MATURE

Progress report December 1993

Theme 4: Higher trophic levels, partim Hyperbenthos

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Occurrence and feeding ecology of *Neomysis integer* in the maximum turbidity zone of European estuaries

Introduction

This report presents the preliminary results of the research conducted in the framework of the MATURE project (first year). The study is divided into 4 major parts. First we report on the completion of the data base on the field occurrence of hyperbenthic animals in the three estuaries. The species composition and the distribution and densities of the dominant species in Elbe, Westerschelde and Gironde are briefly discussed. Next, data on the feeding ecology of the dominant hyperbenthic species, the mysid *Neomysis integer*, are presented. In a third chapter the progress in the experimental part of the research is discussed. Finally, the biology and dynamics of the *Neomysis integer* population in the Westerschelde are described in detail.

1. The field occurrence of hyperbenthos in Elbe, Westerschelde and Gironde

1.1. Sampling

The distribution and density of hyperbenthic animals (mainly the mysid *Neomysis integer*) in Elbe, Westerschelde and Gironde was studied during the joint sampling campaigns in spring 1993. Data on the summer distribution of the hyperbenthos in the Gironde and Westerschelde estuaries were already available from the JEEP project. No data on the hyperbenthos of the Elbe were available to date. The hyperbenthos was collected in all three estuaries in order to complete the data base on field occurrence of mysids. In each estuary at least 5 stations were sampled along a longitudinal transect: 2 stations were located in the MTZ, 2 downstream and 1 upstream. The Elbe was sampled on April 22nd (5 stations), the Westerschelde on May 6th (8 stations) and the Gironde on May 23rd (6 stations). The location of the stations is shown in Fig. 1.1. All samples were taken during daytime. Each station was sampled once with a sledge (a haul of 1000 metres with the tide at a ship speed of 4.5 knots, sampling the lower 1 m of the watercolumn) and twice with a high speed plankton sampler (2 hauls of 1000 metres against the tide at 1.5 knots, one haul near the bottom, the other near the surface).

In Gironde and Elbe a 24 hour sampling was conducted. The hyperbenthos of an anchor station in the MTZ was sampled every hour at 2 different depths using a high speed plankton sampler. In the Elbe, the anchor station was located in the vicinity of Brunsbüttel (Fig. 1.1). Sampling started on April 23rd, 11 a.m. and ended on April 24th, 11 a.m. In the Gironde, the anchor station was situated near Paulliac (Fig. 1); it was sampled from May 20th, 8.30 a.m. to May 21st, 8.30 a.m.

All samples were immediately preserved with a buffered formaldehyde-solution (7% final concentration). In each station, the following environmental variables are measured: temperature, salinity, dissolved oxygen and Secchi-disc depth.

1.2. Processing of samples

In the laboratory all animals were identified, if possible to species level, and counted. For the analyses, different developmental stages of decapods (zoeae, megalopae, postlarvae and adults) were treated as separate species. Animals with continuous growth were measured (standard length: from the rostral tip to the end of the last abdominal segment for crustaceans, from the tip of the nose to the base of the caudal fin for fish) and their biomass was derived from length - ash-free dry weight regressions. Discrete developmental stages were given a mean biomass value. For each sample, about one thousand individuals of *Neomysis integer* (500 from the upper net and 500 from the lower net) were sexed and categorized in six life cycle stages according to Mauchline (1980). Density is expressed as number of individuals per m^2.

Biomass will be calculated using length-ashfree dry weight regressions derived from the Westerschelde population (see below, chapter 4). Net efