 67% (27)	1.38 ± 0.22 79% (29)	1.87 ± 0.29 83% (30)
Phytoplankton solitary	2.53 ± 0.42 77% (30)	33.73 ± 6.72 100% (30)	22.63 ± 6.03 97% (30)	3.40 ± 0.50 87% (30)	4.30 ± 0.78 93% (30)	2.67 ± 0.39 90% (30)	6.93 ± 2.35 85% (27)	7.45 ± 3.86 66% (29)	3.40 ± 0.47 93% (30)
Phytoplankton colonial	42.50 ± 17.63 77% (30)	10.80 ± 4.85 47% (30)	26.90 ± 8.69 63% (30)	34.40 ± 12.75 83% (30)	19.57 ± 9.27 57% (30)	35.20 ± 7.23 97% (30)	24.89 ± 6.52 96% (27)	28.24 ± 5.40 97% (29)	11.83 ± 3.33 73% (30)
Pollen	5.93 ± 0.53 97% (30)	4.50 ± 0.51 97% (30)	3.47 ± 0.44 90% (30)	15.80 ± 1.41 100% (30)	9.80 ± 1.05 100% (30)	9.07 ± 0.92 100% (30)	14.00 ± 1.02 100% (27)	13.31 ± 0.79 100% (29)	14.20 ± 1.18 100% (30)

Table 3a: Results of the Kruskal-Wallis tests (KW) and subsequent multiple comparisons for the different dietary items for adult, subadult and juvenile *Neomysis integer* of Elbe, Westerschelde and Gironde (latitudinal and ontogenic effects). See table 2 for the explanation of the abbreviations. (with: *: p < .05; **: p < .01; ***: p<.001; NS: p > .05)

		LATITUDE									ONTOGENY								
	KW	ELad *vs* WSad	ELad *vs* GIad	WSad *vs* GIad	ELsub *vs* WSsub	ELsub *vs* GIsub	WSsub *vs* GIsub	ELjuv *vs* WSjuv	ELjuv *vs* GIjuv	WSjuv *vs* GIjuv	ELad *vs* ELsub	ELad *vs* ELjuv	ELsub *vs* ELjuv	WSad *vs* WSsub	WSad *vs* WSjuv	WSsub *vs* WSjuv	GIad *vs* GIsub	GIad *vs* GIjuv	GIsub *vs* GIjuv
Unidentifiable detritus Surface (mm²)	***	***	***	*	***	***	***	***	***	***	***	***	*	***	***	***	NS	***	***
Unidentifiable detritus Number	***	***	***	NS	***	***	***	*	***	***	**	***	NS	***	***	*	NS	***	***
Macrophyte detritus Surface (mm²)	***	*	**	NS	*	***	***	NS	***	***	***	***	NS	***	***	*	NS	**	NS
Macrophyte detritus Number	***	***	**	NS	**	***	***	NS	***	***	***	***	NS	***	***	**	**	***	**
Calanoidea Adult & copepodite	***	***	***	*	***	***	**	NS	**	NS	***	***	NS	***	***	***	NS	***	***
Harpacticoidea Adult & copepodite	***	***	**	NS	***	***	NS	*	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Calanoidea & Harpacticoidea Nauplii	**	NS	NS	*	**	**	NS	NS	NS	NS	**	NS	*	NS	NS	NS	*	NS	NS
Cladocera	***	***	NS	***	**	***	***	**	NS	**	***	**	NS	NS	NS	NS	NS	***	***
Rotifera	***	***	***	***	***	***	***	***	***	***	NS	NS	NS	NS	NS	NS	NS	NS	NS
Phytoplankton solitary	***	NS	*	NS	***	***	NS	***	***	NS	***	***	*	NS	NS	NS	NS	NS	NS
Phytoplankton colonial	***	NS	NS	NS	NS	***	***	***	NS	***	**	NS	*	*	*	***	NS	*	**
Pollen	***	***	***	NS	***	***	***	***	***	***	NS	**	NS	***	***	NS	NS	NS	NS

Table 3b: Results of the Kruskal-Wallis tests (KW) and subsequent multiple comparisons for the different dietary items for adult and subadult male (mal) and female (fem) *Neomysis integer* of Elbe, Westerschelde and Gironde (sexual effect). See table 2 for the explanation of the abbreviations. (with: *: $p < .05$; **: $p < .01$; ***: $p<.001$; NS: $p > .05$)

		SEX					
	KW	ELad mal *vs* ELad fem	ELsub mal *vs* ELsub fem	WSad mal *vs* WSad fem	WSsub mal *vs* WSsub fem	GIad mal *vs* GIad fem	GIsub mal *vs* GIsub fem
Unidentifiable detritus Surface (mm^2)	no data	no data	no data	no data	no data	no data	no data
Unidentifiable detritus Number	no data	no data	no data	no data	no data	no data	no data
Macrophyte detritus Surface (mm^2)	no data	no data	no data	no data	no data	no data	no data
Macrophyte detritus Number	***	***	*	***	NS	*	NS
Calanoidea Adult & copepodite	***	***	NS	NS	NS	NS	NS
Harpacticoidea Adult & copepodite	***	NS	NS	NS	NS	NS	NS
Calanoidea & Harpacticoidea Nauplii	**	NS	NS	NS	NS	**	NS
Cladocera	***	NS	NS	NS	NS	NS	NS
Rotifera	***	NS	NS	*	NS	**	NS
Phytoplankton solitary	***	NS	NS	NS	NS	NS	NS
Phytoplankton colonial	***	NS	NS	NS	NS	NS	NS
Pollen	***	NS	NS	NS	NS	NS	NS

Also some phytoplankton was consumed by *Neomysis integer* in the three estuaries. Solitary (Figure 1k, l) and colonial phytoplankton (Figure 1h, i, j) species could be recognized in the stomachs with a mean frequency of occurrence of 82 %. Colonial and filamental algal strands were the most abundant (table 2). For adults, *e.g.* 42.5, 34.4 and 24.9 cells (*i.e.* 4.6, 5.1 and 4.8 strands) were found per mysid in Elbe, Westerschelde and Gironde respectively, while solitary cells only amounted to 2.5, 3.4 and 6.9 counts per adult. Still, most of the differences were not significant (table 3a). Similar trends were found for subadults and juveniles in the Westerschelde and the Gironde. In the Elbe, these ontogenic groups had consumed significantly higher amounts of solitary phytoplankton cells (table 3a) as compared to the other estuaries.

Pollen was found in 90 to 100 % of the stomachs. Average numbers per stomach were 4.6, 11.6 and 13.8 in Elbe, Westerschelde and Gironde, respectively. Two general types were distinguished. Pollen with an air sac on either side (Figure 1n) were recognized as originating from gymnosperms. These were especially abundant in the Gironde (62.53 %) and the Westerschelde (37.3 %), while in the Elbe only 6.3 % of the pollen originated from gymnosperms. The round forms (Figure 1m, o) could not be identified and possibly resting stages of mesozooplankton or cysts of microzooplanktonic species are included in the counts.

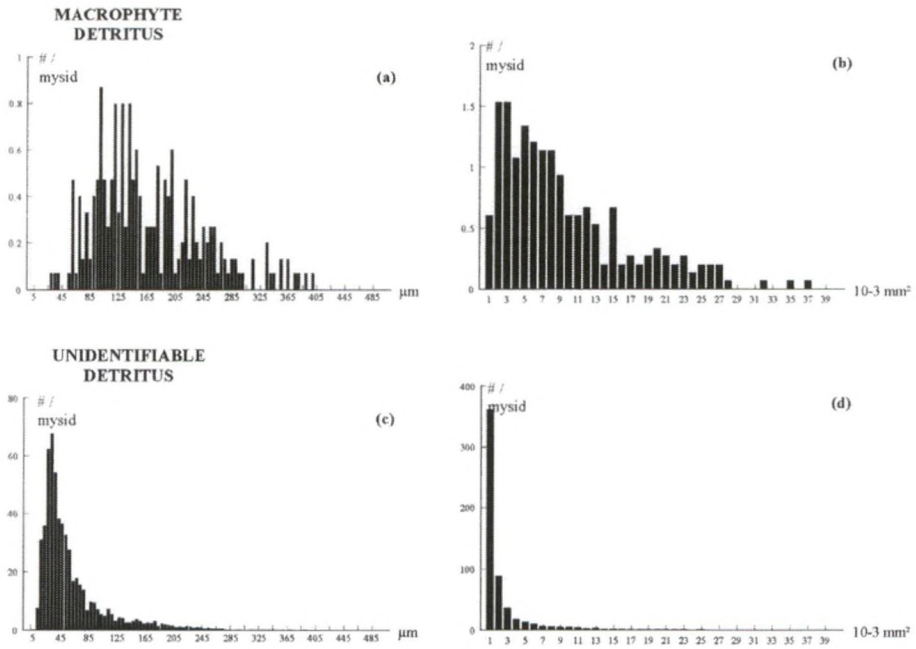

Figure 3: Length- (left) and surface area (right) frequency distribution of macrophyte detritus (top) and 'unidentifiable' detritus (bottom) present in the stomach of adult *Neomysis integer* in the MTZ of the Gironde.

The frequency with which macrophytal detritus was consumed in the three estuaries was always higher than 60 %, although macrophyte detritus accounted for less than 5 % of the total number of detritus particles consumed. The size distributions of the macrophytal detritus in the stomachs (*e.g.* for adults in the Gironde Figure 3a, b) were comparable over the estuaries: the majority (> 90 %) was smaller than 0.020 mm² for adults, 0.013 mm² for subadults and 0.015 mm² for juveniles. Maximal particle sizes of 0.039 mm², 0.061 mm² and 0.064 mm² were recorded in Elbe, Westerschelde and Gironde, respectively. Highest numbers of macrophyte detritus particles were found in adults in the Westerschelde (36.50 ind^{-1}), while subadults and juveniles in the Gironde contained significantly higher numbers (12.62 and 6.57 ind^{-1} respectively) as compared to the same ontogenic stages in the other estuaries.

The size frequency distributions of the unidentifiable fraction of the detritus (Figure 3c, d for adults in the Gironde) showed the same patterns in all estuaries. The bulk (90 %) of the particles found in adult stomachs were smaller than 85 µm (Elbe), 90 µm (Westerschelde) and 125 µm (Gironde). In subadults and juveniles, the bulk of the particles had smaller sizes. Particles with a maximal length up to 300 µm (Elbe), 500 µm (Westerschelde) and 600 µm (Gironde) were regularly found in the stomachs. In the three estuaries, the modes of the size frequency distributions were located around 30 – 35 µm. Adult *Neomysis integer* of the Westerschelde consumed the highest number of unidentifiable detrital particles (704 particles ind^{-1}), while for subadults and juveniles highest numbers were found in the Gironde (496 and 308 ind^{-1} respectively). Mean total numbers of detritus particles consumed by the three ontogenic stages were 153, 363 and 458 ind^{-1} in Elbe, Westerschelde and Gironde.

A more detailed analysis of the 'unidentifiable fraction' of the detritus was made using a petrographic optical microscope. The particles showed a high mineral content. The elemental composition of the detritus was assessed by EDAX analysis. In back-scattered electron (BSE) images of the samples, the flocs were easily identified as aggregates with much lighter grey values, indicating a major difference in composition between the flocs and other components (photograph on Figure 4).

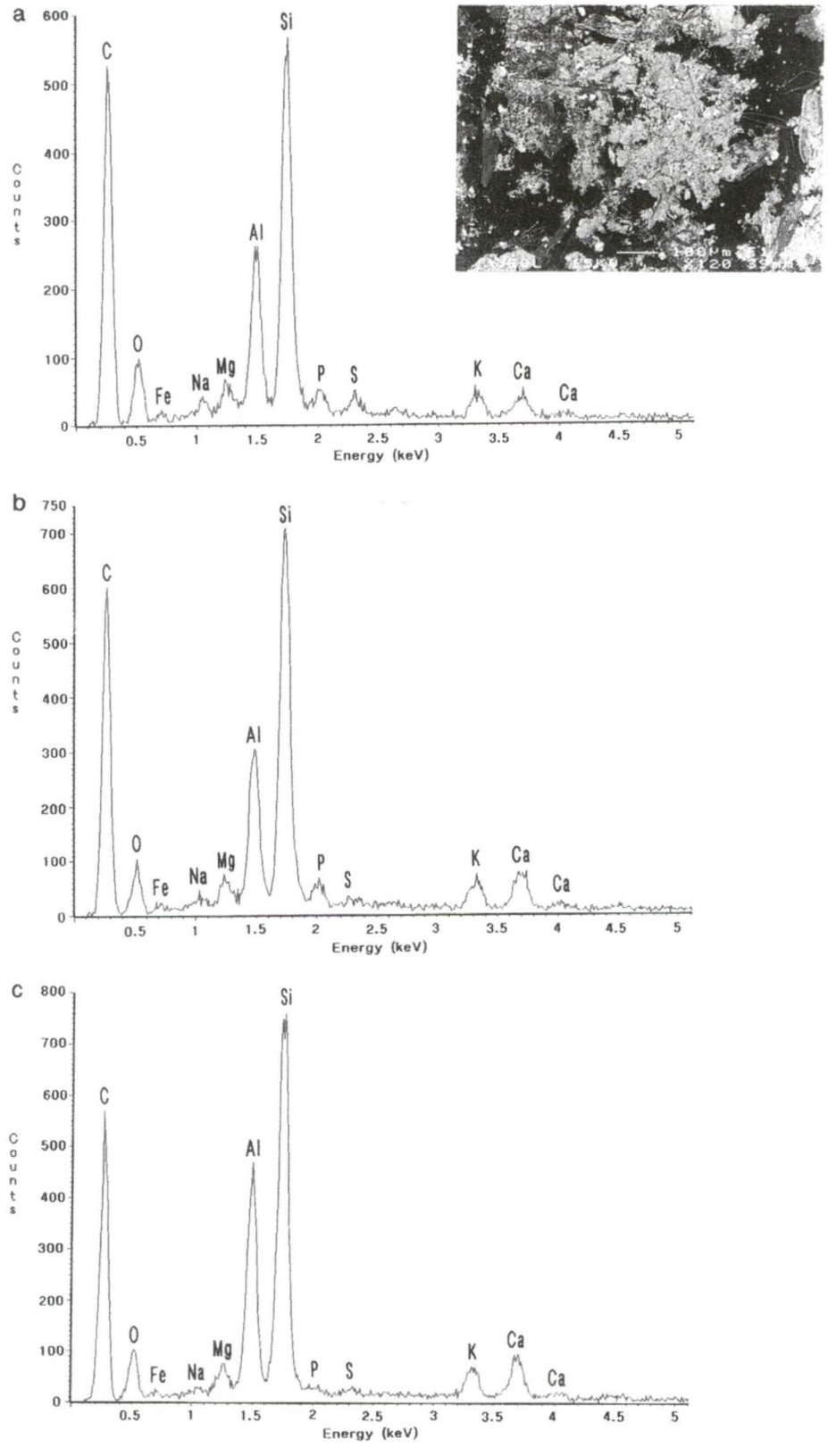

Figure 4: Output of an EDAX analysis on 'unidentifiable' detritus particles from the stomachs of adult *Neomysis integer* in Elbe (a), Westerschelde (b) and Gironde (c). The X-axis was cut off short of the primary Fe-peaks (located around 6.4 and 7.1 keV). Upper right corner: back-scattered electron (BSE) image of an analyzed floc.

Table 4: Relative elemental composition (weight percentage ± standard error) of the flocs found in the stomachs of adult *Neomysis integer* from Elbe, Westerschelde and Gironde. Note that the relative abundance was calculated with the eight most abundant elements, excluding C and O.

Element	ELad	WSad	GIad
Mg	2.65 ± 0.51	1.30 ± 0.48	2.09 ± 0.35
Al	15.56 ± 0.82	13.52 ± 0.85	20.56 ± 0.70
Si	41.87 ± 1.16	43.61 ± 1.28	44.71 ± 0.99
P	4.87 ± 1.21	5.57 ± 1.38	1.45 ± 0.89
S	5.05 ± 0.82	1.78 ± 0.90	1.71 ± 0.59
K	5.59 ± 0.73	5.85 ± 0.93	6.76 ± 0.67
Ca	6.10 ± 0.86	13.24 ± 1.20	11.13 ± 0.85
Fe	18.20 ± 1.91	15.13 ± 2.36	11.58 ± 1.60

For EDAX analyses, only flocs with lengths of 150 – 300 µm were used. The composition of the flocs was very similar in the three estuaries (Figure 4 and Table 4). The elemental composition, dominated by silicon and aluminium (around 60 % by weight) and with lower amounts of magnesium, potassium and iron, demonstrate that the flocs mainly consist of clay minerals. Part of the iron occurs in the form of pyrite (FeS_2), whose presence as individual crystals or grains was often directly observed. Because a carbon coating was used, the carbon content of the flocs could not be quantified, but the EDAX spectra and BSE images show that their carbon content is not high. No diatoms or other unicellular organisms could be found attached to the flocs.

Qualitative diet analysis: Comparison of developmental stages

The diet of subadult and juvenile *Neomysis integer* consisted of the same prey categories as that of adults, but generally a lower number of particles was consumed by the smaller mysids (Figure 2; Tables 2, 3a). Stomachs of juveniles in the Westerschelde and Gironde contained significantly less detritus, calanoid copepods and colonial phytoplankton cells, as compared to these of adults and subadults. In the Elbe the diet of juveniles resembled that of the subadults, whereas in the Gironde the diet of adults rather resembled that of the subadults. Ontogenic differences in the number of solitary phytoplankton consumed were only found in the Elbe. The number of pollen consumed only differed in the Elbe and the Westerschelde, and Cladocera only in the Elbe and the Gironde.

Adults and copepodites of *Eurytemora affinis* were the most important zooplankters consumed by all the mysid stages, except in the Elbe where rotifers were the most abundant taxon in the diet. In the Westerschelde and the Gironde, the smaller mysids selected significantly smaller copepods, whereas in the Elbe no significant difference in copepod size selection was found between the ontogenic stages (Figure 5; Tables 2, 5). This is possibly correlated with the fact that in the Elbe the number of mandibles measured was significantly lower than in the other estuaries.

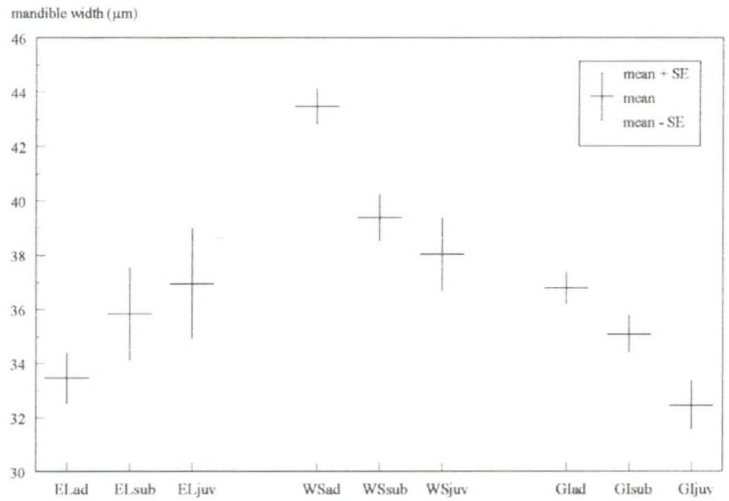

Figure 5: Mean mandible width (and standard error) of the calanoid copepods *Eurytemora affinis* consumed by adult, subadult and juvenile *Neomysis integer* in Elbe, Westerschelde and Gironde. See table 2 for the explanation of the abbreviations.

Table 5: Results of the Kruskal-Wallis tests (KW) and subsequent multiple comparisons for the mandible width of the copepods consumed by adult, subadult and juvenile *Neomysis integer* of Elbe, Westerschelde and Gironde (ontogenic and sexual effects). See table 2 for the explanation of the abbreviations. (with: *: $p < .05$; **: $p < .01$; ***: $p<.001$; NS: $p > .05$)

ONTOGENY									
KW	ELad *vs* ELsub	ELad *vs* ELjuv	ELsub *vs* ELjuv	WSad *vs* WSsub	WSad *vs* WSjuv	WSsub *vs* WSjuv	Glad *vs* GIsub	Glad *vs* GIjuv	GIsub *vs* GIjuv
***	NS	NS	NS	***	***	NS	NS	***	*

SEX						
KW	ELad mal *vs* ELad fem	ELsub mal *vs* ELsub fem	WSad mal *vs* WSad fem	WSsub mal *vs* WSsub fem	Glad mal *vs* Glad fem	GIsub mal *vs* GIsub fem
***	NS	NS	NS	NS	NS	NS

The total number of detritus particles consumed was comparable for adults and subadults in the Gironde (593 and 509 particles ind^{-1}), while the stomachs of juveniles contained significantly less particles (315 ind^{-1}). In the Westerschelde the three ontogenic stages consumed different amounts of detritus: 741, 236 and 158 particles ind^{-1} for adults, subadults and juveniles respectively. In the Elbe, adults consumed significantly higher numbers of detritus particles (254.10 ind^{-1}) than subadults and juveniles (116.90 and 105.47 ind^{-1}). Macrophytal particles only accounted for a minor part of the total detrital fraction in the diet (3 – 5 % for adults, 2 – 3 % for subadults and juveniles). The mean size of the macrophyte detritus particles was independent of the size of the mysid: for all ontogenic stages the mean surface area per macrophyte particle was around 0.004 mm^2 in Elbe and Westerschelde and 0.007 mm^2 in the Gironde. The size range of the unidentifiable detritus was comparable for the different mysid stages in the Gironde: all stages mainly contained particles with a surface area smaller than 0.005 mm^2 (modal length 35 µm). In the Elbe and Westerschelde the size of the unidentifiable detritus particles found in the stomach decreased with the size of the mysid: modal length of 30 µm in adults, 15 µm in subadults and 10 µm in juveniles.

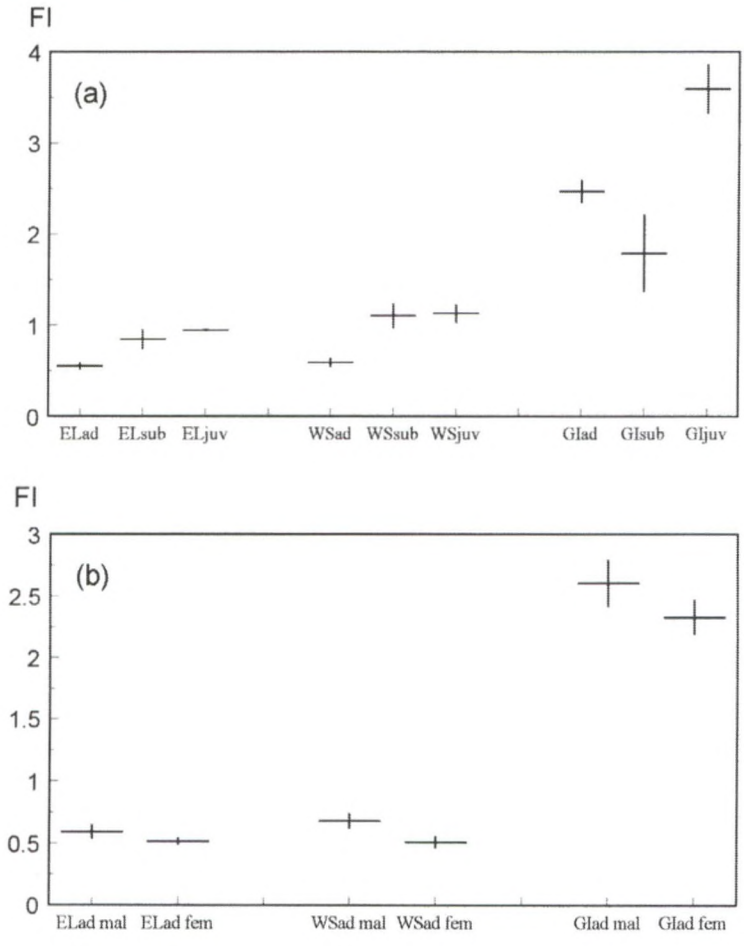

Figure 6: The mean fullness index FI (and standard error) for (a) adult, subadult and juvenile and (b) adult male and female *Neomysis integer* in Elbe, Westerschelde and Gironde. See table 2 for the explanation of the abbreviations.

Table 6: Results of Kruskal-Wallis tests and subsequent multiple comparisons on the fullness index (FI) for the latitudinal-ontogenic and latitudinal-sexual effects. See table 2 for the explanations of the abbreviations. (with: *: $p < .05$; **: $p < .01$; ***: $p < .001$; NS: $p > .05$)

LATITUDE: ***		ONTOGENY: **		SEX (adults): NS	
EL vs WS	NS	ad vs sub	NS		
EL vs GI	***	ad vs juv	**		
WS vs GI	***	sub vs juv	NS		
LATITUDE & ONTOGENY: ***				LATITUDE & SEX (adults): ***	
ELad vs WSad	NS	ELad vs ELsub	**	EL mal vs EL fem	NS
ELad vs GIad	***	ELad vs ELjuv	**	WS mal vs WS fem	**
WSad vs GIad	***	ELsub vs ELjuv	NS	GI mal vs GI fem	NS
ELsub vs WSsub	NS	WSad vs WSsub	***	EL mal vs WS mal	NS
ELsub vs GIsub	NS	WSad vs WSjuv	***	EL mal vs GI mal	***
WSsub vs GIsub	NS	WSsub vs WSjuv	NS	WS mal vs GI mal	***
ELjuv vs WSjuv	NS	GIad vs GIsub	*	EL fem vs WS fem	NS
ELjuv vs GIjuv	***	GIad vs GIjuv	NS	EL fem vs GI fem	***
WSjuv vs GIjuv	**	GIsub vs GIjuv	**	WS fem vs GI fem	***

Qualitative diet analysis: Comparison of sexes

For most dietary items, no difference was found between the sexes (table 3b). An exception is that in all estuaries the numbers of macrophytal detritus particles were higher in the stomachs of adult females compared to adult males (a factor of 2.3, 3.1 and 1.5 in the Elbe, Westerschelde and Gironde, respectively). Another exception is that adult males in the Elbe consumed only 3.0 calanoid copepods per individual versus 7.1 for the adult females.

Quantitative diet analysis

The dry weight of the empty stomachs could be derived from the following regression equation, after which the dry weight of the stomach content could be calculated by substraction.

$$\ln DW_{empty} = 5.04722 + 0.513386 \ln DW_{mysid} \qquad (N = 89; r = 0.672; p < 0.000)$$

The fullness indices (FI) were compared between the estuaries, ontogenic stages and sexes with Kruskal-Wallis tests and subsequent multiple comparisons (see Figure 6 and Table 6). General latitudinal effects in the stomach fullness could not be demonstrated between Elbe and Westerschelde, while in the Gironde significantly higher fullness indices were recorded for adults and juveniles. In the three estuaries, adult *Neomysis integer* had a significantly lower FI than juveniles. In Elbe and Westerschelde, the FI's of subadult *N. integer* were comparable to those of juveniles. In the Gironde, the subadults had a lower FI than adults and juveniles. No sexual effect could be detected in the fullness index.

DISCUSSION

Methodology

The size range of adult, subadult and juvenile *Neomysis integer* used for stomach analysis comparison were different in the three estuaries (table 2). This is not surprising, since the three populations were sampled at different stages of the annual population dynamic cycle. The different length-frequency distributions and population compositions (table 1) found in the three estuaries can be explained by a seasonal temperature effect. The average water temperature in Elbe, Westerschelde and Gironde at the time of sampling was 9 °C, 15 °C and 17 °C, respectively. Studies on the population dynamics of *N. integer* in the Westerschelde (Mees *et al.*, 1994) and the Gironde (Mees and Sorbe, unpublished) have shown that the adults of the overwintering cohort are larger than those belonging to the summer generations and that the species does not reproduce when water temperature is lower than 10 °C. In the Elbe samples, few juveniles were found and many of the females were gravid, indicating that the population was still in a 'winter phase'. The relatively higher abundance of juveniles in the Westerschelde (13 % of the total density) and the Gironde (15 %) samples was due to the fact that reproduction by the overwintering cohort took place before the time of sampling. Although the sampling campaigns in the three estuaries were executed within a one month period, the latitudinal temperature effect was amplified by the North to South sequence of sampling. Also, the length at maturity has been found to increase with increasing latitude (Mees *et al.*, unpublished).

For the preparation of the microscopic slides the mysids were dehydrated from a formaldehyde solution to glycerin, in which the stomach content was subsequently embedded. This procedure yields semi-permanent slides in which artifacts are avoided. The microscopic slides contained parts of the stomach and oesophagus tissue, but their cell structure and armature made them easily distinguishable from parts of zooplanktonic prey categories.

Generally, the gut passage time of mysids has been reported to be in the order of 30 – 90 minutes (Zagursky and Feller, 1985), so the particles present in the stomach will give a good idea about the recently ingested food. The frequencies of occurrence (table 2) of the ingested prey items were usually higher than 60 %, except for calanoid nauplii, harpacticoids and cladocerans. Therefore, the within variation of a sample is low and the analysis of 30 animals per sample sufficed to describe the diet of *Neomysis integer* (Hyslop, 1980).

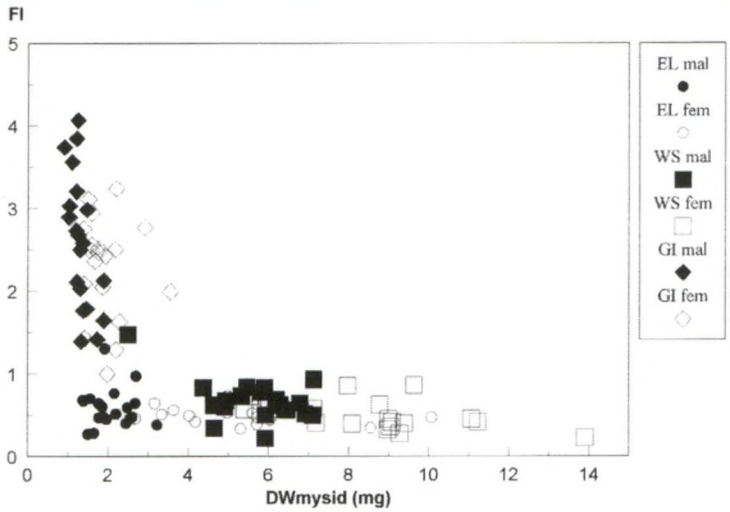

Figure 7: Relationship between the fullness index (FI) and the dry weight of the mysids (DW_{mysid}) for adult male and female *Neomysis integer* in Elbe, Westerschelde and Gironde.

General latitudinal, ontogenic and sexual effects [1]

The fullness indices of *Neomysis integer* from the Elbe and the Westerschelde were comparable (0.55 and 0.59 respectively), while in the Gironde a significantly higher FI was measured (2.46). The adults from the former two estuaries showed quite a wide size range distribution (table 2), but a rather constant FI. Adult *N. integer* from the Gironde had a significantly smaller standard length, a smaller size variation, and a large variation in their fullness index. This phenomenon is demonstrated graphically in Figure 7.

Irrespective of the ontogenic stage, consumption of detritus particles and pollen by *Neomysis integer* increased from the Elbe, over the Westerschelde, to the Gironde. The numbers of calanoids, harpacticoids and phytoplankton consumed were comparable in the Westerschelde and the Gironde. In both estuaries, calanoids were consumed in higher numbers compared to the Elbe, while harpacticoids and phytoplankton were found in lower quantities. No Cladocera, Rotifera or copepod nauplii were found in the stomachs of *N. integer* of the Westerschelde. In the two other estuaries, nauplii were always rare in the diet, while Cladocera were most important in the Gironde and Rotifera in the Elbe.

Irrespective of the estuary, large adults had a lower fullness index than smaller individuals. Adults consumed significantly higher amounts of detritus, calanoids, cladocerans, colonial phytoplankton cells and pollen. No ontogenic effects could be demonstrated for harpacticoids, copepod nauplii, solitary phytoplankton cells and rotifers.

An ontogenic shift in diet composition has been reported for *Neomysis mercedis* (Kost and Knight, 1975; Siegfried and Kopache, 1980). The main changes were found for the smallest juveniles (2 – 3 mm), a size class which was not efficiently sampled in this study (Mees *et al.*, 1994). The mandible widths of the calanoids consumed decreased with the size of the mysid in the Gironde and the Westerschelde. Although juvenile *Neomysis integer* consumed the smallest calanoid copepodite stages, spermatophores were frequently found in the stomachs. Since these are attached to the gonopores of adult females only, this suggests that juvenile mysids at least hunt adult calanoid copepods. In the Elbe, *N. integer* did not show a size selectivity for *Eurytemora affinis*, but here the number of mandibles measured was significantly lower than in the other estuaries.

[1] The tidal phase was not taken into account at the moment of sampling. Diurnal and tidal feeding rhythms were investigated (Fockedey, unpublished) by determining stomach fullness index of *Neomysis integer* for several 24h cycles in the MTZ of the Schelde, Gironde and Elbe. Although variations in the fullness index were difficult to relate to diurnal and tidal cycles, the results indicate a continuous feeding over the day (fullness index between 0.8 and 1.4 in Elbe, 1.0 and 1.5 in Schelde, and between 1.8 and 2.9 in Gironde).

No sexual differences could be found in the fullness index, nor in the amount of dietary items consumed. The only exceptions were calanoids and macrophytal detritus particles, which were sometimes consumed in significantly higher amounts by adult females.

Food items

Neomysis integer mainly fed upon mesozooplankton. Late copepodite stages and adults of *Eurytemora affinis* were the most important prey item. On average 5.07, 10.27 and 8.00 calanoids per adult mysid were consumed in the MTZ's of Elbe, Westerschelde and Gironde, respectively. Subadults and juveniles consumed less copepods (table 2). Siegfried and Kopache (1980) calculated that the relative importance of carnivory amounted to 90% of the total nutritional uptake of *N. mercedis*. 90 – 100 % of the diet of *Mysis mixta* consisted of copepods and Cladocera (Rudstam *et al.*, 1989). In both studies, however, the detritus was not included in the calculations, thus overestimating the importance of carnivory.

Although the gut passage time of mysids is in the order of one hour, it has been shown that rigid zooplankton parts (*e.g.* mandibles) can stay in the stomachs for more than 12 hours (Rudstam *et al.*, 1989). The counting of the number of mandibles present in the stomach can therefore result in an overestimation of the actual number of copepods consumed.

During the sampling campaigns, the densities of adult and copepodite stages of *Eurytemora affinis* in the MTZ of the Elbe and Westerschelde were in the order of 10000 and 40000 ind m^{-3}, respectively (Castel, personal communication). No data were available on copepod densities in the MTZ of the Gironde for spring 1993, although a density between 5000 and 15000 ind m^{-3} can be expected in March – April (Castel and Veiga, 1990). The results of the qualitative stomach analysis indicate a positive correlation between these densities and the predation by *Neomysis integer* on *E. affinis*. Similar results were found for other mysid species: the predation rate was found to increase with copepod densities (*e.g.* Siegfried and Kopache, 1980; Bowers and Vanderploeg, 1982). No other calanoid copepod species were found in the stomachs of *N. integer*, although some were recorded in the watercolumn in low densities (*Diaptomus* species in the Elbe: 25 ind m^{-3}; *Acartia* species and *Temora* species in the Westerschelde: 400 ind m^{-3}). In all three estuaries, cyclopoid copepods were abundant in the MTZ (300 –1000 ind m^{-3}). Nevertheless they were never found in the stomachs of *N. integer*, probably due to a higher escape response of the cyclopoids as compared to *E. affinis* (Tackx, personal communication).

Although Harpacticoida were very abundant in the meiobenthos communities of the subtidal sediments in the MTZ (2000 – 9000 ind m^{-2}) (Vincx, personal communication), they were rarely consumed by *Neomysis integer*. Other meiobenthic animals and microphytobenthic diatoms were rarely found in the stomachs. This indicates that the mysids feed in the hyperbenthic layer of the water column and do not scrape the bottom while foraging.

In spring 1993, high densities of calanoid nauplii were recorded in the watercolumn (23 – 79.10^3 ind m^{-3}). *Neomysis integer* seems to show a negative selection for nauplii. Also *N. mercedis* (Murtaugh, 1981b; Siegfried and Kopache, 1980) and *Mysis relicta* (Siegfried and Kopache, 1980; Bowers and Vanderploeg, 1982) do not consume nauplii in large amounts. Nauplii can be underrepresented in the diet of the mysids because adult and copepodite stages of calanoids, which are energetically more valuable, are positively selected. Or the nauplii might be more successful in avoiding the mysid feeding current than are the later life stages. Another explanation can be the high digestion rate of the nauplii (Rudstam *et al.*, 1989), which can result in an underestimation of the predation on this prey by means of stomach analysis.

Neomysis integer consumed filamentous algae rather than solitary phytoplankton cells. Siegfried and Kopache (1980) reported a higher selectivity of *N. mercedis* for larger algae and filamentous cells, while small phytoplankton were not consumed in high numbers although they were very abundant in the environment. In all estuaries, some ten algal cells per *N. integer* (Figure 2, Table 2) were consumed. Still, the quantitative importance in the diet of the mysids is negligible, although phytoplankton might qualitatively be important for the provision of oligo-elements. Moreover one has to keep in mind that in the turbid zone of estuaries, where peak densities of *N. integer* are encountered, phytoplankton concentrations are generally low (Heip *et al.*, 1995; Muylaert and Sabbe, 1999). In most cases, it was impossible to identify the phytoplankton up to genus or species level. Still, a distinction could be made between specimens from fresh, brackish or marine origin. In all three estuaries, mainly algae from the brackish and freshwater parts of the system were consumed: *Thalassiosira proschkinae*, *Nannochloris coccoides*, *Paralia sulcata*, *Pediastrum species* and colonial chlorophyta (Figure 1h) were the most common. Phytoplankton from the more marine reaches of the estuary (*e.g. Skeletonema species*) were rarely encountered in the stomachs. Filamental phytobenthic strands from the brackish zone (*Vaucheria species*: Figure 1j) could be recognised in the stomachs from the three estuaries, indicating a possible horizontal migration of *N. integer* to intertidal areas for feeding.

Pollen of gymnosperms was mainly found in the stomachs of Westerschelde and Gironde individuals. The rivers Schelde and Garonne run through extensive pine forests. It is not known if the pollen is selectively ingested, nor if they can be digested by *Neomysis integer*. Pine pollen was also found in the stomachs of the euryhaline mysid *Mysis mixta* from the Baltic Sea (Rudstam *et al.*, 1989) and was thought to be digested. The round pollen could not be identified and possibly resting stages of mesozooplankton or cysts of microzooplanktonic species are included in the counts.

3 – 5 % of the total number of detrital particles consumed by *Neomysis integer* in the three estuaries was clearly from macrophytal origin. It is possible that the mysid fragments larger macrophyte detritus particles to a size between 1000 and 20000 μm^2 before ingestion. *N. integer* possesses cellulase enzymes (Zagursky and Feller, 1985), so the species can theoretically digest the macrophytal detritus. It is not known if they are capable of deriving substantial nutrition directly from macrophyte detritus either via digestion with its own cellulases or by an associated gut microflora. *Mysis stenolepis* has an assimilation efficiency of 30 – 50 % on sterile cellulose (Foulds and Mann, 1978; Wainwright and Mann, 1982). Artificially made macrophytal detritus of *Spartina alterniflora* (Zagursky and Feller, 1985) contains 42.7 % C and 2.4 % N of the total dry weight. This detritus can serve as a nutritionally significant food item for *N. americana*, especially in periods of low availability of other nutritionally more valuable food items. Hence mysids can be an important link between (marsh-) macrophyte production and higher trophic levels.

The bulk of the 'unidentifiable detritus' originated from sediment flocs suspended in the watercolumn. However, some of the particles counted as unidentifiable detritus probably were partly digested zooplankton and phytoplankton or originated from the stomach contents of ingested prey species. According to Eisma (1987), two size-groups of flocs can be found suspended in the watercolumn in the MTZ of estuaries. Microflocs are firmly held together and have lengths between 1 and 125 µm. Together with single mineral particles these microflocs are the basic units of the more loosely bound, fragile macroflocs. The latter can reach sizes of 3 – 4 mm in turbid water. The 'unidentifiable detritus' particles in the stomachs of *Neomysis integer* were within the range of 10 to 500 µm length and the fraction smaller than 125 µm was dominant.

EDAX did not allow for quantifications of the relative concentration of carbon in the flocs (because of the carbon coating), although analysis of the particles with diffracted electronic beams suggested that carbon concentration in the flocs was low. The particulate organic carbon (POC) of the river suspended matter is on average between 1 and 5 % (Eisma, 1985). If the carbon content of the unidentifiable detritus is assumed to be of the same order, the importance in the energy balance of *Neomysis integer* is negligible. The reason why so many flocs are present in the stomach can not be explained. The uptake might occur accidentally when feeding on other prey items. No associated bacteria, fungi, nanoflagelates, Protozoa or diatoms were found on the detritus flocs in the stomachs, but this is probably due to the conservation method used.

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Chapter 3

Temperature and salinity effects on post-marsupial growth of *Neomysis integer* (Crustacea: Mysidacea)

ABSTRACT

There has been an increasing interest in using the brackish water mysid *Neomysis integer* as a toxicological test species for Western European estuarine systems. In this respect, more data on growth, moulting and development in this species is needed. The influence of prevailing environmental variables (*e.g.* temperature, salinity) and age on these processes, as well as their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically-induced variability and natural variability in toxicity testing.

Individual post-marsupial growth (size, intermoult period, growth factor) was studied from first day neonates until adulthood at eight environmentally relevant temperature-salinity conditions. Three salinities (5, 15 and 30 psu) were tested at 15 and 20 °C, and two more extreme temperatures (8 and 25 °C) were tested at a salinity of 5 psu.

Survival and growth of *Neomysis integer* were detected within the whole range tested, but sexual maturation was only possible in the narrower range of 15 – 25 °C and 5 – 15 psu. The size at maturity of *N. integer* increased with decreasing temperature and increasing salinity. Salinity seems to have a stronger effect than temperature on the duration of maturation. The sigmoid von Bertalanffy growth model was fitted to the individual and pooled data, except for the 8°C experiment where growth was linear. Estimates from pooled data were comparable with individually-based estimates, but generally underestimated the asymptotic length. Temperature was negatively correlated with the asymptotic length and positively correlated with the growth constant K. Higher temperatures caused smaller intermoult periods but had no effect on the growth increment, while salinity effects were less straightforward and dependent on the water temperature. A tool is provided to estimate the age, moult number, intermoult period, growth factor and growth rate from the body standard length of *N. integer*. Experimentally-derived von Bertalanffy parameter estimates resulted in a higher growth performance index compared with field-based estimates for the Schelde estuary and Galgenweel populations of *N. integer*.

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INTRODUCTION

Recently, there has been increasing interest in using the brackish water mysid *Neomysis integer* (Leach, 1840) as a toxicological test species for estuarine systems (Roast *et al.*, 1998a; Verslycke *et al.*, 2004 – Addendum 3). It is an alternative to *Americamysis bahia* (formerly *Mysidopsis bahia*) for use in European water quality testing (Roast *et al.*, 1998a; Verslycke *et al.*, 2004). Due to its relatively high temperature requirements, the subtropical species *A. bahia* can not be used in temperate regions and its low tolerance for low salinities of 0.1 to 5 psu makes it an inappropriate test species to use in the turbid upper reaches of estuaries or oligohaline inland water bodies (Roast *et al.*, 2000a; 2001).

Ideally, chemical risk should be assessed by standardized endpoints that cover the molecular, individual and population level. For mysids, this implies that, in addition to evaluating mortality, acute, chronic and multigenerational bio-assays have to be developed for testing chemical effects on growth and moulting, reproduction, biochemical composition, metabolism and physiological processes, behaviour and morphologic aberrations (as reviewed by Verslycke *et al.*, 2004). In this respect, baseline data on growth, development and reproduction, and more specifically on intermoult period, size increment at moulting, age/size at maturity, time to the first brood release, brood size, fecundity, etc. of mysids is invaluable. The influence of prevailing environmental variables (e.g. temperature, salinity, food quality and quantity) on these endpoints and their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically-induced variability and natural variability in toxicity testing (a.o. De Lisle and Roberts, 1988; Verslycke *et al.*, 2004).

The estuarine environment is characterized by strong fluctuating conditions of salinity and temperature. Both are considered dominant 'ecological abiotic master factors', which may act either singly or in concert to modify the population dynamics and distribution of estuarine organisms (McKenney and Celestial, 1995). As a typical estuarine species, the brackish water mysid *Neomysis integer* must be able to functionally adapt to this dynamic environment. The species is euryhaline and tolerates salinities of 1 to 40 psu (Vlasblom and Elgershuizen, 1977; Roast *et al.*, 2001). In the field it can be found at salinities between 0.1 and 38 psu, although it is rare in waters of more than 18 psu (Tattersall and Tattersall, 1951; Vlasblom and Elgershuizen, 1977). The isosmotic point of *N. integer* is described to vary between populations from 16 psu to higher than 20 psu (Ralph, 1965; McLusky and Heard, 1971; Moffat, 1996). *N. integer* is highly tolerant to large, acute salinity fluctuations between 1 and 30 psu (Moffat and Jones, 1992; Roast *et al.*, 1998b). In the Schelde estuary, *N. integer* was recorded at salinities ranging from 8 to 25 psu with a maximal abundance at around 15 psu (Mees *et al.*, 1994). Ongoing studies suggest that the population is shifting towards the more oligohaline zone of the estuary as a consequence of improved oxygen conditions in the upstream reaches (Fockedey, unpublished). In other, more oxygenated western European estuaries such as the Guadalquivir (Spain), Gironde (France), Elbe (Germany) and Ems (The Netherlands) the abundance peak is typically found around 5 psu (Mees et al, 1995; Drake *et al.*, 2002; Fockedey, unpublished).

Neomysis integer is a eurythermic species that occurs in brackish waters along the western European coast at longitudes between 36°N and 68°N and in the Baltic Sea (Deprez *et al.*, 2004; http://intramar.ugent.be/NeMys). Its temperature tolerance measured under laboratory conditions ranges from 0 to 30 °C (Arndt and Jansen, 1986; Mauchline, 1980). Within the range Elbe - Guadalquivir, the summer water temperature of the brackish estuarine zone varies from 25 °C in the North to 29 °C in the South, while winter water temperatures range from 1 °C in the North to 10°C in the South (Drake *et al.*, 2002, Zimmermann, 1997).

Temperature is generally thought to overshadow salinity in its effects on growth and reproduction in crustaceans. Still, temperature can influence the salinity tolerance of a species (Vlasblom and Elgershuizen, 1977; Arndt and Jansen, 1986) and interaction effects of both on the survival and growth of a species can change with age (Kinne, 1955; McKenney, 1994; McKenney and Celestial, 1995).

Most studies on the population dynamics of *Neomysis integer* are exclusively based on field data (see Mees *et al.*, 1994 and references herein). Generally, length frequency distributions are obtained through regular (once or twice per month) sampling of the population for at least one year. Cohorts can then be segregated by modal progression analysis, but this is often complicated by the occurrence of overlapping generations and prolonged reproductive periods (Astthorsson and Ralph, 1984; Mauchline, 1985; Irvine *et al.*, 1995). Growth curves have been derived from field data for several populations of *N. integer*, e.g. Mauchline, 1977; Bremer and Vijverberg, 1982; Astthorsson and Ralph, 1984; Mauchline, 1985; Mees *et al.*, 1994. To date, these growth parameters have rarely been validated with laboratory observations. Schrotenboer (1980), Astthorsson and Ralph (1984), Irvine *et al.* (1995) and Winkler and Greve (2002) all performed growth experiments with *N. integer*, but only at very few temperature-salinity combinations. Kuhlmann (1984) studied the short-term effects of 16 temperature-salinity combinations on the daily growth rate of juvenile *N. integer*, but post-juvenile growth, mortality, intermoult period and growth factor were not reported.

The general objectives of the present study are (1) to describe the growth of *Neomysis integer* under laboratory conditions and (2) to investigate the effects of salinity and temperature on growth in *N. integer*. For this purpose, mysid growth (size, intermoult period, growth factor) was recorded over a whole life span in individually-based experiments at 8 environmentally relevant temperature-salinity conditions.

MATERIAL AND METHODS

Field sampling and stock cultures

Neomysis integer was collected from the brackish pond Galgenweel (salinity ± 5 psu), which is situated on the left bank of the Schelde estuary close to Antwerpen, Belgium. A handnet (L x W: 0.3 x 0.2 m; mesh size 1 mm) was pushed over the bottom during short hauls of 2 – 3 minutes. Mysids were transported to the laboratory within 2 hours after sampling in 15 litre bins containing environmental water.

Stock cultures were maintained as reported by Verslycke *et al.* (2003). In short, mysids were kept in a static system in 200 l glass aquaria equipped with a circulating under-gravel filter. The culture medium was filtered (1.2 µm) seawater diluted with aerated tap water until a final salinity of 5 psu. Every two weeks, 50 % of the culture medium was renewed. A 12h:12h light-dark photoperiod was used and water temperature was kept at 20 ± 2 °C. Cultures were fed twice a day with 24 – 48 h old *Artemia* nauplii at a feeding rate of 150 nauplii mysid^{-1} d^{-1}. Mysid culture density was 20 organisms per litre. The under-gravel filter was replaced every six months.

Gravid females were transferred at regular intervals to 10 l aerated static incubators, in which the culture medium was renewed every day for 50 %. In these incubators, animals were fed twice a day with 24 – 48 h old *Artemia* nauplii *ad libitum* and were checked daily for the release of juveniles from the marsupium. These juveniles were separated from the adult females using a netted brood chamber to prevent the adults from cannibalizing their young.

Growth experiment procedures

Neonates (< 24h old, standard lengths of 2.18 to 2.86 mm) were individually placed in a glass container of 400 ml filled with 350 ml of artificial seawater (different experimental treatments of salinity and temperature, see below) and reared to the late adult stage or until mortality occurred. These experiments lasted between 2 months for the higher temperature experiments and 4 months for the lower temperature experiments. No gradual adaptation from stock to experimental salinity and temperature was done, since it is known that estuarine mysids adapt within 1.5 – 3 hours to changes in salinity and temperature (De Lisle and Roberts, 1987; Dormaar and Corey, 1973). The artificial seawater (Instant Ocean®, Aquarium Systems, France) was diluted with distilled water to the respective test salinity and aerated for at least 24 hours prior to being used. The experimental containers were not aerated, but at least half of the content was renewed daily. The four experimental temperatures (8, 15, 20 and 25 °C) were kept constant by using warm-water baths in temperature-controlled climate rooms at 4 and 15 °C. Salinity and temperature were monitored daily with an YSI salinity meter, but variations were small, i.e. 8.5 ± 0.2 °C; 15.0 ± 0.4 °C; 20.1 ± 0.8 °C; 25.0 ± 0.6 °C and 5.1 ± 0.4 psu; 15.2 ± 0.5 psu and 30.2 ± 0.7 psu.

The containers were checked daily for exuvia (moults). These were carefully harvested with a wide-mouthed glass pipette and transferred to a 4 % formaldehyde solution. Since mysids moult at night, this was preferably done early in the morning to reduce the risk of disintegration or scavenging of the moults. At the same time, freshly hatched nauplii of *Artemia* were added. The number of < 24h nauplii were counted in a 0.2 ml subsample to calculate food concentration. Juveniles were fed approximately 250 nauplii mysid^{-1} d^{-1}, subadults 500 to 750 nauplii mysid^{-1} d^{-1}, and adults 1000 nauplii mysid^{-1} d^{-1}. This corresponded to an *ad libitum* feeding regime, without excessive accumulation of left-over food in the containers. Every 4 to 5 days the individuals were transferred to new, clean jars. The mysids were handled and transferred using a conical plastic measuring spoon to avoid physical stress.

Experimental design

Eight temperature-salinity combinations were selected based on their relevance to European estuarine mysid populations. For the core experiment, temperatures of 15 and 20 °C and salinities of 5, 15 and 30 psu were tested. These temperatures correspond to spring and summer temperatures in mid-European estuaries (Mees *et al.*, 1994). The salinities correspond to the upper, middle and lower reaches of an estuary. Originally, experiments included a 1 psu treatment, however, mortality was extremely high (80 % before fifth moult) in this treatment. An additional experiment was set up to test the more extreme lower and higher temperatures of 8 and 25 °C at a salinity of 5 psu. These temperatures correspond to winter temperature in the Schelde and summer temperature in the Gironde estuary, respectively.

Only mysids that survived at least 5 moults were used for further analyses; mysids dying at an earlier stage were replaced by new < 24h juveniles. An overview of the total number of introduced and successful (i.e. those surviving for more than 5 moults) individuals per treatment is shown in Table 1.

Table 1: Mortality statistics (n.d.: not sexually differentiated)

Temperature (C°)	Salinity (psu)	max. # days	max. # moults	Total started	Died before 5th moult / injured	# Individuals in analysis	Gender		
							♀	♂	n.d.
15	5	123	18	28	3	25	9	16	0
15	15	123	18	30	6	24	11	13	0
15	30	122	19	29	8	21	13	8	0
20	5	62	14	36	10	26	12	14	0
20	15	98	17	34	12	22	12	10	0
20	30	98	19	34	14	20	10	7	3
8	5	122	12	43	19	24	8	16	0
15	5	123	18	28	3	25	9	16	0
20	5	62	14	36	10	26	12	14	0
25	5	61	15	46	27	19	8	10	1

Measurements

Measurement of standard length of exuvia is impossible because of the elasticity of the moult. Furthermore, the collected moults were generally broken into two or more parts. Therefore, well-defined rigid parts of the moults were measured using a microscope with drawing tube (magnification 110 x), with the moult mounted temporally (in water) under a glass cover slip (Figure 1). Preferably, the lengths of the left and right exopodites of the uropod were used. The standard length of the mysid was calculated from the mean length of the exopodites using a linear regression (Table 2), based on measurements of 100 randomly selected individuals from the source population (in spring and autumn). If the exopodites were absent or broken, other body parts were used (length of the endopodite of the uropod, length of the antennal scale or length of the telson) and exopodite length was derived using morphometric linear regressions (Table 2). These were derived from measurements made on all moults collected within the experiment.

Table 2: Results of the allometric regression analyses to estimate (1) the standard body length from the mean length of the exopodites of the uropods and (2) the length of the exopodite of the uropod from the mean length of the endopodites of the uropods, the mean length of the antennal scales and telson length ($X = a + b*Y$). For comparison with other data, allometric regressions are added to calculate total body length and carapace length from the standard body length (Mees et al., 1994).

X	Y	a	b	R^2	p	N
Standard body length	Length $_{exopodites}$	1.0856	4.0818	0.9569	< 0.0001	97
Length $_{exopodite}$	Length $_{endopodites}$	- 0.2235	1.5420	0.9939	< 0.0001	1798
Length $_{exopodite}$	Length $_{antennal\ scales}$	- 0.0059	0.7642	0.9965	< 0.0001	1660
Length $_{exopodite}$	Length $_{telson}$	- 0.0210	1.3320	0.9947	< 0.0001	1779
Total body length	Standard body length	-0.080	1.165	0.997	< 0.001	112
Carapace length	Standard body length	0.439	0.266	0.908	< 0.001	112

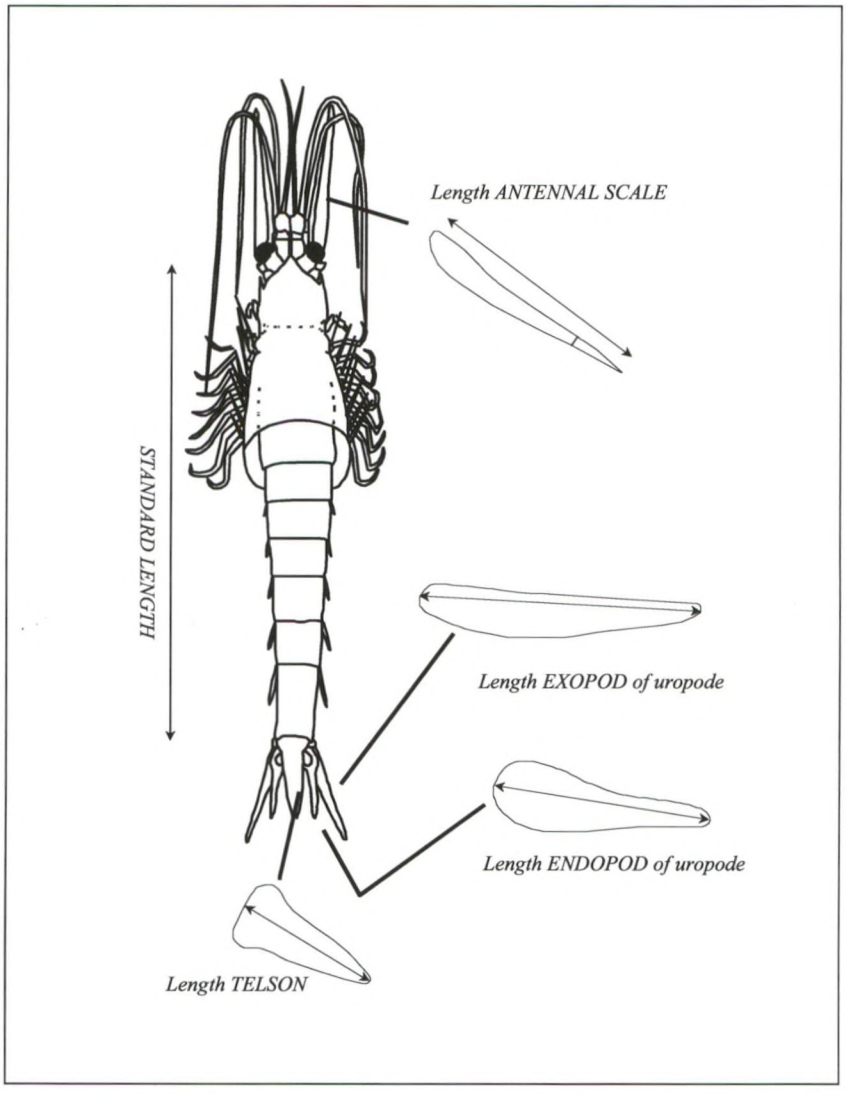

Figure 1: Schematic representation of *Neomysis integer* with indication of the parts of the moults that were measured: length of antennal scale, lengths of the endopod and exopod of the uropod and telson length. Standard length (from the rostrum in between the eye stalks to the end of the last abdominal segment) and mean uropodal exopod length were measured on 100 individuals collected in the field for the linear regression.

Description of growth

Growth is described as the increase in body length over time. For each experimental treatment, the generalized version of the von Bertalanffy growth curve was fitted to the pooled data points within each treatment. The model was also used to describe the growth of each individual:

$$L_t = L_{inf}\,(1 - e^{-K\,(t - t_0)})$$

where L_t is the predicted standard body length (in mm) at age t (in fractions of the year), L_{inf} is the asymptotic length, K is a growth constant and t_0 is the (theoretical) age at a standard length zero. The fitting was done with the non-linear estimation module in Statistica™, using the least squares loss function and the Levenberg-Marquardt estimation method. The individually-based estimates of the growth parameters were tested between the sexes with a Mann-Whitney U-test. Comparison of the growth parameters between treatments and available field data (Schelde: Mees *et al.*, 1994; Galgenweel: Soselisa, 1994; Fockedey, unpublished) was approached from a multivariate perspective in which both K and L_{inf} were taken into consideration. The growth performance index (Φ') was estimated by applying the equation derived by Munro and Pauly (1983) in the form of $\Phi' = \log_{10}(K) + 2\log_{10}(L_{inf})$, with K in $year^{-1}$ and L_{inf} in cm.

Growth in crustaceans is a discontinuous process, i.e. the succession of moults (= exuvia, ecdyses) is separated by intermoult periods. Each time an individual moults, the old integument is shed and a rapid, extensive growth occurs during the short period before the new integument hardens (Hartnoll, 1982). The standard length at subsequent moults was tested in function of both temperature and salinity using a repeated measures analysis of variance (ANOVA). In addition, a repeated measure ANOVA with temperature as independent variable was applied to the more extended temperature experiment at 5 psu. Both tests were performed with a complete design until the 11^{th} moult. Standard length was linearized by a logarithmic transformation.

According to Mauchline (1977), the stepwise growth of mysids can be described as the duration of the intermoult period (IMP, in days) and the increase in length at each moulting event (the growth factor (GF) in % of the pre-moult length) as illustrated in Figure 2. The IMP and GF data were analyzed by (1) a two-way analysis of covariance (ANCOVA) for the combined salinity (5, 15, 30 psu) and temperature (15, 20 °C) effect and (2) a one-way ANCOVA for the temperature effect (8, 15, 20, 25 °C) at 5 psu, both with standard length as the covariable. The intermoult period and standard length were logarithmically transformed, the growth factor was subjected to an arcsine transformation to fulfil the ANCOVA assumptions. Using moult number as the covariable in the respective ANCOVA's yielded the same results, but these are not presented here.

Age and size at maturity

Data on the size and age at sexual differentiation and maturity was collected during all experiments. An animal was identified as a subadult male as soon as the second ramus of the fourth pleopod and the lobus masculinus could be identified on the moult. Animals were classified as adult males when the lobus masculinus became setose and/or the fourth pleopod stretched to the end of the last abdominal segment. Females gradually develop a marsupium between the thoracopods. Since the oostegites were never found attached to the moult during the experiments, the same moult number was used as for males within the same treatment to classify subadult and adult females.

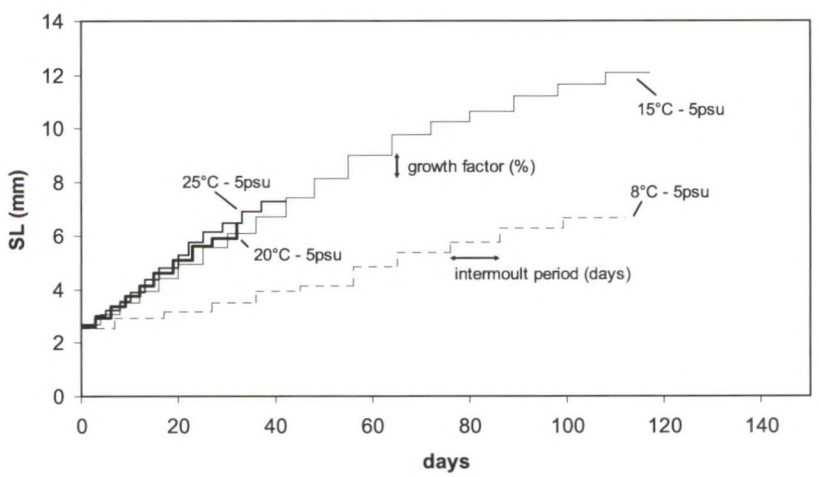

Figure 2: The stepwise growth as observed for 'typical' long-living individuals at four experimental temperatures and a salinity of 5 psu, with indication of growth factor (growth rate) and intermoult period.

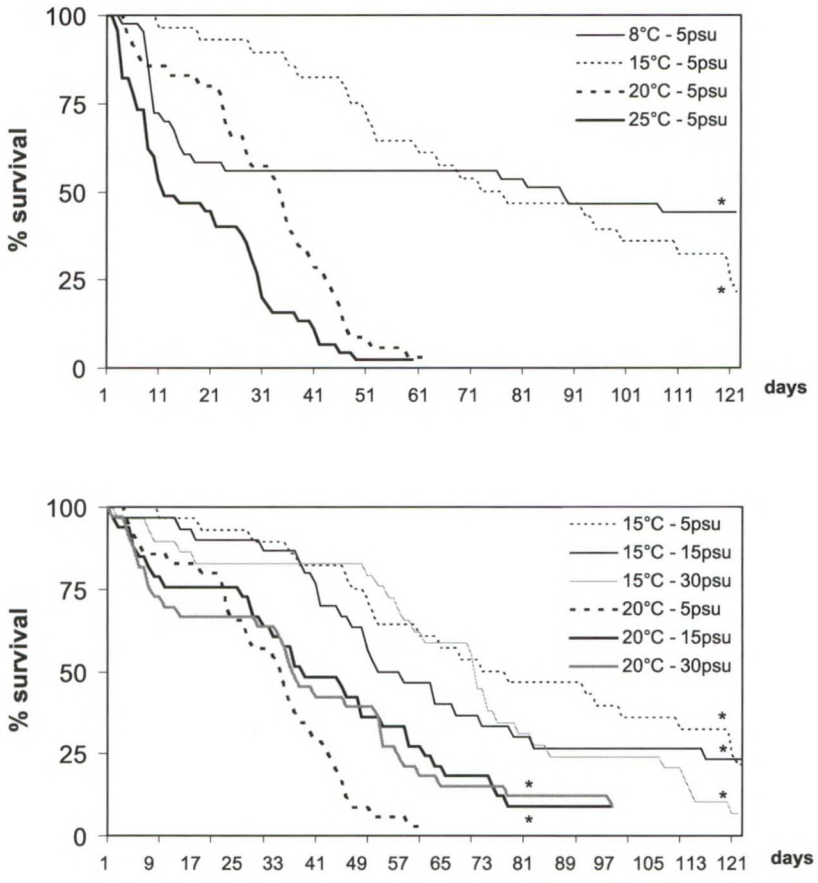

Figure 3: Survival curves at the different salinity-temperature combinations. * indicate that the data include censored data with animals still alive at the end of the experiment.

RESULTS

Mortality

Only mysids that survived at least 5 moults were used for further analyses; mysids dying at an earlier stage were replaced by new < 24h juveniles. Per salinity-temperature combination 28 to 46 mysids were introduced into the experiments and between 19 and 26 individuals were available for the description of the growth (Table 1). Overall survival was markedly higher at 15 °C when compared to the other temperatures (Figure 3). The life cycle of *Neomysis integer* was shorter in the higher temperature treatments (20 and 25 °C), i.e. individuals reached adulthood fast and died after 61 – 62 days, but mortality was relatively high over the whole lifespan. At 8 °C, initial mortality was also high; a trend that stabilized after 24 days (44 – 56 % survival).

Growth curves

Generalized von Bertalanffy growth curves were fitted to the pooled data for each treatment (Figure 4). The growth parameters, asymptotic length (L_{inf}), growth constant (K) and the theoretical age at a standard length zero (t_0) were calculated and presented in Table 3. The overall goodness-of-fit for the different growth curves was high (R^2: 0.92 – 0.98). No von Bertalanffy growth model was fitted to the 8°C data. In this treatment, growth was slow and linear for the duration of the experiment (122 days) and was therefore described using a linear model; SL = 2.259 + 0.042*age (N = 206, R^2 = 0.93, $p < 0.00001$). The asymptotic length (L_{inf}) was larger at 15°C (L_{inf} between 14.60 and 16.81 mm) compared to higher temperatures (10.53 - 12.39 mm at 20 °C and 8.55 mm at 25 °C). The growth rate increased with temperature; K of 11.95 at 25 °C; K of 5.37 – 7.30 at 20 °C and K of 3.54 - 4.64 at 15 °C. Salinity had a less straightforward effect on the growth parameters L_{inf} and K. At 15 °C, the highest L_{inf} was reached at 15 psu (16.81 ± 0.48 mm), whereas at 20 °C L_{inf} was highest at 5 psu (12.39 ± 0.83 mm). K values were highest at 5 psu at 15 °C (4.64 ± 0.25) and at 15 psu at 20 °C (7.30 ± 0.41).

Figure 4: The generalised von Bertalanffy growth curve (——) fitted to the pooled data of females (◆) and males (◇) of each salinity-temperature combination; (Δ) indicate the age at sexual differentiation and (▲) the age at sexual maturity.

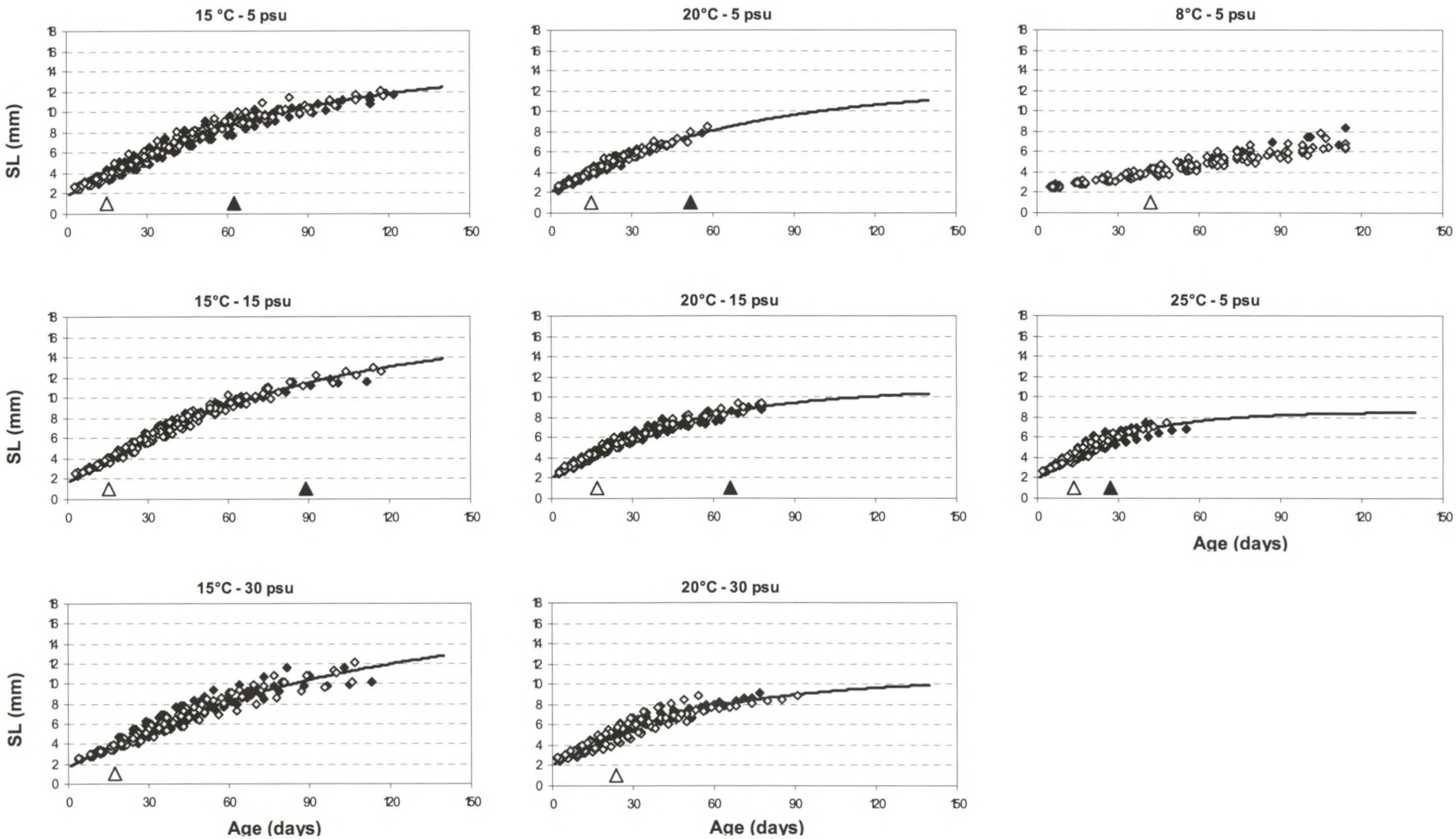

Table 3: Pooled and individually-based von Bertalanffy growth parameter estimations (± standard error) and performance index Φ' (-: linear growth). Gender differences using Mann-Whitney U-test are shown in between brackets (ns: not significant; *: significant; °: test not allowed)

Temperature (°C)	Salinity (psu)		# individuals	# moults	L_{inf} (mm)	K (day-1)	t_0 (fraction of the year)	t_0 (days)	p	R^2	Φ'
15	5	pooled	24	310	14.60 ± 0.39	4.64 ± 0.25	0.027 ± 0.002	10 ± 1	< 0.001	0.974	1.558
		individually	15	8 - 18	15.93 ± 0.85 (ns)	4.55 ± 0.47 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	< 0.0001	0.988 - 0.999	1.625
15	15	pooled	24	258	16.81 ± 0.48	4.25 ± 0.21	0.023 ± 0.001	8 ± 1	< 0.001	0.984	1.642
		individually	10	10 - 18	18.12 ± 0.98 (ns)	4.07 ± 0.32 (ns)	0.024 ± 0.002 (ns)	9 ± 1 (ns)	< 0.0001	0.992 - 0.998	1.688
15	30	pooled	21	254	16.66 ± 1.07	3.54 ± 0.38	0.030 ± 0.003	11 ± 1	< 0.001	0.952	1.555
		individually	5	8 - 18	15.75 ± 1.65 (°)	4.89 ± 0.49 (°)	0.019 ± 0.003 (°)	7 ± 1 (°)	< 0.0001	0.994 - 0.998	1.646
20	5	pooled	27	223	12.39 ± 0.83	5.37 ± 0.59	0.033 ± 0.002	12 ± 1	< 0.001	0.974	1.478
		individually	11	7 - 13	12.85 ± 0.91 (ns)	5.63 ± 0.49 (ns)	0.033 ± 0.002 (ns)	12 ± 1 (ns)	< 0.0001	0.998 - 0.999	1.531
20	15	pooled	22	261	10.88 ± 0.28	7.30 ± 0.41	0.028 ± 0.002	10 ± 1	< 0.001	0.976	1.499
		individually	14	7 - 16	10.55 ± 0.35 (*)	7.82 ± 0.52 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	< 0.0001	0.989 - 0.999	1.502
20	30	pooled	20	228	10.53 ± 0.52	6.85 ± 0.73	0.032 ± 0.003	12 ± 1	< 0.001	0.922	1.443
		individually	9	10 - 18	10.68 ± 0.72 (ns)	7.98 ± 0.66 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	< 0.0001	0.996 - 0.999	1.521
8	5	pooled	-	-	-	-	-	-	-	-	-
		individually	-	-	-	-	-	-	-	-	-
15	5	pooled	24	310	14.60 ± 0.39	4.64 ± 0.25	0.027 ± 0.002	10 ± 1	< 0.001	0.974	1.558
		individually	15	8 - 18	15.93 ± 0.85 (ns)	4.55 ± 0.47 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	< 0.0001	0.988 - 0.999	1.625
20	5	pooled	27	223	12.39 ± 0.83	5.37 ± 0.59	0.033 ± 0.002	12 ± 1	< 0.001	0.974	1.478
		individually	11	7 - 13	12.85 ± 0.91 (ns)	5.63 ± 0.49 (ns)	0.033 ± 0.002 (ns)	12 ± 1 (ns)	< 0.0001	0.998 - 0.999	1.531
25	5	pooled	19	162	8.55 ± 0.43	11.95 ± 1.48	0.021 ± 0.003	8 ± 1	< 0.001	0.920	1.504
		individually	7	7 - 13	10.01 ± 0.52 (ns)	10.25 ± 1.25 (ns)	0.023 ± 0.004 (ns)	8 ± 1 (ns)	< 0.001	0.995 - 0.999	1.574

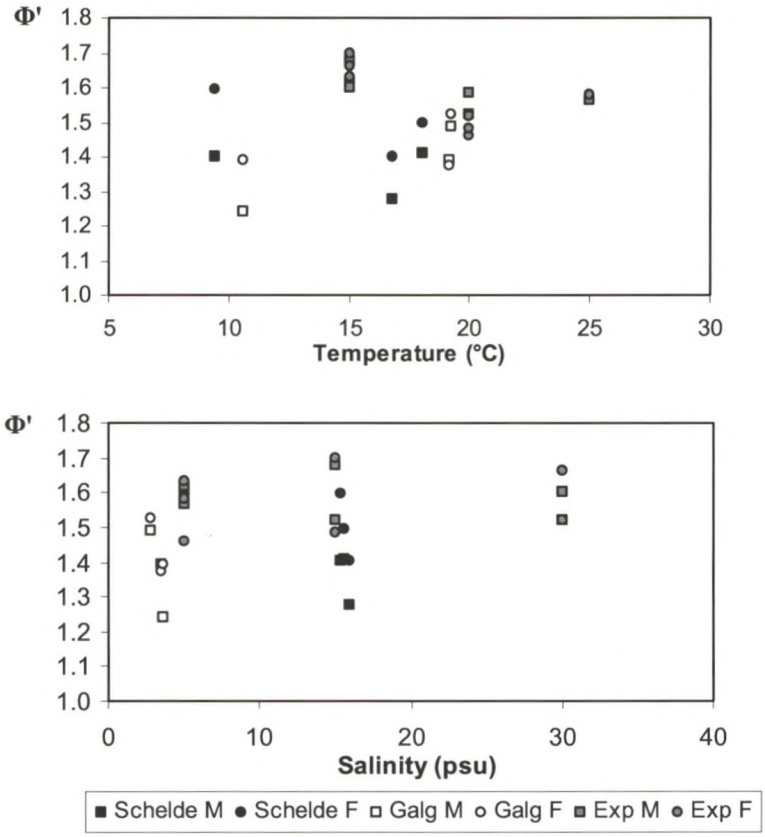

Figure 5: Growth performance index (Φ') for field and experimentally-derived growth for male and female *Neomysis integer* in function of temperature and salinity (Schelde: Schelde population, Galg: Galgenweel population, Exp: present experiments; M: males, F: females).

The growth performance index Φ' (Table 3, Figure 5) showed analogue trends, with the highest growth performance at 15 °C in comparison with higher temperatures (Spearman Rank R: -0.65; p=0.02). Slightly higher values were obtained at 15 psu in comparison with other salinities at 15 and 20 °C, although no correlation could be demonstrated. The growth performance index Φ' in all our experimental treatments was significantly higher (t-test: p=0.000064) than in all cohorts of the field populations of the Schelde estuary and Galgenweel (Table 4, Figure 5).

Table 4: Comparison of field and experimentally derived von Bertalanffy growth parameter estimations and growth performance index Φ' for male and female *Neomysis integer*. Mean temperature and salinity are indicated with their range within parentheses.

	Temperature (°C)	Salinity (psu)	Generation	♂ L_{inf} (mm)	♂ K (day-1)	♂ Φ'	♀ L_{inf} (mm)	♀ K (d^{-1})	♀ Φ'
Schelde population	9 (1-22)	15 (9-21)	winter	16.0	2.7	1.40	19.0	3.0	1.60
(Mees et al., 1994)	18 (10-23)	16 (10-19)	spring	14.3	3.4	1.41	16.0	3.4	1.50
	17 (9-23)	16 (10-21)	summer	13.1	3.0	1.28	14.3	3.4	1.40
Galgenweel population	11 (4-19)	4 (3-5)	winter	16.5	3.1	1.49	17.5	3.0	1.53
(Fockedey, unpublished)	19 (17-23)	3	spring	15.5	2.8	1.39	15.5	2.7	1.37
	19 (17-23)	3 (3-5)	summer	12.6	3.0	1.24	14.5	3.2	1.39
Laboratory experiments	8	5							
(present study)	15	5		16.3	4.3	1.62	15.5	4.9	1.63
	15	15		17.7	4.2	1.68	18.7	3.9	1.70
	15	30		14.0	5.6	1.60	16.6	4.6	1.66
	20	5		13.7	5.6	1.59	11.8	5.7	1.46
	20	15		11.7	6.7	1.52	9.9	8.4	1.48
	20	30		10.4	8.5	1.52	10.8	7.7	1.52
	25	5		9.7	10.7	1.56	10.3	9.9	1.58

Individually-based estimates of the von Bertalanffy growth parameters were only significant for the long living animals. The growth of shorter living individuals, comprising 36 to 76 % of the total of all tested individuals, behaved linearly. The number of animals and moults used for the individual estimates of L_{inf}, K and t_0 are presented in Table 3. The goodness of fit was always higher (> 0.99) for the individually-based data than for the pooled data. The asymptotic length was generally underestimated based on the pooled data in comparison with the individually-derived estimates. Overestimated K values are compensated for, however, by underestimated t_0 values and vice versa. Gender had no significant effect on the estimates of the growth parameters in our experiments.

Standard length in function of moult number

Temperature, salinity and moult number were tested in a 2-way repeated measures ANOVA (Figure 6, Table 5a). Generally, subsequent moults resulted in significantly larger animals and this at least until the 11th moult. Temperature significantly affected the standard length of *Neomysis integer* and the largest individuals were found at 15 °C. This temperature effect on standard length was significant from the 3rd moult on. In addition, salinity had a significant effect on the standard length of *N. integer*. At 15 psu, animals were larger than at 5 or 30 psu, and this from the 3rd moult onwards. The combined effects of salinity-temperature, salinity-moult number and temperature-moult number were all significant, whereas the effect of both temperature and salinity with the within-subject factor (moult number) was borderline significant ($p = 0.049652$).

The effect of a larger temperature range (8, 15, 20 and 25 °C) on mysid standard length at subsequent moults was tested using a repeated measures ANOVA (Figure 6; Table 5b). Again, temperature had a significant effect on the standard length of *Neomysis integer* with larger individuals at 15 °C as compared to the other tested temperatures. This effect was significant from the 2nd – 4th moult onwards. For example, at 5 psu an average individual at its 10th moult measured 6.64, 7.90, 6.34 and 6.26 mm at 8, 15, 20 and 25 °C, respectively.

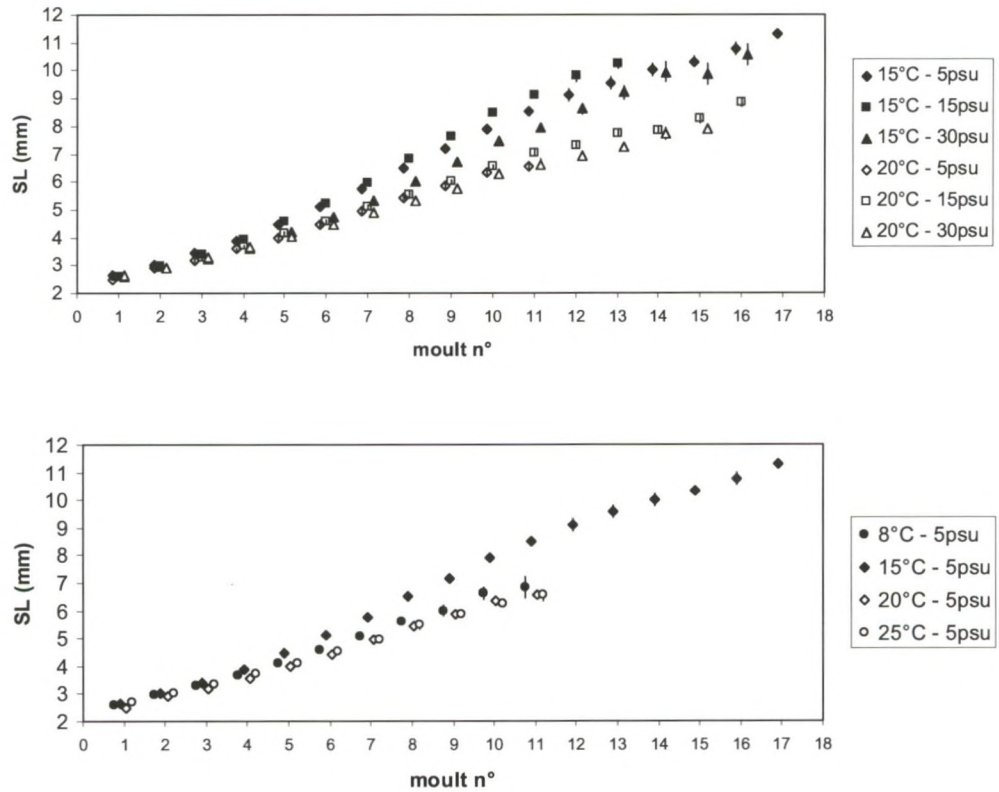

Figure 6: Standard length (± standard error) in function of the moult number at the different salinity and temperature combinations.

Table 5a: Results of the 2-way repeated measures ANOVA testing the effect of temperature and salinity on the standard length at subsequent moults (15 and 20°C – 5, 15 and 30 psu).

	F	p-value	Description of effect
Temperature	36.21	0.000002	15°C > 20°C
Salinity	5.31	0.011091	15 psu > (30 psu = 5psu)
Moult number	4133.52	< 0.0000001	log SL ~ moult number
Salinity x Temperature	4.00	0.029589	15°C: (5 psu = 15 psu) > 30 psu 20°C: 15 psu > (5 psu = 30 psu)
Temperature x Moult number	52.23	< 0.0000001	From 3rd moult: 15°C > 20°C
Salinity x Moult number	4.84	< 0.0000001	From 3rd moult: 15 psu > (5 psu = 30 psu)
Salinity x Temperature x Moult number	1.61	0.049652	---

Table 5b: Results of the repeated measure ANOVA testing the effect of temperature on the standard length at subsequent moults (8, 15, 20 and 25°C at 5 psu).

	F	p-value	Description of effect
Temperature	14.409	0.000083	15°C > (8 = 20 = 25°C)
Moult number	1086.659	< 0.0000001	log SL ~ moult number
Temperature x Moult number	9.770	< 0.0000001	From 2-4th moult: 15°C > (8 = 20 = 25°C)

Intermoult period

The intermoult period (IMP) is positively related to the standard length and to the moult number (Figure 7). The combined effect of temperature and salinity on the intermoult period at 15 and 20 °C was tested in an ANCOVA using standard length as the covariable. Temperature ($p<0.0001$) and salinity ($p<0.006$) both had a significant effect on the IMP, although the salinity effect was less important. The IMP was significantly ($p<0.001$) shorter at the highest temperatures (5.35 ± 0.02 d at 15 °C; 4.28 d ± 0.03 at 20 °C). IMP was significantly ($p = 0.0016$) shorter at 15 psu (4.71 ± 0.03 days) in comparison to the other tested salinities (4.92 ± 0.03 at 5 psu and 4.81 ± 0.03 at 30 psu). The combined effect of salinity and temperature on the IMP was not significant ($p = 0.365$).

A second ANCOVA, aimed at studying the effect of a larger temperature range (8 – 25°C) on mysid growth confirmed that temperature has a significant effect on growth, in this case the IMP ($p < 0.001$): the IMP was longest at 8 °C (10.61 ± 0.06 d) and gradually decreased at higher temperatures (5.05 ± 0.05 at 15 °C, 4.14 ± 0.06 at 20 °C and 3.42 ± 0.07 d at 25 °C). The intermoult period of immature *Neomysis integer* was very similar (2 – 4 days) at 15, 20 and 25 °C, but it was markedly longer at 8 °C (7 – 10 days). For late subadult and adults stages, the IMP was temperature-dependent and ranged between 7 – 10 d at 15 °C, 5 – 7 d at 20 °C and 4 – 5 d at 25 °C.

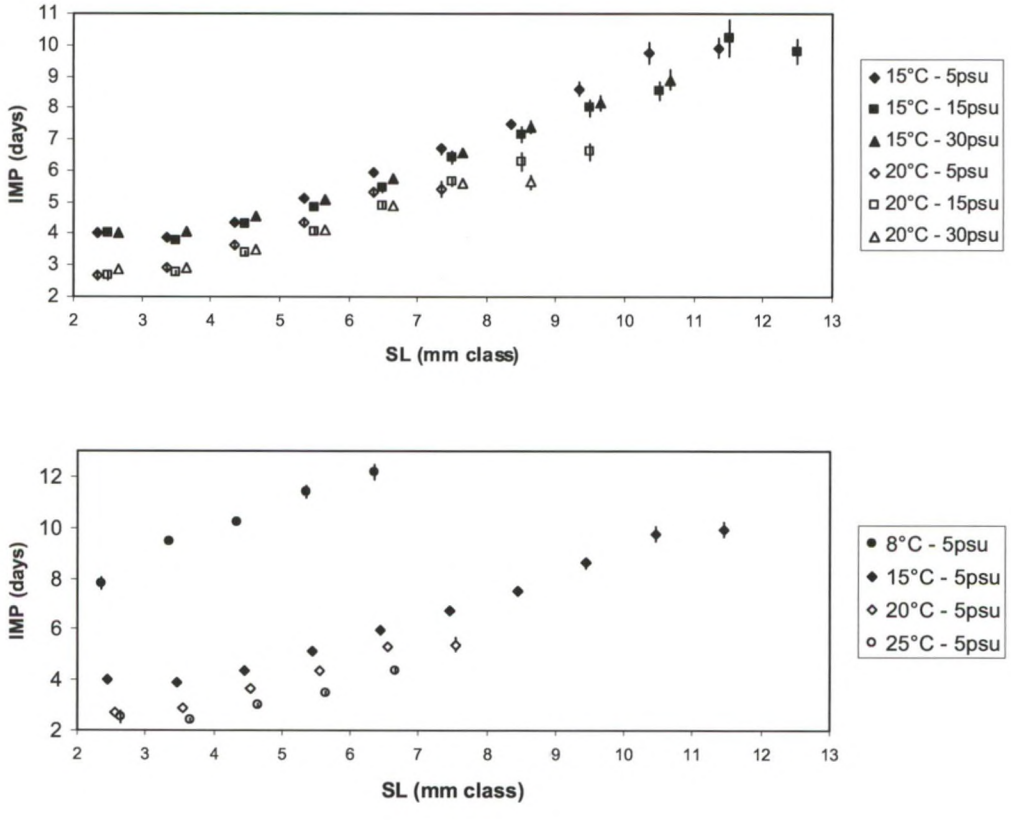

Figure 7: Intermoult period (IMP ± standard error) in function of standard length

Growth factor

The growth factor (GF) is inversely correlated with standard length (SL) and moult number (Figure 8). Temperature and salinity in combination had a significant effect on the growth factor of *Neomysis integer* (ANCOVA: $p < 0.001$). The GF at 15 °C is significantly ($p < 0.0001$) larger (12.31 ± 0.11 %) than at 20 °C (8.82 ± 0.12 %). Salinity also had a significant ($p < 0.0001$) effect on the GF, but this effect was different between the two temperature treatments. At 15 °C, the GF was significantly higher at 15 psu (13.36 ± 0.19 %) than at 5 and 30 psu (12.07 ± 0.17 and 11.49 ± 0.19 %, respectively). At 20 °C, the highest GF was observed at 5 psu (9.34 ± 0.21 %) and was subsequently lower at 15 and at 30 psu (9.08 ± 0.19 and 8.02 ± 0.21 %, respectively).

To study the effects on mysid growth over the full range of temperatures observed in the field (8 – 25 °C), a second ANCOVA was performed using 4 temperature treatments at 5 psu ($p < 0.001$). In these experiments, no obvious temperature effect on the GF was observed, except at 25 °C where sub-optimal growth was caused by a significantly lower GF. In immature *Neomysis integer*, the GF varied between 9 and 16 %, while late subadult and adult mysids increased 4 – 8 % in size at each moult.

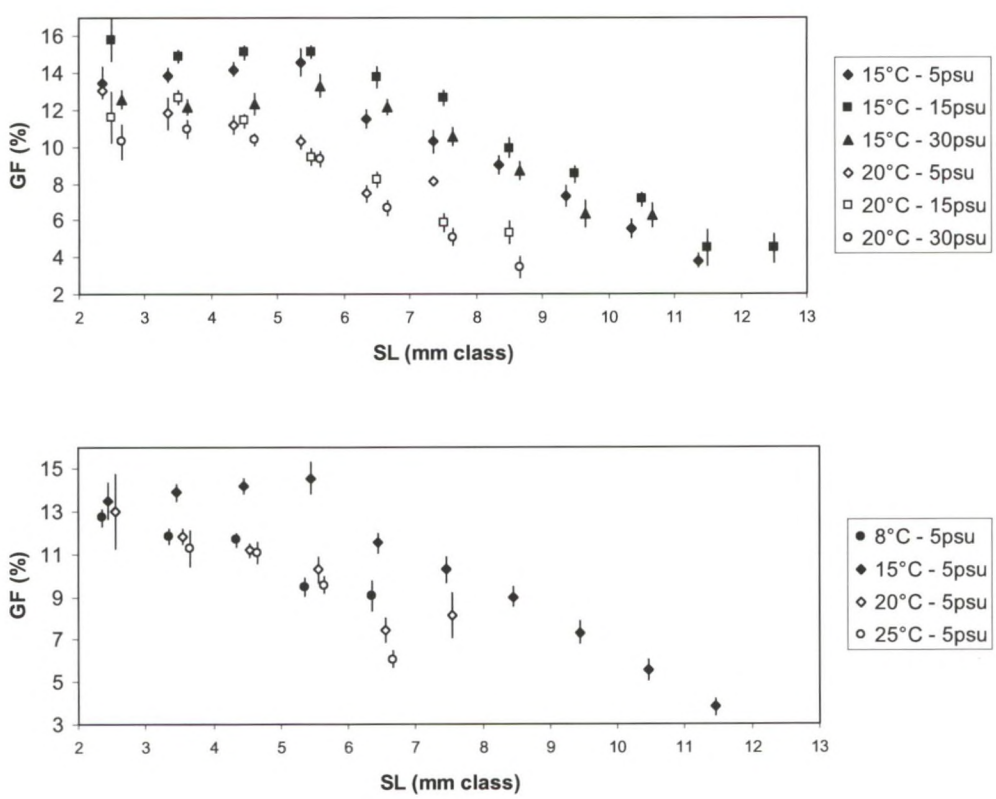

Figure 8: Growth factor (GF ± standard error) in function of standard length

Age, Moult Number, Intermoult Period (IMP), Growth Factor (GF) and Growth Rate (GR) per 1mm length class

Generally, animals are collected from the field or a laboratory stock culture without knowing the exact age. Table 6 can serve as a tool to estimate the age, moult number, intermoult period, growth factor and intermoult growth rate of *Neomysis integer* based on the standard length (for the 8 temperature-salinity combinations) which is useful in ecotoxicological experiments. Variation on these values (not shown) is small, as is also demonstrated by the good fit of the von Bertalanffy growth curve on the data. The maximal size (at maturity) observed was 18 – 35 % lower than the asymptotic length L_{inf} as calculated by the von Bertalanffy model on the pooled data and 16 – 41 % lower based on individual estimations. Therefore, additional data on 'age' was extrapolated from the respective growth models (in bold) for the largest size classes.

Sexual development and maturity

Sexual differentiation, i.e. secondary sexual characteristics appearing (Δ in Figure 4) was reached in all treatments, while sexual maturity, i.e. secondary sexual characteristics in adult form (▲ in Figure 4) was only reached by mysids within the range 15 – 25 °C and 5 – 15 psu (Figure 4, Table 7). In the different 15 and 20 °C treatments, the animals became subadult at a later point in their life with increasing experimental salinity. Sexual differentiation was observed after 15 d at 5 psu; after 15 – 17 d at 15 psu and after 18 – 24 d at 30 psu, at 15 and 20 °C respectively. The number of moults needed to reach subadulthood was 5 at 15 °C in all salinity treatments, but this number increased with salinity at 20 °C (6, 7 and 8 at 5, 15 and 30 psu, respectively).

Maturity was not reached at all in the 30 psu treatments. The limited number of observations on age and size at sexual maturity in the 20 °C treatments hinders firm conclusions. However, some trends could be observed, i.e. at higher temperatures the age at maturity decreased, while at higher salinities it increased (Figure 4).

Table 7: Size of the juvenile *Neomysis integer* at the start of the experiment and size, age and moult number at sexual differentiation and maturity

T	S	Juvenile	Subadult			Adult		
(°C)	(psu)	size (mm)	size (mm)	age (days)	# moults	size (mm)	age (days)	# moults
15	5	2.6 ± 0.1	4.3 ± 0.1	14.9 ± 0.4	4.8 ± 0.1	9.4 ± 0.2	62.4 ± 1.3	12.8 ± 0.2
15	15	2.6 ± 0.1	4.5 ± 0.1	15.3 ± 0.5	4.9 ± 0.1	11.9 ± 0.2	89.3 ± 3.7	16.0 ± 0.4
15	30	2.6 ± 0.1	4.5 ± 0.1	17.6 ± 0.4	5.3 ± 0.1	-	-	-
20	5	2.5 ± 0.1	4.4 ± 0.1	15.0 ± 0.6	6.1 ± 0.2	8.5	52.0	14
20	15	2.6 ± 0.1	5.0 ± 0.1	16.7 ± 0.4	6.8 ± 0.1	8.9	66.3 ± 6.2	15.7 ± 0.7
20	30	2.6 ± 0.1	5.6 ± 0.2	23.6 ± 1.4	8.5 ± 0.1	-	-	-
8	5	2.6 ± 0.1	4.4 ± 0.1	42.0 ± 1.3	5.7 ± 0.1	-	-	-
15	5	2.6 ± 0.1	4.3 ± 0.1	14.9 ± 0.4	4.8 ± 0.1	9.4 ± 0.2	62.4 ± 1.3	12.8 ± 0.2
20	5	2.5 ± 0.1	4.4 ± 0.1	15.0 ± 0.6	6.1 ± 0.2	8.5	52.0	14
25	5	2.7 ± 0.1	4.6 ± 0.1	13.6 ± 0.8	6.1 ± 0.1	6.3 ± 0.2	27.0 ± 3.0	10.5 ± 0.50

Table 6: Estimated age, moult number, intermoult period, growth factor and intermoult growth rate in function of the standard length of *Neomysis integer* (in between brackets: less than 5 observations; in bold: estimated age from von Bertalanffy growth curve; -: no observations)

T (°C)	S (psu)		Standard length (mm); 1mm class – median														
			2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5
		Age (days)	11	31	55	76	95	(103)	(114)	-	-	-	-	-	-	-	-
		Moult n°	1	4	6	8	10	(10)	(11)	-	-	-	-	-	-	-	-
8	5	IMP (days)	8	9	10	11	12	(14)	(14)	-	-	-	-	-	-	-	-
		GF (%)	13	12	12	9	9	(8)	(11)	-	-	-	-	-	-	-	-
		GR (mm.day^{-1})	0.04	0.04	0.05	0.04	0.04	(0.04)	(0.06)	-	-	-	-	-	-	-	-
		Age (days)	5	12	21	28	37	46	56	70	88	**112**	**145**	**194**	**386**	-	-
		Moult n°	1	3	5	7	8	10	11	13	15	17	(18)	-	-	-	-
15	5	IMP (days)	4	4	4	5	6	7	8	9	10	10	(9)	-	-	-	-
		GF (%)	13	14	14	15	12	10	9	7	6	4	(4)	-	-	-	-
		GR (mm.day^{-1})	0.09	0.11	0.13	0.14	0.11	0.11	0.09	0.08	0.06	0.04	(0.05)	-	-	-	-
		Age (days)	6	12	20	26	34	41	51	62	**76**	**91**	**108**	**131**	**162**	**210**	**335**
		Moult n°	1	3	5	6	8	9	10	12	13	15	17	-	-	-	-
15	15	IMP (days)	4	4	4	5	5	6	7	8	9	10	10	-	-	-	-
		GF (%)	16	15	15	15	14	13	10	9	7	4	4	-	-	-	-
		GR (mm.day^{-1})	0.10	0.12	0.14	0.15	0.15	0.13	0.11	0.09	0.08	0.05	0.05	-	-	-	-
		Age (days)	6	14	24	32	40	50	60	75	**92**	**110**	**132**	**161**	**200**	**265**	**475**
		Moult n°	1	3	6	7	9	10	12	14	15	(16)	(18)	-	-	-	-
15	30	IMP (days)	4	4	5	5	6	7	7	8	9	(11)	(8)	-	-	-	-
		GF (%)	13	12	12	13	12	11	9	6	6	(7)	(6)	-	-	-	-
		GR (mm.day^{-1})	0.08	0.09	0.11	0.13	0.12	0.11	0.09	0.07	0.07	(0.07)	(0.09)	-	-	-	-
		Age (days)	5	11	19	27	38	49	**67**	**87**	**116**	**167**	-	-	-	-	-
		Moult n°	2	4	6	8	10	12	(14)	-	-	-	-	-	-	-	-
20	5	IMP (days)	3	3	4	4	5	5	(6)	-	-	-	-	-	-	-	-
		GF (%)	13	12	11	10	7	8	(7)	-	-	-	-	-	-	-	-
		GR (mm.day^{-1})	0.14	0.13	0.13	0.12	0.08	0.10	(0.09)	-	-	-	-	-	-	-	-
		Age (days)	4	9	16	25	35	48	**66**	**93**	**158**	-	-	-	-	-	-
		Moult n°	2	4	6	8	10	12	15	16	-	-	-	-	-	-	-
20	15	IMP (days)	3	3	3	4	5	6	6	7	-	-	-	-	-	-	-
		GF (%)	12	13	11	9	8	6	5	(5)	-	-	-	-	-	-	-
		GR (mm.day^{-1})	0.13	0.15	0.14	0.11	0.10	0.08	0.07	(0.07)	-	-	-	-	-	-	-
		Age (days)	4	11	19	27	38	51	**76**	**112**	-	-	-	-	-	-	-
		Moult n°	1	4	6	8	11	13	16	(18)	-	-	-	-	-	-	-
20	30	IMP (days)	3	3	4	4	5	6	6	(6)	-	-	-	-	-	-	-
		GF (%)	10	11	10	9	7	5	3	(5)	-	-	-	-	-	-	-
		GR (mm.day^{-1})	0.10	0.12	0.12	0.12	0.09	0.07	0.05	(0.07)	-	-	-	-	-	-	-
		Age (days)	4	9	17	23	34	**56**	-	-	-	-	-	-	-	-	-
		Moult n°	1	3	6	8	11	(13)	-	-	-	-	-	-	-	-	-
25	5	IMP (days)	3	2	3	3	4	(5)	-	-	-	-	-	-	-	-	-
		GF (%)	(12)	11	11	10	6	(6)	-	-	-	-	-	-	-	-	-
		GR (mm.day^{-1})	0.16	0.15	0.16	0.14	0.09	(0.08)	-	-	-	-	-	-	-	-	-

The age where individuals became subadult at 5 psu and 8°C was retarded (42 days) in comparison with the other temperatures tested at the same salinity (14 – 15 days). At 15 °C, subadults were observed after 5 moults, while at other temperatures generally one more moult was required to reach differentiation. Within 11 moults, animals at 25 °C were fully sexually developed, while at 15 and 20 °C animals required two to three more moults to reach maturity. Adulthood was not reached in any of the animals at 8 °C within the 4 months duration of these experiments. Clearly, temperature shortens the time to reach sexual maturation in mysids (62, 52 and 27 days at 15, 20 and 25 °C, respectively).

DISCUSSION

Optimizing culture protocols for Neomysis integer

Maintaining a laboratory stock culture of *Neomysis integer* under standardized conditions (e.g. methodology according to Verslycke *et al.*, 2003) has the advantage that all experimental specimens are born from adults living at the same temperature, salinity and food conditions. Especially when experiments run over an extended period of time (two years in this case), variation that might be caused by different stock animals are kept at a minimum. In addition, the exact age of all individuals is known when cultures are checked daily for newly released young.

Based on the present study, some adaptations of the culture protocol for *Neomysis integer* are suggested to enhance culture yield and/or quality. The laboratory stock culture was kept at 20 ± 2 °C. In the individually-based experiments, however, this relatively high temperature resulted in a substantially higher mortality (Figure 3). Therefore, a lower culture temperature is suggested for *N. integer*. On the other hand, lower temperatures result in slower growth. Animals reach maturity in about 2 months at temperatures of 20 °C or higher. At 15 °C, maturity is only reached after twice that time (~ 4 months), but animals are significantly larger. Since size at maturity is directly linked with fecundity (e.g. Mees *et al.*, 1994), larger females at maturity result in a substantially higher number of offspring. Alternatively, when using a temperature lower than 20 °C, it might be advisable to increase salinity to 15 psu to increase the growth performance of *N. integer*. Culturing can also be optimized by feeding mysids at a higher ration. Subadult *N. integer* (5 – 9 mm) shows a growth limitation when fed with less than 200 nauplii.mysid^{-1} d^{-1} (Fockedey, unpublished). The rations given in the individual growth experiments were 1.7 to 6.7 times higher than given to the stock culture and assured a good growth without excessive accumulation of left-over food in the containers.

Optimizing exposure protocols for Neomysis integer

In the present study, *Neomysis integer* was successfully reared form the first day of release from the brood pouch until adulthood under most of the tested temperature-salinity combinations. Their growth was followed in detail using individually-based experiments under steady-state conditions in relatively small vessels of 400 ml. Clutter and Theilaker (1971), Gaudy and Guerin (1979) and Cuzin-Roudy et al. (1981) concluded that it is impossible to study individual growth of mysids using static systems, as the IMP is highly variable and some individuals have indefinitely delayed moults in comparison to others. Constant renewal of the water seems to be a crucial factor for maintaining normal growth and moulting in mysids. Bulk experiments have the disadvantage that detailed information on the IMP and GF cannot be obtained. Recently, Winkler and Greve (2002) published individually-based growth data of *N. integer* with a high success rate in a flow-through construction. In our experiments, water was partly (50 – 80 %) renewed daily and the exposure jars were cleaned every 4-5 days.

IMP was relatively stable for individuals of the same age within one treatment (standard error generally less than 5 % of the mean value).

For a small amount of individuals (8), growth was aberrantly delayed. However, this could always be linked to an injury of the exoskeleton. These animals were regenerating the damaged part over a few moults, but this was associated with a delayed growth. The injury-induced effect on growth has previously been described for euphausiids (Murano *et al.*, 1983; Nicol and Stolp, 1990). The aberrant individuals were not used in further analyses in the present study. Injuries were generally avoided by transferring mysids in a conic measure spoon containing a small volume of water.

von Bertalanffy growth model

The von Bertalanffy growth model was originally used to describe fish growth, but has been applied to crustaceans and more specifically to mysids (Schnute and Fournier, 1980; Cuzin-Roudy *et al.*, 1981; Mees *et al.*, 1994; Fockedey, unpublished). It assumes an asymptotic growth and this sigmoid growth pattern has been confirmed in other studies with mysids (Astthorsson and Ralph, 1984; Winkler and Greve, 2002). Although the model is derived for a single individual, it has mostly been used to model data collected from a group of animals. The growth constant K and asymptotic length L_{inf} vary among individuals in a group, as in most populations of animals for genetic, phenotypic and behavioural reasons (Xiao, 1994). However, as supported by the findings of the present study, growth parameter estimations derived from pooled data were only moderately biased in comparison with individual estimations. More specifically, the L_{inf} was underestimated when using the pooled data (by including the short living animals in the pooled dataset).

Temperature was negatively correlated with L_{inf} and positively correlated with K. The effect of salinity on the different growth parameters was less straightforward and was dependent on the water temperature in the treatment. Highest asymptotic lengths were achieved in the 15 psu/15 °C treatment and in the 5 psu/20 °C treatment. The highest growth rate was found in the 5 psu/15 °C and 15 psu/20 °C treatments.

Standard length in function of moult number

Contrary to the findings of Astthorsson and Ralph (1984), we found the standard length to be affected by temperature (and salinity) at a specific moult number. Mysids growing at 15 °C had a larger standard length in comparison with the other temperatures from the 3rd moult onwards. The effect of salinity on the standard length-moult number relation was also significant, however less so than the temperature effect.

Intermoult period, Growth factor and Growth rate

The duration of the first intermoult period was equal for all animals in one treatment and lasted 3 – 4 days after leaving the marsupium in the same night. From the second moult onwards, there was individual variability on the stage duration and the moults were no longer synchronous between different individuals in one treatment. This effect became more obvious with increasing moult numbers which corroborates observations by Cuzin-Roudy *et al.* (1981) for the mysid *Siriella armata*. Within a life cycle, when animals become larger and pass through a number of moults, the intermoult period generally increases while the growth factor decreases. Astthorsson and Ralph (1984) and Mauchline (1985) described these growth parameters as logarithmically for *Neomysis integer* and other mysids. Winkler and Greve (2002) found an initial increase and a later decrease of the growth factor from maturity on.

The GF in the 8 treatments, in contrast, was almost constant for the first 5 – 6 moults before gradually decreasing. The IMP behaved similarly over the first 5 moults. Both GF and IMP responses result in a general faster growth rate than would be expected from a logarithmic response (Mauchline, 1985). For *S. armata* the IMP was constant over the first 10 moults until the reproductive cycle started (Cuzin-Roudy *et al.*, 1981). However, no relationship was found in the present study between the variation in IMP/GF and sexual development.

The intermoult period in the experiments was strongly temperature dependent and became smaller at higher temperatures, similar to previous studies with mysids (Astthorsson and Ralph, 1984; Winkler and Greve, 2002) and other crustaceans (Nicol and Stolp, 1990). To a lesser degree, we also found the IMP to be salinity dependent, with the shortest IMP at 15 psu. The growth increment (expressed as GF) was affected by temperature, especially at 25 °C where the GF was substantially lower (Hartnoll, 1982). Thus the fast growth at higher temperatures is caused by a higher moulting frequency and not by a higher size increment at moulting as reported by Astthorsson and Ralph (1984).

Neomysis integer is described as thermophobic (Arndt and Jansen, 1986) with optimal resistance to salinities higher and lower than its isosmotic point (16 – >20 psu) in the lower temperature range. Temperatures of 20 °C and higher have an adverse effect on the respiration, especially in juveniles (Arndt and Jansen, 1986). Largest animals, i.e. with maximal asymptotic length, and low mortality were indeed obtained at 15 °C in comparison with higher temperatures. At 15 °C, the highest growth rate was obtained at 15 psu, being the salinity closest to the isosmotic point. It is however difficult to explain why at 20 °C the highest growth rates were observed at 5 psu.

Size at sexual differentiation and maturity

In contrast to male mysids, it is difficult to determine maturity or the development of secondary sexual characteristics in females by looking at the exoskeleton. Even in early subadults, it remains hard to distinguish the small marsupium on the living animal while it is swimming around. Therefore, the moult number at the transition from juvenile to subadult and from subadult to adult in females was extrapolated from the information collected for males. This was possible as the von Bertalanffy growth parameters were not significantly different between genders.

In general, increasing temperature shortens the time to reach sexual differentiation and maturation in general. This was very obvious in animals from the lowest temperature treatment which did not sexually develop during the course of the experiment. A cessation of growth of *Neomysis integer* during the colder winter months was reported previously based on field data (Astthorsson and Ralph, 1984; Arndt and Jansen, 1986; Mees *et al.*, 1994). From experiments with this species at 9°C (Astthorsson and Ralph, 1984) the duration to maturity (15 mm total length) was extrapolated to be at least 277 days.

Within the range 15 – 20 °C, salinity seems to have a stronger effect than temperature on sexual maturation. The highest tested salinity of 30 psu retarded the development (in age and moult number) at 20 °C. In both the 30 psu treatments (15 and 20 °C) maturity was never reached. Sexual differentiation was generally reached at a length of 4.3 – 4.5 mm, except at higher salinities at 20 °C (5.00 – 5.59 mm). Size at maturity is smaller at higher temperature and lower salinity.

Interpopulation effects

Salinity-temperature conditions for optimal growth might vary between different populations of the same species for genetic and phenotypic reasons (Lee, 1999). *Neomysis integer* used in the present experiments originated from the Galgenweel, a brackish pond with a relatively constant salinity of 5 psu. Population genetic analysis (based on mitochondrial cytochrome oxidase I sequences) revealed no differentiation between *N. integer* from the experimental source population (Galgenweel) and the Schelde estuary population (Remerie *et al.*, submitted a). The results of the present experiments can therefore be considered representative for the *N. integer* population of the Schelde estuary.

It is unknown how growth responses to temperature-salinity conditions vary between populations from different latitudes (temperature effect). *N. integer* of the Baltic Sea population died within 2.5 weeks when held at 20 °C and showed increased respiration rates. These animals rarely experience temperatures above 15 °C in their natural environment and never for periods exceeding a few days at a time (Laughlin and Lindén, 1983). Kuhlman (1984) reported an optimal growth at 19 – 21 °C and 16 – 20 psu for juvenile *N. integer* from the Kiel Canal.

Winkler and Greve (2002), working with *N. integer* collected from the Elbe estuary, reported a faster maturation (110 days at 10 °C; 45 days at 15 °C – 20 psu) at a smaller size (standard length 8 – 9 mm at 10 °C and 7 mm at 15 °C) than our population. In our most comparable treatment (15 °C and 15 psu) to the Winkler and Greve study, adulthood was reached after 89 days at a length of 12 mm. *N. integer* from the Ythan estuary cultured at 16°C and ± 10 psu (~ 30 % seawater) were mature after 188 days at a standard length of 12.9 mm (Astthorsson and Ralph, 1984). This variation might indicate inter-population variation, although other factors (like food quality and quantity, flow regime in tanks, size of the recipients, etc.) might also be at the basis of the variation between experimental results.

Validation of field-derived growth parameters

To date, field-derived von Bertalanffy growth parameters have rarely been validated with laboratory observations. This can be done by means of comparing the multivariate growth performance index Φ' (Munro and Pauly, 1983). Φ' is expected to be basically equal within different populations of the same species and within different stocks of the same population, but can differ because of pollution, environmental stress or differences in habitat (Moreau *et al.*, 1986).

The growth performance index Φ' in all our experimental treatments was significantly higher than in all cohorts of the field populations of Schelde and Galgenweel. This is probably the effect of the *ad libitum* feeding with the high-energy containing *Artemia* nauplii and little energy loss by restricted swimming activity in the static experimental conditions. Abiotic stress, as reflected in the growth performance, was primarily caused by temperature and only secondarily by salinity (both in the field and in the experimental treatments). In the highly dynamic estuarine habitat tidal, daily and seasonal variation of these environmental factors may have a great (adverse) effect on the growth of *N. integer*.

Conclusions

Based on the present study, it can be concluded that higher temperatures caused a smaller intermoult period in *Neomysis integer*. However, temperature does not seem to have an effect on the growth factor, except at 25 °C where suboptimal growth occurred. Salinity had a secondary effect on the growth (IMP and GF) of *N. integer* in comparison to temperature, and was temperature dependent. At 8 °C, *N. integer* grew slowly because of long intermoult periods and a relatively low growth factor. At 15 °C, mysids had a larger GF, but also a larger IMP in comparison with 20 °C. Consequently, they took longer to grow, but grew to a larger body length. At 25 °C, animals had the shortest IMP, but also had a significantly lower growth increment at moulting. At 15 °C, the optimal salinity for growth was 15 psu, whereas at 20 °C the shortest IMP and largest GF were found at 5 psu.

Survival and growth of *Neomysis integer* was possible within the tested range of temperatures (8 – 25 °C) and salinities (5 – 30 psu), but maturation was only possible in a smaller range of 15 – 25 °C and 5 – 15 psu. Within this range, the size at sexual differentiation was constant, but the size at maturity increased with decreasing temperature and increasing salinity.

In comparison with field populations of *Neomysis integer* of the Schelde estuary and Galgenweel the growth performance was higher in all the experimental treatments. Abiotic stress was primarily caused by temperature and only secondarily by salinity.

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Chapter 4

Survival, growth and feeding rate of the mysid *Neomysis integer* (Crustacea: Mysidacea) on laboratory-made estuarine aggregates

ABSTRACT

Laboratory-generated aggregates (flocs) made from natural estuarine water of the oligohaline part of the Schelde estuary (Belgium) were administered to the brackish water mysid *Neomysis integer* in order to determine their value as a dietary item. Survival, growth, intermoult period, growth factor and intermoult growth rate of subadult mysids (4 – 10 mm standard length) were monitored over 4.5 weeks in a roller table. In a first experiment, the effect of tidal dynamics on the floc formation process, as well as on the floc size and shape was followed. Also, we evaluated the effect of continuous rotation in the roller tanks on the growth of *N. integer* to be negligible. Subsequently, we performed a growth experiment with *N. integer* reared on the laboratory-made aggregates and estimated the feeding rate.

The estuarine aggregates are a valuable food source for the mysids as they showed good survival (80 %) and grew substantially on this dietary item (0.08 ± 0.01 mm d^{-1}), although growth is slower than on *Artemia* nauplii (0.11 ± 0.01 mm d^{-1}). The high feeding rate of subadult *Neomysis integer* on the laboratory-made flocs (38 flocs ind^{-1} h^{-1}), may compensate for their low energetic value. The roller table is an adequate tool for feeding experiments with *N. integer* on the fragile estuarine aggregates.

For *Neomysis integer* living in the maximum turbidity zone of estuaries, the estuarine flocs may be an important additional food source, especially in periods when mesozooplankton prey (mainly calanoid copepods) is scarce. The rich bacterial and protozoan communities associated to the flocs as well as the incorporated amorphous organic matter, normally too small to be efficiently consumed by mysids, become part of their diet. This pathway thus constitutes a short-cut in the estuarine food chain.

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INTRODUCTION

The brackish part of estuaries is characterized by high concentrations of suspended aggregates composed of a large mass of fine sediment particles, biogenic debris and fluffy organic material (Chen *et al.*, 1994). These microflocs (< 125 µm) collide with each other, and with mineral grains, detritus, phytoplankton, faecal pellets and macrophytal debris to form subsperical macroflocs with a length up to 3 – 4 mm (Eisma, 1986; Zimmermann and Kausch, 1996). These macroflocs are very fragile, and easily deflocculate and reflocculate in the water column (Eisma, 1986; Maldiney and Mouchel, 1995). The components of estuarine micro-aggregates are strongly held together within a matrix of exopolymers released by bacteria, algae and higher plants, or carbohydrates mobilized from the suspended and dissolved fractions at low salinity (Eisma, 1986; Zimmermann-Timm *et al.*, 1998; Artolozaga *et al.*, 2002).

The aggregates are intensively colonized by micro-organisms and sometimes metazoans (Alldredge and Silver, 1988; Shanks and Edmondson, 1990). In comparison with the surrounding water, estuarine flocs are enriched with bacteria, amoebae, ciliates and rotifers and contain 50 – 80 % of the total densities of these organisms in the water column (Zimmermann and Kausch, 1996; Zimmermann, 1997; Zimmermann-Timm *et al.*, 1998), even though the living biomass contributes less than 3 % to the total organic carbon in the flocs (Muylaert *et al.*, 1999). Respiration of macrofloc-associated bacteria and protozoa amounts to 84 – 94 % of the total respiration in the water column of the upper Elbe (Ploug *et al.*, 2002). The densities of the micro-organisms on flocs are of the same order as in the sediments (Wörner *et al.*, 2000).

The number of large macro-aggregates (> 400 – 3000 µm) in the water column of the Elbe estuary (Germany) was estimated with an *in situ* camera at 20 to 4000 flocs l^{-1} (Zimmermann and Kausch, 1996; Ploug *et al.*, 2002). The aggregates sink out of the water column at a faster rate than their individual components (Wolanski, 1995), thus forming a potentially important energetic link between pelagic and benthic communities (Eisma, 1993a). However, flocs in a turbid estuary probably undergo several sedimentation and re-suspension events (Ploug *et al.*, 2002).

The catchment basin of the Schelde estuary (The Netherlands – Belgium) is a strongly industrialized and densely populated area (Wollast, 1988). As a consequence, the river is highly polluted by domestic and industrial waste water and agricultural run-off (Heip, 1988; Baeyens *et al.*, 1998). The estuary acts as a bio-filter that changes the organic contamination qualitatively and quantitatively (Soetaert and Herman, 1995b). The highest turbidity in the Schelde is generally measured in the oligohaline and freshwater zone between Doel and Temse (Baeyens *et al.*, 1998; Chen, 2003), but the maximum turbidity zone (MTZ) shifts its position along the longitudinal axis of the estuary depending on the tides and the freshwater run-off (Eisma, 1986).

In general, marine snow and estuarine aggregates can be considered as sediment-like, nutrient rich micro-patches within the pelagic environment (Silver *et al.*, 1978; Alldredge and Silver, 1988). The numerous aggregates may be grazed upon directly by larger mesozooplankton, mysids and fish, as is demonstrated for marine snow (Lampitt *et al.*, 1993; Artolozaga *et al.*, 2002). Aggregates (< 65 µm) have been shown to be an important item present in the stomachs of the brackish water mysid *Neomysis integer* in the MTZ of the Schelde, Elbe and Gironde as demonstrated by gut content analyses combined with Energy Dispersive Spectroscopy (EDS) X-Ray Microanalysis (EDAX) (Fockedey and Mees, 1999 – Chapter 2). However, it is not clear if *N. integer* actively search for and feeds on the aggregates or if they accidentally swallow them while preying on other prey items (mainly calanoid copepods).

The aim of this study was to identify the nutritional importance of flocs for the brackish water mysid *Neomysis integer* by assessing their survival and possible growth when feeding solely on estuarine aggregates. Because estuarine macroflocs fall apart upon sampling in strongly bounded microflocs, we modified an experimental device (Shanks and Edmondson, 1989) – originally designed for the formation of marine snow – in which flocs are continuously regenerated from estuarine water. We first tested effects of tidal dynamics on the process of floc formation. Also we evaluated the effect of continuous rotation in the roller tanks on the growth of *N. integer*. Subsequently, we performed a growth experiment with *N. integer* reared on the laboratory-made aggregates and estimated the feeding rate.

MATERIAL AND METHODS

Water sampling and site description

The aggregates used in the experiments were made of water collected from the Schelde estuary. The sampling station Sint-Anneke (51°14.0' N – 4° 23.8' E) is situated all year through in the MTZ of the estuary (Baeyens *et al.*, 1998). Samples of the 0.5 m surface layer were taken by bucket from a pontoon above an intertidal mud flat. Portable conductivity and oxy-meters (type WTW) were used for the measurement of water temperature, salinity and dissolved oxygen concentration. Turbidity was measured with a portable microprocessor turbidity meter (type HANNA; in Formazine Turbidity Units or ftu). The collected water was transported to the laboratory in plastic containers of 50 l, where it was aerated while being stored at 15 °C in the dark (for a maximum of 3 days).

Sampling of Neomysis integer

Due to logistical restrictions, no mysids could be sampled from the estuarine sampling station, but were instead collected in the brackish pond Galgenweel (51°12.7' N – 4° 22.1' E) near Sint-Anneke. The mean annual salinity of the pond is 4 psu. A large population of *Neomysis integer* was present in the pond and its population dynamics and production have been formally studied (Fockedey, unpublished). The mysids were collected from the shallow south-western edge of the pond with short hauls using a hand net with a mouth of 0.3 x 0.2 m and a mesh size of 1 x 1 mm. The organisms were transported and kept in the laboratory in ambient water with aeration at 15 °C prior to the experiments.

Laboratory-made aggregates

Aggregates were generated using the adapted device of Shanks and Edmondson (1989). Ten plexi-glass cylindrical containers (diameter 14 cm; depth 5.5 cm; volume 0.847 l) with estuarine water were placed on two parallel rotating bars on a roller table (Figure 1a) and rotated at a velocity of 10 – 11 rpm. Before filling, the water was passed through a 250 µm sieve to exclude larger plankton and detritus. While filling the cylinders, the water was mixed at low speed with a paint mixer to homogenize the suspended matter.

The tanks were photographed while rotating (Leica camera with 1/2.8/60 mm macro-objective; diaphragm 8; closing time 1/60; distance 25 cm; indirect flash). It was impossible to focus on the complete depth of the rolling tank. Therefore, a movable small, white plastic plate (diameter 4.8 cm) was mounted in the container to use as a background to focus on a limited part of the water column (Figure 1b). Depending on the floc density, it was positioned at a depth of 1 or 2 cm, estimates of floc densities based on photographs taken at either depth not differing significantly.

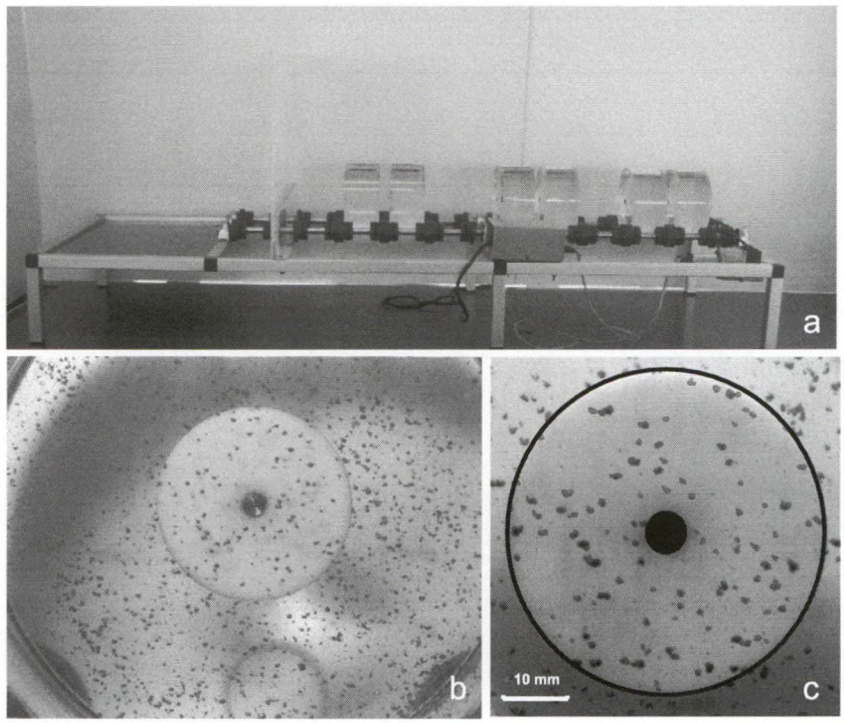

Figure 1: (a) Roller table adapted from Shanks and Edmondson (1989); (b) detail of a plexi-glass cylinder photographed while rotating; (c) a movable white plastic plate (diameter 48 mm) creates a background (1 or 2 cm deep) for the detailed picture used for counting and sizing of the generated flocs by image analysis.

Before and after taking the pictures, this plate was pushed against the container wall to prevent interference with floc-formation. Three pictures were taken of each tank per observation (Figure 1c). Black and white photo film of 100 ASA gave the best results for later measurement of the number and size of the aggregates by means of image analysis (Leica Quantimet 500+). The surface area, length, width and perimeter of all individual macro-flocs, with a length larger than 250 µm (> 2 pixels), were measured. The number of aggregates present in the water volume in front of the white plate was recalculated to number per litre.

Measuring growth

The individual growth of the mysids was indirectly assessed from the size increments of successive moults shed by an individual (Fockedey *et al.*, in press – Chapter 3). Moulting in Mysidacea mainly takes place during the dark and mysids are known to eat their moults rapidly. Therefore, the experimental containers were checked daily at the beginning of the light period. The moults produced by the mysid in the roller tanks quickly fell apart due to the rotation. The waste water of the former day was sieved over a 106 µm sieve to find the uropode exopodites, which were then preserved in 4 % formaldehyde. With a drawing mirror and a digitising tablet, the length of the exopod (EXO) of the uropod was measured and recalculated to pre-moult standard length (SL) by means of the regression (Fockedey *et al.*, in press):

$$SL = 1.085566 + 4.081793\ EXO \qquad (p<0.0001;\ R^2: 0.9569;\ N=97)$$

At the start of each experiment, subadult animals of a specific size range were selected (as specified further). This was done by laterally measuring the standard length (*i.e.* the distance between the eye bases and the basis of the telson) under a stereomicroscope equipped with a drawing mirror. Little illumination was used to prevent stress during handling and measuring. The growth was followed for 3.5 weeks (experiment 2) or 4.5 weeks (experiment 3). Although the overall growth of *Neomysis integer* at 15 °C is best described by the generalized von Bertalanffy growth model (Fockedey *et al.*, in press), the subadult individuals of the selected size classes grew linearly during the course of the experiment. Linear regression analysis was applied and the slope and elevation of the linear regression equations was tested between treatments (Zar, 1996).

The intermoult growth rate (GR; in mm d^{-1}), the intermoult period (IMP; in days) and the growth factor (GF; in %) were calculated (Mauchline, 1977) and compared between experimental treatments at each moult event by pairwise comparisons (Mann-Whitney U-tests).

Experiment 1 – Effect of tidal dynamics on the floc formation process

Flocculation is a dynamic process with the state of flocculation changing with time (Lick *et al.*, 1992; Chen *et al.*, 1994). The aim of the first experiment was (1) to determine the time needed to obtain a stable situation in the floc formation process within the roller tanks, and (2) to study the effect of the tidal phase at the moment of water sampling on this process. Estuarine water was sampled on 9/4/1998 at 2 hours and 1 hour before high tide, at high tide (HT) and 1 and 2 hours after high tide and treated as described before. For each tidal situation, 2 containers were set up on the roller table: one for the measurement of dissolved oxygen concentration during the floc formation process and another not-manipulated replicate for the quantification and sizing of the aggregates (with photography and image analysis) by monitoring at time 0, 10 min, 20 min, 35 min, 1h, 1.5h, 2h, 3h, 4h, 6h, 8h and 18h after setup. Next to the number of macro-aggregates, the surface area S, length L, width W, perimeter P and the spherical equivalent diameter SED, the elongation L/W, circularity $4\pi(S/P^2)$ and roundness $4S/\pi L^2$ (Billiones *et al.*, 1999) were also calculated to describe the shape of the flocs. The experiment was performed in a climatized chamber at 15 °C and under constant illumination.

Experiment 2 – Impact of rotation on growth of Neomysis integer

A comparison was made between the growth performance of subadult *Neomysis integer* individuals in rotating tanks (content 847 ml; 8 replicates) with individuals in glass jars under static conditions (content 350 ml; 10 replicates). Both treatments were performed with artificial seawater of 5 psu (Instant Ocean) and were given freshly hatched (< 48 h) *Artemia* nauplii as food (4000 l^{-1}). The selected *N. integer* individuals (4 – 6 mm standard length) were adapted for 2 days to a diet of *Artemia* nauplii prior to the start of the experiment. The experiment was performed at room temperature (16 – 22 °C) with a 14h light:10h dark photoperiod. Salinity and temperature were monitored daily and any shed moults were collected. No aeration was provided, but the water was replaced and fresh food supplied daily. The experiment lasted for 23 days (24/10/1997 – 15/11/1997).

Experiment 3 - Survival and growth

The survival and growth of *Neomysis integer* on a diet of laboratory-made flocs were studied over a period of 32 days (from 19/02/1998 till 22/03/1998). As a control, the survival and growth of identically sized individuals feeding on *Artemia* nauplii (4000 l^{-1}) were followed. The experiment was set up in a climatized chamber of 15 ± 1 °C and a light regime of 12 h light:12 h dark. The selected *N. integer* individuals (4 – 6 mm standard length) were allowed to adapt for 3 days to either a diet of natural suspended matter from the maximum turbidity zone of the Schelde estuary or *Artemia* nauplii (control). Afterwards, they were put individually in rotating tanks (847 ml; 10 replicates) with estuarine water on the roller table or in static jars (content 350 ml; 15 replicates) in artificial seawater of 5 psu (control).

The water in the rolling containers was changed daily and the waste water checked for shed moults. Every day the salinity, temperature, dissolved oxygen, suspended particulate material (SPM), particulate organic material (POM) and C:N ratio (Carlo Erba CHN elemental analyser) were monitored. The number and size of the flocs were estimated by means of 3 pictures taken after at least 3 hours of rotation.

Every two to three days, new Schelde water was collected at the predicted high tide (± 30 minutes) and kept in the dark under stable temperature conditions (15 ± 1°C) while aerated for later use in the experimental units.

Experiment 4 – Feeding rate

Using the same photographic techniques as described above, a conservative estimate of the feeding rate of *Neomysis integer* on laboratory-made aggregates was established. The cylinders were filled with estuarine Schelde water collected on 29/04/1998 and floc formation was started. After 3 hours the generated aggregates were photographed (t_0) for counting and sizing. Five *N. integer* individuals with a standard length between 7 and 10 mm were then put into each rolling tank (845 ml; 10 replicates) and allowed to feed on laboratory-made aggregates for 24 hours after which the remaining aggregates were quantified and sized again (t_{24h}). The animals had first been adapted to this diet for 4 days. The experiment was performed in a climatized room of 15 ± 1 °C and under 12h light:12h dark conditions. The feeding rate on laboratory-made aggregates (in # aggregates ind^{-1} h^{-1}) was estimated by:

$$\text{Feeding rate} = \frac{\#\text{ aggregates at } t_0 - \#\text{ aggregates at } t_{24h}}{24\text{h} \cdot 5\text{ ind}}$$

The decrease in the floc concentration (overall and within each 0.2 mm length class) over the course of the experiment were statistically tested using a non-parametric Wilcoxon matched pair test.

RESULTS

Experiment 1 – Effect of tidal dynamics on floc formation in roller tanks

The salinity of the water mass sampled varied between 0.5 psu (2h before HT) and 2.6 psu (1h after HT). The turbidity was maximal at high tide (66 ftu) and generally higher before (28 –34 ftu) than after (20 – 22 ftu) high tide. The water temperature recorded in the field was 11.9 – 12.2 °C. Dissolved oxygen concentrations generally dropped by 1 to 1.5 mg l^{-1} during the course of the experiments in comparison with the initial values at start-up (4.4 – 5.8 mg l^{-1}).

The formation of macro-aggregates in the rolling tanks started within 20 to 120 minutes after the start of the experiment. A stable situation, referring to the number of flocs (Figure 2), floc size and shape (Figure 3), was encountered after ± 3 – 4 hours of rotation (as indicated with the vertical line on all graphs). The highest number of flocs is generated with water taken at high tide (28000 – 38000 l^{-1}), and generally higher with water from before (7000 – 14000 l^{-1}) than from after (2000 – 6000 l^{-1}) high tide.

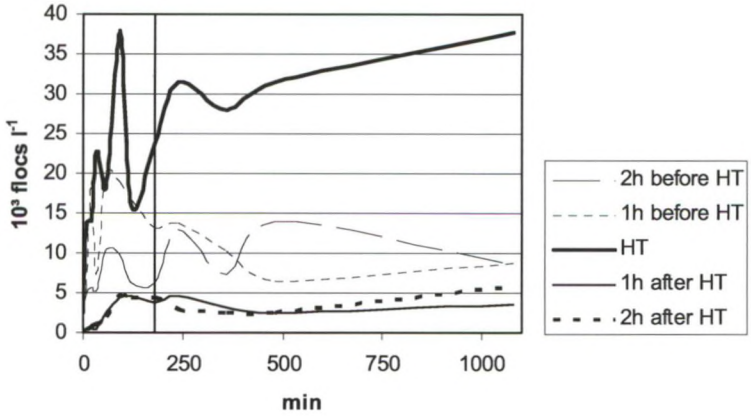

Figure 2: Number of macro-aggregates generated over 18 hours with water from 5 sampling occasions within the tidal cycle. The vertical line visualises the time (180 – 240 min) of obtaining a relative steady state.

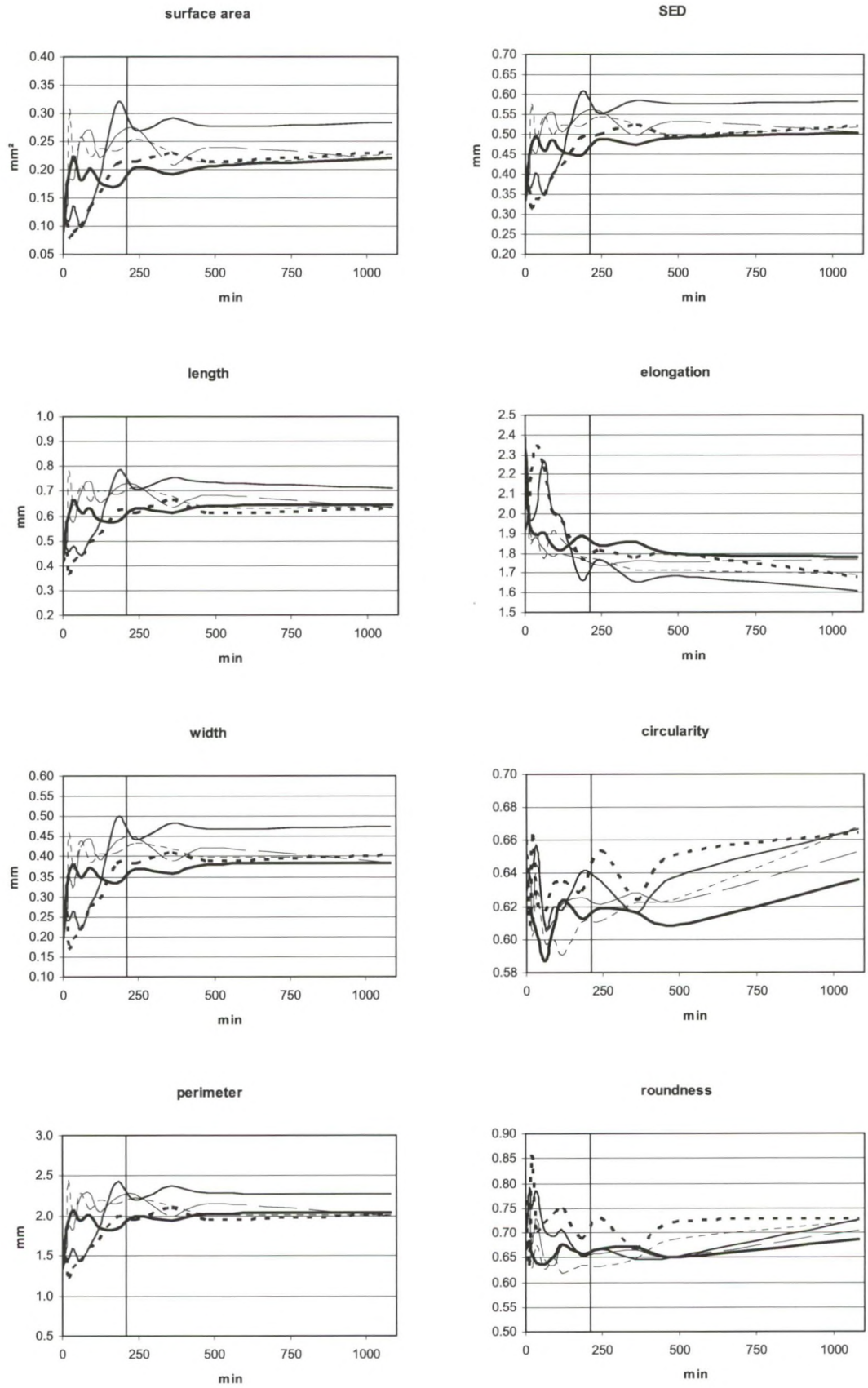

Figure 3: Size and shape factors of the macro-aggregates generated over 18 hours with water from 5 occasions within the tidal cycle. The vertical line visualises the time (180 – 240 min) when a steady state is assumed. Legend as in Figure 2.

In steady state (> 4 h) the mean floc surface area for each treatment ranged between 0.19 and 0.29 mm², with a mean length between 0.61 and 0.76 mm and a mean width between 0.36 and 0.48 mm. The mean SED varied between 0.47 and 0.58 mm, and the mean perimeter between 1.94 and 2.37 mm. The length of the aggregates equalled 1.6 to 1.9 times their width (elongation). The circularity varied between 0.61 and 0.67 and increased with time in all treatments, as the aggregates rounded up. Roundness ranged between 0.63 and 0.73 and followed the same pattern as circularity, although not so pronounced. The variation (SE) on the number, size and shapes of the flocs between the 3 pictures per situation was generally smaller than 5 % of the mean value.

The length frequency distributions of the flocs were generally unimodal with the modus relatively stable over time in all treatments (0.4 – 0.6 mm). Only in the situation where water from 1h after high tide was used, the length frequency distribution became bimodal after 3h of rotation with modes at 0.4 – 0.7 and 0.9 – 1.0 mm length.

Despite the absence of replication, the observed size and shape of the generated flocs differ only moderately according to the tide. Therefore, only high tide water was used in subsequent experiments, since this yielded the highest floc numbers in the roller tanks.

Experiment 2 – Impact of rolling tanks on growth of Neomysis integer

Individuals of *Neomysis integer* kept in the cylinders on the roller table were constantly moving. They were swimming against current to keep their position or hung on the side wall of the tank, rotating at the same speed as the containers. In the rotating cylinders, all individuals except one survived the experiment. The individual growth (as the increase in standard length) was linear in both the rotating tanks and the static containers. The inclination and elevation of both linear regression equations based on all measuring points within each treatment (Figure 4) were not significantly different (respectively p=0.052 and p=0.071).

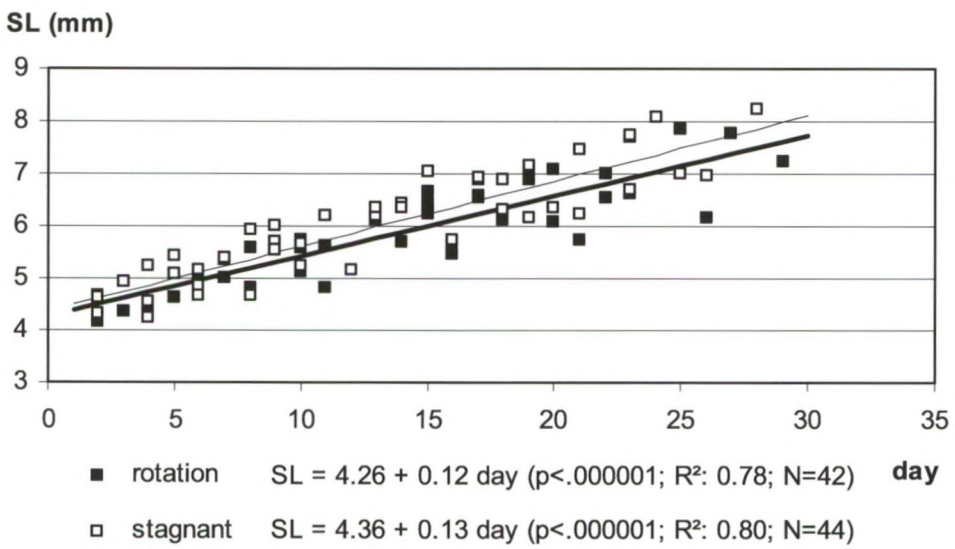

Figure 4: Growth of *Neomysis integer* in the rotating cylinders and static containers. Linear regression analyses performed with all measurement points within each treatment.

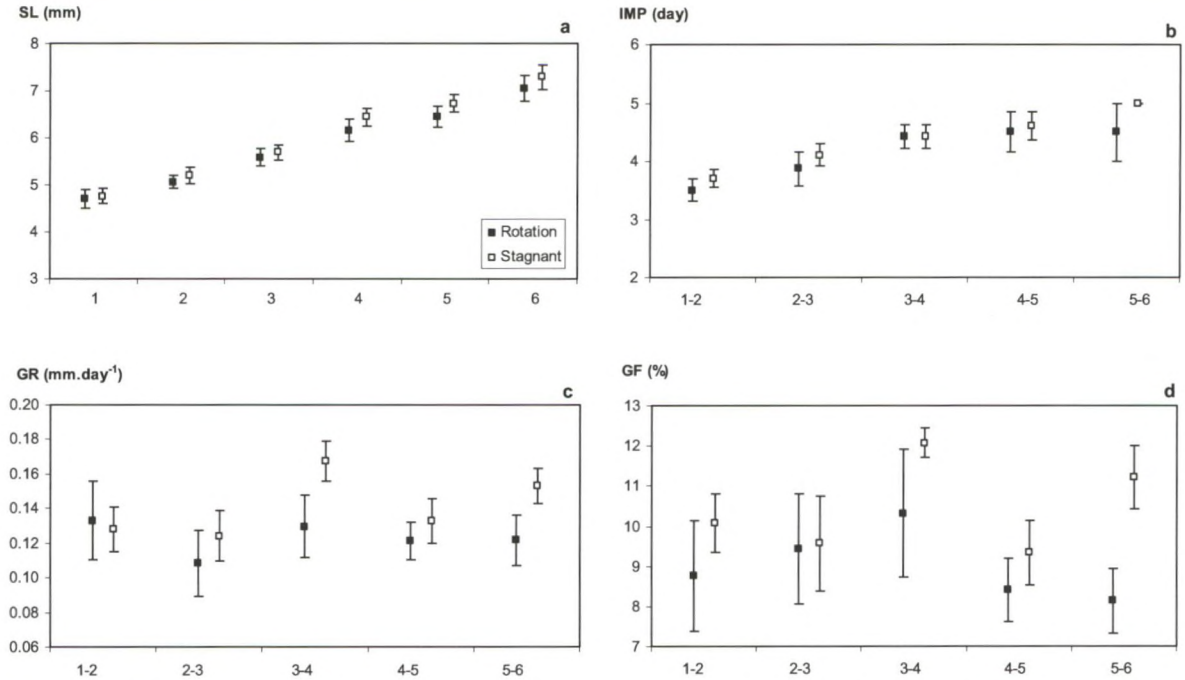

Figure 5: Pre-moult standard length SL at each moulting event (a), intermoult period IMP (b), intermoult growth rate GR (c) and growth factor GF (d) in between successive moults in the rotating cylinders and the static containers.

The animals grew on average from 4.69 ± 0.20 to 7.06 ± 0.27 mm in the rotating cylinders and from 4.76 ± 0.17 to 7.29 ± 0.26 mm in the static jars (Figure 5a). The pre-moult standard length for each moult did not differ significantly between the two treatments (all pairwise comparisons: NS). No significant differences were found in the IMP between the two treatments (all pairwise comparisons: NS) and a gradual increase in the IMP from 3.61 ± 0.12 to 4.67 ± 0.33 days was observed (Figure 5b). In between the successive moult stages the mean GR varied between 0.11 ± 0.02 and 0.17 ± 0.01 mm d^{-1} and the mean GF between 8.1 ± 0.8 and 12.1 ± 0.37 %. No consistent patterns could be detected over the course of the experiment, nor between the treatments (Figure 5c, d; all pairwise comparisons: NS).

So, the effect of rotation on the growth performance of *Neomysis integer* (size class 4 – 8 mm) is negligible. In further experiments, the static jars were used to conduct the control treatments.

Experiment 3 - Survival and growth

Over the course of the experiment, the salinity of the collected estuarine water ranged between 1.3 and 6.5 psu, the lower values being caused by heavy rainfall between day 11 and 25 (Figure 6a). The oxygen concentration of the estuarine water *in situ* ranged between 3.4 and 5.4 mg l^{-1} over the 4.5 weeks. Direct use of this O_2-poor water in the growth experiment caused a 100 % mortality of *Neomysis* individuals within 1 day (preliminary experiment). Thus, aeration was necessary before use (start concentrations: 6.1 – 8.7 mg l^{-1}). After one day in the rolling tanks with 1 mysid, the dissolved oxygen concentration reduced maximally with 2.8 mg l^{-1}. The SPM and POM concentrations of the administered water varied according to a semi-lunar cycle (Figure 6b): periods after spring tides were generally associated with high SPM (> 140 mg l^{-1}) and high POM values (> 26 mg l^{-1}); periods following neap tides with lower SPM (< 100 mg l^{-1}) and lower POM concentrations. Despite the high variation in suspended matter concentration, the ratio POM:SPM did not show a relationship with the spring-neap tide cycle and was fairly constant during the whole experiment; *i.e.* POM varied between 15 and 32 % of the SPM.

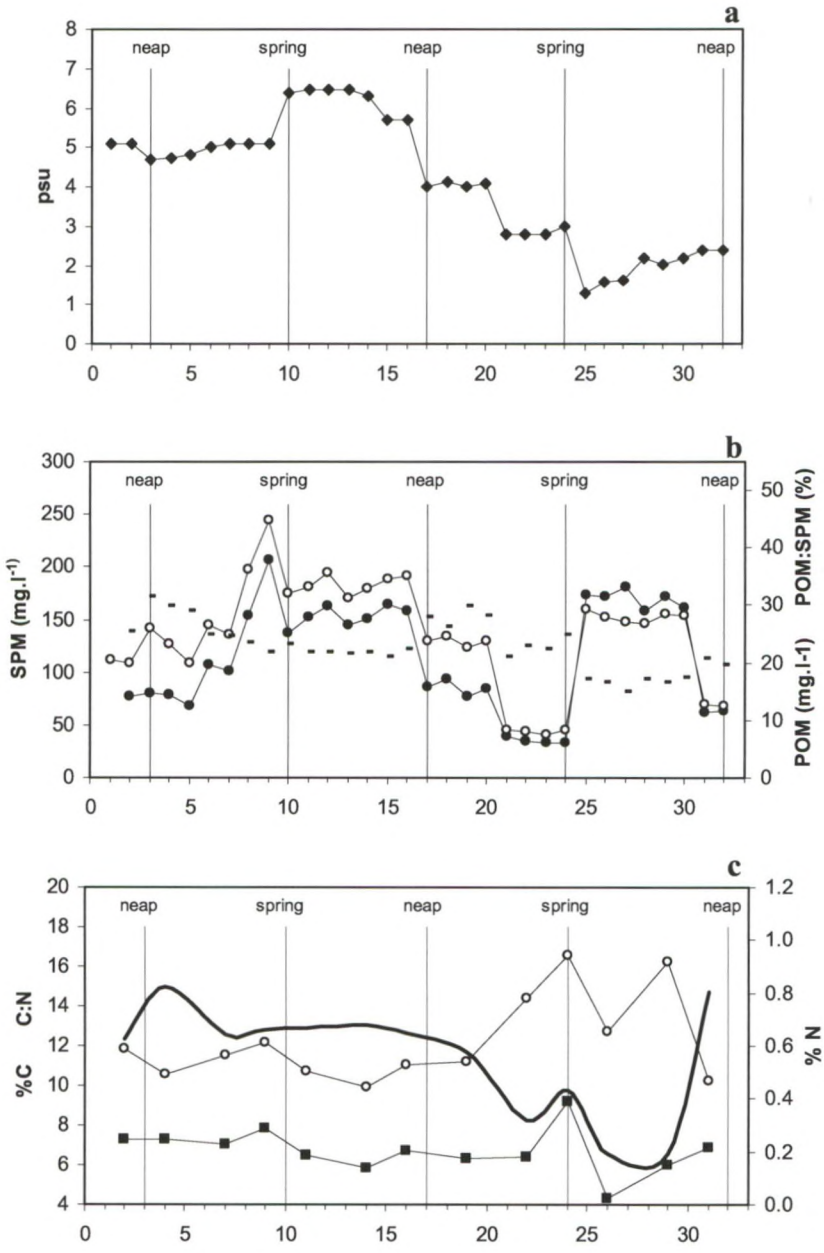

Figure 6: Environmental variables of the estuarine water sampled over the course of the growth experiment with *Neomysis integer* feeding on laboratory-made flocs: (a) high tide salinity; (b) SPM (black dot), POM (white dot) and POM:SPM ratio (hyphen); (c) Carbon content (black square), nitrogen content (white dot) and C:N ratio (smooth line) of the suspended particulate matter. Standard error generally less than 5 % of the measured value.

Storage of environmental water in the laboratory (in a dark 15 °C climatized room with constant aeration) for up to 3 days did not affect the SPM or POM concentrations. The total carbon in the SPM (expressed as % of the dry weight) of the administered water varied between 4.3 and 9.2 %, while the total nitrogen accounted for 0.45 to 0.94 % (Figure 6c). The C:N ratio stayed relatively stable until the 19th day of the experiment (mean C:N = 12.9 ± 0.3). Between day 19 and 29, the C:N ratio was substantially lower (C:N = 7.8 ± 0.8).

The number of laboratory-made aggregates in the roller tanks varied between 744 ± 101 and 32989 ± 372 aggregates l^{-1} (Figure 7a) and was highly correlated with the SPM (Spearman R: 0.8851; N = 41; p = 0.0000) and POM values (Spearman R: 0.8727; N = 42; p = 0.0000). The mean size of the flocs was relatively constant (Figure 7b, Table 1) and was not related to the amount of suspended material. The maximal dimensions, though, were related to the SPM concentrations (Figure 7b) and thus to the number of aggregates l^{-1}.

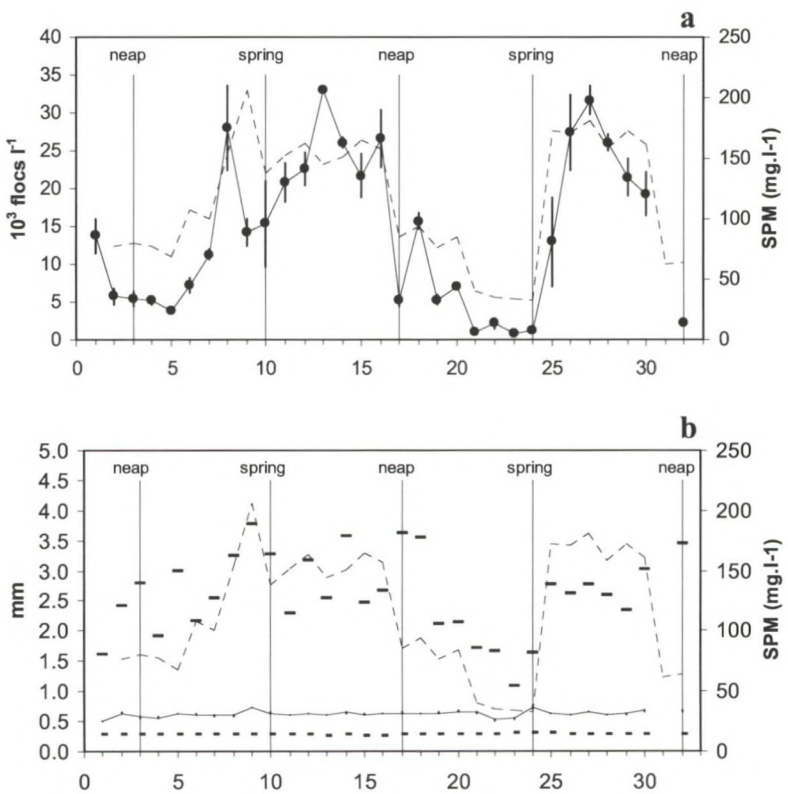

Figure 7: Concentration (a) and maximal length (large hyphen in b) of the laboratory-generated aggregates is strongly correlated to the SPM values over the course of the experiment. Mean length (black line in b) and minimal length (small hyphen in b) are not related to SPM concentrations. Error bars indicate standard errors.

Table 1: Mean number (± SE) and dimensions of the laboratory-made flocs over the course of the growth experiment

		mean ± SE	min	max
Number of aggregates	number l^{-1}	12349 ± 1586	522	32989
Length	µm	0.615 ± 0.007	0.255	3.770
Width	µm	0.369 ± 0.006	0.127	2.504
Surface area	mm^2	0.209 ± 0.005	0.065	3.691
Perimeter	mm	1.972 ± 0.023	1.019	19.114
Spheric equivalent diameter (SED)	mm	0.490 ± 0.6	0.288	2.168
Elongation		1.852 ± 0.015	1.000	11.031
Circularity		0.632 ± 0.000	0.097	0.836
Roundness		0.696 ± 0.005	0.113	1.284

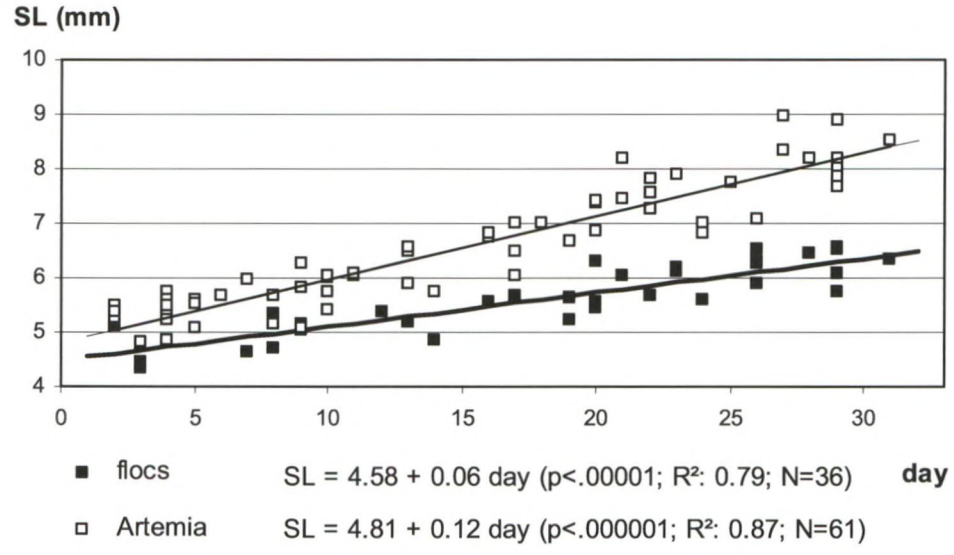

Figure 8: Growth of *Neomysis integer* feeding on laboratory-made flocs and *Artemia* nauplii. Linear regression analyses performed with all measurement points within each treatment.

Neomysis integer fed laboratory-made flocs showed a good survival over the course of the 32 day experiment (80 %); only at day 26 and day 28 single individuals died. Mortality in the controls was comparable (73 %) and appeared on day 2, 14, 20 and 23. The surviving individuals moulted minimally 5 times. The animals fed laboratory-made aggregates grew significantly, although with a slower rate compared to the control individuals fed *Artemia* nauplii (Figure 8), as indicated by a significantly lower inclination of the linear regression ($p = 0.00074$). The animals grew on average from 4.84 ± 0.13 to 6.28 ± 0.15 mm on flocs and from 5.36 ± 0.08 to 8.21 ± 0.13 mm on *Artemia*.

The standard length at each moult (Figure 9a) of *Artemia* fed *N. integer* was always significantly larger than the ones feeding on flocs (all pairwise comparisons, except 2nd moult: $p < 0.01$). Unfortunately, the initial standard length of the selected individuals of the *Artemia* treatment was significantly larger (5.36 ± 0.8 mm) than for the floc treatment (4.84 ± 0.13 mm), and was an unforeseen error within the experimental set-up. A gradual increase in the IMP was observed over the course of the experiment from 5.38 ± 0.10 to 6.83 ± 0.27 days (Figure 9b), but no significant differences were found between the two treatments (all pairwise comparisons: NS). In between successive moult stages, the mean GR varied between 0.064 ± 0.015 and 0.095 ± 0.024 mm d^{-1} and 0.078 ± 0.004 and 0.130 ± 0.014 mm d^{-1}, respectively for the treatments 'flocs' and '*Artemia*' (Figure 9c). The GR of the animals fed *Artemia* was significantly higher in the last two intermoult periods ($p < 0.05$). The mean GF varied between 7.0 ± 1.6 and 10.2 ± 1.6 % in the floc treatment and between 7.8 ± 0.4 and 13.4 ± 1.3 % in the *Artemia* treatment. No patterns could be detected over the course of the experiment, nor between the treatments (Figure 9d).

It can be concluded that *Neomysis integer* can survive and substantially grow when feeding on laboratory-made aggregates, although with a slower growth rate than when fed *Artemia* nauplii.

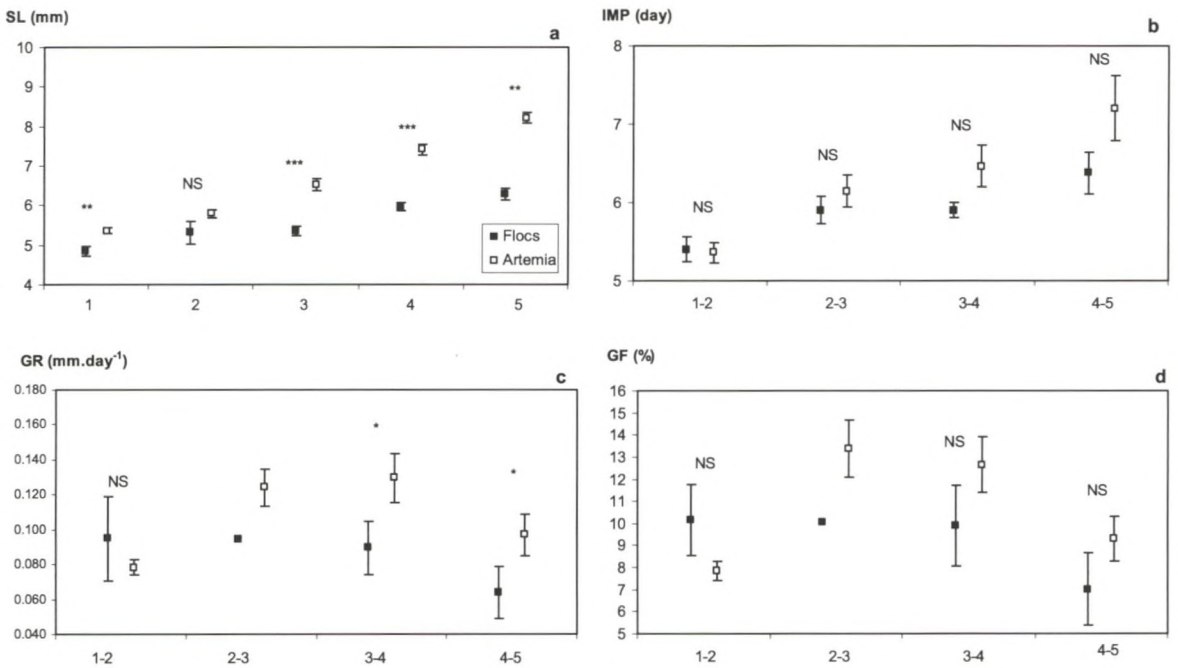

Figure 9: Pre-moult standard length SL at each moulting event (a), intermoult period IMP (b), intermoult growth rate GR (c) and growth factor GF (d) in between successive moults in the floc and *Artemia* fed *Neomysis integer* individuals. Mann-Whitney U-test results are indicated (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; NS: not significant).

Experiment 4 – Feeding rate

In 2 out of 10 replicates, mortality of 1 – 2 mysids occurred in the course of the feeding experiment and these were omitted from further analyses. A significant feeding of *Neomysis integer* individuals on laboratory-made flocs was demonstrated (Wilcoxon matched pair test: $p = 0.012$) with a feeding rate of 38 ± 4 flocs ind^{-1} h^{-1}. In none of the 0.2 mm size classes was there a significant decrease in the number of flocs l^{-1} observed after 24 hours of feeding, indicating no size selection on aggregates by *N. integer* (9.6 ± 0.2 mm standard length).

DISCUSSION

Comparison between laboratory-made and natural estuarine flocs

The vast majority of the laboratory-made flocs were in the 0.4 to 0.8 mm length class, *i.e.* the 0.4 to 0.5 mm diameter class. The maximal diameter of *in situ* aggregates in the Schelde estuary is ± 0.8 mm (Eisma *et al.*, 1983), with the highest frequency of flocs in the diameter class 0.15 – 0.5 mm (Eisma *et al.*, 1990). Wartel and Francken (1998) however, measured a smaller floc diameter in the Schelde (outside the MTZ), between 0.02 and 0.25 mm. The maximal length (3.8 mm) of the flocs generated in the roller table is in good agreement with the maximal length generally reported for estuarine flocs (*i.e.* 3 – 4 mm, Eisma, 1986). The size and shape of the laboratory-generated flocs differ only moderately according to the tide. In the field, the floc size changes over a tidal cycle in relation to the current velocity and turbulence (Chen *et al.*, 1994). Largest flocs form at slack tide due to the reduced current velocity and turbulence (Chen *et al.*, 1994; Eisma *et al.*, 1994). These larger flocs start to settle out to the bottom, resulting in a reduced floc size at the surface layer (Eisma, 1986). The size and firmness of estuarine aggregates are also much related to the nature of the organic matter in the flocs (Eisma *et al.*, 1983; 1985).

Macro-aggregates reach concentrations of 20 – 4000 flocs l^{-1} in the Elbe estuary (Germany) (Zimmermann and Kausch, 1996; Zimmerman, 1997; Ploug *et al.*, 2002). No comparable data are available for the Schelde estuary. In the laboratory, floc concentration was on average 14000 ± 1100 flocs l^{-1}, with a range of 700 and 33000 flocs l^{-1}. The highest number of aggregates was generated with water collected at high tide in the period after spring tide.

One can conclude that the size of the laboratory-made flocs is comparable with those naturally occurring in the Schelde. The number of flocs generated in the roller tanks is highly variable according to the spring-neap cycle, but exceeding the numbers reported for the upper estuarine zone in the Elbe estuary by one order of magnitude. *In situ* floc concentration measurements have spatial and/or seasonal variation, as discussed by Zimmermann (1997). Other studies are restricted to the floc size variation over a tidal, spatial and seasonal scale (Chen *et al.*, 1994; Eisma *et al.*, 1994; Eisma, 1986; Wartel and Franken, 1998; Chen, 2003). Tidal or neap-spring tide variation on *in situ* floc concentration has hitherto not been considered. Shanks and Edmondson (1989) originally developed the roller table for the generation of marine snow and compared field aggregates with laboratory made ones. The laboratory-prepared aggregates had a significantly greater axis and a significantly larger volume than field aggregates. However, they had the same density, porosity and composition. The roller table is therefore considered an adequate tool for feeding experiments with fragile aggregates (Larson and Shanks, 1996).

Impact of variability in salinity and ration on the growth of Neomysis integer

Although the sampling of the estuarine water was standardised according to the tide, the salinity decreased substantially from 6.5 to 1.3 psu due to heavy rainfall. According to Eisma *et al.* (1991), salinity does not influence the *in situ* macrofloc size, but it alters the firmness of the flocs through a differential mobilisation of polymers.

A maximum turbidity zone occurs in the low salinity region of estuaries (< 5 psu), but its geographical position shows a large spring-neap tide variation (Loring *et al.*, 1983; Syvitski *et al.*, 1995). Concentrations of SPM at the MTZ can vary between 30 and 280 mg l^{-1} in the upper Schelde estuary (Baeyens *et al.*, 1998). Turbidity is highest at high tide (Chen *et al.*, 1994; Baeyens *et al.*, 1998) and is between one and two orders of magnitude higher at spring tides than at neap tides (Morris *et al.*, 1982). In the present study, the spring-neap tide variations in SPM and POM had respective ranges of 33 – 207 mg l^{-1} and 6 – 45 mg l^{-1}. The number of aggregates formed in the roller tanks varied greatly between 700 and 33000 flocs l^{-1} and was correlated with the SPM concentrations, which in turn varied according to the semi-lunar cycle and to salinity. These large quantitative variations in the ration were not reflected in the growth performance of *Neomysis integer*. Taking the estimated feeding rate of 38 flocs h^{-1} ind^{-1} and assuming a continuous feeding over 24 hours, the maximal daily ration of 7 – 10 mm sized *N. integer* approximates ca. 1000 flocs d^{-1}. Hence, the concentration in the roller tanks provided ample food to the mysids, except at day 21 where less than 1000 flocs l^{-1} were recorded.

Nutritive value of the flocs for Neomysis integer

Wörner *et al.* (2000) studied the succession of bacteria and protists within laboratory-made macro-aggregates generated from Elbe water. On the first day, more than 10^9 cells ml^{-1} were counted within the aggregates. Flagellates and ciliates were also much more abundant than in the surrounding water. The first protozoan colonizers were small heterotrophic flagellates (such as choanoflagellates and euglenoids).

Per aggregate, 1 – 24.10^6 bacterial cells were counted (Ploug *et al.*, 2002) in the upper Elbe estuary, so that bacteria on flocs account for 80 – 90 % of the total number of bacteria in the water column (Zimmermann, 1997; Zimmermann and Kausch, 1996). The estimated biomass of bacteria and protozoa on 0.5 – 1.5 mm sized aggregates from the Elbe was highly variable (20 – 400 ng C per aggregate) and showed poorly correlated with aggregate size (Ploug *et al.*, 2002). Protozoa, dominated by nanoflagellates, varied between 25 and 1500 cells per aggregate (Ploug *et al.*, 2002).

In a laboratory study with water from the MTZ of the Schelde, 35 % of the bacteria, 10% of the flagellates and 25 % of the ciliates present in the water column were associated with laboratory-made flocs (Muylaert *et al.*, 1999). Numbers of micro-organisms per volume unit of flocs were 10 (flagellates and ciliates) to 100 times (bacteria) higher than those in the surrounding water. 30 – 50 % of the POC in the water column of the MTZ of the Schelde was associated to the estuarine flocs (Muylaert *et al.*, 1999), but living biomass contributes only 3.2 % of the total particulate organic carbon in the flocs. The majority of the organic carbon in flocs consists of mucus and detritus particles embedding the inorganic sediment (Eisma, 1986; Zimmermann-Timm *et al.*, 1998; Artolozaga *et al.*, 2002). The volume ratio of mineral matter versus organic matter in flocs varies from 7:1 to 2:1 (Eisma, 1986). The organic matter content of flocs amounts to 5 – 15 % of the dry weight (Eisma 1986) or 10 – 20 mg POC l^{-1} aggregates (Muylaert *et al.*, 1999).

Fockedey and Mees (1999 – Chapter 2) demonstrated that *Eurytemora affinis* and estuarine aggregates are the main items in the stomachs of *Neomysis integer* in the MTZ of Elbe, Schelde and Gironde estuaries. The size of the unidentifiable detritus particles encountered in the stomachs of the mysids is an order of magnitude smaller than the aggregates administered in the present experiment and the ones found *in situ* (Eisma *et al.*, 1990). The macro-aggregates (0.4 – 0.8 mm) possibly fall apart or are broken down (mechanically or enzymatically) into micro-aggregates (< 65 µm) during uptake or/and digestion.

The mean dimensions of the laboratory-made macro-aggregates are comparable with those of calanoid copepods (Billiones *et al.*, 1999): length, width, surface area, SED, elongation, roundness and circularity are all in the same order as in this prey. An aquatic animal generally feeds on items 1 – 10 % its own body size (Kiørboe, 1993). For *Neomysis integer* used in the feeding experiment (9 – 10 mm), this means between 10 and 100 µm. In experiment 4 no size selectivity could be detected, however.

Feeding rate

The present study is the first to make a direct estimate of the feeding rate on estuarine flocs, as expressed in number of flocs consumed per unit time. Other studies determine ingestion rate on estuarine aggregates or marine snow indirectly by measuring either egestion, decrease in POC concentrations in the experimental units, natural tracers like chlorophyll or artificial tracers like dyes or polystyrene beads (Bochdansky and Herndl, 1992; Lampitt *et al.*, 1993; Larson and Shanks, 1996; Dilling *et al.*, 1998).

The consumption rate of *Neomysis integer* on estuarine flocs (38 flocs h^{-1}) is one order of magnitude higher in comparison with the experimentally derived feeding rate on similar sized mesozooplankton (Billiones *et al.*, 1999). *Eurytemora affinis* copepodites and adults are preyed upon at a rate of 0.2 – 8 ind h^{-1} (Irvine *et al.*, 1993; Aaser *et al.*, 1995; Winkler and Greve, 2004). The higher feeding rate on flocs may compensate for their comparatively lower energetic value.

As the gut passage time of *N. integer* is only 30 minutes when feeding on estuarine flocs (Fockedey et al., submitted d – Addendum 2), the mysids must have produced a large amount of faecal pellets during the course of the feeding experiment (24h). These pellets mixed and coagulated with the flocs. As no correction was performed for this increase, one should consider the feeding rate estimate as a minimal estimate.

Selectivity experiments were not performed here, but one can assume that when mesozooplankton is available it is more beneficial to *N. integer* to feed on calanoid copepods than on the energetically less valuable flocs. On the other hand, the availability of the aggregates is high in comparison with the calanoid prey (<10 – 250 l^{-1}; Tackx *et al.*, 2004) and it is not known how the energy requirements for catching the calanoid prey compare to those for feeding on the suspended flocs.

Estuarine aggregates suspended in the water column (present study) or deposited on the sediment surface (Roast *et al.*, 2000b; 2004) may be an important additional food source to *Neomysis integer*, especially in periods or areas with low densities in mesozooplankton food sources. The present study provides first direct evidence that *N. integer* can indeed survive and grow on a diet of estuarine macro-aggregates only, although growth is slower than on *Artemia* nauplii. Larson and Shanks (1996) found that juvenile mullets consumed both marine aggregates and *Artemia* nauplii in the laboratory, although marine snow alone was not sufficient to allow growth.

Estuarine food web

Feeding by *Neomysis integer* on aggregates has implications for understanding the heterotrophic estuarine food webs described in the low saline region of estuaries (Hummel *et al.*, 1988; Hall and Raffaelli, 1991; Soetaert and Herman, 1995b). The estuarine microbial food web plays an important role in nutrient cycling, as well as in the transfer of nutrients to higher trophic levels via bacterivorous protists towards the metazoan zooplankton and subsequent higher trophic levels (Azam *et al.*, 1983; Crump and Baross, 1996; Gasparini and Castel, 1999; Muylaert *et al.*, 2000b).

The rich bacterial and protozoan communities as well as the incorporated amorphous organic matter in the relatively large macro-aggregates (>250 µm) makes them a highly edible food source (Alldredge and Silver, 1988). In this way the organic material, normally too small to be efficiently consumed by mysids and considered to constitute the diet of the protist community, becomes part of the diet of organisms higher up in the food-chain (Silver *et al.*, 1978; Lampitt *et al.*, 1993). The uptake of macro-aggregates by *Neomysis integer* thus constitutes a short-cut in the estuarine microbial food chain. The process is also likely to play a role in the bio-sedimentology (Uncles, 2002) by the biodeposition of the suspended organic matter and sediment into faecal pellets numerously produced by the mysids when feeding on the estuarine aggregates (Fockedey *et al.*, submitted d – Addendum 2).

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Addendum 1

Composition of estuarine macro-aggregates and their importance as concentration sites for micro-organisms in the Schelde estuary (Belgium)

ABSTRACT

In November 1997 and June 1998, the composition of estuarine macro-aggregates in the Schelde estuary was investigated at 4 sites situated in the estuarine turbidity maximum. The flocculation process was simulated *in vitro* using rolling cylinders and macro-aggregates were separated from the surrounding water by sedimentation and decantation. 47 – 90 % of the suspended particulate matter, 29 – 67 % of the particulate organic carbon, 6 – 57 % of the chlorophyll *a*, 1 – 39 % of the bacteria, 5 – 14 % of the heterotrophic nanoflagellates and 5 – 25 % of the ciliates in the water column were found to occur in association with the macro-aggregates. The fraction of total chlorophyll *a* that was associated with the macro-aggregates was at all sites lower in June when compared to November. The fraction of total bacteria that was associated with the macro-aggregates was highest in the freshwater tidal reaches and tended to decrease in downstream direction. Concentrations of bacteria, heterotrophic nanoflagellates and ciliates in the macro-aggregates were generally one to two orders of magnitude higher than in the surrounding water. Despite high concentrations of micro-organisms in the macro-aggregates, living biomass contributed at most to 3.2 % of total organic carbon of the macro-aggregates.

Published as and manuscript in preparation:

Muylaert, K., Fockedey, N., Mees, J., Vijverman, W., 1999. Association of microorganisms with estuarine flocs. In: Muylaert, K., 1999. Distribution and dynamics of protist communities in a freshwater tidal estuary. Ph.D. Thesis, Ghent University: 137-147.

INTRODUCTION

Macro-aggregates in aquatic systems are sites of intense microbial activity. The presence of particulate organic carbon within the aggregates makes them attractive substrates for bacteria (Vetter *et al.*, 1998). Attached bacteria are in turn a food source for heterotrophic protists, nanoflagellates and ciliates (Albright *et al.*, 1987; Caron, 1987; Sibbald and Albright, 1988). In addition, attachment to aggregates may also be advantageous to heterotrophic protists feeding on freely suspended bacteria: an attached lifestyle has been shown to result in increased contact rates with suspended prey items (Shimeta *et al.*, 1995) or it may provide them with a refuge from grazers (Layborn-Parry *et al.*, 1994). Macro-aggregates can be seen as parcels in which a large part of the total suspended particulate organic matter is concentrated into a small volume. They may thus constitute nutritious food particles for metazoan organisms (Silver *et al.*, 1978; Alldredge and Silver, 1988). Fish (Larson and Shanks, 1996; Grossart *et al.*, 1998), mysids (Fockedey and Mees, 1999 – Chapter 2), euphausiids (Dilling *et al.*, 1998) and calanoid copepods (Lampitt *et al.*, 1993; Dilling *et al.*, 1998) have recently been shown to ingest and digest macro-aggregates.

Since the discovery of 'marine snow' in the 1950's (Suzuki and Kato, 1953), macro-aggregates have been observed in a wide range of pelagic ecosystems including the open ocean (*e.g.* Silver *et al.*, 1984), lakes (*e.g.* Grossart and Simon, 1993) and estuaries (*e.g.* Eisma, 1986). In estuaries, macro-aggregates are often referred to as 'estuarine flocs'. Estuarine macro-aggregates are formed by coagulation of sediment particles and organic matter (Eisma, 1993b). This process is greatly enhanced by the resuspension of sediment by strong tidal currents and the high concentrations of allochtonous and *in situ* produced organic matter in estuarine water columns (Eisma, 1993b; Kies, 1995; Herman and Heip, 1999). Estuarine macro-aggregates contain a large fraction of inorganic material which is embedded in an organic matrix derived from phytoplankton or terrestrial macrophytes (Zimmerman, 1997). They are highly unstable and are formed from and decompose into smaller particles called microflocs at a time-scale of hours during each tidal cycle (Eisma *et al.*, 1991; Eisma *et al.*, 1994). Due to their large size and their high inorganic sediment content, sedimentation rates of estuarine macro-aggregates are high (10 – 100 m d^{-1}, Largier, 1993; Pejrup and Edelvang, 1996). These high sedimentation rates together with the specific hydrodynamics of estuaries result in their accumulation in estuarine turbidity maxima (Largier, 1993).

Both hydrodynamic trapping in non-tidal circulation at the freshwater salt water interface (Postma and Kalle, 1955; Schubel, 1968) and periodic settling and resuspension during ebb and flood currents ('tidal pumping', Salomons *et al.*, 1988; Wolanski, 1995; Guézennec *et al.*, 1999) are important in the formation of these estuarine turbidity maxima. The result of these processes is an increased residence time of sediment, organic matter and organisms associated with macro-aggregates in the estuary. As macro-aggregates accumulate in the turbidity maxima, densities of macro-aggregates in estuaries are much higher than in other aquatic systems.

In this paper we describe the composition of estuarine macro-aggregates in the turbidity maximum of the Schelde estuary in terms of living biomass and non-living organic and inorganic particulate matter. We also evaluate the importance of estuarine macro-aggregates as concentration sites for micro-organisms, organic carbon and suspended matter in the estuarine water column.

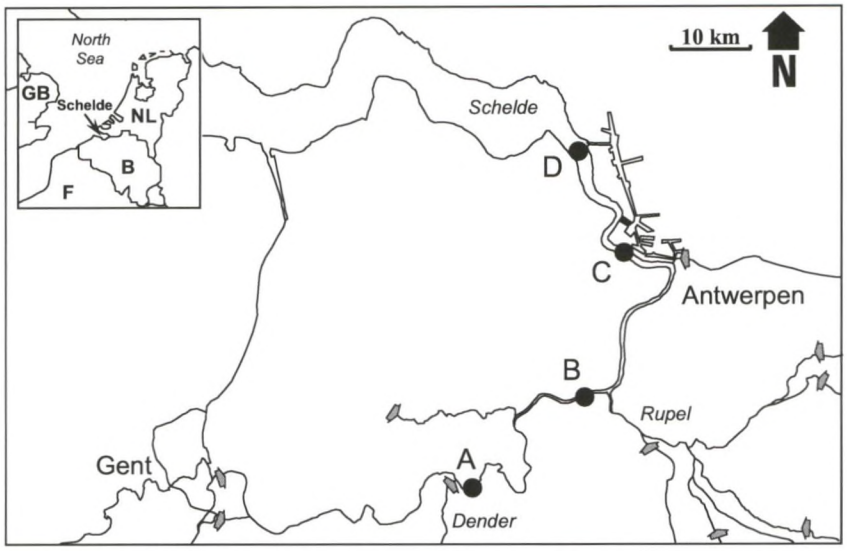

Figure 1: Map of the Schelde estuary showing the position of the sampling stations.

MATERIAL AND METHODS

Study site

The Schelde estuary is a macro-tidal coastal plain estuary situated in Western Europe (Figure 1). River runoff is low compared to the total volume of the estuary and, as a result, residence time is relatively long and the salinity gradient is gradual and stable in time and space (Soetaert and Herman, 1995a). This study focuses on the mesohaline to freshwater tidal reaches of the Schelde estuary where the estuarine turbidity maximum is situated (Herman and Heip, 1999) and where the largest macro-aggregates or estuarine flocs are observed (Eisma *et al.*, 1991). In this part of the estuary, tidal range is about 5 m and the water column is vertically well mixed. Dense phytoplankton blooms dominated by centric diatoms (mainly *Cyclotella* spp. and *Actinocyclus normannii*) and coccoid green algae (*Scenedesmus* spp.) occur in spring and summer when chlorophyll *a* concentrations exceeding 50 $\mu g\ l^{-1}$ are observed (Muylaert *et al.*, 2000a). The Schelde estuary is heavily antropogenically influenced and inputs of inorganic nutrients and organic matter are very high (Heip, 1988). As a result, bacterial abundance and production are very high ($5 - 25.10^6$ cells ml^{-1} and 96 – 600 $\mu g\ C\ l^{-1}\ d^{-1}$ respectively). Bacterial respiration often results in periods of anoxia in the water column in summer (Goosen *et al.*, 1997). Annual average abundance of heterotrophic flagellates and ciliates is 2100 and 65 cells ml^{-1} respectively (Muylaert *et al.*, 2000b). Biomass of phytoplankton, bacteria and heterotrophic protists is generally maximal in the freshwater tidal reaches and declines towards the mesohaline reaches.

Sampling

Samples were collected at four different stations along the longitudinal estuarine axis in autumn 1997 (November 17th: sites A and C, and November 28th: sites B and D) and summer 1998 (June 10th: sites C and D; June 12th: sites A and B) (Figure 1). All samples were stored overnight at ambient temperature (November) or were processed within 3 hours of sampling (June). Salinity and temperature were measured *in situ*. Salinity in stations A and B varied between 0.2 and 0.4 psu and these stations can therefore be considered to be entirely freshwater. In stations C and D, salinity was respectively 4.9 and 9.7 psu in November and 3.8 and 9.2 psu in June. Temperature varied little between the sites and was on average 10 °C in November and 18 °C in June.

In vitro formation of estuarine macro-aggregates

Macro-aggregates are very fragile particles and are notoriously difficult to sample and study. In estuaries, as in other aquatic systems, the use of traditional sampling methods to collect macro-aggregates results in their almost immediate disintegration. Upon sampling or during slack tide each tidal cycle, estuarine macro-aggregates of up to 3 – 4 mm in size break up into microflocs with a size of about 125 µm. During ebb or flood tide, when current velocities and suspended matter concentrations are maximal, macro-aggregates are formed again from microflocs (Eisma *et al.*, 1991; Eisma and Li, 1993; Chen *et al.*, 1994; Eisma *et al.*, 1994).

We used a roller table described by Shanks and Edmondson, (1989) to simulate *in situ* flow conditions in order to artificially re-create macro-aggregates from microflocs in estuarine water samples under controlled circumstances. Estuarine water was transferred to a series of 900 ml Plexiglas cylinders which were rotated at a speed of 10 rounds per minute. Macro-aggregates were rapidly formed and appeared to be stable in size and abundance after 3 hours of incubation. As flocculation and de-flocculation occurs within the same time-scale under natural circumstances in the estuary, we assumed that our artificially created macro-aggregates are representative for those present in the estuarine water column (Fockedey et al., submitted c – Chapter 4).

After 3 hours of incubation, the macro-aggregates were allowed to settle for 5 min and were separated from the supernatant by careful decantation until about 10 ml of suspension containing the macro-aggregates was retained. The exact volume of concentrate containing the macro-aggregates was measured and the concentrate, as well as the supernatant, was used in subsequent analyses. As the cylinders used had a diameter of 14 cm, our method will quantitatively collect particles with settling rates of 40 m d^{-1} or more in the concentrate. This is within the range of sedimentation rates of particles accumulating in estuarine turbidity maxima (10 – 100 m d^{-1}, Largier, 1993; Perjup and Edelvang, 1996). In this study, we separated the macro-aggregates from other suspended matter based on differences in sedimentation rates, which is ecologically meaningful as it is their sedimentation rate which determines the behaviour of macro-aggregates in estuaries, *i.e.* their accumulation in estuarine turbidity maxima.

Analyses

All analyses were performed on three replicate incubations unless stated otherwise in the graphs. The suspended particulate matter (SPM) concentration in both fractions was determined gravimetrically after filtration of subsamples on preweighed GF/F filters. For determination of particulate organic carbon (POC) content, samples were filtered on preweighed and precombusted GF/F filters; percentage carbon content of SPM was measured using a Carlo Erba CN analyser after removal of carbonates under acid atmosphere. For chlorophyll *a* analyses, subsamples were filtered onto GF/C filters. Filters were stored at –80°C until extraction in acetone and subsequent analysis by High Performance Liquid Chromatography (protocol according to Mantoura and Llewellyn, 1983).

Subsamples for quantification of bacteria and heterotrophic protists were fixed according to the lugol, formalin and sodium thiosulphate method (Sherr *et al.*, 1989). Bacteria were separated from sediment and detrital particles by means of pyrophosphate and sonication treatment (Velji and Allbright, 1986). Bacteria were stained with acridine orange and filtered onto 0.2 µm pore size Nuclepore polycarbonate filters (Hobbie *et al.*, 1977). Bacteria were counted using epifluorescence microscopy with blue light illumination; a minimum of 150 bacteria was counted in at least 10 random fields.

For quantification of heterotrophic nanoflagellates and ciliates in the supernatant, subsamples were stained with DAPI (4',6'-diamidino-2-phenylindole, 1 µg ml^{-1} final concentration, Sherr *et al.*, 1993) and filtered onto 2 µm pore size Nuclepore polycarbonate filters. Heterotrophic protists were counted using epifluorescence microscopy with UV illumination; at least 50 ciliates and 100 heterotrophic nanoflagellates were counted along radial transects on the filter. Heterotrophic protists in the concentrate with macro-aggregates were separated from sediment and detrital particles using isopycnic density gradient centrifugation (in Percoll, Starink *et al.*, 1994) before staining with DAPI and counting.

Analyses

The concentration of measured variables associated with macro-aggregates in the incubated water sample will be referred to as the bulk concentration in the macro-aggregates. This concentration was calculated as:

$$V_{conc} \cdot (C_{conc} - C_{water}) / V_{incub}$$

with C_{conc} being the concentration in the macro-aggregate concentrate, C_{water} the concentration in the supernatant, V_{conc} the volume of the macro-aggregate concentrate and V_{incub} the volume incubated in the cylinders. This calculation takes into account the concentration of measured variables in the amount of supernatant left in the macro-aggregate concentrate and in the pore-water of the macro-aggregates. As this calculation assumes that the macro-aggregates have no volume, our results provide a conservative estimate for the bulk concentrations of the macro-aggregates.

The concentration of bacteria, heterotrophic nanoflagellates and ciliates per unit volume of the macro-aggregates will be referred to as the specific concentration in the macro-aggregates. This concentration was calculated as:

$$V_{conc} \cdot (C_{conc} - C_{water}) / V_{aggr}$$

with V_{aggr} being the volume of the macro-aggregates. This volume was estimated at 5 ml, which is about half of the average volume of concentrate obtained during fractionation of macro-aggregates and supernatant. This volume is a high-range estimate so that our estimates concerning the specific properties of the macro-aggregates should be treated as conservative. Nevertheless, they should give information on the order of magnitude of bacteria, heterotrophic nanoflagellate and ciliate concentrations per volume unit of macro-aggregates. A similar approach to estimate the specific concentration of micro-organisms in macro-aggregates was used by Caron *et al.* (1982).

We also estimated the contribution of living micro-organisms to total POC in the macro-aggregates. Chlorophyll *a* was converted to C assuming a C to chlorophyll *a* ratio of 50 mg C (mg chl *a*)$^{-1}$ (Geider, 1987). For bacteria, a conversion factor of 2.10^{-11} mg C $cell^{-1}$ was used (Lee and Fuhrman, 1987). Heterotrophic protistan biomass was estimated from biovolume measurements using a volume to carbon conversion factor of 0.15 pg C μm^{-3}, which is within a range of values given for heterotrophic flagellates and ciliates (Fenchel, 1982; Sheldon *et al.*, 1986; Børsheim and Bratbak, 1987; Putt and Stoecker, 1989).

For heterotrophic nanoflagellates and ciliates, conversion factors of 39 and 3000 pg C $cell^{-1}$ respectively were used, which are based on measurements of at least 200 cells of each group from different samples collected during the course of 1996 (Muylaert *et al.*, 2000b). Given the large interspecific differences in biomass among ciliates, heterotrophic protistan biomass estimates should be interpreted with caution. High numbers of ciliates are often caused by a dominance of small forms, which have a cellular biomass significantly lower than the conversion factor used.

RESULTS

In general, measurements carried out on separate incubations yielded similar results, indicating a good reproducibility of our incubation and measurement techniques. In November, total SPM concentration (Figure 2) was maximal in station B. Between 47 and 90 % of the total SPM was contained in the macro-aggregates. In June, total SPM concentrations were generally much higher in all stations but maximal SPM concentrations were observed in station D. In June, 47 to 67 % of the total SPM was associated with the macro-aggregates. Total POC concentrations were highest in June, and, at that time, did not show much variation among the different stations.

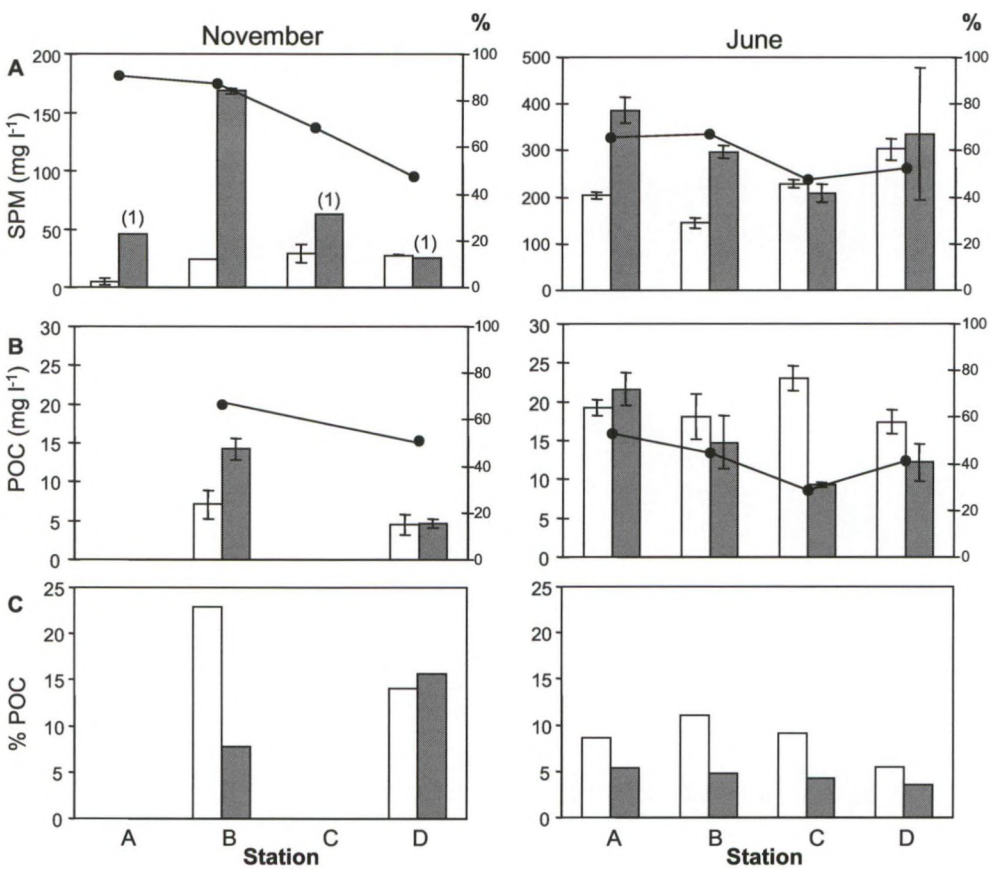

Figure 2: Bulk concentrations of SPM (A) and POC (B) in macro-aggregates (grey bars) and the surrounding water (white bars) at the different sampling stations in November and June. Black dots connected by line indicate the percentage of the total that is associated with the macro-aggregates. The organic carbon content of suspended matter in the macro-aggregates (grey bars) and the surrounding water (white bars) is shown in (C). No data on POC were collected for stations A and C in November. Unless indicated otherwise (in brackets above the data), data on SPM and POC are averages of three measurements; error bars indicate standard deviation.

In November, POC data were only available for two stations and total POC levels were highest in the freshwater station B. The percentage contribution of POC to total SPM was on average 15 % in November but only 6.5 % in June. Except for station D in November, the POC content of the macro-aggregates was substantially lower than that of the SPM in the supernatant.

Total chlorophyll *a* concentrations (Figure 3) were slightly higher in November than in June. While in November, 39 to 57 % of total chlorophyll *a* was associated with the macro-aggregates, this was always less than 12 % in June. Total bacterial densities were comparable in November and June and, in both seasons, a similar fraction of the bacterial population (about 30 %) was associated with the macro-aggregates. The total numbers of bacteria as well as the fractions associated with the macro-aggregates decreased in seaward direction; this effect was much more pronounced in November than in June.

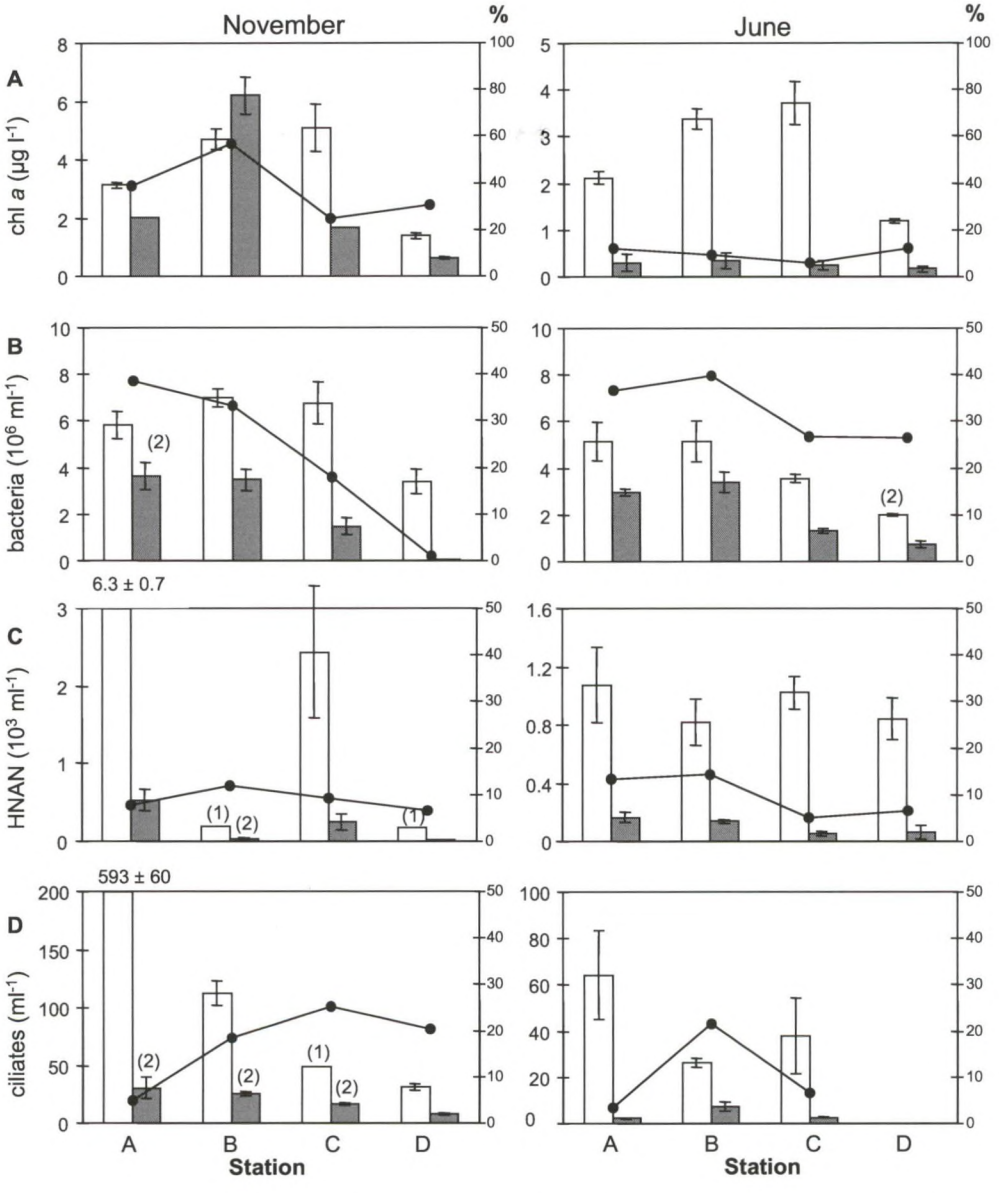

Figure 3: Bulk concentrations of chlorophyll *a* (A), bacteria (B), heterotrophic nanoflagellates (C) and ciliates (D) in macro-aggregates (grey bars) and the surrounding water (white bars) at the different sampling stations in November and June. Unless indicated otherwise (in brackets above the data), data are averages of three measurements; error bars indicate standard deviation. Black dots connected by line indicate the percentage of the total that is associated with the macro-aggregates.

In November, large differences in heterotrophic nanoflagellate abundance were observed between the sampling stations: high heterotrophic nanoflagellate numbers were observed in stations A and C while numbers in stations B and D were very low. In June, heterotrophic nanoflagellate densities were relatively low at all stations.

Despite the large variations in total heterotrophic nanoflagellate abundance, the fraction of the total heterotrophic nanoflagellate population associated with the macro-aggregates was fairly constant, fluctuating around 10 %. In station D in June, ciliate densities were too low to produce reliable counts with the method used (< 5 ciliates ml^{-1}). Ciliate numbers were highest in November, especially in station A. Ciliate densities decreased towards the brackish stations. Overall, less than 20 % of the ciliate population was associated with the macro-aggregates.

Specific concentrations of bacteria in the macro-aggregates were 1 to 2 orders of magnitude higher than in the surrounding water (Table 1). For heterotrophic nanoflagellates and ciliates, differences were smaller (1 order of magnitude). The bulk biomass attained by different groups of organisms and their contribution to total POC in the macro-aggregates and the surrounding water is given in Table 2. The contribution of phytoplankton, bacteria and heterotrophic protists to total living organic matter was comparable. Only in November in station A, the biomass attained by heterotrophic protists was atypically high. This observation, however, should be treated with caution as the high biomass could largely be attributed to the high numbers of ciliates, the biomass of which is only roughly approximated by our methods. Bacteria and auto- or heterotrophic protists contributed only 0.2 to 3.2 % to total POC in the macro-aggregates. In November, living micro-organisms contributed more to total POC than in June. The contribution of living micro-organisms to total POC was highest in the suspended matter in the supernatant and always lower in the macro-aggregates.

DISCUSSION

Bulk properties of the macro-aggregates

47 to 90 % of total SPM in our water samples was found to be associated with macro-aggregates. In the Elbe estuary, Kerner *et al.*, (1995) used a specially designed sedimentation funnel in which, like in our study, fast sinking SPM was separated from slow sinking SPM based on differences in sedimentation rates. In that study, a comparable fraction of total SPM was found in the fast-settling SPM fraction. 29 – 67 % of total POC was incorporated in the macro-aggregates. This fraction was usually slightly lower than the fraction of SPM associated with the macro-aggregates, meaning that the macro-aggregates have a relatively low organic matter content when compared to suspended matter in the surrounding water. This is in agreement with measurements from marine snow (Alldredge, 1979) and the fast-settling fraction of suspended matter in the Elbe estuary (Kerner *et al.*, 1995).

Table 1: Range of specific concentrations of bacteria, heterotrophic nanoflagellates (HNAN) and ciliates (in log cells ml^{-1}) in the supernatant and macro-aggregates of the Schelde estuary.

	plankton water	macro-aggregates
bacteria	6.29 - 6.83	6.73 - 8.82
HNAN	2.24 - 3.80	3.34 - 4.98
ciliates	1.42 - 2.77	2.61 - 3.74

Table 2: Bulk biomass (in μg C l^{-1}) of different groups of micro-organisms in the supernatant (water) and macro-aggregates (aggr.). The percentage contribution of micro-organisms to total POC in the supernatant or macro-aggregates is also given.

	November		June	
	water	aggr.	water	aggr.
phytoplankton				
station A	157	100	106	15
B	235	310	169	17
C	255	84	186	12
D	70	31	60	8
bacteria				
station A	116	73	103	59
B	140	70	103	68
C	135	30	71	26
D	68	1	40	14
heterotrophic protists				
station A	2026	112	235	13
B	345	77	111	27
C	242	59	153	10
D	101	24	33	2
percentage contribution of micro-organisms to total POC				
station A	-	-	2.3	0.4
B	10.1	3.2	2.1	0.8
C	-	-	1.8	0.5
D	5.3	1.2	0.8	0.2

The fraction of total chlorophyll *a* associated with macro-aggregates varied between 25 and 57 % in November and between 6 and 12 % in June. In the Elbe estuary, the majority of the chlorophyll *a* was part of the slow-sinking fraction of the SPM (Wolfstein and Kies, 1995) and was thus not associated with macro-aggregates. In the Elbe estuary, however, most green algae were found in the slow sinking SPM while diatoms appeared to be enriched in the fast-settling fraction of SPM. Several estuarine diatoms are known to occur in association with sediment and detritus particles (Ernissee and Abbot, 1975; Smetacek, 1985; Muylaert and Sabbe, 1996) and may, therefore, easily become incorporated in estuarine macro-aggregates. Previous studies have shown that, in the Schelde estuary, green algae become relatively more important towards the summer while diatoms dominate phytoplankton biomass throughout the rest of the year (Muylaert *et al.*, 2000a). This might explain why a larger fraction of total chlorophyll *a* was associated with the macro-aggregates in November when compared to June.

On average 28 % of the bacterial community was associated with the macro-aggregates. In other turbid estuaries, this fraction was similar or slightly higher (Goulder, 1976; Boetcher *et al.*, 1995; Crump and Baross, 1996; Zimmermann and Kausch, 1996; Crump *et al.*, 1998). Although this trend was most pronounced in November, the fraction of bacteria associated with macro-aggregates tended to decrease in downstream direction and in station D in November, only 1 % of the bacterial population was found associated with the macro-aggregates.

This may indicate that during downstream transport, macro-aggregates become depleted in carbon and are thus no longer a suitable substrate for bacteria. Although previous observations indicate a decrease in organic matter content of the total SPM when going from the freshwater tidal to the brackish reaches (Billiones, 1998), this was not apparent from our measurements. More important than the total organic matter content, however, is the quality of organic matter and its bio-availability, which in estuaries may change significantly during downstream transport (Hollibaugh and Wong, 1999).

Heterotrophic protists (ciliates and heterotrophic nanoflagellates) are known to be the dominant grazers on bacteria in planktonic environments (Azam *et al.*, 1983). Although a large part of the bacterial population was associated with the macro-aggregates, only 5 – 14 % of the heterotrophic nanoflagellates and 5 – 25 % of the ciliates were found in the macro-aggregates. In the Elbe estuary, Zimmermann and Kausch (1996) found the majority of ciliates but only 10 % of heterotrophic nanoflagellates to be associated with macro-aggregates. On the contrary, in the Clyde estuary, 80 % of all heterotrophic nanoflagellates were closely associated with macro-aggregates while ciliates were only transient visitors (Rogerson and Laybourn-Parry, 1992). The reason why we found a larger fraction of the bacterial population to occur in association with the macro-aggregates when compared to the heterotrophic protists may be related to the methodology we used. We assume that during sampling, all macro-aggregates in the samples had disintegrated into microflocs of about 125 µm in size (Eisma *et al.*, 1991; Eisma and Li, 1993; Chen *et al.*, 1994; Eisma *et al.*, 1994). These microflocs are probably large enough to provide a bacterium with enough carbon to support growth during several generations and as such represent a stable substrate for bacteria to attach to. We can therefore assume that they were colonized by bacteria prior to the incubation. During the incubation in the rotating cylinders, the microflocs coagulated to form macro-aggregates. The majority of the bacteria that were found on the macro-aggregates after the 3-hour incubation were presumably bacteria that were already attached to the microflocs.

The same microflocs, however, are probably too small to serve as sites for permanent attachment for heterotrophic protists and most of the heterotrophic protists that were in our study found on the macro-aggregates had probably colonized these macro-aggregates during the course of the incubation. It is therefore likely that after a longer incubation time, a larger fraction of the protistan population would become associated with the macro-aggregates. Under natural circumstances, however, estuarine macro-aggregates disintegrate into and are formed from microflocs within a similar time span as the incubation time used in our experiment. Therefore, it is conceivable that, in the estuary like in our experimental incubations, a larger fraction of the bacteria is associated with the macro-aggregates when compared to heterotrophic protists as the macro-aggregates constitute a relatively stable substrate for the bacteria but an unstable micro-environment for the protists.

Specific properties of the macro-aggregates

Concentrations of bacteria and heterotrophic protists in the macro-aggregates were 1 to 2 orders of magnitude higher than in the surrounding water, which corresponds to enrichment factors of 10 to 100. These enrichment factors are generally lower than those reported in studies of non-estuarine macro-aggregates (Silver *et al.*, 1978; Caron *et al.*, 1982; Grossaert and Simon, 1993; Artolozaga *et al.*, 1997). In contrast to lake and marine snow, estuarine macro-aggregates are relatively unstable (see above). This instability may prevent the colonisation and development of dense microbial communities on the macro-aggregates.

Organic carbon content of macro-aggregates in the Schelde estuary ranged between 3.5 and 16 %. This is within the range found for marine snow (Alldredge, 1979), lake snow (Grossart and Simon, 1993) and macro-aggregates in the Elbe estuary (Zimmermann, 1997).

Macro-aggregates in lakes or marine environments represent micro-patches where organic matter concentrations are much higher than in the surrounding water. In estuaries in general and in the Schelde estuary in particular, organic matter concentrations are already high in the water surrounding the macro-aggregates while organic carbon content of the macro-aggregates is similar to that of marine or lake snow. This greater discrepancy in organic matter concentrations between the macro-aggregates and the surrounding water in marine or lake snow when compared to estuarine macro-aggregates may provide an alternative explanation to the lower enrichment factors for bacteria and heterotrophic protists in macro-aggregates of estuaries as opposed to those found in lakes or marine systems.

Despite the high concentrations of organisms in the macro-aggregates, they only contributed marginally to the organic carbon in the aggregates. Phytoplankton, bacteria and heterotrophic protists all contributed a comparable fraction to living biomass in the macro-aggregates but at most 3.2 % of all organic carbon in the macro-aggregates was attributable to living organisms. Most of the POC in the macro-aggregates can thus be considered to be detrital material. In the Elbe estuary, phytoplankton was on some occasions found to contribute significantly to the organic matrix of estuarine macro-aggregates (Kies, 1995; Zimmermann, 1997). In the Schelde estuary, however, POC is generally mainly composed of detritus while phytoplankton rarely contributes significantly to the total POC pool (Hellings *et al.*, 1999). It should be noted that chlorophyll *a* concentrations measured during this study were relatively low (< 11 μg chl *a* l^{-1}). In spring and summer, chlorophyll *a* concentrations exceeding 50 μg l^{-1} are frequently observed (Muylaert *et al.*, 2000a) and during those blooms phytoplankton may be a more important component of estuarine macro-aggregates.

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Chapter 5

Effect of food quality on the growth of the brackish water mysid *Neomysis integer* (Crustacea: Mysidacea)

ABSTRACT

The growth of the brackish water mysid *Neomysis integer* can be described in three alternative ways: (1) by the increase in standard length (SL), (2) by the intermoult period (IMP) and growth factor (GF), or (3) by the intermoult growth rate (GR). Individual variation of these growth parameters in growth experiments is small. These endpoints can thus be used to evaluate the effects of environmental variables, food quality and quantity, and toxic substances on the growth of the mysid species.

The present study evaluates to what extent diets of *Artemia salina* (nauplii), *Eurytemora affinis* (copepodites and adults), laboratory-made estuarine flocs and macrophytal detritus (*Scirpus maritimus* and *Spartina anglica*), all administered *ad libitum*, influence the survival and somatic growth of subadult *Neomysis integer*.

Detritus originating from non-leached *Spartina anglica* was toxic to *Neomysis integer*, leading to morphologic aberrations and a high mortality. The growth of *N. integer* individuals was slower on a diet of *Scirpus maritimus* detritus than on a diet of animal food items or laboratory-made flocs. *Artemia* nauplii were the highest quality food for *N. integer*: a relatively smaller IMP and higher GF and GR resulted in a significantly higher SL at the end of this experiment. When fed with laboratory-made flocs, *N. integer* moulted as frequently as when fed *Artemia*, but GR decreased over the course of the experiment. A *Eurytemora affinis* diet resulted in a significantly elongated IMP from the first moult onwards as compared to mysids fed *Artemia* or flocs. The mean associated growth rate however, was comparable with the flocs treatment and significantly lower than fed *Artemia*.

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INTRODUCTION

Neomysis integer is a typical inhabitant of the oligohaline, turbid reaches of European estuaries (Mees *et al.*, 1995). It plays a key role in the local food web as food for fish and macrocrustaceans (*e.g.* Hostens and Mees, 1999). In this part of the estuary, the food web is based on detritus, and heterotrophic processes dominate (Hamerlynck *et al.*, 1993; Soetaert and Herman, 1995b; Muylaert *et al.*, 2000b). Due to a shallow euphotic zone in the highly turbid water column, the local primary production is limited, despite the high nutrient concentrations (Hummel *et al.*, 1988; Relexans *et al.*, 1988). Except for a dense diatom bloom in summer (Muylaert *et al.*, 1997), autotrophic phytoplankton is not considered to play a substantial role in the food web in this part of the estuary (Bernát *et al.*, 1994; Irigoien and Castel, 1997). Still the upper estuary is highly productive and characterised by high abundances of zooplankton, hyperbenthos, epibenthic macrocrustaceans and fish (Hummel *et al.*, 1988; Soetaert and Van Rijswijk, 1993; Hamerlynck *et al.*, 1993; Mees *et al.*, 1995; Hostens and Mees, 1999; Maes *et al.*, 2003).

Suspended sediment and particulate organic matter flocculate in the presence of specific dissolved organic compounds (*e.g.* polysaccharides, humic acids and fluvic acids) to estuarine aggregates (Eisma *et al.*, 1991). Due to the estuarine circulation, these aggregates accumulate at the head of the estuary (at the freshwater-brackish water interface), and form a so called maximum turbidity zone (MTZ) or estuarine turbidity maximum (ETM) (Eisma, 1986). Biota can also contribute to the turbidity in this zone through active re-suspension of sediments or increased sediment erosion due to burrowing and feeding behaviour (Widdows and Brinsley, 2002; Roast *et al.*, 2004). Because of the entrapment and associated increased residence time in the MTZ (Siegfried *et al.*, 1979; David *et al.*, 2005), the reactive organic detritus (in suspension or aggregated in the flocs) is quickly remineralised by bacterial activity and/or is directly grazed upon by higher trophic levels (Heinle and Flemer, 1975; Hummel *et al.*, 1988; Fockedey *et al.*, submitted c – Chapter 4). As a result, little material is exported to the downstream reaches of the estuary and the coastal zone and it consists of a large refractory fraction (Soetaert and Herman, 1995b).

The brackish water mysid *Neomysis integer* is known to be omnivorous. Populations living in the MTZ of Western European estuaries predominantly feed on the calanoid copepod *Eurytemora affinis*, estuarine aggregates and macrophytal detritus (Fockedey and Mees, 1999 – Chapter 2). Also, aggregations of sediment collected at the substratum surface have been demonstrated to be a relevant food item in the shallow estuarine areas (Roast *et al.*, 2000b). In laboratory experiments, *Eurytemora affinis* is an adequate prey item to fulfil the species' energy requirements (Irvine *et al.*, 1993; Aaser *et al.*, 1995; Winkler and Greve, 2004), but the impact of this mono-specific diet on growth and reproduction of *N. integer* is not known. Macrophytal detritus, imported from the fluvial part of the estuary and/or from the local tidal marshes, is also hypothesized to be an important food source in the oligohaline food web, especially in periods when mesozooplankton is scarce (Sorbe, 1980; Irvine *et al.*, 1993; Fockedey, unpublished). The nutritional and energetic value of this material for *N. integer* is still uncertain, but the species possibly represents a trophic link between salt marsh macrophyte production and higher trophic levels (Zagursky and Feller, 1985; Cattrijsse *et al.*, 1994). Estuarine macro-aggregates seemed to be an adequate food item for *N. integer* as demonstrated by laboratory growth experiments (Fockedey *et al.*, submitted c – Chapter 4).

Studies with euphausiids, gammarid amphipods and copepods demonstrate that the growth and reproduction rates are determined by environmental variables (mainly temperature and salinity), food quantity, food quality and genotype (*e.g.* Willoughby and Sutcliffe, 1976; Heinle *et al.*, 1977; Koski *et al.*, 1998; Haywood and Burns, 2003). The relationship between an animal's production and the food concentration in the field is often obscured by variations in the food quality (Koski *et al.*, 1998). Until now, few studies have examined the survival and growth rates of mysids in relation to food quality (Lehtiniemi *et al.*, 2002) and little information is available on the impact of food quality on their reproduction (Domingues *et al.*, 2002). For *Neomysis integer*, only Ferguson (1973) performed experiments comparing growth efficiencies of selected size classes of mysids feeding them an animal diet and two detrital diets.

In the present study it was evaluated to what extent diets of *Eurytemora affinis* (copepodites and adults) and macrophytal detritus, administered *ad libitum*, are able to support the survival and somatic growth of subadult *Neomysis integer*. The growth is described by the increase in standard length over time, and by the intermoult period, the growth factor and the intermoult growth rate (Fockedey *et al.*, in press – Chapter 3). The obtained values of the growth parameters are compared with results from (simultaneously performed) experiments on *N. integer* growth performance when feeding on *Artemia salina* nauplii and laboratory-generated estuarine aggregates (Fockedey *et al.*, submitted c – Chapter 4). In addition, a starvation experiment was conducted to observe the effect on the survival and the growth parameters of *N. integer* when deprived from food.

MATERIAL AND METHODS

Neomysis integer

Specimens of *Neomysis integer* were collected with a handnet (opening: 29.0 x 18.5 cm; mesh size of 1 x 1 mm) in the brackish water pond Galgenweel (4 psu; 5 °C) situated at the left bank of the Schelde estuary. Short hauls were taken using a handnet. The animals were transported to the laboratory in environmental water within 2 hours, where they were gradually adapted to a water temperature of 15 °C (over 2 days) under continuous aeration. Before the start of the experiments they were kept for 2 days in artificial seawater (Instant Ocean®, Aquarium Systems, France) of 5 psu and given the food that they would be exposed to in the growth experiments (see later).

The standard length of *Neomysis integer* individuals was determined as the distance from the tip of the rostrum to the end of the last abdominal segment measured laterally on individuals using a drawing mirror mounted on a stereomicroscope. For each treatment, 15 individuals (10 in the case of estuarine flocs) with a standard length between 4 and 6 mm were selected. During the experiments the animals were kept individually in 400 ml glass jars with 350 ml aerated artificial seawater of 5 psu, except for the experiments with estuarine aggregates where the rolling experimental containers have a volume of 845 ml. Fockedey *et al.* (submitted c – Chapter 4) demonstrated that the growth performance of individual *N. integer* was comparable in the two experimental setups. All experiments were performed in a climate-controlled room at 15 °C with a light regime of 12h light:12h dark. Each day the mysids were gently transferred to a new jar using a conical measuring spoon and they were offered daily fresh food *ad libitum*.

Table 1: Food items and ration administered to subadult *Neomysis integer* individuals.

Food type	Food concentration (l^{-1})	Administered daily $mysid^{-1}$		N
		DW (mg)	C (mg)	
Artemia nauplii (<24h)	5700	2.4 †	1*	15
Eurytemora affinis (copepodites + adults; > 250µm)	143	2.1	1 **	15
Macrophytal detritus				
Scirpus maritimus (<400µm) – fresh	70 mg DW	25	29 ***	15
Spartina anglica (<400µm) – fresh	115 mg DW	40	48 °	15
Laboratory-made estuarine aggregates *	16000 ± 2000	30 – 175	2 – 11 ***	10

†: conversion factor according to Paffenhöfer (1967); * conversion according to Evjemo and Olsen (1999); ** conversion according to Parsons et al. (1984); *** conversions according to Fockedey et al., submitted d – Appendix 2; ° conversion according to De Mesel (personal communication)

Food quality

To study the effect of the food quality on the growth of *Neomysis integer*, *5* different food items were supplied at an excess concentration (Table 1): *Artemia salina* nauplii, adults and copepodite stages of the calanoid copepod *Eurytemora affinis*, laboratory-made estuarine flocs, and detritus of two species of estuarine macrophytes (*Spartina anglica* and *Scirpus maritimus*). An additional treatment was set up where *N. integer* was starved (no food added). Except for the *Artemia* nauplii, the food items chosen are relevant in the diet of *N. integer*, as demonstrated by Fockedey and Mees (1999 – Chapter 2) by stomach content analyses and by Gorokhova and Hansson (1999) by isotopic composition analysis. No attempt was made to estimate feeding rates on the different food items. The food was offered *ad libitum* and the growth performance (see later) was monitored for 32 days (4.5 weeks).

Artemia salina cysts (San Fransisco Bay) were hatched daily in a 2 litre conical glass container (25 psu; 28 – 32 °C) under continuous aeration. Less than 36 hours old *Artemia* nauplii (ART) were supplied to the mysids in a concentration of 2000 ± 200 *Artemia* ind^{-1} d^{-1} (± 2.4 mg DW). Adult and large copepodite stages of *Eurytemora affinis* (Copepoda, Calanoidea) were collected in the pond Galgenweel by filtering surface water through a 250 µm sieve. The copepods were transferred to the laboratory in environmental water and kept in the laboratory without additional feeding for maximally 3 days. We assumed that the environmental water contained enough phytoplankton to keep the copepods in optimal condition in this period. Every 3 days new copepods were sampled in the field. Daily, 50 copepods (EURY) were supplied to the mysids (± 2.1 mg DW). They were counted according to the spotting technique of Reeve (1970).

Macrophyte detritus was artificially made in the laboratory using two plant species that are abundant in the marshes of the brackish water zone of the Schelde. *Spartina anglica* (living plant) and *Scirpus maritimus* (died-off plant) were collected from the banks of the Schelde river at Doel. After washing off the sand and epiphytes, the plants were oven dried (60 °C) for 2 days and ground to 400 µm particles. Each detritus type was supplied daily as a constant volume corresponding to 40.1 ± 2.6 mg DW for *S. anglica* (SP-0) and 24.6 ± 2.4 mg DW of *S. maritimus* (SC-0).

The estuarine aggregates (FLOC) administered to *Neomysis integer* in the experiment were re-assembled in the laboratory, out of filtered (250 µm) water that was collected from the Schelde river at Antwerpen, by rotation on a roller table (for a detailed description see Fockedey *et al.*, submitted c – Chapter 4). The mysids were kept individually in the rolling tanks (10 – 11 rpm). The water was changed daily. The floc formation process was in equilibrium after three to four hours.

Measuring the growth performance

Survival and growth of the *Neomysis integer* individuals were followed as described by Fockedey *et al.* (in press – Chapter 3; submitted c – Chapter 4). The containers were checked daily at the start of the light period. Moults were collected and the length of the uropodal exopods (EXO) measured. Standard body length (SL) was calculated using the regression

$$SL = 1.085566 + 4.081793\ EXO \qquad (p < 0.0001;\ R^2 = 0.9569;\ N = 97).$$

The overall growth of *Neomysis integer* can be described by the von Bertalanffy growth curve (Fockedey *et al.*, in press – Chapter 3), but the growth of individuals of the size class 4 – 6 mm SL over a period of 4.5 weeks is linear. Thus, linear regression analysis was applied to the results. The slopes of the linear regression equations were tested between treatments using an ANCOVA and subsequent multiple comparison with a Tukey test (Zar, 1996). Growth was expressed as intermoult period (days), growth factor (%) and intermoult growth rate (mm d^{-1}) according to Fockedey *et al.* (in press – Chapter 3). Differences between experimental treatments were tested at each consecutive moult event by using a Kruskal-Wallis test and multiple comparisons (Conover, 1980).

RESULTS

Survival

All *Neomysis integer* survived the experiment when feeding on *Eurytemora affinis* (Figure 1). Survival on *Artemia* nauplii, estuarine flocs and on *Scirpus* detritus ranged from 73 – 80 % at the end of the experiment. When feeding on *Spartina* detritus, and when administered no food (STARV), mortality was 100 % after 26 and 20 days respectively.

The animals fed *Spartina* detritus moulted 0 to 2 times during the experiments, but morphological deformations of the uropods and the presence of external spherical tumours could be observed. The muscles became white coloured and mysids had a copious growth of an epizootic protozoan on their exoskeleton. Occasionally, the starved individuals moulted twice (13 %), but most of the individuals died after 15 days after moulting only once (40 %) or without having moulted (46 %). The individuals surviving the 32 day time span of the experiment in the other treatments moulted 3 – 5 times when feeding on *Scirpus* detritus, 4 – 5 times on *Artemia* or *Eurytemora*, and 5 – 6 times on laboratory-made flocs.

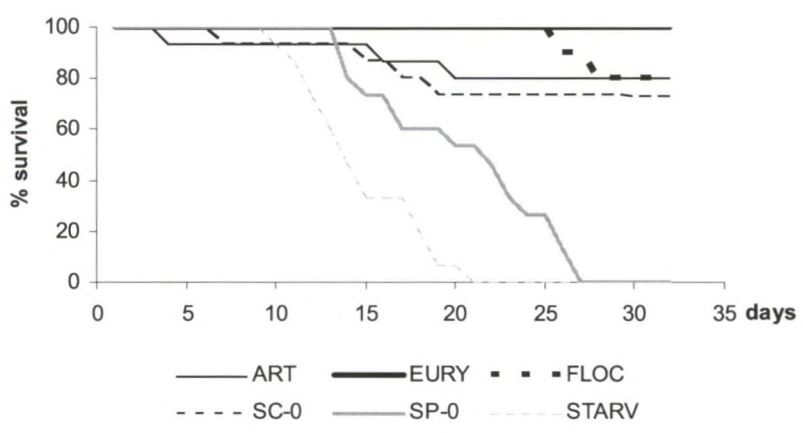


Figure 1: Survival functions of all treatments over the course of the experiment (ART: *Artemia salina* nauplii; EURY: *Eurytemora affinis*; FLOC: laboratory-made estuarine flocs; SC-0: *Scirpus maritimus* detritus; SP-0: fresh *Spartina anglica* detritus; STARV: starved).

Table 2: Linear regression analyses performed with all measurement points within each treatment.

Food type		Regression	#moults	R^2	p
Artemia nauplii	ART	SL = 4.8127 + 0.1162 day	61	0.87	$p < 0.00001$
Eurytemora	EURY	SL = 4.9035 + 0.0615 day	52	0.76	$p < 0.000001$
Laboratory-made flocs	FLOC	SL = 4.4778 + 0.0623 day	36	0.79	$p < 0.000001$
Scirpus detritus	SC-0	(SL = 5.2472 + 0.0216 day)	41	0.19	$p < 0.00473$
Spartina detritus	SP-0	(SL = 5.7156 + 0.0224 day)	31	0.18	$p < 0.01627$
Starvation	STARV	-	17	0.08	$p < 0.28618$

Increase in SL over time

All individuals within one treatment grew linearly and a linear regression analysis was applied to all data points for each treatment (Figure 2; Table 2). A significant regression could be fitted to all treatments, except for STARV ($p < 0.05$). The regressions for *Neomysis integer* feeding on both macrophyte detritus types gave a relatively low R^2 value (0.18 – 0.40). In the other three regression equations, the R^2 ranged between 0.76 and 0.87. The data on the growth of *N. integer* feeding on *Spartina* detritus and when starved are not considered further.

The slopes of the 4 remaining significant linear regression equations were significantly different ($p < 0.001$). The Fisher post-hoc test indicated that the mean length of *Neomysis integer* during the experiment was significantly higher on a diet of ART than respectively EURY, FLOC and SC-0 (all pairwise comparisons with $p < 0.001$). The mean length in the EURY treatment was also significantly higher than on FLOC and SC-0 (all pairwise comparisons with $p < 0.001$), while the latter two did not significantly differ.

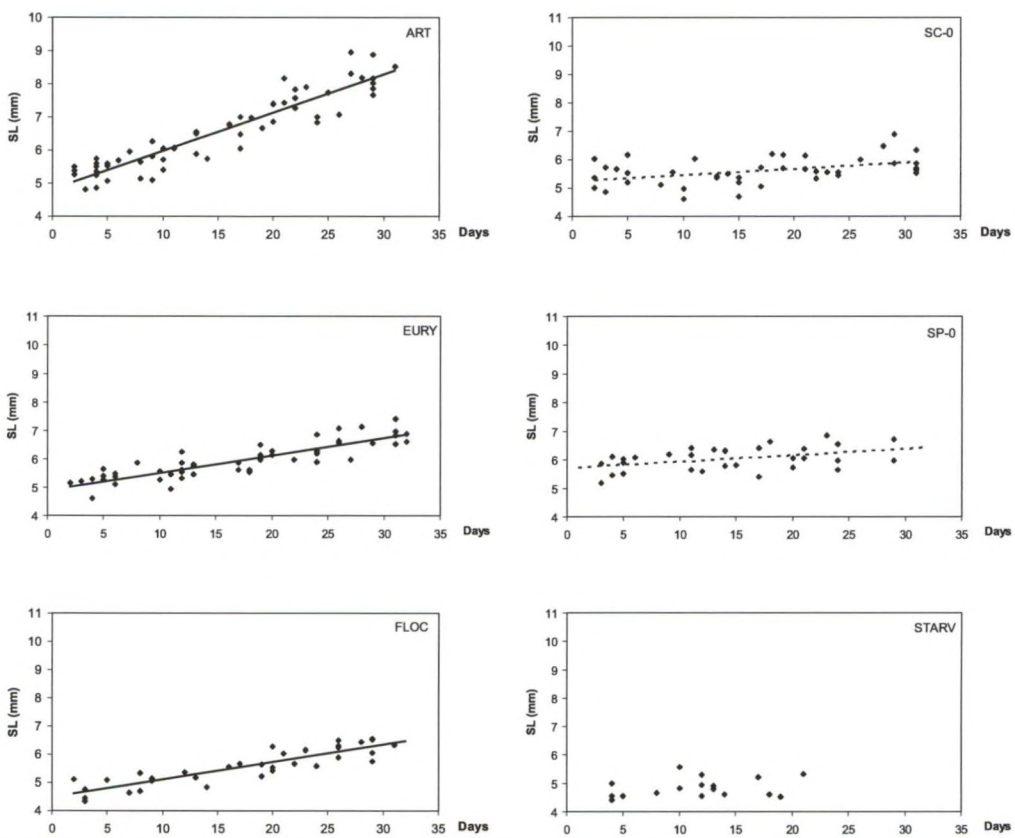


Figure 2: Linear regressions representing the growth of *Neomysis integer* in the six treatments over the course of the experiment (ART: *Artemia salina* nauplii; EURY: *Eurytemora affinis*; FLOC: laboratory-made estuarine flocs; SC-0: *Scirpus maritimus* detritus; SP-0: fresh *Spartina anglica* detritus; STARV: starved).

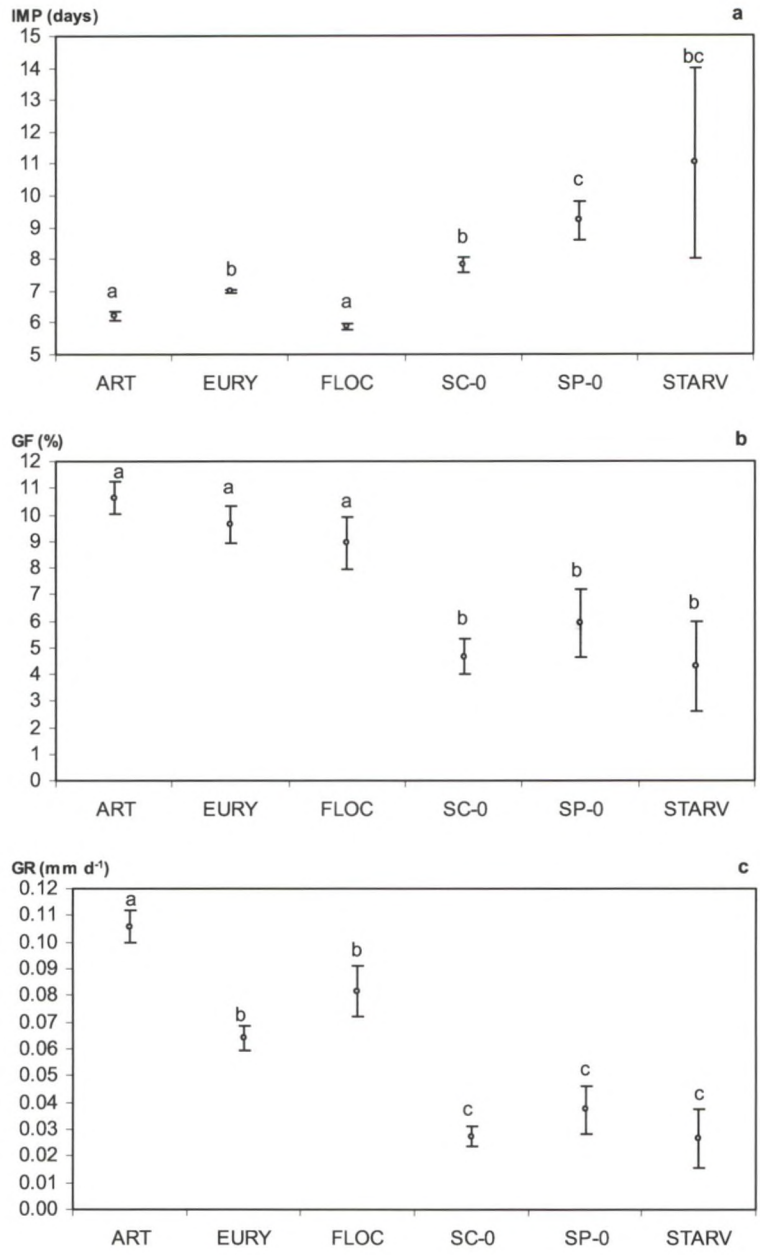
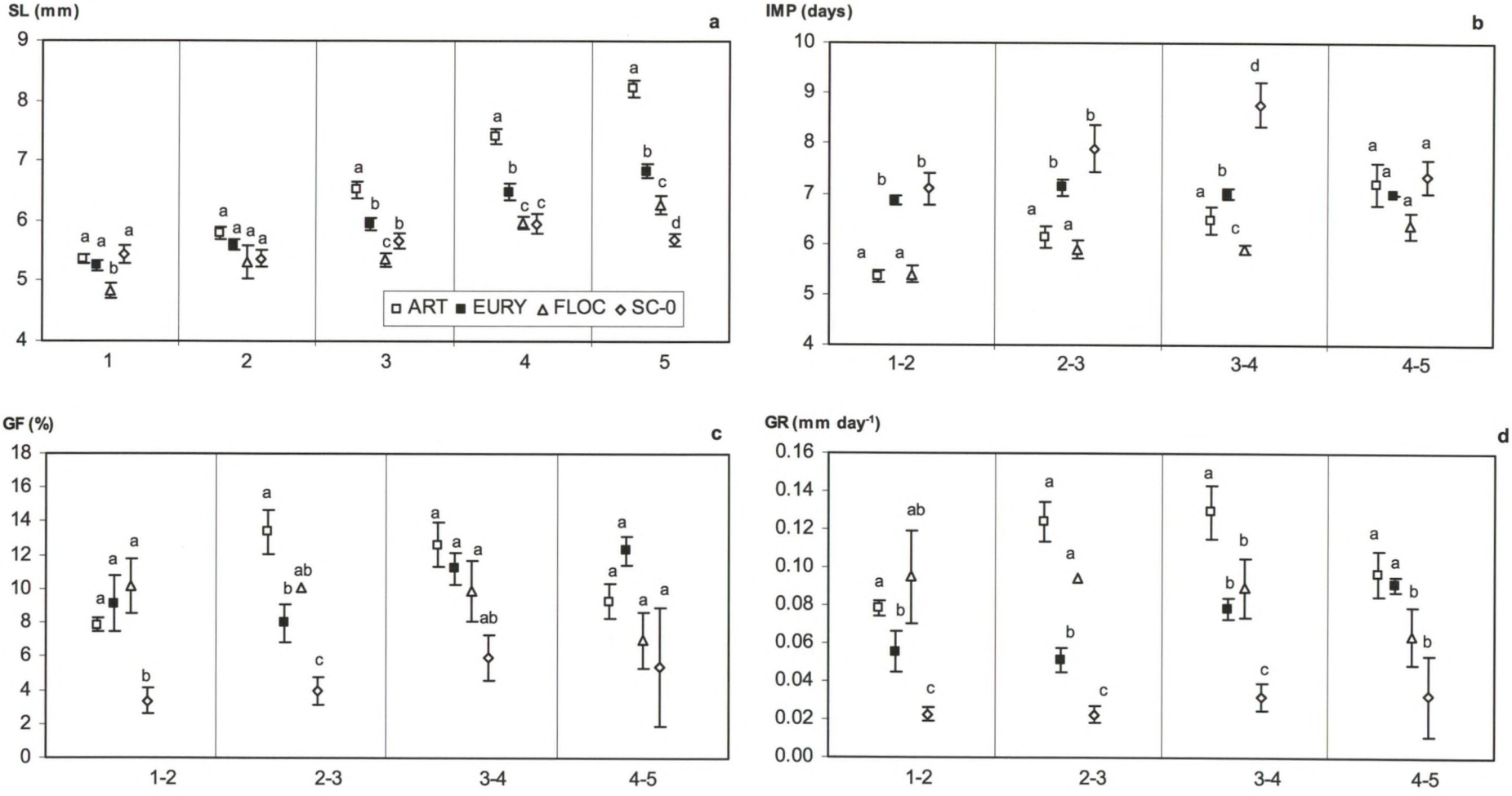


Figure 3: Mean (a) intermoult period IMP, (b) growth factor GF and (c) growth rate GR of all treatments. Different letters indicate statistically significant different groups (multiple comparison $p<0.05$).

Overall IMP, GF and GR

Neomysis integer moulted most frequently (Figure 3) when feeding on ART and FLOCS (mean IMP of 6.07 ± 0.10 days). The longest IMP was measured when feeding on EURY, both types of macrophyte detritus and when starved (7.71 ± 0.17 days). The mean GF was highest when given an animal diet or estuarine flocs (9.97 ± 0.42 %) and was significantly smaller when feeding on macrophyte detritus or when being starved (5.06 ± 0.60 %). When expressed as mean length increase per day within an intermoult period, the growth was highest in ART (0.106 ± 0.006 mm d^{-1}), followed by EURY and FLOC (0.070 ± 0.005 mm d^{-1}), and SC-0, SP-0 and STARV (0.031 ± 0.004 mm d^{-1}).

Figure 4: (a) Pre-moult standard length (SL) at each moulting event (1 – 5), (b) intermoult period (IMP), (c) growth factor (GF), and (d) intermoult growth rate (GR) in between successive moults in the treatments ART, EURY, FLOC and SC-0. Different letters indicate significant differences (multiple comparison $p<0.05$).



SL at each moult event

Within a period of 32 days, subadult *Neomysis integer* grew on average 2.79 ± 0.13, 1.90 ± 0.24, 1.44 ± 0.15 and 0.65 ± 0.01 mm when feeding on *Artemia* nauplii, *Eurytemora*, flocs and *Scirpus* detritus, respectively. This means a relative length increase of 51.7 ± 2.7, 38.0 ± 5.7, 30.4 ± 3.2 and 13.2 ± 0.5 %, respectively. The mean initial standard length of the individuals (Figure 4a) selected for the FLOC treatment was biased and significantly smaller (4.48 ± 0.13 mm) than for the other treatments (5.35 ± 0.05 mm).

Significant differences in SL (Figure 4a; $p < 0.05$) between the different diets were observed from the 3rd moult onwards, when the standard length of ART fed *Neomysis integer* became significantly larger compared to all other treatments. From the 4th moult onwards, a significant gap between the individuals feeding on animals prey (ART and EURY) and individuals feeding on FLOC and macrophyte detritus (SC-0) became apparent. At the fifth moult, a significantly lower SL was observed in the SC-0 treatment in comparison to FLOC as well.

IMP at each moult event

Neomysis integer moulted most frequently (Figure 4b) in the treatments ART (6.2 ± 0.2 days) and FLOC (5.9 ± 0.1 days); while the mean IMP was subsequently longer in EURY (7.0 ± 0.1 days) and SC-0 (7.8 ± 0.2 days). The intermoult period generally increased during the course of the experiment in ART (+34 %), FLOC (+18 %), and SC-0 (+24 %), but it remained more or less constant in EURY (+2 %). From the first IMP onwards, the frequency in moulting is significantly different ($p < 0.05$) between the diets although these differences disappeared after the 4th moult.

GF at each moult event

Over the course of the experiment, the GF (Figure 4c) increased in EURY and SC-0 by 35 % and 65 %, respectively. In the ART treatment, the GF increased over the first 3 moults (+71 %) and then decreased again (-44 %). In *Neomysis integer* feeding on FLOC, the GF generally decreased by 31 % according to the initial GF.

From the first moult onwards, the GF was different between ART, EURY and FLOC on the one hand and in SC-0 on the other hand. After the second moult, the GF of ART-fed mysids increased to 13.38 %, while the other 2 groups remained at a value similar to the first moult (respectively 9.33 and 3.67 % for ART – FLOC and SC-0). After the 3rd moult, the GF of all treatments became more and more alike.

GR at each moult event

Over the course of the experiment, the mean growth rate during each intermoult period (Figure 4d) generally increased in ART (from 0.078 to 0.130 mm d^{-1}), EURY (from 0.056 to 0.091 mm d^{-1}) and SC-0 (from 0.023 to 0.033 mm d^{-1}). Only in the FLOC treatment the GR decreased over time (from 0.095 mm d^{-1} to 0.064 mm d^{-1}), mainly due to the short IMP in this treatment.

From the first moult onwards the GR differed between ART, EURY and FLOC on the one hand and a lower valued GR in SC-0 on the other hand. After the 3rd moult the GR was significantly larger in ART and significantly lower in SC-0 than EURY or FLOC. After the 4th moult the GR on an animal diet was significantly higher than on the detrital diets.

DISCUSSION

Starvation

The capacity to withstand starvation in *Neomysis integer* is dependent on salinity, temperature and size (Vlasblom and Elgershuizen, 1977; Winkler, 2000). At 9 °C, juvenile and subadult *Neomysis integer* survived starvation for maximally 7 weeks (Gorokhova and Hansson, 1999). Armitage *et al.* (1978) starved *N. integer* on kaolin and obtained a mortality of 60 % after 6 to 8 days (8 psu at 15 °C). In the present experiment, the subadult *N. integer* survived starvation for maximally 20 days at 15 °C (5 psu), with 50 % mortality occurring after 14 days. In experiments performed under identical conditions as the ones presented here, subadult *N. integer* could survive for at least 4.5 weeks when fed only 10 *Artemia* nauplii per day (Fockedey, unpublished).

The mysids produced faecal pellets until the last day of their survival. The water was renewed daily, so animals were prevented from eating their cast exuvia and faecal pellets. However, *Neomysis integer* has been described to feed directly on its own faecal string (Molloy, 1958; Ferguson, 1973; Parker and West, 1979) and this could have provided a source of energy for some time. Faecal pellets derived from organically rich food sources still have a carbon content of 40 – 60 % (Ferguson, 1973).

Starvation in *Neomysis integer* causes a decrease in dry weight and a reduction in total body carbon (Gorokhova and Hansson, 1999), indicating a substantial loss of organic reserves. The actual amount of sugars in *N. integer* is small, and allows the mysid to live for only a few hours (Raymont *et al.*, 1968). The low amount of lipid reserves of *N. integer* is enough to maintain respiration rates and activity levels for at least up to 48 h (Weisse and Rudstam, 1989). When starved for a longer period, the species can deaminate body proteins (Raymont *et al.*, 1968). In their natural estuarine habitat, *N. integer* probably never have to overcome periods of food shortage, as the omnivorous mysids can easily take advantage of a wide range of food items in the estuary (Winkler, 2000; Fockedey and Mees, 1999 – Chapter 2).

Excess food concentration

The *Neomysis integer* individuals were offered food *ad libitum*, so the mysids were assumed to feed at a maximum feeding rate in all treatments. Food quality, as measured in the present experiment by the ability of *N. integer* to grow and moult on a certain food item, actually reflect the ability of *N. integer* to assimilate that food item and thus, its nutritional value to the mysid.

The maximum feeding rate of subadult and adult *Neomysis integer* on *Artemia* nauplii, is respectively 200 and 600 – 800 nauplii d^{-1} (Astthorsson, 1980; Fockedey, unpublished). Thus, *N. integer* was fed *Artemia* nauplii well in excess in the current experiment (2000 d^{-1}).

Neomysis integer is known to prey very efficiently on *Eurytemora affinis*. Laboratory experiments in relatively small beakers and with a copepod density an order of magnitude higher than in the field resulted in a maximal daily feeding rate of 20 – 40 *E. affinis* copepodites per mysid (Winkler and Greve, 2004). When fed *E. affinis* nauplii as well (Irvine *et al.*, 1993; Aaser *et al.*, 1995), predation rates were even higher (up to 55 nauplii and 44 copepodites). In the present experiment we did not aim to study predation rates, however, the remaining number of copepods was counted daily.

Over the course of the experiment only 18 % of the supplied copepods were consumed on average (6 – 37 %) and therefore assumed to be administered *ad libitum*. Since feeding rates were substantially smaller (3 – 18 mysid^{-1} d^{-1}) than the ones reported in literature for subadults (20 – 40 mysid^{-1} d^{-1}), it is possible that some food limitation occurred.

Each subadult *Neomysis integer* received 50 copepodite per 350 ml daily (143 l^{-1}). This food concentration is double the maximal density in the Schelde estuary (61 ind l^{-1}; Soetaert and Van Rijswijk, 1993), but smaller than natural *E. affinis* concentrations in the Elbe estuary (220 ind l^{-1}; Köpcke, 2002). During the day, no replacement of eaten prey was done and prey concentration decreased gradually over time. As *N. integer* does not attempt to hunt at low concentrations of calanoid copepod prey (Irvine *et al.*, 1993), this could have had an impact on the ingestion rate and subsequently on growth.

The concentration of flocs produced in the roller tanks varied according to the neap-spring tide cycle with an average of 16000 ± 2000 aggregates l^{-1} (Fockedey *et al.*, submitted c – Chapter 4). Considering a feeding rate of ± 40 flocs h^{-1} ind^{-1} (Fockedey *et al.*, submitted c) and a continuous feeding intensity over 24 hours, the maximal daily ration of *Neomysis integer* on flocs approximates 1000 flocs. Hence, the concentration in the roller tanks provided ample food to the mysids.

In the present experiments, the mysids survived and grew when feeding on detritus from *Scirpus maritimus*. The macrophytal detritus was administered well in excess (25 mg DW ind^{-1} d^{-1}) and a large fraction of the ration was left each day. Generally, detritivory is associated with low assimilation and is be partly compensated by a high ingestion rate (Marchant and Hynes, 1981; Zagursky and Feller, 1985).

Growth performance

Although survival was comparable, the growth of subadult *Neomysis integer* individuals was slower on a diet of macrophytal detritus (*Scirpus maritimus*) than when feeding animal food or laboratory-made flocs. This difference reflects the poor nutritional value of the macrophytal detritus. *Artemia* nauplii were the best quality food for subadult *N. integer*, as a combination of relatively small IMP and relatively high GF and GR gave rise to a significantly higher standard length at the end of the experiment. When fed laboratory-made flocs, *N. integer* moulted as frequently as in *Artemia*-fed mysids, but GR decreased relatively over the course of the experiment. A *Eurytemora affinis* diet resulted in a significantly elongated IMP from the first moult onwards in comparison with mysids fed *Artemia* or flocs, and was associated with a significantly lower growth rate in comparison with the *Artemia* treatment.

The individual variation on the growth parameters (SL, IMP, GF, and GR) was small, thus making it easy to distinguish the effects of food quality (Fockedey *et al.*, submitted c – Chapter 4; present study), food quantity (Fockedey, unpublished) and toxicological effects (Ghekiere *et al.*, submitted) on these endpoints. IMP, GR and GF were affected by the food quality from the first moult onwards. In the present experiment, the IMP increased significantly and hence growth rate (GR, GF) decreased significantly with decreasing food quality, as demonstrated for gammarid amphipods (Willoughby and Sutcliffe, 1976; Delong *et al.*, 1993; Pöckl, 1995). At 15 °C and 5 psu (conditions of the present experiment), *Neomysis integer* feeding *ad libitum* on *Artemia* nauplii needs 13 moults to become mature (Fockedey *et al.*, in press – Chapter 3). Any increase in the IMP due to poor food quality, will result in a considerable prolongation of the maturation time at this temperature and salinity. A decreased growth rate at moulting, associated with the consumption of low quality food, resulted in a

smaller size at the end of the experiment and probably a smaller size-at-maturity associated with a lower fecundity.

Field relevance

The intertidal salt marshes on the margins of the Schelde estuary are massively occupied by *Neomysis integer* at flood during each tidal cycle (*e.g.* Mees *et al.*, 1993a; Cattrijsse *et al.*, 1994; Hampel *et al.*, 2003; 2003b). The areas are favoured by the estuarine mysids for reproductive purposes (Cattrijsse *et al.*, 1994; Mees *et al.*, 1993a), even though residence in the marsh is coupled to a high predation pressure by fish and shrimp (Cattrijsse *et al.*, 1994; Dean *et al.*, 2005). The large amounts of macrophytal detritus available inside the marsh creeks are also assumed to attract the mysids (Zagursky and Feller, 1985; Cattrijsse *et al.*, 1994).

Spartina anglica and *Scirpus maritimus* are abundant vascular plants in the brackish marshes of the Schelde estuary (Beeftink, 1977; Adam, 1990). Gut contents of animals living in marshes often include large quantities of vascular plant detritus, although there is little evidence that a strict diet of this material can sustain the populations of the high-order consumer-species like fish (Zagursky and Feller, 1985; Kneib, 1997). *Neomysis integer* could survive on a diet of *S. maritimus* detritus in the present experiment, although the somatic growth was low. The *S. anglica* detritus caused a high mortality and morphologic aberrations to *N. integer*. The latter detritus was made from living plants and the leaves were not leached before application. Some chemical constituents (*e.g.* polyphenols or tannins) released in the water by living macrophytes are toxic for *N. integer* (Lindén and Lehtiniemi, 2005).

Generally, the refractile macrophyte detritus can be digested by detritivore crustaceans through the presence of cellulolytic bacteria in the intestine (Plante *et al.*, 1990) or the availability of specific enzymes in the gut. Cellulases have been identified in *Neomysis integer* (Molloy, 1958), but it is not known if the enzyme is produced by the mysids themselves or with the aid of an associated gut microflora (Foulds and Mann, 1978; Wainwright and Mann, 1982). Although microflora residing in the alimentary track of *N. integer* has been demonstrated (Bradshaw *et al.*, 1989), specific cellulolytic bacteria have not yet been found for the species. Other authors (*e.g.* Hargrave, 1970) suggest that the main energy value of the macrophyte detritus for crustaceans lies in the associated bacteria and protozoans (trophic upgrading), while the detritus itself is egested unchanged. Observations of the faecal pellets produced when feeding on *Scirpus maritimus* detritus showed that the plant remains were digested themselves (Fockedey et al, submitted d – Addendum 2).

The detritus was supplied without aging. The extensive growth of fungi, bacteria and protozoans on decaying plant remains makes the resistant detritus acceptable to shredders by softening the leaves and by raising their protein content (Hargrave, 1970) and result in a higher growth performance in gammarid amphipods and copepods (Willoughby and Sutcliffe, 1976; Heinle *et al.*, 1977). Preliminary results (Fockedey, unpublished), however, showed that a relatively short decay of this detritus for 3 – 5 days did not alter the growth rate of the mysid *Neomysis integer* in comparison with the unconditioned detritus.

In the subtidal reaches of the MTZ of the estuary, the availability of estuarine flocs is high in comparison with calanoid prey. *Neomysis integer* encounters at best one *Eurytemora affinis* copepodite or adult per 10 macro-flocs (Soetaert and Van Rijswijk, 1993; Zimmerman, 1997; Tackx *et al.*, 2004). The escape reaction of the copepods makes them a less easily preyed item than flocs. The lower energetic value of the flocs is compensated for by a higher

consumption rate as demonstrated by egestion rates (Fockedey *et al.*, submitted d – Addendum 2), but still resulted in a significantly lower growth rate in comparison with a diet of *E. affinis*.

Food selection experiments were not in the scope of the present study and nothing is known about the *in situ* preference of *N. integer*. Also we did not aim to perform growth experiments with mixed diets. It is possible that a combination of animal and plant food stimulates growth more than given one type of diet (Heinle *et al.*, 1977; Roman, 1984). Some items may not be consumed massively, but do deliver essential nutrients like essential fatty acids and vitamins necessary for optimal growth, development and/or reproduction (Koski *et al.*, 1998).

ACKNOWLEDGEMENTS

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Addendum 2

Some experimental observations on gut passage time, egestion rate and faecal pellet production of the brackish water mysid *Neomysis integer* (Mysidacea: Crustacea) feeding on different food iems

ABSTRACT

Gut passage times in *Neomysis integer* were calculated by measuring voided faecal pellets when feeding *ad libitum* on a variety of environmentally relevant food types and a reference diet of *Artemia salina* nauplii. When feeding on *A. salina* nauplii, gut passage times were variable (from 4.1 to 12.9 h), but significantly longer than when feeding on the post-naupliar stages of the calanoid copepod *Eurytemora affinis* (2.6 h). Estuarine flocs passed through the intestine within 0.5 hour after ingestion, and *N. integer* produced daily up to twice its own body length in compact faecal material. The gut residence time of macrophytal detritus was 1.9 h and no difference was found between fresh and aged detritus.

The egestion rate of *Neomysis integer* feeding on estuarine flocs (0.163 ± 0.001 mm^3 h^{-1}) was significantly higher than in all other treatments (0.011 ± 0.001 mm^3 h^{-1}). The faecal pellet production rate, when feeding on flocs, amounted to 0.044 mgDW mgDW^{-1} h^{-1}.

Preliminary results on the C:N ratio of food and faecal pellets demonstrated a general enrichment in nitrogen in the faecal pellets, probably due to bacterial growth on the pellets, the peritrophic membrane and disintegrating cells of the mysids intestine. The faecal pellets produced by *Neomysis integer* are still potential sources of energy themselves. Scanning electron micrographs of faecal pellets produced on the different food types give details about the peritrophic membrane and pellet content.

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INTRODUCTION

Stomach fullness data and quantitative gut content analyses of *Neomysis integer* living in the maximum turbidity zone of several European estuaries were presented by Fockedey and Mees (1999 – Chapter 2). In this zone of the estuary, *N. integer* is a consumer of mesozooplankton, estuarine aggregates and macrophytal detritus (Fockedey and Mees, 1999). In laboratory studies, these food items were tested on their nutritive value for *N. integer* using the growth performance of the animals (Fockedey *et al.*, submitted a and submitted c – Chapters 4 and 5). Additional *in situ* stomach fullness analyses were performed for *N. integer* sampled during several 24h cycles in the Elbe, Westerschelde and Gironde estuaries (Fockedey, unpublished - MATURE). However, feeding rate and daily ration could not be calculated, since information on the gut residence time was lacking.

In any study concerning the feeding ecology of a species, there is a need to estimate the gut passage time (also called gut residence or retention time or gastric evacuation rate), *i.e.* the time that a food particle needs to pass through the complete length of the intestine. Especially when estimating *in situ* feeding rates based on the gut fullness, one cannot make firm interpretations without the knowledge of the gut passage time (Heyraud, 1979; Murtaugh, 1984; Perissinotto and Pakhomov, 1996).

Previous studies have shown that invertebrates can exercise some kind of control over their gut passage time, and adapt the retention to the nature and abundance of the food particles (Stålberg, 1933; Murtaugh, 1984). A poor quality food or a small number of ingested prey may be retained for a longer period in the gut to maximize food assimilation (Murtaugh, 1984). High quality or abundant food can pass through the gut quickly with a limited extraction in the gut (Willoughby and Earnshaw, 1982). Gut passage time is also inversely related to the environmental temperature (*e.g.* Welton *et al.*, 1983; Chipps, 1998) and the size of the animal (Zimmerman and Wissing, 1978; Uye and Kaname, 1994).

A method often used for determining gut passage times in invertebrates is to starve animals taken from the field and measure the time to empty their gut (Moore, 1977; Marchant and Hynes, 1981; Murtaugh, 1984). Another method is to starve the animals in order to empty their gut, where after the animals are fed a specific dietary item at a specific concentration in the laboratory and to monitor the time to the first faecal pellet production (Foulds and Mann, 1978). However, starvation increases the retention time of the food (Murtaugh, 1984) and results in a non-linear emptying of the gut (Marchant and Hynes, 1981). Also, feeding rates may be artificially high after a period of starvation by compensatory feeding.

In the case of the brackish water mysid *Neomysis integer*, gut clearance experiments are difficult since the mysid retains food in its stomach for at least 7 days under starvation conditions (Molloy, 1958). Furthermore, the species becomes coprophagous and/or cannibalistic when there is shortage of food (Raymont and Krishnaswamy, 1960; Parker and West, 1979; Weisse and Rudstam, 1989; Roast *et al.*, 2000b). Alternative methods are the use of coloured food as a marker to follow its passage in the intestine through the translucent cuticle (Monk, 1977; Zimmerman and Wissing, 1978; Murtaugh, 1984; Chipps, 1998) or the use of the "faecal pellet production method" (Willoughby and Earnshaw, 1982). This latter method measures the length of the faecal pellets, produced by the animals within a certain time span, in relation to the length of the intestine.

The aim of the present study was to measure and compare the gut passage time of *Neomysis integer* for different environmentally relevant dietary items and a reference diet of *Artemia salina* nauplii. Egestion rates were estimated (as volume or dry weight of faeces produced per time unit). Additionally, preliminary data are presented on (1) the carbon and nitrogen content of the food and the faecal pellets, and (2) scanning electron microscopic observations on the different types of faecal pellets produced.

MATERIAL AND METHODS

Gut passage time experiment

Neomysis integer was sampled in the brackish water pond Galgenweel situated on the left bank of the Schelde estuary. Short hauls were taken with a 1 x 1 mm meshed handnet (0.3 by 0.2 m) and the animals were taken to the laboratory in environmental water within 2 hours sampling. Subadult *N. integer* individuals (standard length between 7 and 11 mm) were adapted for 2 to 4 days on the following experimental diets: *Artemia salina* nauplii (< 36 h old), adult and copepodite stages of the calanoid copepod *Eurytemora affinis*, estuarine flocs, fresh *Scirpus maritimus* detritus and aged *S. maritimus* detritus (Table 1).

Experiments were performed in artificial seawater of 5 psu, except for the treatment with estuarine flocs. The methodology for the formation of the flocs in the laboratory with natural estuarine water is described in detail in Fockedey *et al.* (submitted c – Chapter 4). In short, surface water was collected in the maximum turbidity zone of the upper part of the Schelde estuary (10 and 14/4/1998), sieved (250 µm) and rotated (11 rpm) in cylindrical containers (0.85 l) to allow floc formation. After 3 hours a steady state in flocs numbers and size was obtained and 1 mysid was added per container.

Died-off plant material of *Scirpus maritimus* was collected from the banks of the Schelde. After carefully washing off the sediments and epiphytes, the *S. maritimus* was air-dried (15 – 20 °C) for 2 days and ground to 400 µm particles. The mysids were fed with 40 ± 3 mg DW of this powder (further named fresh detritus SC-0) that was suspended in the medium (350 ml of artificial seawater per container). To age the detritus (SC-A), the same DW of particles was suspended in artificial seawater of 5 psu, and was allowed to decompose for 3 – 5 days in a climate room (25 °C) with aeration before being administered to *Neomysis integer*.

Artemia salina nauplii (San Francisco Bay Brand) were hatched from cysts in the laboratory. After harvesting, an aliquot was quantified, and 2000 nauplii were administered to the experimental jars (350 ml).

Table 1: Set-up and results of the experiments to determine gut passage time (GPT) of subadult *Neomysis integer* feeding on different food items.

	Concentration Food (l^{-1})	N	Duration (h)	SL mysids (mm)	Length intestine (mm)	Egestion rate length (mm h^{-1})	Egestion rate volume (mm^3 h^{-1})	GPT (h) Mean ± SE
Artemia sp.	5700	10	18.0	8.5 – 10.8	7.6 – 9.7	0.6 – 2.3	0.001 – 0.016	6.8 ± 1.1
Eurytemora affinis	143	9	20.17	7.2 – 9.2	6.4 – 8.2	2.0 – 3.7	0.008 – 0.015	2.6 ± 0.1
Estuarine flocs *	16000 ± 2000	10	21.0	7.5 – 10.0	6.3 – 9.3	6.9 – 20.3	0.057 – 0.217	0.5 ± 0.1
Fresh detritus	115 g DW	8	6.67	7.5 – 8.9	6.6 – 7.9	2.2 – 5.9	0.005 – 0.027	2.0 ± 0.3
Aged detritus	115 g DW	8	6.0	8.1 – 9.6	7.2 – 8.5	1.7 – 14.4	0.004 – 0.023	1.8 ± 0.5

* See Fockedey et al. (submitted) for a detailed description of the floc formation and quantification methodology

Copepodites and adults of the calanoid copepod *Eurytemora affinis* were collected in Galgenweel by filtering surface water through a 250 µm sieve. A ration of 50 post-naupliar stages was quantified using the spot method of Reeve (1970) and transferred to each experimental unit (350 ml).

The gut passage time, defined as the time a food item needs to pass through the intestine channel, was measured according to the technique of Willoughby and Earnshaw (1982) developed for gammarids. The faecal pellets are considered to be cylindrical. Before the start of each experiment the following parameters were measured for each mysid using a drawing mirror mounted on a stereomicroscope: standard length SL (as the length between the base of the eye stalks and the base of the telson), the length of the intestine (posterior of the stomach until the anus) and the length and width of the faecal pellets present in the intestine. One mysid was introduced per experimental unit (8 – 10 replicates per treatment) and was allowed to feed *ad libitum*. The duration of the experiments was adapted for each dietary item according to the amount of faecal material produced (Table 1). All experiments were performed in a climate-controlled chamber at 15 °C under continuous lighting. At the end of each experiment the faecal pellets present in the intestine and the faecal pellets that were produced during the course of the experiment were measured (length of all pellets, width of 30 pellets randomly selected). No attempt was made to sort the pellets out from between the remaining food, as it was too time-consuming to collect all faecal material (especially in the detritus and flocs trials). It was assumed that the mysids were not feeding on the pellets. The gut passage time GPT (h) was calculated as:

$$\text{GPT} = \text{duration of the experiment (h)} \times \frac{\text{length of the intestine (mm)}}{\text{total length of faecal pellets produced (mm)}}$$

The GPT and the egestion rate (as mm^3 faecal pellets produced h^{-1}) when feeding on the different diets was compared with an ANOVA and the Fisher post-hoc test (log transformed data).

Egestion rate: faecal pellet production method

An additional experiment was set-up with *Neomysis integer* feeding on laboratory-made flocs with the aim to collect all faecal pellets produced and measure egestion rate (as dry weight). Estuarine water was sampled on 5/5/1998 and *N. integer* was adapted for 2 days to a diet of laboratory-made flocs. Groups of mysids of a specific size class were selected and allowed to feed on the (not-quantified) aggregates for 24 hours (Table 2). All faecal pellets were collected from between the remaining aggregates with needle and pipette. However, it was a very time consuming process as pellets were often coagulated within the aggregates and had to be washed in distilled water for several times. Pellets were oven-dried (60°C, 48h). Mysid standard length was measured with a drawing mirror mounted on a stereomicroscope and converted to dry weight (Irvine *et al.*, 1995). Egestion rate was calculated as mg dry weight of faecal pellets produced per mg mysid dry weight per hour.

C:N in the food and faecal pellets

Additional experiments were set up for the analysis of the C and N content of the food and faecal pellets produced by *Neomysis integer* feeding on the 5 diets. Faecal pellets were collected with a needle under stereomicroscope. Special attention was paid not to collect food remains together with the pellets.

Since this process is very time consuming the experimental units were placed at 8 °C until processing (maximally 6 h), to avoid the formation of bacteria or other micro-organisms. At regular time intervals (± each 15 min) the picked faecal pellets were frozen (in bulk per treatment). The numerous faecal pellets produced when feeding on estuarine flocs were first concentrated (with loss) by sieving the medium over a 106µm sieve. No attempt was made to collect all faecal pellets produced by *N. integer*, but a sample large enough to perform a C:N analysis with the Carlo Erba elemental CHN elemental analyser (N1500), *i.e.* minimally 2 mg dry weight was collected. The samples of faecal pellets (1 replicate) and food (2 – 3 replicates) were weighed with a microbalance (METTLER M3). The C and N content are expressed as a % of the dry weight. Additional samples of floc food material were collected to compare total carbon content and the organic carbon content of the flocs after acidification.

Scanning electron microscope (SEM)

Some individual faecal pellets were picked out for scanning electron microscopy (SEM, JEOL840). They were carefully washed with distilled water, critical point dried from liquid CO_2, and coated with gold. Some pellets were broken prior to coating to examine its content at the fractured surface.

RESULTS

Gut passage time experiment

The gut passage time of *Neomysis integer* (Table 1, Figure 1) feeding on *Artemia salina* nauplii varied between 4.1 and 12.9 h (mean 6.8 ± 1.1 h), and only a small amount of faecal pellets was produced per hour (< 2.3 mm or < 0.016 mm³). When feeding on post-naupliar *Eurytemora affinis*, the mysids had a significantly lower gut passage time (2.6 ± 0.1 h). This was comparable to the gut residence time when the mysids were feeding on fresh and aged macrophytal detritus (respectively 2.0 ± 0.3 h and 1.8 ± 0.5 h). Aging of the macrophyte detritus did not result in different egestion rates or gut passage times. Estuarine flocs passed the intestine of *N. integer* at a significantly higher speed (0.5 ± 0.1 h). The mysids produced a large amount of faecal pellets up to 20.3 mm, or 1 to 2 times their own body length per hour, corresponding to an egestion rate of 0.06 – 0.22 mm³ h^{-1}.

The cross section area of faecal pellets varied between 0.002 and 0.007 mm² (average of 0.004 mm²) when feeding on animal diets or macrophyte detritus. The diameter of the pellets was substantially larger when feeding on estuarine flocs (0.008 – 0.013 mm²; mean 0.010). The egestion rate (Figure 2) of *Neomysis integer* feeding on flocs (0.163 ± 0.001 mm³ h^{-1}) was significantly higher than in all other treatments (0.011± 0.001 mm³ h^{-1}).

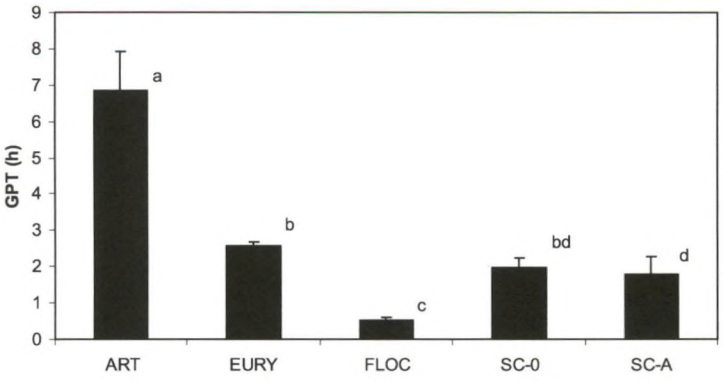

Figure 1: Gut passage time (GPT ± standard error) of subadult *Neomysis integer* feeding *ad libitum* on 5 different diets. Different letters indicate significant differences (multiple comparison $p<0.05$). ART: *Artemia salina* nauplii, EURY: post-naupliar stages of *Eurytemora affinis*, FLOC: laboratory-made estuarine flocs, SC-0: fresh macrophyte detritus and SC-A: aged macrophyte detritus of *Scirpus maritimus*.

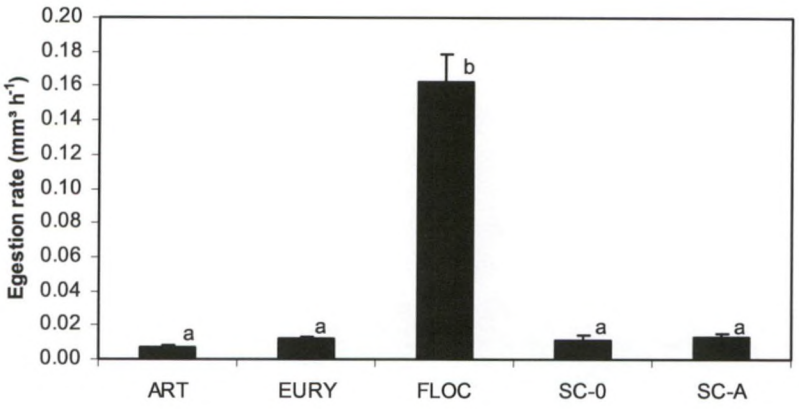

Figure 2: Egestion rate (± standard error) of subadult *Neomysis integer* feeding ad libitum on 5 different diets. Different letters indicate significant differences (multiple comparison $p<0.05$).

Egestion rate

The method described to collect all faecal pellets from between the remaining aggregates was a very time consuming process (1 sample processed per 6 hours!). Pellets were often coagulated within the aggregates and had to be washed in distilled water for several times, with the risk of damaging the pellets. The technique was too impractical to perform on a large scale and in a statistically robust way. However, some results are presented in Table 2. The egestion rate ranged between 0.30 and 0.57 mg DW mysid^{-1} d^{-1} in small subadults and 1.67 and 1.84 mg DW mysid^{-1} d^{-1} in larger subadults. Weight-specific egestion rates were estimated as 0.035 – 0.044 mgDW mgDW^{-1} h^{-1} for small subadults and 0.022 – 0.029 mgDW mgDW^{-1} h^{-1} for larger subadults.

C:N in the food and faecal pellets

The carbon content (Figure 3) of animal food (6 – 7 %) was lower than that of macrophytal detritus (41 %). The nitrogen content of animal food (1.2 – 1.4 %) was double the value of the macrophyte detritus (0.7 %). No effect of aging could be observed in the C or N content of the plant remains. The carbon content of estuarine flocs was 6.4 % and mainly consisted of organic carbon, as acidification caused the C content to decrease only moderately to 5.1 %. The nitrogen content of the flocs was very low (0.4 %).

Although measurements of faecal pellets are based on one sample only, some trends in the C and N ratio between food and faecal pellets could be observed. When feeding on *Artemia salina* and *Eurytemora affinis*, the mysids produced faecal pellets that were clearly enriched in C (+13 % and +10 %) and N (+1.6 % and +1.9 %).

Table 2: Set-up and results of the experiment to determine egestion rate of subadult *Neomysis integer* feeding on flocs. SS: small subadult; LS: large subadult. (*) According to standard length – dry weight conversion of Irvine et al., 1995.

		Mysids		Egestion rate	
# ind	Stage	SL mysid (mm)	DW mysid $^{-1}$ (*) (mg)	mgDW ind^{-1} d^{-1}	mgDW mgDW^{-1} h^{-1}
8	SS	5.48 ± 0.30	0.56 ± 0.07	0.57	0.044
10	SS	4.74 ± 0.17	0.36 ± 0.04	0.30	0.035
2	LS	10.54 ± 0.40	3.48 ± 0.38	1.84	0.022
3	LS	9.22 ± 0.21	2.37 ± 0.15	1.67	0.029

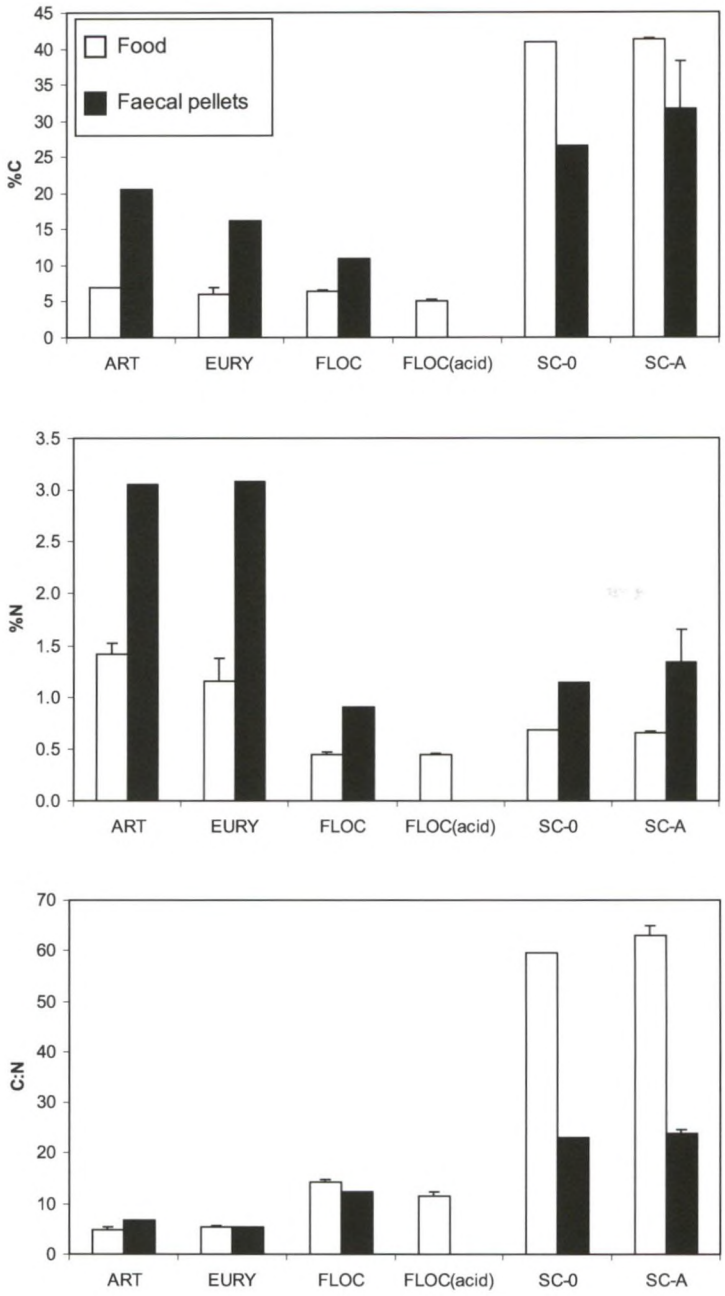

Figure 3: Carbon (% C) and nitrogen (% N) content, and C:N fraction of food and faecal pellets produced by *Neomysis integer* when feeding on the different diets. The error bars indicate standard error.

The faecal pellets produced when feeding on estuarine flocs are also enriched, though more moderately (+5% C and +0.5% N). When feeding on fresh and aged macrophyte detritus, the faecal pellets are enriched in N as well (+0.5 % and +0.7 %), but depleted in carbon (-14 % and -9 %).

Scanning electron microscope (SEM)

When feeding on animal food, the faecal pellets were closed units surrounded with a firm and elastic peritrophic membrane (Figure 4a). Pellets produced when feeding on the other food types were longer and fragile. The peritrophic membrane of the pellets produced on macrophyte detritus was not so firm and the membrane was ruptured on several locations (Figure 4c). When the mysids fed on estuarine flocs, the peritrophic membrane was completely lacking (Figure 4e).

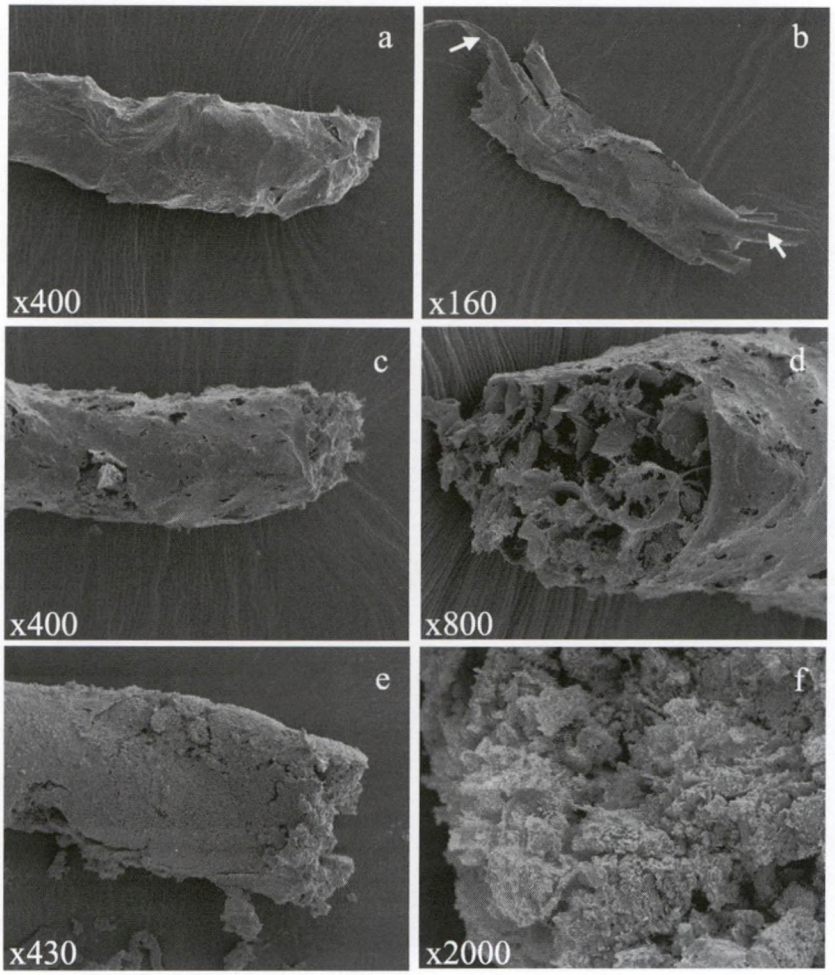

Figure 4: Scanning electron micrographs of faecal pellets produced after feeding post-naupliar stages of *Eurytemora affinis* (a, b), artificially made macrophyte detritus of *Scirpus maritimus* (c, d) and laboratory-made flocs (e, f). Left side: general appearance of the pellet; right side: detail of the fractured surface of a broken pellet. Arrows in b: extending copepod remains.

Within the pellets produced on animal food, one could recognize undigested parts of the exoskeleton of *Artemia salina* or *Eurytemora affinis* (indicated with arrows in Figure 4b). The faecal pellets produced on estuarine flocs were made up of very fine sediment material closely packed together (Figure 4f). In some cases mineral grains or remains of diatom frustules could be recognised at the fractured surface (not shown). The content of pellets produced on macrophyte detritus was not that compact, and remains of the detritus were visible, although no tracheae were recognisable as was the case in macrophyte detritus recuperated from stomach contents of the mysids (Fockedey and Mees, 1999 – Chapter 2). Micro-organisms were not observed on or within quickly recuperated faecal pellets, but the peritrophic membrane of older faecal pellets (> 24 h) was intensively colonized with bacteria which started to biodegrade the membrane (Figure 5).

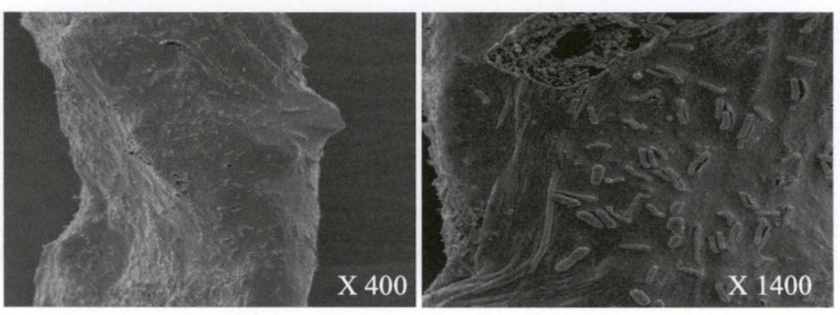

Figure 5: Peritrophic membrane of an older faecal pellet (> 24h) produced after feeding on *Artemia salina* nauplii. The pellet's surface is colonized by bacteria beginning to biodegrade the peritrophic membrane.

DISCUSSION

Gut passage time

Several studies have confirmed that macrophytal detritus, especially when conditioned with micro-organisms, is an excellent food to detritivorous invertebrates that results in high growth rates (*e.g.* Sutcliffe *et al.*, 1981). The gut passage times of *Neomysis integer* on macrophyte detritus are in line with those calculated for detritivorous amphipods (Bärlocher and Kendrick, 1975; Marchant and Hynes, 1981; Willoughby and Earnshaw, 1982). The laboratory-made detritus of *Scirpus maritimus* proved to be a nutritive food for *N. integer* (Fockedey *et al.*, submitted a – Chapter 5). Preliminary results (Fockedey, unpublished) showed that aging of this detritus for 3 – 5 days did not alter the growth rate of the mysids in comparison with the unconditioned detritus. Although not quantified, microscopic observation of the aged detritus revealed abundant micro-organisms to be present on the pieces of plant material after 3 – 5 days conditioning at 25 °C. In the present study, administering fresh and aged detritus did not cause a significant difference in the gut passage time of *N. integer*. Other studies offering laboratory-made macrophytal detritus as food to invertebrates often use a longer incubation time of several weeks or work with already decaying material collected in the field (Marchant and Hynes, 1981; Willoughby and Earnshaw, 1982; Gorokhova and Hansson, 1999). A time span of 3 to 5 days is probably too short to develop extensive aquatic bacteria and/or fungi, as reflected in the unaltered nitrogen and carbon content of the aged detritus.

Murtaugh (1984) found the gut residence time of the mysid *Neomysis mercedis* to be highly variable and negatively related with the ingestion rate, and concluded that stomach fullness data are completely unreliable as an indicator of *in situ* feeding rates. The highly variable gut residence time observed may have been caused by the very low prey concentrations. In the current study the dietary items were offered *ad libitum*. Whether a negative relationship of gut passage time and ingestion rate also holds for *Neomysis integer* (when offering more realistic food densities), remains to be tested.

Highly variable gut passage times, like in the treatment with *Artemia salina* nauplii in this study, are indicative of an intermittent rather than a continuous feeding (Willoughby and Earnshaw, 1982). In this case the gut is not kept full and the gut passage time is possibly overestimated. It is possible that the actual rate of passage of material through the gut, once it has been ingested, is as rapid as with the other dietary items (Willoughby and Earnshaw, 1982).

The extremely short gut passage time of only 30 minutes when feeding on estuarine flocs is comparable with amphipods feeding on sediments (Hargrave, 1970). Molloy (1958) noted that carmine particles require 30 – 40 min to pass along the complete length of the alimentary track of *Neomysis integer*.

Egestion rate

The egestion rate on a variety of diets is quantified as mm^3 pellets produced per hour. Egestion rates on *Daphnia* sp. by *Neomysis mercedis* (Murtaugh, 1984) are comparable with the egestion rates measured in the current study, except for the egestion rate on estuarine flocs that is on average one order of magnitude higher.

The egestion rate of *Neomysis integer*, when feeding on laboratory-made aggregates, was additionally estimated with a quantitative recovery of faecal pellet produced (expressed in DW). The egestion rates obtained (0.022 – 0.044 mgDW $mgDW^{-1}$ h^{-1}) are comparable with the ones described for *N. integer* feeding on organically poor sediments (0.017 – 0.049 mgDW $mgDW^{-1}$ h^{-1}; Roast *et al.*, 2000b). The method however, was too impractical and time-consuming to perform a statistically robust experiment.

Although gut residence time is variable, egestion rates have been used to calculate feeding rates of mysids (Murtaugh, 1984; Roast *et al.*, 2000b). The present study demonstrated that *Neomysis integer* needed to consume estuarine aggregates at a much faster rate than when feeding on animal food. *N. integer* fed rapidly on the estuarine flocs, with mysids passing up to two times their own length in faecal material in one hour. These faecal pellets are significantly wider than on other food types, resulting in a production of 0.163 ± 0.015 mm^3 faecal material per hour.

The biodeposition effect of *Neomysis integer*, compacting suspended macro-flocs by consumption and depositing them in C-enriched faecal pellets is hard to quantify. Assuming maximal reported densities of *N. integer* in the MTZ of the Schelde of 240 ind m^{-2} (Mees and Jones, 1997), and a continuous feeding of the mysid uniquely on estuarine flocs without any food limitation, the total faecal pellet production was estimated to be 10 – 50 mg C m^{-2} d^{-1}.

Peritrophic membrane

The ingested food passes from the stomach into the lumina of the digestive glands and is digested and absorbed there (Molloy, 1958). *Neomysis integer* has a single, large dorsal diverticulum that arises as an extension of the midgut in the posterior dorsal area of the stomach. It continuously secretes the peritrophic membranes into the intestine and packs up the faecal material (Molloy, 1958), that consists of indigestible food remains and cellular components of disintegrating epithelial cells of the intestine and gut microflora. The function of this chitinous peritrophic membrane in crustaceans remains unclear (Brunet *et al.*, 1994).

When feeding on macrophyte detritus and on estuarine flocs, the faecal pellets of *Neomysis integer* have an incomplete to lacking peritrophic membrane. Therefore, the pellets break apart more easily into smaller pieces after egestion. Shortage of specific dietary proteins or amino-acids in the detrital food may result in this poor quality membrane. On the other hand, the digestion of chitinous material (*Artemia salina*, *Eurytemora affinis*) probably involves chitinases (Molloy, 1958). Possibly, the peritrophic membrane is a protection against enzymatic abrasion of the chitinous lining of the endgut of *N. integer* when enzymatically active faecal material passes (Brunet *et al.*, 1984). This mechanism is not needed in the case of a chitin-free detrital consumption. Molloy (1958) however, assumed that some kind of inhibitor must also be present to prevent digestion of the chitinous lining of the hindgut and the chitinous peritrophic membrane surrounding the faeces.

Chitinases and cellulase were identified in whole animal extracts of *Neomysis integer* and Molloy (1958) expected these to be present in the intestinal channel. In the pellets produced when feeding on a crustacean diet (*Artemia*, *Eurytemora*), the chitinases were not very active, as the exoskeleton of the prey is still easily recognisable in the faecal pellets. Large macrophyte detritus is not recognisable any more as such in the pellets, except for some fibres (not shown). One can assume that cellulases were active in the gut of *N. integer* in the experiments and substantially digested the macrophyte pieces.

C:N in the food and faecal pellets

The general enrichment in nitrogen in the faecal pellets is probably due to the secretion of proteins and lipids of the peritrophic membrane and cellular components of disintegrating epithelial cells of the intestine and gut microflora (Bradshaw *et al.*, 1989; Brunet *et al.*, 1994). The nitrogen enrichment can be caused by an external bacterial growth on the peritrophic membrane in our experiments (Turner and Ferrante, 1979), as can be observed on the SEM observations of aged (> 24h) pellets.

The depletion of carbon in the faecal pellets when feeding on macrophyte detritus indicates the actual digestion of the carbon of the plant remains. Carbon enrichment in faecal pellets is generally explained by the preferential selection of the organic fraction of the food source supplied (Ferguson, 1973) and might explain the (possible) increase in the carbon content of the faecal pellets on estuarine flocs. The carbon enrichment in the pellets when feeding on *Artemia* or *Eurytemora* cannot be explained.

The faecal pellets produced by *Neomysis integer* are potential sources of energy themselves (for *N. integer* and other coprophagous invertebrates); especially when derived from organic rich food sources the faecal pellets still have a high carbon content (Ferguson, 1973). *N. integer* feeds on its own faeces when shortage of food is apparent (Molloy, 1958; Weisse and Rudstam, 1989; Roast *et al.*, 2000b) with an assimilation efficiency of 10 – 25 %. Although this is not particularly high, it indicates that some nourishment can be derived from the faecal pellets (Ferguson, 1973).

The carbon and nitrogen content of the animal food items, measured in the present study, are much lower than those found generally in literature (e.g. Parsons *et al.*, 1984; Evjemo and Olsen, 1999). The carbon content of *Eurytemora affinis* and *Artemia* sp. is reported by the other authors as respectively 48 and 45.5%; while the nitrogen content amounts respectively 12.5% and 10.1%. Our measurements are considerably lower (6.9% and 6.0% for carbon and 1.4% and 1.2% for nitrogen). The measurements of fresh *Scirpus* detritus though, are realistically (as compared to fresh *Spartina* detritus – De Mesel, personal communication). For flocs the values compare to the POC values of Muylaert et al. (1999 – Addendum 1). We do not know what artefacts caused these low results in the case of animal dietary items.

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Chapter 6

Effect of salinity and temperature on the intra-marsupial of the brackish water mysid *Neomysis integer* (Crustacea: Mysidacea)

ABSTRACT

The brackish water mysid *Neomysis integer* has been proposed as a toxicological test species for the low saline reaches of Western European estuaries and brackish inland water bodies. The embryonic/larval development is a critical time window within the life history of an organism and has high potential to serve as a tool for assessing endocrine disruptive effects. A protocol is developed to examine the intra-marsupial development of *Neomysis integer in vitro* and a morphological description of the embryonic and larval developmental stages was made. Daily survival percentage, percentage survival days, hatching success, total development time, duration of each developmental stage and the size increment of the embryos and larvae were evaluated as potential endpoints, and their response to temperature and salinity was investigated.

The survival and hatching success are highly dependent on the salinity conditions, while the development time is strongly affected by temperature. High temperatures (21 °C) shorten the development time in comparison with low temperatures (11 °C) from 22 to 10 days, but have an opposite effect on survival. Optimal salinity for *in vitro* embryonic/larval development of *Neomysis integer* is 14 – 17. Living in lower or higher salinities thus implies suboptimal conditions for the juvenile recruitment to the population, unless the species can actively regulate the concentration of its marsupial fluid.

The developed *in vitro* technique may be used for testing the effect of both abiotic factors and (endocrine) disrupting chemicals on the intra-marsupial development of *N. integer*. Survival, hatching success and development time appeared to be adequate endpoints, while size and growth increment of the embryos/larvae seemed to be unsuitable.

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INTRODUCTION

In the last decades concerns have been expressed about the potential effects of low levels of (natural and anthropogenic) endocrine disrupting chemicals to man and the environment. The effects on wildlife and especially on invertebrate species might have far reaching adverse consequences for biodiversity and the sustainability of natural ecosystems (e.g. Guillette and Guillette, 1996; Edmunds et al., 2000; Santos et al., 2002). Industry and environmental management agencies urgently need new tools to assess the potential of effluents and chemicals to perturb the hormonal system (Depledge and Billinghurst, 1999). Because of the high contamination loads in estuaries, the need to use estuarine organisms in ecotoxicological studies is stressed (Lawrence and Poulter, 2001). Through their adaptation to the dynamic estuarine environment, these animals may either be pre-adapted to tolerate pollution stress or be more susceptible to any additional stress (Lawrence and Poulter, 1996).

The brackish water mysid *Neomysis integer* has been put forward as a test organism for the evaluation of environmental endocrine disruption in the brackish reaches of Western European estuaries and inland water bodies (Verslycke et al., 2004). Since *N. integer* has a key function in the estuarine ecosystem (Mees et al., 1994; Hostens and Mees, 1999; Maes et al., 2003), an alteration in the intra-marsupial development due to changing abiotic environmental conditions or pollution, might have an impact at the population level on the species' recruitment and thus on the sustainability of the estuarine ecosystem (e.g. Depledge and Billinghurst, 1999; Lawrence and Poulter, 2001).

As reproduction and embryonic/larval development are critical time windows within the life history of an organism (Depledge and Billinghurst, 1999; Lawrence and Poulter, 2001), they have high potential to serve as sensitive indicators of endocrine disruption (Wittmann, 1984). They are the critical stages in the hierarchical levels of response by an organism to pollution that link molecular, sub-cellular and physiological responses to population and community impact. The number of endocrine disrupting chemicals identified to specifically affect the development, fecundity and reproductive output of aquatic invertebrates is increasing (a.o. Sundelin and Eriksson, 1998; Lawrence and Poulter, 2001; Billinghurst et al., 2001; Kast-Hutcheson et al., 2001; Nice et al., 2003; Roepke et al., 2005; Forget-Leray et al., 2005).

Neomysis integer, like all other mysid species, carries its embryos in a marsupium where the entire larval development takes place from oviposition to the release of free swimming juveniles (Wittmann, 1984). It allows the embryos/larvae some degree of protection against predation. The marsupium is a chamber formed by thin-walled, concave plates fringed with long setae that interlock ventrally to form a closed chamber (Mauchline, 1980). Studying the intra-marsupial development *in vivo*, *i.e.* through the semi-transparent oostegites, is difficult (Fockedey, personal observation) or requires anaesthetization of the test specimens (Irvine et al., 1995). These difficulties emphasize the need for the development of a protocol to study intra-marsupial development *in vitro*. Although some *in vitro* data are available on the development time and the hatching success of *N. integer* embryos at a salinity range of 0.4 to 16 at 15°C (Vlasblom and Elgershuizen, 1977), detailed information is lacking for a wider salinity range in combination with a wide temperature range. *N. integer* is known to be euryhaline, tolerating salinities of 1 to 40 (Vlasblom and Elgershuizen, 1977), and eurythermic, tolerating temperatures between 0 and 30°C under laboratory conditions (Arndt and Jansen, 1986; Mauchline, 1980).

The combined influence of temperature and salinity on the intra-marsupial development as well as their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically-induced variability and natural variability in toxicity testing. The *in vitro* embryogenesis and larval development have been described in other mysids and pericaridans and the technique is used as a bioassay to evaluate changing environmental conditions (Vlasblom and Bolier, 1971; Morritt and Spicer, 1996a), toxicity and endocrine disruption (Lawrence and Poulter, 2001).

The aim of this study was to develop and optimize a methodology to study the intra-marsupial development of *Neomysis integer in vitro*. A detailed description of the embryonic mortality, morphology and the duration of subsequent developmental stages are presented. The combined impact of salinity and temperature on the intra-marsupial development was studied on endpoints like survival, hatching success, duration of development and size of the embryos and larval sub-stages. These results are essential for the development of a bioassay to assess the effects of endocrine disrupting chemicals on the intra-marsupial development of *N. integer*.

MATERIAL AND METHODS

Sampling

The brackish water mysid *Neomysis integer* used in the experiments originates from dock B3 in the harbour of Antwerp (Belgium), situated at the right bank of the Schelde river and connected to it through the Berendrecht and Zandvliet sluices. A chemical factory pumps up water from the dock for use as cooling water. Animals and debris are extracted from this incoming water by sieving over a 1x1 mm sieve and collected in a reservoir. Living *N. integer* were collected with a hand net (2x2 mm mesh size) from this reservoir and transported to the laboratory within 2 hours. Salinity and temperature conditions in the reservoir during the sampling period (weekly from 16 March to 20 April 2004) were on average 5 psu and 11 °C. In the laboratory the animals were kept in a 16 °C climatized room for < 7 days at a density of ± 50 ind l^{-1}. They were fed *ad libitum* on <24h old *Artemia* nauplii and the culture water was replaced every 2 – 3 days (5 psu artificial seawater - Instant Ocean®, Aquarium Systems, France).

Description of the embryology

Detailed descriptions of the histology of successive larval stages of *Neomysis integer* are given by Wagner (1896) and Needham (1937). External morphological descriptions of the intra-marsupial development are available by Kinne (1955) and de Kruif (1977). Although these papers give the morphological descriptions, they do not contain any pictures or drawings which could be useful tools for ecotoxicological evaluation. Gravid females, with embryos/larvae at different stages, were selected from the field samples. Using a stereomicroscope, the animals were decapitated and the embryos/larvae were removed from between the lamellae of the marsupium with a fine spatula while submerged. The embryos/larvae were then individually transferred using a Pasteur pipette to a Petri dish (diameter 38 mm, height 4 mm) containing 4 ml of aerated artificial seawater (salinity 5) at 16 °C. Preliminary experiments indicated a significantly higher survival of the embryos when placed on an orbital shaking table (80 rpm); this approach was used for all subsequent testing. Daily, half of the water was renewed and photographs were taken to aid in the description of the intra-marsupial development.

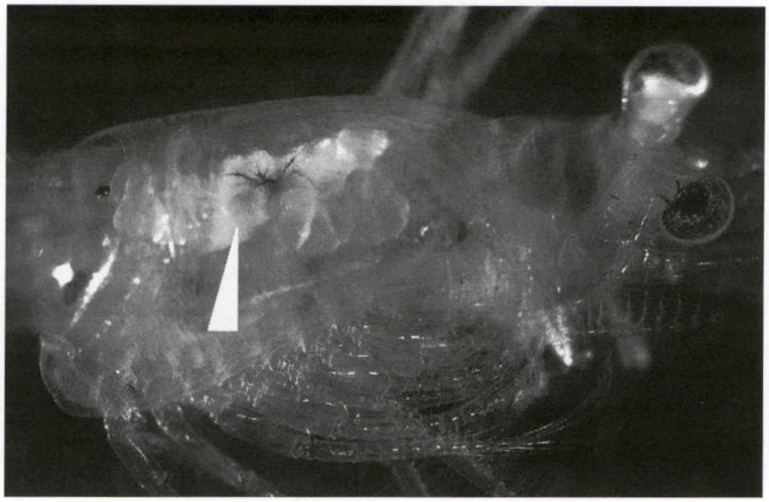


Figure 1: The ripe ovary fills the posterior dorsal lateral regions of the thorax (white arrow) and can easily be observed under a stereomicroscope (12x) as a white mass with clear egg contours.

Short-term survival

To select the adequate temperature and salinity range for the experimental design, a multi-factorial experiment was performed to evaluate the embryo's survival during a 3 day period. Spherical shaped (stage I) embryos of 24 field-collected gravid females were taken from the brood pouches (between 18 and 105 embryos per brood) and randomly distributed to Petri dishes (25 embryos per dish) containing 4 ml aerated artificial seawater. Embryo survival was monitored for 3 days. The following test-design was used: 13, 16 and 19°C; each of these temperatures was tested at salinity 2.5, 5, 10, 15, 20 and 25 (2 replicates per treatment). Petri dishes were placed on an orbital shaking table (80 rpm). No prior acclimation to the experimental salinity or temperature was done. Half of the culture medium was renewed daily and dead (i.e. white and/or shrivelled) embryos removed. After 72h, survival was noted and expressed as the mean percentage survival.

Mating and fertilization

Non-gravid females, with a standard length (from the tip of the rostrum in between the eye stalks to the end of the last abdominal segment) of 11 – 15 mm and with a large ovary, were selected from the field samples. The mature ovary fills the posterior dorsal lateral regions of the thorax (Mauchline, 1980, Figure 1). The whitish eggs present in the ovary can easily be observed through the carapace. Mature males (standard length 11 – 13 mm) were distinguished by their elongated 4th pleopods that are stretched to the end of the last abdominal segment and the paired penes.

To allow fertilization, one female was placed with 2 males in a 400 ml glass container filled with 350 ml artificial seawater (salinity 5). The jars were placed in a climatized room at 16 °C under a 12h light:12h dark light regime. The following was performed daily: excess food, faeces and possible moults were removed, dead mysids taken away and replaced by new individuals; 80% of the medium was renewed and fresh food was added (1000 *Artemia* nauplii mysid^{-1}).

Mating takes place at night (Mauchline, 1980) and coincides with the moulting of the female (Wittmann, 1984). Females with full marsupia were isolated for 2 days prior to removal of the embryos from the marsupium on day 3.

Table 1: Percentage survival days (± standard error), median percentage survival, mean percentage hatching (± standard error) and percentage of replica leading to hatching for all treatments of the Central Composite Design.

	Block	Temp (°C)	Sal (psu)	Percentage survival days (% day^{-1})	Median percentage survival (days)	Hatching success (%)	Replica hatched (%)
A	1	16.0	15.0	55.6 ± 7.7	7.3	52.2 ± 9.1	83.3
F	1	16.0	15.0	29.3 ± 7.5	5.1	37.5 ± 14.5	41.7
I	2	16.0	15.0	64.5 ± 5.5	14.6	53.9 ± 4.6	91.7
J	2	16.0	15.0	71.7 ± 5.0	16.0	48.4 ± 4.6	100.0
Average control		16.0	15.0	55.3 ± 3.9	15.6	49.5 ± 3.6	79.2
B	1	13.0	8.0	24.1 ± 3.3	5.8	25.8 ± 7.6	16.7
C	1	19.0	22.0	28.1 ± 4.2	4.7	16.0 ± 4.3	28.6
D	1	13.0	22.0	38.3 ± 9.5	5.9	49.9 ± 12.0	46.2
E	1	19.0	8.0	21.2 ± 2.2	4.5	20.0 ± 0.0	6.3
G	2	16.0	5.1	40.9 ± 1.8	4.2	0	0
H	2	16.0	24.9	33.8 ± 2.8	6.4	0	0
K	2	11.7	15.0	71.6 ± 6.1	25.4	57.4 ± 6.2	100.0
L	2	20.2	15.0	66.6 ± 3.9	10.3	36.4 ± 4.5	100.0

Effect of salinity and temperature on embryonic development

The evaluation of the effects of salinity and temperature on the *in vitro* intra-marsupial development of *Neomysis integer* was made using a (circumscribed) 'Central Composite Design' (STATISTICA 6.0). For the two independent factors – i.e. salinity and temperature – 12 treatments were tested in two blocks of 6 with each block comprising 2 central points (Table 1). Subsequently, a response surface model was fitted to the data and the optimal temperature/salinity combination for the different endpoints tested was derived.

To achieve randomization, the three day old embryos of one brood were distributed in as many treatments as possible and transferred to the 5 ml wells of a 12-cell multiwell plate. All treatments were performed in 12 replicates; each replicate containing 7 to 17 embryos. No adaptation period to the experimental salinity or temperature was made prior to the test, but animals surviving < 3 days were excluded from the analyses. Multiwell plates were placed on an orbital shaking table (80 rpm) and covered from the light. The following was performed daily: the survival and developmental stage were noted, dead embryos/larvae were removed, half of the medium was replaced with freshly prepared medium and the salinity and temperature was monitored. Every day (maximally 8) embryos/larvae were measured per replicate using a drawing mirror mounted on a stereomicroscope (25 – 50x). The following measurements were performed with ImageJ 1.32e (http://rbs.info.nih.gov/ij/Java_1.3.1_03 public domain): the maximum diameter (i.e. Feret's diameter) of stage I embryos, the total length (TL) excluding the abdominal setae in stage II larvae and the total length (from the eye basis to the tip of the uropods) in stage III larvae. Hatched juveniles were fixed in 4 % formaldehyde and their standard length (SL), from the eye basis till the last abdominal segment, measured dorsally.

Statistics

The cumulative survival function was plotted as percentage survival. The percentage survival days (Jones, 1972) and the median survival time (50 % survival) were calculated for each treatment. Survival percentage data of the 4 control treatments were submitted to a two-factor ANOVA (treatment x age of embryo/larvae) without meeting the normality assumptions (Mann and Harding, 2003). Other endpoints, like percentage survival days, hatching success and development time, were tested between treatments using a one-factor ANOVA. Fisher's multiple comparison test was used for post-hoc comparison when appropriate.

Table 2: Morphological and activity characteristics of the embryonic stages of *Neomysis integer*.

Morphology	Yolk	Activity	PRESENT STUDY Kinne (1955) Mauchline(1973)	De Kruif (1977)	Wittmann (1981)
• Egg-like, (sub)spherical (Figure 2a); first 2 days in 2 packages within a tertiary egg membrane • Later: cephalic and abdominal rudiments developing (Figure 2b)	Yolk granules spread all over embryo	Inactive	stage I = 'egg'	stage I	E1 – half E5
• Shedding of egg membrane					
• Comma-shaped habitus with rudimentary pointed abdomen clearly distinguished from rounded anterior; appearance of two pair of rudimentary thoracic appendages and abdominal setae (Figure 2c) • Later: the beginning of abdominal segmentation, without appendages (Figure 2d)	Yolk granules homogeneously spread all over embryo	Inactive	stage II = naupliar stage = 'eyeless' larva	stage II stage III stage IV	Half E5 + N1 to N4
• Further extension of the body and elongation thoracic appendages (Figure 2e); appearance of cleft at optic rudiment (Figure 2f)	Yolk migrates dorsally	Inactive			
• Development of head; optic lobes with pigmented eye rudiments; rudiments of telson and uropods visible; further segmentation of abdomen; brown chromatophores appearing laterally (Figure 2g and 2h)	Yolk diminishes and migrates dorsally in the anterior part	Rhythmic contractions of the gut and beating of the heart		stage V	
• Moulting from naupliar cuticle					
• Distinct eye projections; development of uropods and pleopods; developing 8 thoracic appendages, mouthparts and antennae; developing carapace and elongated abdomen (Figure 2i and 2j)	Yolk disappears	Very active flexing and stretching of the body; moving of the appendages	stage III = post-naupliar stage = 'eyed' larva	stage VI	P1 – P3
• Moult					
• All (except sexual) characteristics similar to adult (Figure 2k)	No yolk left; actively feeding	Freely swimming	Juvenile	Juvenile	Juvenile

The size of the embryos, larvae and juveniles, and their relative growth (in % day^{-1}) was tested at each substage using a two-way ANOVA taking age into account. All replicate values of the dependent variables (percentage survival at a certain day, percentage survival days, hatching percentage, duration of stages I, II, III and total development time and standard length of the offspring) were used to fit a response surface model including the linear and quadratic main effect and the two-way interactive effects (STATISTICA 6.0). The significance of the effects (including the factor 'block') was tested with an ANOVA. Since in each block the control treatments were replicated, the pure (random) error could be quantified and the residual variance tested using a Lack of Fit test.

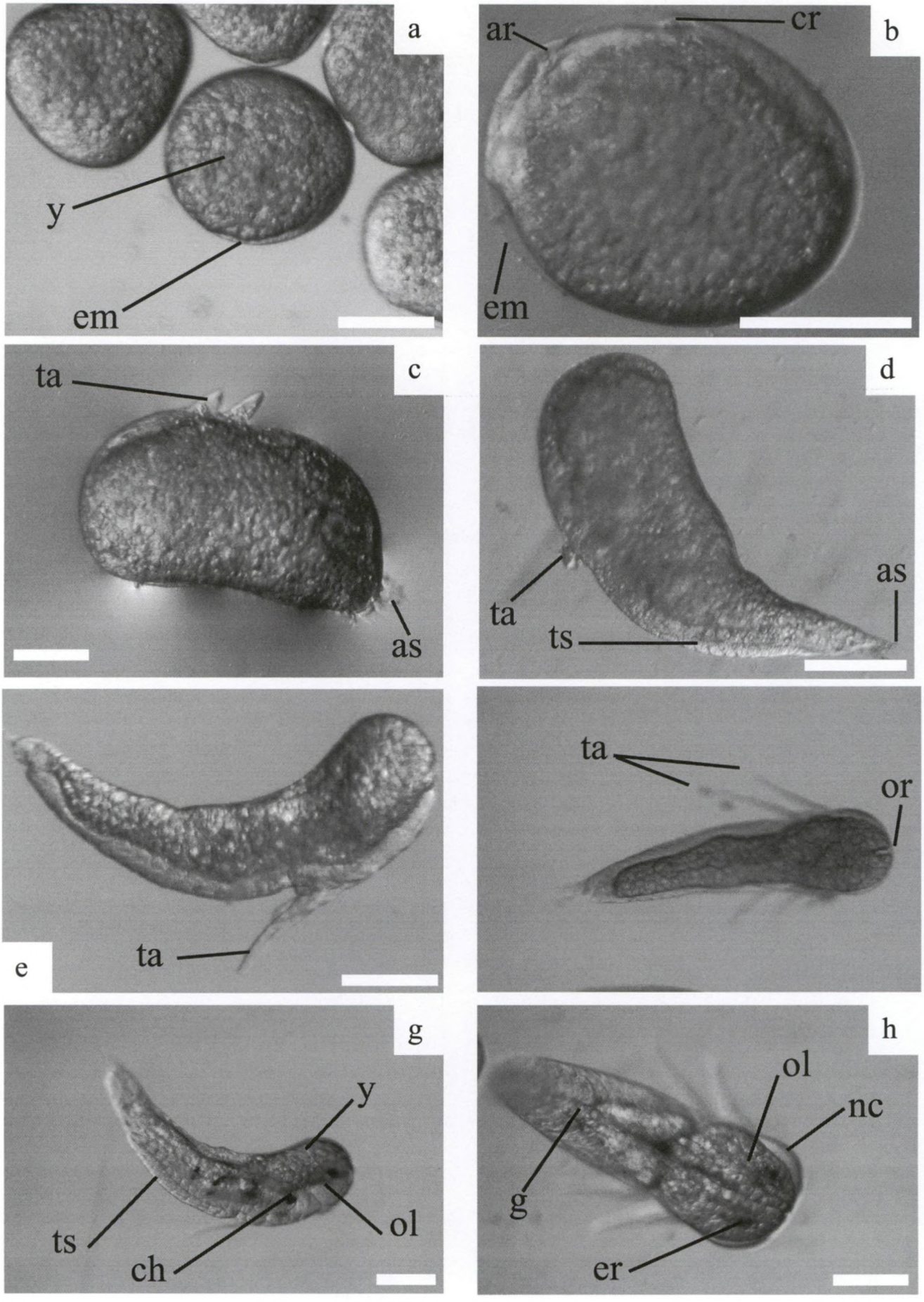
RESULTS

Description of the embryology

The intra-marsupial development of *Neomysis integer* was divided into 3 substages in the present study, while generally for mysids a subdivision into 3 to 12 substages is common (Berrill, 1969; Mauchline, 1973; de Kruif, 1977; Wittmann, 1981). Table 2 and Figure 2 summarize the terminology used by the different authors, including the one used in the present study, and applied to the observed morphology in the intra-marsupial development of *Neomysis integer* (with supporting pictures).

The early embryos (stage I) are spherical or sub-spherical (Figure 2a). Rudiments of antennae and abdomen are developing (Figure 2b) and observable under low magnification (25x) as a lighter coloured disk. The abdominal rudiment is ventrally bent and develops anteriorly towards the cephalic appendix. Stage I ends with the hatching from the egg membrane by puncturing it with the developing abdomen. The shed egg membrane quickly disintegrates, but is sometimes visible in the wells.

The stage II larvae are dorsally bent and have a comma-like appearance. Initially, a rudimentary abdomen with a clear distinction between the rounded anterior and the pointed posterior of the larva can be observed together with two thoracic appendages (Figure 2c). In a later phase, the abdomen shows the clear beginning of segmentation, however, without any appearance of appendages (Figure 2d). Later on, the body is further extended and the thoracic appendages more elongated (Figure 2e). The larvae have globules of the yolk protein vitellin within their tissues. These globules are homogeneously distributed throughout the body in stage I embryos and early stage II larvae, but as the yolk volume decreases relative to the body volume, the yolk becomes more concentrated in the anterior dorsal regions at the end of stage II. Dorsally the optical rudiment is visible as an anterior cleft (Figure 2f). As the larva grows, the naupliar cuticle is stretched and the uropods and telson are formed. Eight abdominal segments are clearly visible. Lateral chromatophores appear, mainly in the anterior part (Figure 2g). The optical lobes are visible with pigmented eye rudiments (Figure 2h). A rhythmic beating of the heart and contractions of the gut are visible. The naupliar stage II terminates with the moulting from the naupliar cuticle.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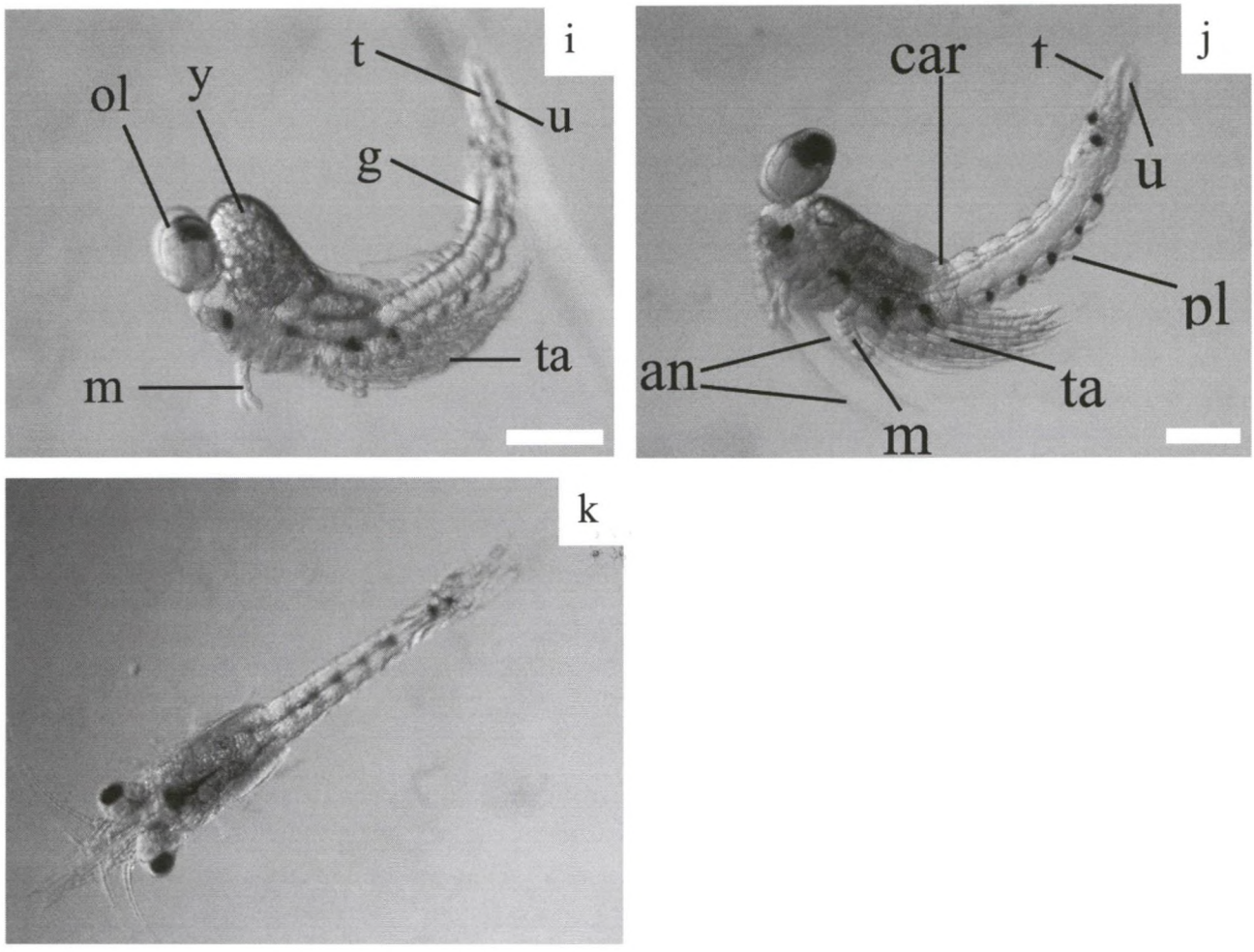

Figure 2: Embryonic and larval stages of *Neomysis integer*: stage I (a and b), stage II (c to h), stage III (i and j) and the free-living juvenile (k). (an: antennae; ar: abdominal rudiment; as: abdominal setae; car: carapace; c: naupliar cuticle; cr: cephalic rudiment; ch: thoracic chromatophore; er: eye rudiment; em: egg membrane; g: gut; m: mouth parts; nc: naupliar cuticle; or: optic rudiment; ol: optic lobe; pl: pleopods; t: telson; ta: thoracic appendages; ts: thoracic segmentation; u: uropods; y: yolk granules). Scale bar = 250µm.

Table 3: Mean survival percentage (± standard error) of stage I embryos after 72h.

	2.5 psu	5.0 psu	10.0 psu	15.0 psu	20.0 psu	25.0 psu
13.0 °C	10 ± 6	20 ± 4	32 ± 4	60 ± 8	58 ± 2	48 ± 0
16.0 °C	8 ± 0	16 ± 8	46 ± 10	64 ± 4	40 ± 0	28 ± 4
19.0 °C	2 ± 2	8 ± 8	26 ± 10	28 ± 4	26 ± 6	20 ± 12

The post-naupliar stage III larvae (Figure 2i) have stalked eyes, a developed telson and uropods without lith in the statocyst of the inner ramus. The thoracic appendages, mouth parts and antennae are developing. All over the body, darkly pigmented chromatophores appear. Near the end of this stage a carapace can be observed (Figure 2j). The larvae are very actively moving by a longitudinal dorsal flexing and stretching of the body. Also an active rhythmic moving of the thoracic appendages is observed. Stage III terminates in a moult, leading to free-living young juveniles (Figure 2k) that are, except for the sexual characteristics, morphologically similar to the adults. The gradually disintegrating yolk is completely consumed.

Short term survival experiment

The mean percentage survival of the stage I embryos after 72h at all tested salinity and temperature combinations is shown in Table 3. At all temperatures tested, the survival was ≤ 10 % at salinity 2.5 and ≤ 20 % at salinity 5.0. At the other salinities, survival was substantially higher and was maximal at 15 and 16 °C (64 %). These results inferred the selection of the temperature range (13 – 19 °C, centred at 16 °C) and the salinity range (8 – 22, centred at 15) used in the Central Composite Design (Table 1).

Survival

The cumulative survival functions were plotted as percentage survival (Figure 3). Table 1 shows the percentage survival days and the median survival time of the 12 treatments. The percentage survival days of treatment F was significantly lower than in the other control treatments ($p < 0.0001$). However a two-factor ANOVA (treatment x age) of the percentage survival did not show a significant interactive effect ($p = 0.960$). For comparative purposes the survival of the 4 centre points were therefore treated as equal and plotted as the average percentage survival (grey line in Figure 3).

In the treatments G (salinity 5.1) and H (salinity 24.9) at 16 °C, an extreme high mortality was noted and all embryos/larvae had died at the age of 6 and 13 days, respectively. Note that treatment G has salinity and temperature conditions that were identical to the conditions in which the adults were kept at and mating took place. At 19 °C, both at salinity 8 (E) and 22 (C), a high initial mortality occurred.

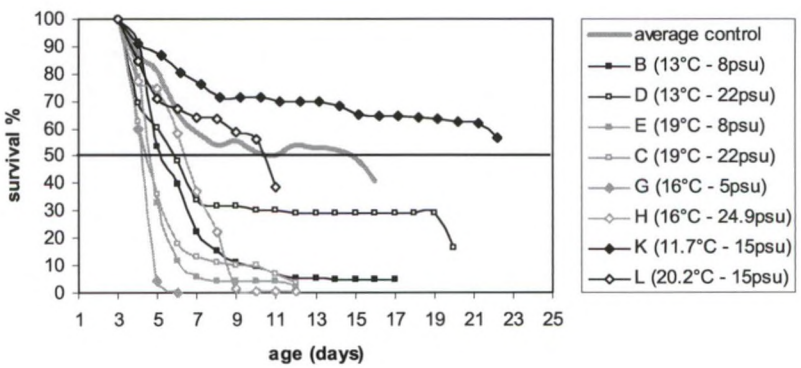

Figure 3: Survival functions of all treatments (temperature °C – salinity). In grey: mean of the centre point treatments.

However, some larvae in a low number of replicates did survive and hatched (respectively 6 and 29 %). At 13 °C a higher survival was observed at the higher salinity (salinity 22, D) in comparison with the low salinity treatment (salinity 8; B). In general, the highest mortality occurred within the first 6 days of the embryonic development, i.e. during stage I. An ANOVA demonstrated significant differences in the percentage survival days within all treatments ($p < 0.0001$). A significantly higher mean value (> 55 % d^{-1}) of the percentage survival days was observed at salinity 15 at temperatures between 11.7 and 20.2 °C (centre points, K and L). This is reflected in the higher median percentage survival (> 10 days).

Figure 4a to 4i plots the response profiles of the age-specific survival at day 4 through day 12 (beyond this latter later age the dataset became too incomplete as a result of hatching or mortality). The number of temperature and salinity combinations with the highest survival (> 60 %, indicated in the darkest colours) decreases with increasing age. Figure 5 shows the response surface of the survival at age 6, 9 and 12 days and the percentage survival days. Highest survival was associated with the lowest temperatures (< 14 °C) and medium salinities (salinity 12 – 22). The regression coefficients and the ANOVA effect estimates (Table 4) indicate a relatively poor fit of the response surface models ($R^2 = \pm 0.5$). Note the highly significant effect of the block ($p < 0.0001$). For the survival at day 6 significant variation is observed which is due to unexplainable error. Despite the relatively poor fit, the models indicate that the survival is mainly affected by the quadratic salinity effect and to a lesser degree by the linear effect of salinity and temperature (especially at the beginning of the incubation period).

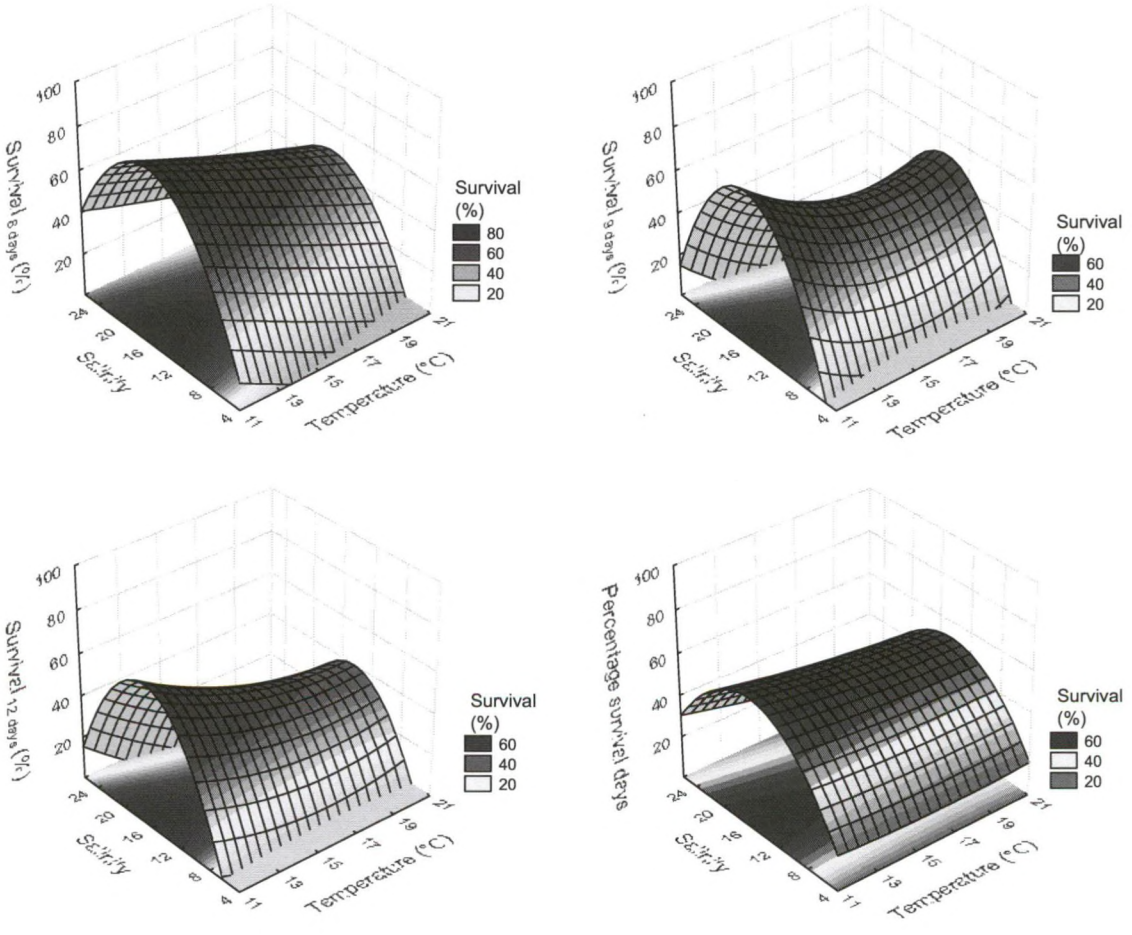

Figure 5: Response surface plot of the fitted models for survival at age 6 days (a), 9 days (b), 12 days (c) and percentage survival days (d).

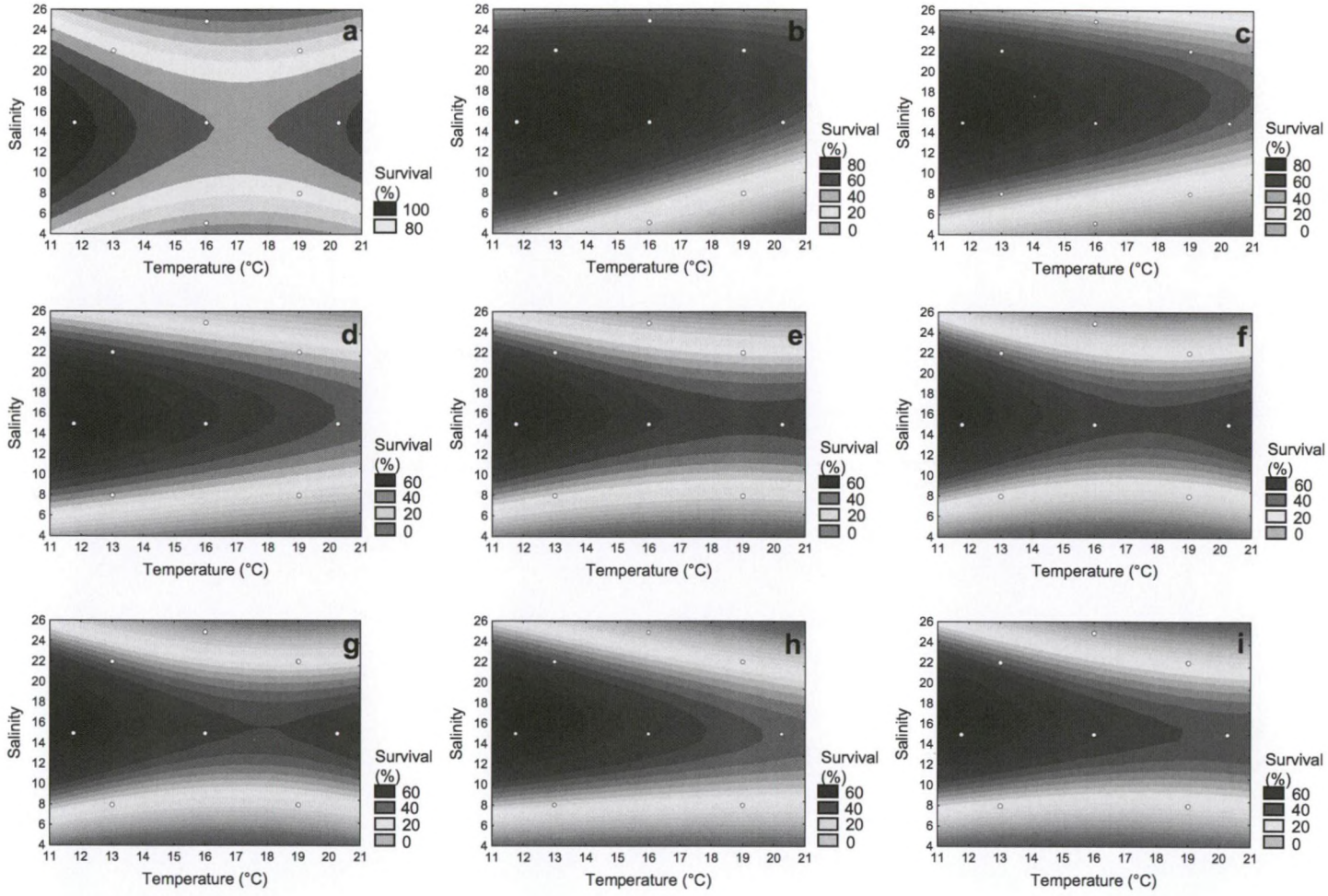

Figure 4: Response profiles of the survival at age 4 (a) to 12 (i) days

Table 4: Regression coefficients (± standard error) of the response surface model fitted to the survival at age 6, 9 and 12 days and the percentage survival days with their p-values; ANOVA effect estimates with their p-values for the fitted model including the Lack of Fit test (T: temperature; S: salinity; ns: not significant).

		Regression coefficient	SE	p-value	Anova F-value	p-value	R^2
Survival age 6 days	Intercept	26.469	101.974	ns			
	T	-6.287	11.570	ns	15.927	< 0.001	
	T^2	0.032	0.349	ns	0.008	ns	
	S	13.582	3.483	< 0.001	21.329	< 0.001	
	S^2	-0.451	0.064	< 0.001	50.139	< 0.001	0.461
	TxS	0.108	0.177	ns	0.374	ns	
	Block				33.956	< 0.001	
	Lack of fit				4.585	< 0.001	
Survival age 9 days	Intercept	49.773	73.703	ns			
	T	-19.084	10.987	ns	5.822	< 0.05	
	T^2	0.594	0.334	ns	3.154	ns	
	S	21.207	3.754	< 0.001	3.437	ns	
	S^2	-0.609	0.077	< 0.001	63.021	< 0.001	0.493
	TxS	-0.129	0.163	ns	0.629	ns	
	Block				17.595	< 0.001	
	Lack of fit				1.221	ns	
Survival age 12 days	Intercept	-49.215	97.690	ns			
	T	-7.927	12.108	ns	6.664	< 0.05	
	T^2	0.290	0.379	ns	0.585	ns	
	S	22.810	3.636	< 0.001	3.268	ns	
	S^2	-0.597	0.076	< 0.001	62.142	< 0.001	0.519
	TxS	-0.255	0.155	ns	2.699	ns	
	Block				18.404	< 0.001	
	Lack of fit				1.660	ns	
Percentage survival days	Intercept	-6.125	73.541	ns			
	T	-1.749	8.344	ns	1.865	ns	
	T^2	-0.061	0.252	ns	0.058	ns	
	S	10.771	2.512	< 0.001	0.735	ns	
	S^2	-0.312	0.046	< 0.001	46.182	< 0.0001	0.446
	TxS	-0.073	0.128	ns	0.329	ns	
	Block				61.390	< 0.0001	
	Lack of fit				1.383	ns	

Table 5: Regression coefficients (± standard error) of the response surface model fitted to the hatching percentage with their p-values; ANOVA effect estimates with their p-values for the fitted model including the Lack of Fit test (T: temperature; S: salinity; ns: not significant)

		Regression coefficient	SE	p-value	Anova F-value	p-value	R^2
Hatching percentage	Intercept	-78.913	73.546	ns			0.524
	T	0.069	8.344	ns	10.296	< 0.01	
	T^2	0.014	0.252	ns	0.003	ns	
	S	17.519	2.512	< 0.001	2.194	ns	
	S^2	-0.477	0.046	< 0.001	108.010	< 0.001	
	TxS	-0.174	0.128	ns	1.865	ns	
	Block				25.794	< 0.001	
	Lack of fit				0.855	ns	

Hatching success

The centre point treatments at 16 °C and salinity 15 (A, F, I and J) did not differ in their hatching success (ANOVA, p = 0.569) and were further treated as one (Table 1): 79 % of the control treatments replica resulted in hatching and 49.5 ± 3.6 % of the initial embryos led to free living juveniles. Note that in treatment G and H no hatching was observed due to complete mortality. Significant differences in the hatching percentage between all treatments were noted (p = 0.010). This was mainly due to the significant lower hatching in the C treatment (19 °C – salinity 22) of only 16 % of the larvae. Hatching at a salinity of 15 was significantly affected by temperature as demonstrated by a hatching success at 11.7 °C and 20.2 °C of 57 % (K) and 36 % (L), respectively.

Regression coefficients and the ANOVA results of the response surface modelling indicate a poor fit (Table 5, Figure 6). The hatching success was significantly affected by the quadratic salinity effects and the linear temperature effect. Hatching percentage was highest at the moderate salinity (± 16 psu) and low temperature (< 15 °C) combinations (Figure 6). Hatching success was 10 to 20 % lower at higher temperatures (> 15 °C).

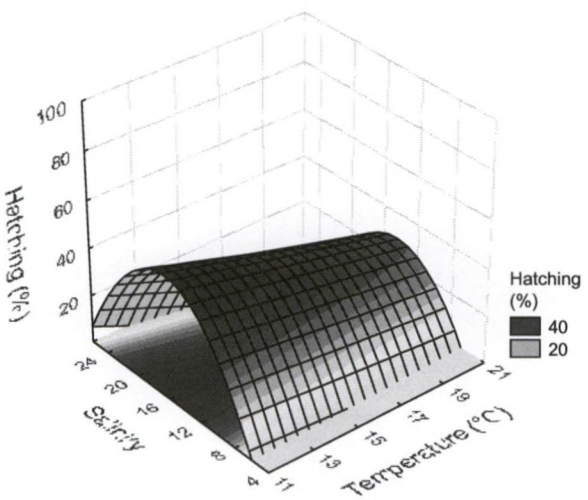

Figure 6: Response surface plot of the fitted model for hatching success.

Table 6: Mean duration of the embryonic development and sub-stages (in days, ± standard error) for all treatments of the Central Composite Design.

	Temperature (°C)	Salinity (psu)	Total	Stage I	Stage II	Stage III
A	16.0	15.0	15.3 ± 0.2	5.0 ± 0.3	7.0 ± 0.0	3.6 ± 0.2
F	16.0	15.0	15.8 ± 0.2	4.4 ± 0.2	7.4 ± 0.2	3.2 ± 0.2
I	16.0	15.0	15.8 ± 0.1	5.3 ± 0.3	7.2 ± 0.1	3.8 ± 0.1
J	16.0	15.0	16.2 ± 0.1	4.8 ± 0.2	7.8 ± 0.2	3.6 ± 0.2
Average control	16.0	15.0	15.8 ± 0.1	4.9 ± 0.2	7.4 ± 0.1	3.6 ± 0.1
B	13.0	8.0	18.0 ± 0.0	5.0 ± 0.3	9.5 ± 0.5	4.0 ± 0.0
C	19.0	22.0	12.0 ± 0.0	4.8 ± 0.2	4.4 ± 0.2	2.8 ± 0.3
D	13.0	22.0	19.7 ± 0.2	5.5 ± 0.3	9.3 ± 0.2	4.2 ± 0.2
E	19.0	8.0	12.0 ± 0.0	4.0 ± 0.0	5.0 ± 0.0	3.0 ± 0.0
G	16.0	5.1	—	—	—	—
H	16.0	24.9	—	6.8 ± 0.7	—	—
K	11.7	15.0	21.8 ± 0.1	5.8 ± 0.2	10.7 ± 0.1	5.3 ± 0.1
L	20.2	15.0	11.2 ± 0.1	4.3 ± 0.2	4.2 ± 0.1	2.4 ± 0.2

Duration of development

The duration of the intra-marsupial development varied between 11 and 22 days, of which stage I took on average 31 % of the time, stage II 45 % and stage III 23%. Again the centre point treatments were further considered as one (Table 6). Due to the extremely poor survival in treatments G and H, these data were excluded from the analyses. ANOVA revealed significant differences between the treatments for the stages I, II and III, and the total duration of the development (all at least with $p < 0.001$). In general, the duration of the intra-marsupial development decreased with increasing temperature as demonstrated in the 15 psu treatments: at 20.2 °C (L), 16 °C (Control) and 11.7 °C (K), total development duration was 11.2, 15.8 and 21.8 days, respectively. This was mainly due to a reduction of stage II with increasing temperature from 11 to 4 days (61 %) at the lowest and highest tested temperature, while stage I reduces on average from 6 to 4 days (26 %) and stage III from 5 to 2 days (55 %).

The response surface models (Table 7 and Figure 7) revealed a good fit for the total development time ($R^2 = 0.984$) and the duration of stage II ($R^2 = 0.951$) and III ($R^2 = 0.701$). It was mainly the linear temperature component that controlled the duration of the intra-marsupial development, although salinity also had some minor influence in the observed patterns (Table 7). Note that the factor 'block' also had a significant effect.

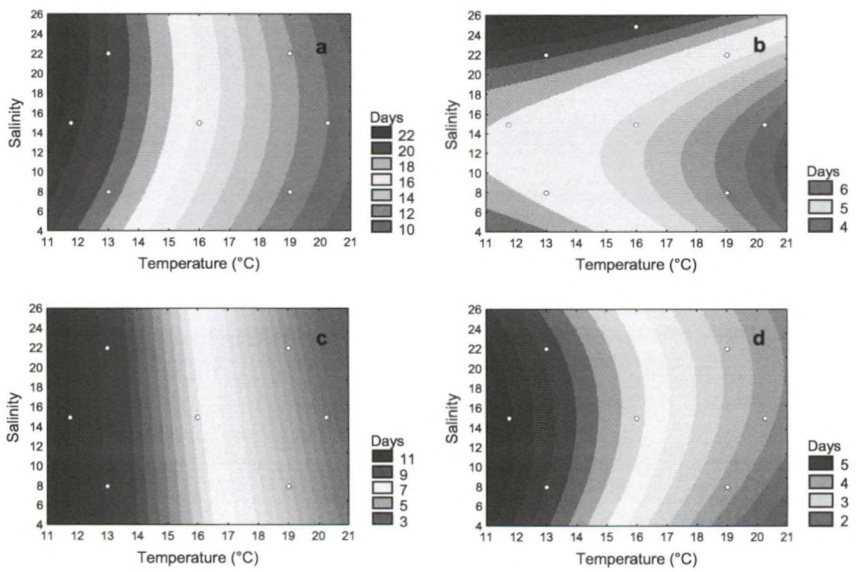

Figure 7: Response profiles for the total development time (a), and duration of stage I (b), stage II (c) and stage III (d)

Table 7: Regression coefficients (± standard error) of the response surface model fitted to the total development time and the duration of stages I, II and III with their p-values; the ANOVA effect estimates with their p-values for the fitted model including the Lack of Fit test (T: temperature; S: salinity; ns: not significant).

		Regression coefficient	SE	p-value	Anova F-value	p-value	R^2
Total development time	Intercept	37.098	2.272	< 0.001			0.984
	T	-1.967	0.246	< 0.001	3989.548	< 0.001	
	T^2	0.028	0.007	< 0.001	16.002	< 0.001	
	S	0.501	0.153	< 0.01	10.703	< 0.01	
	S^2	-0.008	0.004	< 0.05	4.202	< 0.05	
	TxS	-0.012	0.006	ns	3.804	ns	
	Block				13.779	< 0.001	
	Lack of fit				4.596	< 0.05	
Duration stage I	Intercept	6.833	3.730	ns			0.354
	T	0.094	0.417	ns	24.190	< 0.001	
	T^2	-0.010	0.012	ns	0.608	ns	
	S	-0.231	0.180	ns	4.720	< 0.05	
	S^2	0.008	0.004	< 0.05	4.120	< 0.05	
	TxS	0.003	0.009	ns	0.110	ns	
	Block				7.459	< 0.01	
	Lack of fit				1.381	ns	
Duration stage II	Intercept	16.987	2.584	< 0.001			0.951
	T	-0.492	0.283	ns	1151.915	< 0.001	
	T^2	-0.005	0.008	ns	0.441	ns	
	S	0.085	0.173	ns	1.523	ns	
	S^2	-0.0002	0.005	ns	0.002	ns	
	TxS	-0.007	0.007	ns	0.998	ns	
	Block				5.459	< 0.05	
	Lack of fit				0.124	ns	
Duration stage III	Intercept	10.586	2.959	< 0.001			0.701
	T	-0.591	0.321	ns	156.135	< 0.001	
	T^2	0.006	0.009	ns	0.479	ns	
	S	0.029	0.199	ns	0.085	ns	
	S^2	-0.003	0.005	ns	0.367	ns	
	TxS	0.005	0.008	ns	0.324	ns	
	Block				1.498	ns	
	Lack of fit				3.851	ns	

Size and growth rate of the embryos

Stage I: The Feret's diameter of the stage I embryos (Figure 8) was tested within the 2 subsequently performed blocks, taking age into account. Although the two-factor ANOVA indicated that there was no significant interactive effect (Feret x age; $p = 0.071$), the size of early embryos in block 1 was always larger than the embryos that had contributed to the 2nd block ($p < 0.0001$). Age did not have an effect on the Feret's diameter of the spherical embryos ($p > 0.05$). The stage I embryos did not measurably increase in size (Table 8), except for those in the treatments L, G and H. The latter salinity-temperature combinations did exhibit high mortality. Probably the size increase of > 10 % in the egg-like embryos was an indication of a near dead.

The centre point treatments of the second block (I and J) resulted in embryos with smaller size than those in the treatments covering centre points A and F of the first block ($p = 0.0001$). However, among centre point treatments within one block differences were also observed, with the F embryos being larger than the A ones (Figure 8).

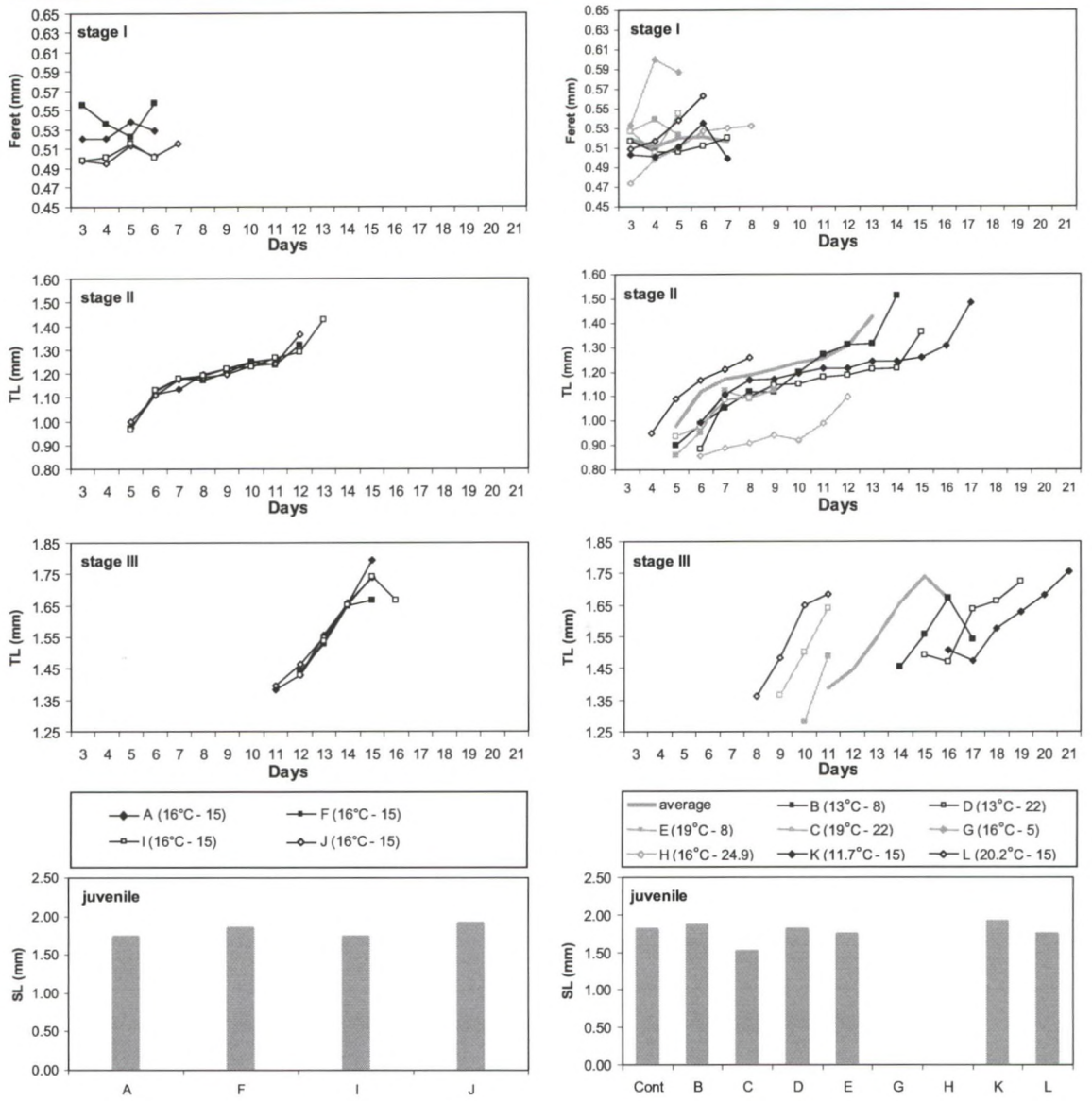

Figure 8: Embryo size related to age of the 4 centre point treatments (left); Embryo size related to age in all treatments of the central composite design with the mean of the centre point treatments in grey (right). Standard errors are generally smaller than 0.05 mm. (TL: total length; SL: standard length).

Despite the randomization protocol used in the test design, the initial size of the embryos was not identical in all treatments. To avoid misinterpretation of the data, the relative growth (in % day^{-1}) was used as an endpoint to evaluate the size of stage I embryos between the 12 treatments, assuming this parameter is not affected by the initial size discrepancy. From this analysis it appears that the mean size of stage I embryos of one replica was either increasing or decreasing (up to +15 to -15 % day^{-1}), indicating that the Feret's diameter is an unsuitable size measure of stage I embryos.

Stage II: The length of stage II larvae of a certain age did not significantly differ between the control treatments A, F, I and J (ANOVA; $p = 0.370$) and were further treated as one control (Figure 8). Stage II larvae increased in size substantially with time ($p = 0.0001$; Table 8); compared to their initial size an increase of 46 % was noted. At the lowest temperatures (B, D, K) the growth of stage II larvae was higher (50 – 68 %) than that in treatments C, E and L at 19 and 20.2 °C (17 – 32 %). However, at lower temperatures the development lasted longer, resulting in a rather uniform growth rate between the treatments (0.04 – 0.08 mm day^{-1}). The larvae at the highest salinity of (24.9 psu; H) did not survive stage II and a suboptimal growth was observed.

Stage III: The total length of stage III larvae at a certain age was not different between all control treatments ($p = 0.635$). A significant increase in size (20%) as a function of time was observed ($p < 0.0001$). Again temperature had a pronounced effect on the size of the stage III larvae. However, here we noted that lower temperatures (B, D and K) reduced the growth rate (0.03 – 0.06 mm day^{-1}) in comparison with the warmer temperatures (C, E and L; 0.11 – 0.21 mm day^{-1}).

Juveniles: The free living juveniles in treatments A and I were significantly smaller than those in F and J ($p < 0.0001$). Also in the comparison with the other treatments, some differences were observed (ANOVA, $p < 0.0001$), but no trend with salinity or temperature could be demonstrated. The fit of the response surface within the central composite design is low (Table 9), and only temperature and the factor 'block' had an adverse effect on the standard length of the hatching offspring.

DISCUSSION

Methodology

The eggs were extruded from the oviducts into the marsupium and closely packed together in two (a left and right) packages, which are enclosed together with the sperm within a very thin membrane (as described by Kinne, 1955 as tertiary egg membranes). Since the jagged-shaped embryos do not have a firm consistency at this time, removal of the eggs from these membranes is impossible without damaging. This is the reason why the *in vitro* experiment starts 3 days after deposition in the marsupium, when the thin membranes are dissolved and the embryos are spherical and firm. An additional advantage is that unfertilized eggs, disintegrating within 24h, are not included into the bioassays.

In a preliminary experiment, improved survival and a shorter development time were observed when the multiwell plates were placed on an orbital shaking table (80 rpm). The continuous movement on a roller table probably simulates the rhythmic lateral moving of the brood lamellae by the gravid female to irrigate and provide oxygen to the embryos/larvae and positively affects the hatching success and shortens the development time (Mauchline, 1980; Fernández et al., 2002). This phenomenon was especially apparent in stage III as the oxygen-demand of crustacean embryos/larvae increases as they develop (Smith and Klieber, 1950; Fernández et al., 2003).

Number and size of embryos in the marsupium

The number of embryos/larvae in the marsupium of female mysids depends upon the body size of the female, the size of the individual eggs, the season of the year and geographic location (latitude) of the mysid populations (Mauchline, 1980). For *Neomysis integer*, the brood size is demonstrated to be highly depended on female body size, which varies seasonally (Kinne, 1955; Parker and West, 1979; Mees et al., 1994): late summer and autumn breeding animals usually have a smaller size-at-maturity compared to those breeding in spring and early summer. The size of the early embryos also varies seasonally (for *Neomysis integer*: Mauchline, 1973; Irvine et al., 1995), with winter embryos being larger than in spring or summer.

The initial size of the stage I embryos from block 1 and 2 (from day 3 to day 6-7) was clearly different, with the block 1 embryos always being significantly larger. Due to logistic limitations 2 subsequent blocks were set off, with embryos from females caught respectively between 16 and 30/03/2004 and between 6 and 20/04/2004.

The water temperature had increased from 9.8 °C to 12.2 °C during this period. *Neomysis integer* has a growth stop in winter time (Mees et al., 1994). From the moment water temperature rises above 10 °C, growth and development is triggered. Larger (spring) animals have a higher fecundity and the size of the embryos is negatively related with the brood size (Mauchline, 1973). This may explain the difference in the initial size of the 2 blocks used in our design. The observed size difference clearly had an impact on the other measured endpoints (as demonstrated by a significant effect of 'block' in the ANOVA's while fitting the response surface models). For future experiments, it is recommended to use *N. integer* from a continuous and well standardized laboratory culture. If this is not possible we advise not working with different blocks in the design, or to keep the time difference between the blocks to a minimum. The size of newly released juveniles was significantly different between treatments. Reduced size or weight at birth may have implications for the future survival and breeding potential of the offspring (Kolding and Fenchel, 1981; Wehrtmann and Lopez, 2003).

The size and growth of the embryos and larvae do not seem to be useful for evaluating the influence of environmental variables on the intra-marsupial development of *Neomysis integer*. This seems to be especially true for the ellipsoid stage I embryos. Variation on the measurement of the Feret's diameter is high because of the ellipsoid form. However, Wittmann (1981) and Lawrence and Poulter (2001) found the maximal width and length of ovaline shaped embryos to be a good endpoint. The sub-spherical embryos were measured with a larger accuracy (light microscope) than the current stereomicroscope measurements (50x). The stage I embryos of treatments G and H showed a significant increase in the Feret's diameter. This was probably due to osmotic swelling of the embryos prior to their eventual disintegration (Morritt and Spicer, 1996b).

Intra-marsupial mortality – Hatching success

The hatching success was highest (max. 58 %) in treatments with an intermediate salinity of 14 – 17 psu combined with a low temperature (< 15 °C). Especially the earliest stages (first 6 days) are susceptible to unfavourable salinity and temperature conditions. The mortality of the embryos and larvae during the *in vitro* experiment ranged from 40 to 100 %. Mauchline (1973) reports a marsupial mortality of about 12 – 13 % for *Neomysis integer*, while Irvine et al. (1995) estimated intra-marsupial mortality of *N. integer* to be in excess of 50 %, and mainly occurring at the beginning of the development. In the field, mortality might also be caused at the end of the development by accidental loss of late stage II and III larvae, when gravid females have distended marsupial lamellae to contain the large larvae and move the marsupial lamellae to irrigate the larvae (Mauchline, 1980).

Intra-marsupial development time

The duration of the incubation period is a key factor in the understanding of the population biology of a species and is related to the timing of the breeding season, age at maturity, frequency of broods, number of young per brood, egg size and adult body size (Wittmann, 1984). Intra-marsupial development time, i.e. from appearance in the brood pouch to the release of young, is highly related to the environmental temperature (Mauchline, 1980; Wittmann, 1984) and not or minimally affected by salinity (Vlasblom and Elgershuizen, 1977; Greenwood et al., 1989). In the present experiment, the development time varied respectively between 22 and 10 days at temperatures between 11 and 21 °C, respectively. Irvine et al. (1995) provide indirect estimates of the intra-marsupial development time of *N. integer* from Hickling Broad, UK (salinity ± 3): 56 days (at 7 - 16.5 °C), 29 days (at 16.5 – 18 °C), and 13 – 14 days (at 19.5 – 20 °C). From their experiments, the same authors concluded that the development time was 42 days at 6 °C and 6 days at 20 °C at a salinity of 3.

The latter development time is extremely short in comparison with the present results and the authors' field derived estimate at the same temperature. Kinne (1955) performed laboratory experiments at 10 psu aimed at establishing the total intra-marsupial development time of *N. integer* and reports 20 days at 11 °C and 14 – 15 days at 19 °C. Vlasblom and Elgershuizen (1977) obtained (experimentally) a constant duration of 16 – 18 days at varying salinities (0.4 – 16 psu) at 15°C. Taking the age-at-maturity into account (Fockedey et al., in press), the intra-marsupial development of *N. integer* takes 15 to 17 % of the generation time at 20 °C and 16 to 21 % of the generation time at 15 °C (respectively at 15 and 5 psu). These values are considered as typical for temperate mysid species (Wittmann, 1984).

Stage II is mainly responsible for the prolongation of the total development time at lower temperatures. At the extremes of the temperatures, stage II is delayed from 4 days at 21 °C to 11 days at 11 °C. The growth of larval structures is possible through the conversion of egg proteins, mainly vitellin. A delay in stage II indicates that temperature has a strong influence on the developmental processes and thus egg protein metabolism. Recently, Ghekiere et al. (2004) purified and characterized vitellin from *N. integer* with the aim to develop an enzyme-linked immunosorbent assay (ELISA) to quantify the yolk protein. Future laboratory and field studies will evaluate the use of this immunoassay for investigating effects of abiotic variables and xenobiotics on *N. integer* vitellogenesis.

In general, a prolongation of the intra-marsupial development time may reduce survival by prolonging a sensitive and vulnerable life stage. In Mysidacea, this is compensated for by the protection provided by the intra-marsupial development of the organisms. Indeed, at lower temperatures (combined with a medium salinity) the final survival and hatching success is even better. We found the experimental salinity to have a minor impact on the duration of larval development of *N. integer*. Earlier findings of Vlasblom and Elgershuizen (1977) indicate that the experimental salinity does not influence the intra-marsupial development, but that animals adapted to a higher salinity generally take longer to develop. In the present study, this adaptation factor was not taken into account (all animals originated from a salinity of ± 5).

All the larvae within a single marsupium are at the same stage of development (Mauchline, 1980). The occasional presence of younger larvae among a brood is usually attributed to adoption (Wittmann, 1978; Mauchline, 1980). However, in our experiments some variation in the time of transition form one stage to another occurred and may explain these observations. A 1 or 2 days delay in transition from stage I to stage II occurred in 48 % and 18 % of the organisms, respectively. In the moulting of stage II to stage III or from stage III to the juvenile, a 1 day delay occurred in 21 % and 14 % of the cases, respectively.

Salinity optimum

The salinity range at which the embryos and larvae develop is more restricted than the salinity range at which the female mysids can survive (Vlasblom and Elgershuizen, 1977; Greenwood et al., 1989). Although fertilization in the laboratory occurred at a salinity of 5 and 16 °C, the embryos cultured *in vitro* at this salinity and temperature combination (G) never developed to free-living juveniles. Complete mortality occurred after 6 days. Vlasblom and Elgershuizen (1977) found a survival of 0 to 30 % of the early embryos at a comparable salinity.

In the subtidal of Gironde, Weser, Tamar and Schelde estuaries (Sorbe, 1980; Schuchardt et al., 1989; Moffat, 1996; Fockedey, unpublished), ovigerous females mainly occur in the low salinity zone (salinity 3 – 10), indicating that no migration from the adverse salinity conditions occurs in *Neomysis integer*.

Also, permanent populations are described in enclosed low saline brackish ponds and lakes (e.g. Irvine et al., 1995). Our experimental *(in vitro)* results on the intra-marsupial survival and hatching success of *N. integer* at this low salinity suggest that the recruitment success of juveniles to the population may thus be seriously affected, unless the gravid female is able to actively regulate the salinity within its marsupium and in this way increase the survival and hatching success of its offspring. The active regulation of the marsupial fluid salinity is described for isopods (Charmantier and Charmantier-Daures, 1994), amphipods (among others Morritt and Spicer, 1996b) and mysids including *N. integer* (Ralph, 1965; McLusky and Heard, 1971), although the technique of freezing point analysis used by the latter authors has been criticized by Morritt and Spicer (1996b). The measurement of haemolymph and marsupial fluid concentration of *N. integer* over a range of salinities with more modern techniques (e.g. direct-reading nanolitre osmometer) is required to confirm or reject the hypothesis of active marsupial salinity regulation for the species.

The mechanism for the (possible) regulation of the marsupial fluid salinity in mysids remains unknown and needs further study. A pair of tubes extending ventrally from the female's thorax into the marsupium is described for *Neomysis integer* (Vorstman, 1951; Kinne, 1955). Although their function is unknown (Mauchline, 1980), they may have a secreting function (Kinne, 1955) and may hence have a role in the regulation of the ionic composition of the marsupial fluid. Morritt & Spicer (1996b) suggested that the marsupial salinity can be actively regulated, as described for amphipods, by redirecting urine from the antennary excretory gland into the brood pouch.

Population differences

Genetically different populations of a species may differ in the way their intra-marsupial development is affected by salinity (Lee, 1999) and temperature (Wittmann, 1984). The *in vitro* development time of embryos of a *Neomysis integer* individuals adapted to higher salinities (salinity 23) is longer than that of embryos taken from a low salinity (salinity 7) population (Vlasblom and Elgershuizen, 1977). The *N. integer* individuals used in the present experiment were sampled from a dock along the Schelde estuary. Although the dock is connected to the estuary, population genetic analysis based on mitochondrial cytochrome oxidase I sequences revealed this population to be significantly distinct from the population living in the subtidal of the Schelde estuary (Remerie et al., submitted a) or other estuaries (Remerie et al., submitted b). The responses to temperature and salinity differences reported in this paper may be population-dependent and need further study.

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Addendum 3

Mysid crustaceans as potential test organisms for the evaluation of environmental endocrine disruption: a review

ABSTRACT

Anthropogenic chemicals which disrupt the hormonal systems (endocrine disruptors) of wildlife species have recently become a widely investigated and politically charged issue. Invertebrates account for roughly 95 % of all animals, yet surprisingly little effort has been made to understand their value in signalling potential environmental endocrine disruption. This omission largely can be attributed to the high diversity of invertebrates and the shortage of fundamental knowledge of their endocrine systems. Insects and crustaceans are exceptions and, as such, appear to be excellent candidates for evaluating the environmental consequences of chemically induced endocrine disruption. Mysid shrimps (Crustacea: Mysidacea) may serve as a viable surrogate for many crustaceans and have been put forward as suitable test organisms for the evaluation of endocrine disruption by several researchers and regulatory bodies (e.g. the U.S. Environmental Protection Agency). Despite the long-standing use of mysids in toxicity testing, little information exists on their endocrinology, and few studies have focused on the potential of these animals for evaluating the effects of hormone-disrupting compounds. Therefore the question remains as to whether the current standardized mysid endpoints can be used or adapted to detect endocrine disruption, or if new procedures must be developed, specifically directed at evaluating hormone-regulated endpoints in these animals. This review summarises the ecological importance of mysids in estuarine and marine ecosystems, their use in toxicity testing and environmental monitoring, and their endocrinology and important hormone-regulated processes to highlight their potential use in assessing environmental endocrine disruption.

Since its content differs from the remainder of the thesis, the chapter has a separate reference list and font.

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INTRODUCTION

Anthropogenic chemicals that disrupt the hormonal systems (endocrine disruptors) of wildlife species recently have become a widely investigated and politically charged issue [1-3]. Invertebrates account for roughly 95% of all animals [4], yet surprisingly little effort has been invested to understand their value in signaling potential environmental endocrine disruption [5-12]. Although growth, reproduction, development, and other aspects of invertebrate physiology are known to be under hormonal control, the endocrine systems and hormones produced and used in invertebrates are not directly analogous to those of vertebrates [13]. In invertebrates, the selection of suitable test methods and species for evaluating endocrine disruption is confounded by diversity. The use of a limited number of species as representative of this diversity is a naive approach destined to failure in the absence of suitable safeguards [2,9]. Hence, the key challenge for environmental assessment is to find invertebrate species, selected from multiple levels of ecosystem function, to efficiently monitor and evaluate the complexity of potential environmental effects of endocrine-disrupting chemicals at a reasonable financial cost [14].

Many anthropogenic pollutants have the world's oceans and seas as a final sink, and are carried there through riverine and estuarine conduits [12,15]. Estuaries are intrinsically and commercially important ecosystems and are amongst the first recipients of endocrine disruptors in their seaward transport. Of the estuarine organisms that could be adversely affected by these compounds, crustaceans are good candidates for the study of potential impacts. Crustaceans are common in freshwater, estuaries, and shallow coastal waters and form vital links in aquatic food webs [16-21]. In addition, crustaceans are susceptible to the effects of endocrine disruptors [13]. An international Society of Environmental Toxicology and Chemistry workshop on endocrine disruption in invertebrates held in The Netherlands in 1998 [7] identified insects and crustaceans as potential organisms for evaluating chemically induced endocrine disruption by virtue of the wealth of information available on their endocrinology compared with other invertebrates [9,12,22-24].

Of the crustaceans, mysid shrimp have been put forward as suitable test organisms for the evaluation of endocrine disruption [7,9,25]. The U.S. Environmental Protection Agency established the Endocrine Disruptor Screening and Testing Standardization and Validation Task Force to coordinate and conduct the scientific and technical work necessary to validate the screens and tests recommended by the Endocrine Disruptor Screening and Testing Committee. The Standardization and Validation Task Force recommended a two-tiered approach for determining whether a chemical is an endocrine disruptor, and mysids were proposed as a suitable invertebrate assay in the tier 2 testing (*in vivo* testing) (http://www.epa.gov/scipoly/ oscpendo) for a two-generation reproductive and developmental toxicity test. Recently, a draft review paper was com piled on mysid life-cycle toxicity testing [13], and the two generation mysid life-cycle assay was proposed to the Organization for Economic Cooperation and Development as a new Organization for Economic Cooperation and Development test guideline. Despite the long-standing use of mysids in toxicity testing, little information on their endocrinology has been published and few studies have focused on the potential of these animals for evaluating the effects of hormone-disrupting compounds. Therefore, the question remains as to whether the current standardized mysid endpoints can be used or adapted to detect endocrine disruption, or if new procedures must be developed, specifically directed at evaluating hormone-regulated endpoints in these animals.

This present review provides an overview of the available information on mysids relevant to the issue of endocrine disruption, including their ecological role in marine and estuarine ecosystems, their use in toxicity testing and environmental monitoring, and their endocrinology. A case is made for their potential use in assessing the environmental consequences of endocrine-disrupting chemicals.

MYSID BIOLOGY AND ECOLOGY

Mysids (Malacostraca: Peracarida: Mysidacea) are relatively small (with the majority of the species being between 5 and 25 mm in length), shrimp like crustaceans, often referred to as opossum shrimp because the oostegites form a ventral female marsupium for carrying the developing embryos. The latter feature distinguishes mysids from other shrimplike crustaceans.

Mysids are identified from other peracarids (Amphipoda, Isopoda, Cumacea, and Tanaidacea) by the presence of a statocyst (containing large endogenous statoliths, the primary equilibrium organs for mysids) on the proximal part of the uropodal endopod. Mysids are distributed from 800N to 800S and occur in various aquatic environments, including freshwater, groundwater, brackish, estuarine, coastal, and oceanic habitats [26-28]. Mauchline and Murano [28] published a world list of mysids in 1977 (765 species distributed between ~ 120 genera); however, this number is ever increasing through improved sampling techniques and exploration of new habitats. The present count is more than 1,000 species belonging to approximately 160 genera (http://crustacea.netJ). A comprehensive database on the world mysid fauna (Nemys, http://intramar.ugent.be/nemys), containing links to relevant information (i.e., taxonomical, morphological, ecological, biogeographic, literature, pictorial, and molecular information), on the species level presently is being constructed (T. Deprez, Ghent University, Section Marine Biology, Ghent, Belgium, personal communication).

In general, mysids are regarded as omnivores and feed on phytoplankton, zooplankton, and organic detritus [26,27,29-31]. Pelagic forms filter particles during swimming, whereas benthic species have been observed actively hunting and grabbing small particles [27]. Mysids form important links in the food webs of aquatic ecosystems and often feed selectively for size or species (or both) of prey [26,32]. Consequently, they have the potential for structuring zooplankton communities [33,34] and influencing the structure of phytoplankton, tychoplankton, and meiofaunal communities [32,35-41]. Most mysids utilize organic detritus to a considerable extent and are capable of remineralizing a substantial portion of the nonrefractory detritus suspended in the water column or buried in the surface sediments [29,37,42,43]. Mysid size is intermediate between mesozooplanktonic (µm) and endobenthis or epibenthic (cm) prey items, and mysids often progressively replace copepods in the diet of many postlarval and juvenile commercial fish species [19,26,44,45]. In addition, mysids may serve as prey for larger crustaceans, marine mammals, or wading birds [26,27,32,46-48].

Estuarine mysids have a flexible physiology that responds to a host of dynamically changing environmental variables, characteristic of the complex chemistry of estuaries. Temperature and salinity are the dominant ecological variables, and may act either singly or in combination to modify the physiological and ecological properties of estuarine organisms as well as responses to xenobiotic exposure. Therefore, empirical determination of the optimal salinity and temperature conditions of estuarine mysids is essential for the development of optimum laboratory culture of these organisms and their use in toxicity and hazard assessment. For example, the optimal salinity and temperature conditions for growth of *Americamysis bahia* (formerly *Mysidopsis bahia)* through its entire life cycle [49] are correlated with resistance patterns to these dominant environmental variables [50] and distribution of this species in estuaries. Moreover, temperature and salinity interact to modify the reproductive capacity of this species [51].

MYSIDS AND TOXICOLOGY

Mysids are sensitive to some chemical contaminants at environmentally relevant concentrations and have been used in regulatory toxicity testing for more than 20 years [15,43,52-66]. The U.S. Environmental Protection Agency and the American Society for Testing and Materials both have adopted the subtropical *A. bahia* as a key testing species for coastal and estuarine monitoring, and standard guides for conducting life-cycle toxicity tests with this species have been developed [13,67-72]. Although a relatively large amount of published toxicity data is available for *Americamysis* species, relatively limited data are available on the sensitivity of other mysid species to toxicants [64]. However, the available evidence suggests that mysids are generally more sensitive to toxic substances than many other test species [43,73-75]. Toxicity test procedures have been published for *Neomysis mercedis* [52,76], *Mysidopsis intii* [59], *Holmesimysis costata* [62], *Americamysis bigelowi* [77], *Neomysis integer* [64,75,78], *Tenagomysis novae-zealandiae* [79], *Praunus flexuosus* [80,81], *Neomysis americana* [82,83], and *Neomysis awatschensis* [84] (Table 1). In addition, methods for maintaining viable populations of different mysid species under laboratory conditions have been described by several researchers [46,59,75,79,85-89]. Recently, a strong correlation was reported between the toxic response of daphnids and mysids *(R2* = 0.941, *n* = 28; 96-h median lethal concentrations for *A. bahia* and 48-h median lethal concentrations for *Daphnia magna)* for pesticides and organics, emphasizing the use of mysids in future toxicity testing [90].

Mysids have been used successfully to measure various sublethal toxicant effects, such as growth, swimming capability, feeding behaviour, moulting, energy budget, reproduction, sexual maturity, and vitellogenesis (described in detail in the following paragraphs and summarized in Table 2). Also, field studies and caging experiments with mysids have been published [17,91-94].

Because of their ecological importance, wide geographic distribution, year-round availability in the field, ease of transportation, ability to be cultured in the laboratory, and sensitivity to contaminants, mysids are appropriate toxicity test organisms. Clearly, field validation of the biomarkers is a strong research need for the future.

CANDIDATE MYSID TEST SPECIES FOR ENDOCRINE DISRUPTION RESEACH

General selection criteria for the most appropriate mysid species for toxicological testing are given by Nimmo and Hamaker [15] and Roast *et al.* [43]. These criteria include available when required; already adapted to laboratory conditions, eliminating an (expensive) conditioning phase; collection for laboratory testing will not decimate field populations (or destroy habitat during collection); easily transported; life history is short, making it possible to study the effects of a pollutant on various aspects of reproduction; diet is known and readily controlled; and ecologically important. In addition, the important characteristics for the selection of a suitable test species for identifying the effects of endocrine disruption in the environment are given by DeFur *et al.* [7] and include primary mode of reproduction, culture in the laboratory, generation time, size, knowledge of endocrinology, and standard methods available. Some attributes described in the latter publication (e.g., mode of reproduction or knowledge of endocrinology) do not allow for discrimination among candidate mysid species. A very useful document in this context is a draft review paper on life- cycle toxicity testing with mysids in which several species *(A. bahia, Americamysis almyra, A. bigelowi, H. costata, M. intii, N. mercedis,* and *N. integer)* are considered for their potential utility in endocrine-disruption testing [13]. From this review, it may be concluded that, although *A. bahia* has many strengths, limited ecological relevance for high-latitude and low-saline systems preclude its general utility. However, given the high degree of standardization in *A. bahia,* progress in development of standardized test protocols for endocrine-disruption testing should be fastest in this species. Table 1 summarizes the distribution, habitat description, and available culture protocols for other candidate mysid test species.

MYSID ENDOCRINOLOGY AND HORMONE-REGULATED ENDPOINTS

The use of hormones to regulate biological processes is a strategy common to vertebrates and invertebrates. Although the endocrine systems of invertebrates regulate many of the same processes in vertebrates (development, growth, and reproduction), some endocrine-regulated processes are unique to specific groups of invertebrates. For example, moulting, diapause, and limb regeneration are endocrine-regulated processes associated with some invertebrate groups that are rare or absent among vertebrates [7].

Most of the current knowledge of crustacean endocrinology is based upon studies with decapods such as crabs, lobsters, crayfish, and shrimp, and has been reviewed previously [7,95-100]. The main biological processes, such as growth, moulting, and reproduction, are cyclic and fairly well understood in benthic and terrestrial malacostracans, such as decapods, isopods, and amphipods [22,101,102]. These biological processes are regulated by a complex endocrine system [96,101]. Basically, inputs from the environment are integrated by the central nervous system; neurotransmitters and neuromodulators govern the release of neuropeptides, which control the production of hormones by the endocrine glands [103]. The main crustacean endocrine centers include the Y-organ, mandibular organ, androgenic gland, X-organ, and sinus gland [7,97]. Unfortunately, endocrine glands or sites of hormone production in mysids are largely undecided.

Table 1: Candidate mysid test species for toxicity testing with details on their natural habitat and culturing.

Species name	Distribution	Habitat description	Commercial culture	Culture protocol
Americamysis bahia (= *Mysidopsis bahia)*	Coastal estuaries and embayments ranging from the Gulf of Mexico to Narragansett (RI, USA) [250]	Marine (> 15‰), < 20-34°C [49-51]	Yes	[46, 89, 251]
Americamysis bigelowi (= *Mysidopsis bigelowi)*	Eastern coast of the USA from MA (Georges Bank) to FL often together with *A. bahia* [250]	Marine (30-35‰), 2-30°C	No	[46]
Americamysis almyra (= *Mysidopsis almyra)*	Eastern coast of the USA, inshore waters along the entire coast of Gulf of Mexico and northward along Atlantic coast to Patapsco River (MD) [250]	Marine (10-20‰), > 20°C	Yes	[85,88,252]
Holmesimysis costata (= *Acanthomysis sculpta)*	Principal species of the genus, from southern California (USA) to British Columbia (Canada) [122, 253]	Marine, planktonic, lives within surface canopy of kelp	No, field-collected animals available (USEPA, 2002)	[122,253]
Mysidopsis intii	Eastern Pacific from South America to the southern California coast of the USA [59, 250]	Marine, epibenthic, optimal temperature 20-22°C, optimal salinity 28-35‰	No	[59]
Mysis mixta	Eastern (from White Sea to Iceland) and Western (Greenland coastal waters down to Cape Cod) Atlantic regions [26]	Brackish, low salinity, cold water	No	[114]
Neomysis awatschensis	Pacific coast of Japan, Korea, and USA [115]	Marine, estuarine	No	[115]
Neomysis mercedis	Northeastern Pacific coast of the USA (southern Alaska to Goviota Bay, CA) [52]	Freshwater, estuaries, and coastal lakes, planktonic/epibenthic, euryhaline <0.5 to > 25‰), 6-22°C	No	[52]
Neomysis integer	Northern European estuaries and coastal waters; oligohaline and freshwater lakes [20, 43]	Marine, estuarine, freshwater, hyperbenthic, euryhaline < 0.5 to > 25‰), cold water < 20°C) [116]	No	[75]
Praunus flexuosus	Northern European coastal waters	Hyperbenthic/planktonic, eury-haline, eurytherm [254, 255]	No	[123]

The effects of organic and inorganic contaminants on crustacean functions regulated by hormones are being investigated with increasing frequency and several show promise as biomarkers of environmental contamination and endocrine disruption [7,8,104,105]. Unfortunately, relatively few data are available on the hormonal control of biological processes in mysids. Having said that, certain endpoints relevant to the testing of suspected endocrine disruptors, such as survival, fecundity, sexual maturation, and biomass increase, are already standardized procedures for some mysids, such as *A. bahia, A. bigelowi* (partly), *A. almyra, H. costata,* and *N. mercedis,* and many other endpoints or species are promising [7]. The use of potential mysid hormone-regulated endpoints as biomarkers of exposure or effects of endocrine disruptors are discussed in detail in the following paragraphs and are summarized in Table 2. Although many, if not all, of these endpoints may indicate a response to an endocrine disruptor, most also vary in response to exposure to other stressors and this is further confounded by the interrelatedness (i.e., nonindependence) of some of these endpoints [13]. The key to the interpretation of these endpoints as indicators of endocrine disruption will be to create for each species a large database of what constitutes the normal unstressed response, and what constitutes a normal reference site or population when working under field conditions.

Growth and moulting

Most commonly, growth is measured either by increases of dry weight or body length per time interval [59,106-108] and, for crustaceans, is often expressed in terms of intermoult period and growth factor (percentage increase in body size at the moult) [26]. Growth curves (such as the von Bertalanffy equation) can be fitted to the growth data [20,109,110], allowing comparisons of the different growth parameters between treatments. Although several studies have focused on growth and moulting in mysids under natural conditions [49,107,111-123], exposure experiments also have confirmed the sensitivity of these endpoints in toxicology [52,56,61,84,92,106,108,124131]. For mysids, reduced growth is the most common sublethal response to toxicant exposure and this has important implications for reproductive success because fecundity is related directly to female body size [20,84,118,123]. In crustaceans, significant growth occurs only as a result of moulting; therefore, disruption of moulting may result in alterations in growth [13,132].

Ecdysteroids (the moulting hormones in crustaceans) also function in the control of reproduction and embryogenesis [97,133]; therefore, the crustacean moult cycle has profound effects on many aspects of organismal function, including physiology, behaviour, and changes in biochemical composition [134,135]. Moulting is regulated by a multihormonal system but is under the immediate control of moult-promoting steroid hormones (ecdysteroids) secreted by an ecdysial gland, called the Y-organ (the homologue of the prothoracic gland in insects [103,136]. The Y-organ secretes ecdysone, which, on release in the hemolymph, is converted into active 20-hydroxyecdysone (synonyms: crustecdysone and ecdysterone). Circulating titers of 20-hydroxyecdysone vary impressively during the moult cycle [103,134]. The Y-organ produces two other ecdysteroids, 3-dehydroecdysone and 25-deoxyecdysone, with he latter forming the immediate precursor to the active ponasterone A [133]. More studies have been done on the effects of contaminants on moulting and limb generation than on any other hormone-mediated process in crustaceans [8,135,137].

Moult staging, based on changes in the integument, has been developed for various crustaceans and is generally divided into four major periods: postmoult, intermoult, premoult, and moult (ecdysis). Mysid moult stages have been described for *Siriella armata* [138,139], *Mysis mixta* [140], and *N. integer* [140]. In mysids, ecdysis is instantaneous, with the entire carapace lifting up and the mysid sliding out of the old cuticle while swimming. For female mysids, integumental development during moult preparation, marsupial brood development, and development of new eggs in the ovary are synchronized, facilitating moult staging [101]. To date, only one study has quantified ecdysteroid titers during the mysid moult cycle and this study was with S. *armata* [101]. However, both ecdysone and 20-0H ecdysone have been identified in *N. integer* (Ghekiere *et al.*, unpublished data) and *A. bahia* (Tuberty and McKenney, unpublished data).

As mentioned previously, moulting is controlled by ecdysteroids [136]. Ecdysteroids control the activity of specific genes at the transcriptional level by interacting with the intracellular ecdysteroid receptor [103,135,141]. In arthropods, the ecdysone receptor is in the same gene family as the vertebrate thyroid receptor but, interestingly, steroidal estrogens do not agonize or antagonize the ecdysteroid receptor [142]. Evidence exists [142] that some nonsteroidal environmental estrogens are ecdysteroid antagonists (e.g. lindane, bisphenol A, diethylphthalate, and *p,p'*-DDT). In addition, several classes of phytochemicals antagonize ecdysone activity [137]. The apparent ubiquity of the antiecdysteroidal activity of environmental chemicals necessitates investigation into their potential effects on crustaceans [143,144]. Many pesticides, generally classed as insect growth regulators, function as ecdysone agonists [7,11].
As suggested by Zou and Fingerman [136], future investigations of moulting in crustaceans should have two emphases, one examining interactions between potential endocrine disruptors and the ecdysone receptor, and one focusing on the possible impairment of ecdysteroidogenesis by these agents. *In vitro* assays can determine quickly whether a chemical has (anti)ecdysteroidal activity [145,146]. Given the current methods for quantifying ecdysteroids by using immunoassay [139,144,147] and the available methods for moult staging [138,140], it should be possible to evaluate the potential interaction of these chemicals with the process of moulting in mysids.

Because ecdysteroids are used as major endocrine-signaling molecules in crustaceans [7], and little is known of their other functions, it may be expected that a chemical with (anti)ecdysteroidal activity also will affect other hormone-regulated processes in crustaceans. Support for this hypothesis is provided by Mu and LeBlanc [144], who demonstrated that the fungicide fenarimol altered embryo development in daphnids by interfering with ecdysteroid metabolism. However, one major advantage of using ecdysteroid metabolism as an endpoint is that it provides a means of evaluating the impact of environmental chemicals on crustaceans (and potentially other arthropods), while not necessarily affecting vertebrates. Because (anti)ecdysteroidal activity has been proven *in vitro* for certain chemicals [145], and disruption of moulting has been observed as a result of chemical exposure, these chemicals should be tested in exposures with mysids. In these exposures, endpoints such as intermoult period, growth, morphological aberrations, ecdysone titers, protein concentrations, integument development, as well as related endpoints such as vitellogenesis, should detect *in vivo* effects of chemicals on ecdysteroid metabolism and moulting in mysids. *In vitro* assays should aid the mechanistic understanding of chemical action to better allow distinction between endocrine-specific and pharmacological effects.

Energy metabolism

Biomarkers linked with physiological energetics provide information on key processes in the organism's energy acquisition and expenditure, and possibly also elucidate the mode of action of the toxicant. Under normal conditions, specific amounts of energy are allocated to basal metabolism, growth, and reproduction and therefore, theoretically, changes in metabolic turnover and specific allocations should be linked to effects at higher levels of ecological organization [148]. A large body of information is available on the neuroendocrine pathways of physiological regulation in macrocrustaceans. Although typical and well-studied challenges to endogenous energy metabolism include environmental hypoxia, functional (internal) hypoxia, changing energetic requirements, disturbance to water balance and ion homeostasis and changes in temperature (for review, refer to Morris and Airriess [149]), exposure to toxicants also will result in an energetic challenge. Because energetic processes are hormone-regulated, they are, by definition, sensitive to hormone disruption and several measurements of energy reserves and consumption may serve as useful biomarkers of endocrine-disrupting substances in crustaceans [11]. However, once again, the utility of bioenergetic endpoints will depend strongly on establishment of a consensus database of what the normal bioenergetic state is for mysids.

Alterations to the energy metabolism of mysids have been used successfully as an indicator of stress to toxicant exposure in *A. bahia* [63,125,126,129,130], *P. fiexuosus* [81], *N. awatschensis* [150-152], and *N. integer* [153-156]. In *A. bahia, P. fiexuosus,* and *N. awatschensis,* weight-specific respiration, ammonia excretion rates, and oxygen to nitrogen ratios have been measured after toxicant exposure. In *N. integer,* Roast *et al.* [153] used scope for growth [157], whereas Verslycke and Janssen [155] and Verslycke *et al.* [156] used the cellular energy allocation assay [158]. Both methods are promising and were recently validated in *N. integer* after exposure to the pesticide chlorpyrifos (Verslycke *et al.*, unpublished data). The cellular energy allocation assay also was recently validated in the field (Scheldt estuary, The Netherlands) [94]. The ecological relevance and utility of short -term bioindicators of metabolic processes in *A. bahia* have been demonstrated after chronic exposure to pesticides [63,125,126,129,130]. In these studies, pesticide-exposed juvenile mysids had a greater reliance on the more energy-rich lipid substrates during maturation to support elevated metabolic demands, resulting in less lipid material available for gamete production and reduced reproductive success. Unexposed mysids shift toward more proteinaceous substrates during maturation, as demonstrated for *A. bahia* [63] and *N. integer* [155,159]. These changes in metabolic substrate usage can be measured by monitoring the oxygen to nitrogen ratio [125,160], the lipid and protein content [155], or the carbon to nitrogen ratio of the test organism [114]. On the other hand, hyperglycemia is a common response to environmental or functional hypoxia and contaminant exposure in numerous decapods, and it is thought to be triggered by the action of crustacean hyperglycemic hormone on various target tissues [8,149]. The amino acid sequence of crustacean hyperglycemic hormone is highly homologous with that of the moult-inhibiting hormone, another product of the sinus glands in crustaceans, indicating possible involvement in the control of moulting and reproduction [149]. Several investigators have examined the effects of metals and organic contaminants on blood glucose concentrations and crustacean hyperglycemic hormone titers in crustaceans [8]. Changes in blood glucose levels in mysids exposed to potential endocrine disruptors may indicate disruption of hormonal activity other than that associated with moulting or reproduction [13].

The methods described above are transferred easily to other mysid species. Endpoints related to energetic processes are relatively easy to measure, but a better and holistic understanding of the role of the different hormones involved in energy metabolism, such as crustacean hyperglycemic hormone, is needed to evaluate the potential impact of hormonemimicking substances on mysids. In this context, new immunoassays for determination of circulating hormones in the hemolymph, such as crustacean hyperglycemic hormone, are promising [161].

Other endpoints related to metabolism in mysids have been studied. High acetylcholinesterase activity in *Siriella clausi,* indicating a high metabolic rate, identified this mysid as particularly suited for research based on biomarkers in the marine environment [17]. In addition, *N. integer* has been used as a viable alternative model for the partial replacement of vertebrate animals in metabolic studies with illegal growth promoters and veterinary drugs [162]. Finally, respiratory responses have been studied in mysids in relation to a variable environment and toxic exposure [63,81,84,115,125,160,163-165].

Table 2: List of potential endpoints for evaluating the effects of endocrine disruptors and their use in mysids

Endpoint	Use in mysids	References/comments
Survival (acute)	*Americamysis bahia* (S)[a]	[69,253]
	Americamysis bigelowi (S)	[69]
	Americamysis almyra (S)	[69]
	Holmesimysis costata (S)	[62,67,253]
	Neomysis mercedis (S)	[52,67,76]
	Mysidopsis intii	[55,59]
	Neomysis integer	[53,64,75,78]
	Other species	[79,80-84]
Life-cycle testing	*A. bahia* (S)	[68,70,72,253]
	A. bigelowi (S), *A. almyra* (S)	[68]
	H. costata (S)	[56,68,71]
	M. intii	[55,59]
	N. integer	[160]
Two-generation testing	*A. bahia, A. bigelowi, A. almyra* (S in prep)	[13,219,256]
	M. intii	Method should be developed
	H. costata, N. mercedis, N. integer	Probably impractical because of long generation time
Fecundity (brood size)	*A. bahia, A. bigelowi, A. almyra* (S)	[68,70]
	H. costata (S)	[71,122]
	N. mercedis	[257]
	M. intii	[55,59]
	N. integer, Praunus flexuosus	[123,258]
	Mysis mixta	[114]
	Neomysis awatschensis	[115]
Embryonic development	*A. bahia*	[205]
	Mesopodopsis slabberi	[208]
Sexual maturity	*A. bahia* (S)	[51,58,60,68,218]
Time to first brood release	*H. costata*	[122]
Egg development time	*N. integer, P. flexuosus*	[123]
	M. mixta	[114]
	M. intii	[55,59]
Sex ratio and intersexuality	*A. bahia* (S)	[68,219,253]
	N. integer	[223-225]
Growth, biomass	*A. bahia, A. bigelowi, A. almyra* (S)	[68,70]
	H. costata (S)	[56,71,122]
	N. mercedis	[52]
	M. intii	[55,59]
	M. mixta	[124]
	N. integer	[107,116]
	N. awatschensis	[84,115,150,151]
	P. flexuosus	[114]
	Tenagomysis novae-zealandiae	[79]
	Other species	[117,119-121,132]
Molt time and success	*A. bahia*	[259]
	N. integer	[111,140]
	M. mixta	[140]
	Siriella annata	[138]
	N. awatschensis	[115]
Energy metabolism	*A. bahia*	[63]
O:N ratio, C:N ratio	*N. mercedis*	[260]
Respiration	*N. integer*	[153,155,164-167,169-175,178-181]
Biochemical composition	*M. mixta*	[114,182]
	N. awatschensis	[150-152]
	P. flexuosus	[81]
	Leptomysis lingvura	[54]
	Mysis relicta	[176,261]
	Gastrosaccus brevifissura	[163]
Ecdysteroid metabolism	*A. bahia*	S.R. Tuberty[b] and C.L. McKenney[c], unpublished data
	N. integer	A. Ghekiere *et al.*[d], unpublished data
	S. *armata*	[139]
Steroid metabolism	*N. integer*	[162,185,198,199]
P450 enzymes	*N. integer*	[162,185,198]
Vitellogenesis	*A. bahia*	[215]
	S. *armata*	[139]
	N. integer	A. Ghekiere *et al.*[d], unpublished data

Table 2 (cont)

Endpoint	Use in mysids	References/comments
Osmoregulation	*A. bahia*	[259,262,263]
	N. integer	[264]
	P. flexuosus	[265]
	Other species	[248]
Morphology, histology	*A. bahia, A. bigelowi*	[77]
	N. integer	[225]
Swimming behavior	*N. integer*	[43,226-230,232]
Feeding behavior	*A. bahia*	[231,241]
	M. mixta	[239,242]
Other behavioral endpoints	Mating, grooming, swarming, burrowing ability, predator-prey dynamics	[233-238,243-247]

[a]S = method is standardized.
[b] Center for Environmental Diagnostics and Bioremediation, University of West Florida, Pensacola, FL, USA.
[c] U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Gulf Breeze, FL, USA.
[d] Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Ghent, Belgium.

Although undoubtedly having environmental relevance and being fairly easily extrapolated to higher levels of biological organization, the major disadvantage of endpoints related to energy metabolism is their difficulty in mechanistically explaining hormone-regulated responses as can be expected from exposure to endocrine disruptors. Many abiotic and toxic stressors affect the energy metabolic processes of organisms [114,127,152-156,164], while not necessarily being related to disruption in normal hormonal regulation. Therefore, the successful use of biomarkers for the evaluation of endocrine disruption will be limited by the amount of background data on natural variation and normal levels of the endpoints in question, and also by the fundamental understanding of the toxicant action at the (sub-)cellular level. In this regard, many studies containing background information on the biochemical composition (e.g., proteins, lipids, and sterols) of mysids have been published [166-182], but information on neuropeptide and hormonal levels in mysids needs development.

Steroid metabolism and cytochrome P450

Pollutants may exert reproductive effects through interference with normal steroid metabolism [183-187]. For invertebrates, several studies have focused on pollutant-induced alterations in steroid metabolism. These chemicals often interfere with the microsomal P450 mono oxygenase system, also called the mixed-function oxygenase system. The mixed-function oxygenase system is involved not only in the metabolism of organic toxicants but also in steroid metabolism; consequently, induction or inhibition of the mixed-function oxygenase system also may have repercussions for the hormonal control of reproduction. In sea stars, a linkage was demonstrated between impaired reproductive success, pollution-modulated endocrine function, and induction of the mixed- function oxygenase system [187]. In gastropods, much work on steroid metabolism has been initiated by the observation of tributyltininduced imposex, a state of pseudohermaphrodism in which females exhibit functional secondary male characteristics. Although the underlying mechanism by which tributyltin causes imposex in gastropods has not been elucidated conclusively, the weight of evidence is in favour of the cytochrome P450dependent aromatase inhibition hypothesis [187-192].

Alterations in steroid metabolism have been studied in *D. magna* [186,193-197] and in the blue crab *Callinectes sapidus* [191]. In daphnids, changes in steroid metabolism could provide an early indication of potential reproductive toxicity after sublethal exposure to suspected endocrine disruptors [186,193,194,197]. Verslycke *et al.* [185] reported testosterone metabolism and the presence of vertebrate-type steroids in *N. integer* and demonstrated the presence of a complex steroid hydroxylase system consisting of different P450 isozymes. The remarkable diversity of testosterone hydroxylation exhibited should stimulate further studies on the induction, stereospecificity, and regulation of the enzyme systems of *N. integer* and other mysids. More recently, alterations in the phase I and II testosterone metabolism in *N. integer* after acute exposure to tributyltin have been demonstrated [198]. In addition, metabolic studies with *N. integer* have been used recently in exposure experiments with other chemicals, such as nonylphenol and methoprene, and also in the field [199].

The presence of sex hormones has been suggested in many, if not all, arthropods [183]. Vertebrate-type steroids (such as 17β-estradiol, testosterone, and progesterone) have been measured in several malacostracan crustaceans [7,184]. Although the lack of a role for vertebrate sex steroid hormones in arthropods has been highlighted [7,183], fragmented evidence suggests that some of these compounds may function as hormones in crustaceans [7,143,185]. Endogenous androgens may be the precursors for other hormones; therefore, exposure to exogenous androgens (or androgen mimics) could elicit activity through receptors other than the androgen receptor. Although this has not been determined in crustaceans, Verslycke *et al.* [185] found evidence of a sex-specific production of androgens, such as testosterone and androstenedione, in *N. integer.* Similarly, LeBlanc and McLachlan [200] reported various rates of testosterone conversion to androstenedione in daphnids. Future studies are needed to reveal if these conversions are affected by age, gender, reproductive state, or changes in the abiotic environment. Note that an androgen receptor has not been found or cloned in crustaceans. Therefore, identification and characterization of the androgen receptor should be a priority for research to explore the usefulness of sex steroids for evaluating endocrine disruption in crustaceans and other invertebrates.

Studies over the last 30 years have established the important role of cytochrome P450 in the biotransformation of xenobiotics and endogenous compounds (such as ecdysteroids) in crustaceans (for a review on crustacean P450, refer to James and Boyle [184]). Although no structural information on cytochrome P450 in crustaceans is available, it is clear that they are involved in several steps in the biosynthesis of ecdysteroids and other physiologically important substrates in crustaceans [201]. More studies are needed to understand the effects, if any, of various classes of environmental and other chemicals that are known modulators of cytochrome P450 expression or activity. New molecular tools, such as primer-based reverse transcription-polymerase chain reaction procedures and expression of P450s in heterologous systems, should result in better insights into the function and expression of P450s in the context of endocrine disruption. In addition, *in vivo* metabolic studies with different substrates (testosterone and ecdysone) will provide valuable tools for evaluating the effects of toxicant exposure, particularly when linked with effects on higher levels of biological organization. Although information on the identity of P450s and their functional role in mysids is, to our knowledge, nonexistent, mysids should be a good model to study these mechanisms. From the preliminary studies with *N. integer* by Verslycke *et al.* [185,198,199], sufficient information is available to suggest that mysids have an enzymatic biotransformation system that rivals that of other invertebrates and vertebrates. Metabolic studies with physiologically relevant substrates that also measure hormone-regulated effects at a higher level of biological organization (i.e., reproductive success) would be valuable in evaluation of environmental endocrine disruption.

Reproduction and vitellogenesis

Although the main neurosecretory centers and the sinus gland in mysids resemble these from decapods, sexual differentiation in juveniles and mysid reproduction are more like those of amphipods and isopods and are strictly linked to the moult cycle [101]. In mysids, embryonic and postembryonic development occurs in the female marsupium and includes five consecutive stages from oviposition to the juvenile stage [26,118,202-205]. Juveniles are liberated immediately before ecdysis of the mother, shortly after which she lays a new batch of eggs in the marsupium. A secondary vitello genic cycle starts for a new batch of oocytes on the second day of the moult cycle. Secondary vitellogenesis is not only cyclical, as in other crustaceans [206], but also strictly linked to the moult cycle, offering an example of the type 2 pattern (e.g., Amphipoda, Isopoda, and Decapoda) for the regulation of simultaneous gonadal and somatic growth in crustaceans [206,207]. Cuzin-Roudy and Saleuddin [101] published an excellent review on the use of the mysid S. *armata* as a biological model for the study of hormonal control of moult and reproduction, which should be extended for other mysid species. In addition, Wortham and Price [205] and Greenwood *et al.* [208] published studies on the *in vitro* culture of mysid marsupial developmental stages at different temperatures. These assays should be evaluated further as a means of detecting effects of contaminants on marsupial development in mysids.

In general, few studies have been conducted on the effects of contaminants on gonadal maturation of crustaceans [8]; however, much attention has been given recently to vitellogenin, the precursor to the yolk protein vitellin in egg-laying invertebrates and vertebrates, as an indicator of exposure to estrogenic xenobiotics [5,209-216]. Control of vitellogenesis is being studied intensively because yolk is an excellent model for studying mechanisms of hormonal control at the cellular and molecular levels [5,215]. To assess the potential adverse effects of xenobiotics on crustacean reproduction, it is important to measure accurately vitellogenin and vitellin in crustacean models (an overview of crustacean species from which vitellin, vitellogenin, or lipovitellin has been isolated or partially characterized is given by Tuberty et ai. [215]). Recently, a quantitative enzyme-linked immunosorbent assay was developed for the mysid *A. bahia* by using polyclonal antisera [215]. In addition, studies are under way to characterize and purify vitellin of the mysid *N. integer* (A. Ghekiere et al., Laboratory of Environmental Toxicology and Aquatic Ecology, Ghent University, Ghent, Belgium, unpublished data). Future laboratory and field studies with mysids are needed to evaluate the use of these immunoassays for investigating effects of xenobiotics on crustacean vitellogenesis. A good example of this is given by Oberd6rster et ai. [213,214], who reported the effects of chronic pyrene exposure on moulting and reproduction assessed in the grass shrimp *Palaemonetes pugio* by using a monoclonal enzyme-linked immunosorbent assay for vitellin. Other studies also have found an impact of xenoestrogens on the production of crustacean proteins (e.g., vitellin and cypris major protein), which are thought to be under estrogen control [5,217]. Future work on sequence determination of vitello genic genes and their hormonal activity will provide interesting insight into the vitellogenic process in mysids. Study of genomic and nongenomic effects of ecdysteroids on ovarian maturation is another potential area of work. Synergistic and antagonistic actions of the different neuropeptides, and the mandibular organ control over moulting and reproduction, are other areas requiring further study as a basis for use of crustaceans for endocrine-disruption testing in the future [13].

Life-cycle testing and population and field studies

Despite superficial resemblance to decapod shrimps, mysids are more closely related to amphipods and isopods, and are grouped together in the superorder Peracarida. All three orders are good candidates for toxicological testing, and amphipods and mysids are used routinely. However, for endocrine-disruption testing, especially for life-cycle tests, mysids offer clear advantages over amphipods. Most marine amphipods used in toxicological testing must be collected from their natural habitats before use in tests. Although they can be held for a few weeks before testing, they generally are not cultured for tests. Conversely, several mysid species have been cultured in the laboratory and used in life-cycle tests [13]. Several measures of reproductive performance can be used to assess sublethal response in life-cycle testing, including sexual maturity, the time to first brood release, the time required for egg development (and its separate phases), fecundity, brood success, and alterations in reproductive characteristics in popu lations [11,58,60,63,108,125,126,128,130,218,219] (Table 2). Inhibited reproduction is the most sensitive, sublethal population response of *A. bahia* chronically exposed to pesticides [63]. Numerous studies have described the use of reproductive endpoints in mysids after toxic exposure and changes in the abiotic environment [15,123,131,220]. Although standard chronic assays, including reproductive endpoints, are described for *A. bahia,* these should be applicable to other mysids, although the longer life cycle in other species may restrict their use in routine testing.

The life history of *A. bahia* is very amenable to demographic modelling because of rapid growth, early sexual differentiation (at 14 d) and reproduction (commencing around 17 d), and frequency of brood production (average of five to seven per female) over the full life span of 90 d [221,222]. These endpoints provide useful information for predicting population-level effects of reproductive toxicants. However, further validation is needed in multigenerational laboratory studies as well as incorporation of other population growth parameters such as density dependence, predation, migration, and competition, before conclusions can be formulated that are relevant for natural environmental conditions. Preliminary transgenerational responses of *A. bahia* to a pesticide acting as a juvenile hormone agonist have been reported [219]. Survival, growth, development, and reproduction of this estuarine mysid were monitored through an entire life-cycle exposure to fenoxycarb and during the second generation without additional exposure. Juvenile mysid growth, and carbon and nitrogen accumulation, as well as mysid survival through the first brood production, were significantly affected by fenoxycarb.

On the other hand, maturation time, sex determination, and young production were not significantly altered during the life-cycle exposure. However, second-generation adults, exposed to fenoxycarb only as developing embryos and juveniles, produced fewer young and contained significantly fewer males. These results demonstrate clearly the need for transgenerational studies with mysids to fully understand the potential chronic impact of endocrine disruptors.

Detailed information and the short life cycle of *A. bahia* clearly favour the use of this species in the initial development and further validation of population models based on reproductive endpoints. A concise draft of a detailed review paper has been produced by U.S. Environmental Protection Agency [13] on a recommended protocol and additional data needs for a two-generation life-cycle test with *A. bahia* in the context of endocrine disruptors. In this review, the following endpoints and their preferred methods for quantification are given: survival, moulting frequency, reproduction (sexual maturity, time to first brood release, brood size, and number of offspring produced), metabolic disruption, disruption in steroid metabolism, vitellogenin induction, cytochrome P450 levels, and blood glucose levels. Table 2 summarizes the potential endpoints for evaluating environmental endocrine disruption in mysids.

The use of mysids in field studies has been extremely limited. McKenney *et al.* [92] and Clark *et al.* [91] performed experiments with caged mysids to evaluate the lethal and sublethal responses of *A. bahia* during field applications of fenthion, an organophosphate insecticide. To our knowledge, these are the only published studies on in situ exposures with caged mysids. In addition, studies that have investigated biomarker responses in field-exposed mysids also are very limited [17,94]. Clearly, field validation of the biomarkers described in this review is a strong research need for the future.

Morphology and histology

Morphological changes resulting from exposure to contaminants have been documented for many taxa, including arthropods, but have not been considered widely in mysid toxicological studies as a measurable endpoint [13]. Gentile *et al.* [77] reported morphological aberrations at the onset of sexual maturity in *A. bahia* and *A. bigelowi* exposed to cadmium in the laboratory. In addition, field observations of intersexuality and variable telson morphology were reported in *N. integer* from different European estuaries and the Baltic Sea [223-225]. Most of the telson differences may be explained by regeneration of parts damaged by predation and cannot be related directly to physiological perturbations during moulting. Still, a genetic or epigenetic basis cannot be ruled out completely [225]. The degree of fluctuating asymmetry in mysids has been proposed as a quantifiable measure of morphological aberrations and is thought to arise from environmental or genetic stress during development [13]. Because the results from earlier studies on morphological aberrations could not give a clear mechanistic explanation for the observed effects, preliminary studies examining different potential characteristics would first have to be performed in mysids, before further considering this endpoint.

Behavioural and other endpoints

Disruption of mysid swimming and position maintenance behaviour has been investigated in laboratory studies with *N. integer* exposed to sublethal concentrations of chlorpyrifos (an organophosporous pesticide) and cadmium [226-230]. Although the mode of action of the toxicant on swimming remains unknown, the authors speculated that the disruption in chlorpyrifos-exposed mysids was probably due to the inhibitory action on acetylcholinesterase. In addition, Cripe *et al.* [231] reported a reduction in the maximum sustained swimming speed of *A. bahia* after exposure to sublethal levels of two pesticides. Other authors have investigated the swarming behaviour of mysids in laboratory or field studies [232-238]. For mysids, disruption of swimming and swarming behaviour may lead to increased predation or displacement from optimum sites in the estuary [43].

Other behavioural responses that have been measured in mysids include feeding activity [39,239-242], grooming behaviour [243], burrowing ability [244] and predator-prey dynamics [245]. The use of behavioural responses as a monitoring tool, however, has little utility unless behavioural changes are understood within an ecological context; that is, how well the patterns are understood within the context of an animal's natural life habits and ecological requirements [246] and if the changes can be related clearly to internal residue levels or environmental levels of specific contaminants [247].

Several studies have been published on osmotic regulation in mysids (Webb *et al.* [248] and references therein) and the interaction between osmoregulation and chemical exposure [66,249]. Other hormonal responses and disturbances in crustaceans, such as color changes (one of the earliest studied phenomena that provided definite proof of a hormone- mediated process in a crustacean), retinal pigments, and limb regeneration are discussed in a review by Fingerman *et al.* [8]. However, the use of these endpoints in mysids awaits further study.

CONCLUSIONS

This review clearly demonstrates the ecological relevance and the potential use of mysid shrimp as test species for the evaluation of environmental endocrine disruption and as a potential surrogate for many other crustaceans. The highly standardized use of mysids in toxicity testing is an important advantage and research should be directed at evaluating the current standardized endpoints, such as survival, growth, and reproduction, preferably through an entire life cycle, with a number of endocrine disruptors. In this context, a number of reference chemicals, chosen for their possible mode of action (i.e., ecdysone agonist, estrogen antagonist, juvenile hormone agonists, and others) was proposed by DeFur *et al.* [7] for evaluating relative endpoint sensitivity to potential endocrine disrupting compounds. In addition, evidence of trans-generational effects has been published and presently a two-generation life-cycle protocol is being investigated with the standard species *A. bahia.* However, an extensive list of nonstandardized endpoints has been published and should be investigated further. Some of these endpoints, such as disruption of ecdysteroid metabolism and embryonic development, might differentiate for invertebrate-specific effects of chemicals. The selection of which mysid species to use will be a balance of its ecological relevance and its ease of use for measuring the selected endpoints. Clearly, the amount of available information and the relatively short life cycle of *A. bahia* favour the use of this species, but its narrow salinity and temperature range limit its use in colder water or low-salinity testing. Various other mysid species are proposed in this review, together with a list of potential endpoints to evaluate the effects of endocrine disruptors in these animals. These should stimulate the scientific community to explore further the use of mysid shrimp as an invertebrate model for the evaluation of environmental endocrine disruption.

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Conclusions

The conclusions on each of the specific aims of the present work, as presented in the preface, are:

1. An extensive literature review on *Neomysis integer* is presented, focussing on its feeding and life history aspects, physiology, behaviour, biochemical composition and energy budgets. All distribution records of the species are added as an appendix.

2. *Neomysis integer* living in the turbid reaches of estuaries is omnivorous, and mainly feed on calanoid copepods (*Eurytemora affinis*), macrophytal detritus and estuarine sediment aggregates. Some variation in the diet was observed between the different investigated populations from Elbe, Schelde and Gironde. The quality of the diet did not differ between the sexes or between different developmental stages, although smaller individuals consumed fewer items.

3. Individually-based laboratory growth experiments over the entire life span of *Neomysis integer* learned us that its post-marsupial growth is possible over a wide temperature- and salinity range, but its maturation is only likely within the narrower range of 15 – 25 °C and 5 – 15 psu. Although survival is lower, the post-marsupial growth and development of *Neomysis integer* are both accelerated at a higher temperature, mainly due to a more frequent moulting of the animals. The size-at-maturity decreased at a higher temperature. Salinity had a stronger effect than temperature on the time needed to become mature.

4. Diets of *Eurytemora affinis*, macrophytal detritus or estuarine aggregates proved to be of sufficiently high quality to provide a good survival for the mysid, although growth rates were significantly higher when *Neomysis integer* fed on animal food in comparison to detrital food. For *N. integer* living in the maximum turbidity zone of estuaries, the estuarine flocs and macrophytal detritus may be important additional food sources, especially in periods when mesozooplankton prey is scarce.

5. *In vitro* experiments with embryos of *Neomysis integer* learned us that the embryonic survival and development are confined to a restricted salinity range of 14 – 17 psu, unless female mysids can actively regulate the salinity of their marsupial fluid. The duration of the embryonic development (between 11 – 22 days) is accelerated at a higher temperature.

In conclusion, *Neomysis integer* living in the turbid reaches of estuaries is omnivorous, and mainly feeds on calanoid copepods, macrophytal detritus and estuarine sediment aggregates. The quality of all of these food items is sufficiently high to provide a good survival for the mysid, but growth rates are significantly higher when *N. integer* feeds on animal food in comparison to detrital diets. For *N. integer* living in the maximum turbidity zone of estuaries, the estuarine flocs and macrophytal detritus may be important additional food sources, especially in periods when mesozooplankton prey (mainly calanoid copepods) is scarce.

Under experimental conditions, the following conclusions on the effect of environmental variables (temperature and salinity) on the growth and development of *Neomysis integer* can be presented: the post-marsupial growth of *N. integer* is possible over a wide temperature and salinity range, but sexual maturation is only possible within the narrower range of 15 – 25 °C and 5 – 15 psu. Intra-marsupial survival (> 50 %) and development are confined to an even more restricted salinity range of 14 – 17 psu, unless female mysids can actively regulate the concentration of their marsupial fluid. The duration of the intra-marsupail development of *N. integer* is strongly affected by temperature, while survival and hatching success are dependent on the salinity conditions. Although survival is lower, the post-marsupial growth and development of *N. integer* are both accelerated at a higher temperature, mainly due to a more frequent moulting of the animals. The size-at-maturity decreased at a higher temperature. Salinity even had a stronger effect than temperature on the time needed to become mature.

The results of the experimental research presented in this thesis contribute to our basic knowledge of the ecology of the mysid *Neomysis integer*, a key species in the brackish water zone of temperate European estuaries. More specifically, we contribute to the understanding and quantification of the species' feeding ecology and population dynamical characteristics, *i.e.* the impact of environmental variables (temperature, salinity and food quality) on processes like growth, moulting and pre- and post-marsupial development. These data are relevant for ecological modelling; and the techniques developed and described for assessing the effects of environmental conditions on individual growth, moulting, and *in vitro* embryology are currently used in bioassays for the evaluation of the effects of toxic substances (mainly endocrine disrupting chemicals) in the estuarine ecosystem (Verslycke et al., 2004 – **Addendum 3**; Bruwiere, 2004; Kregersman, 2005; Ghekiere et al., submitted; Ghekiere et al., in preparation).

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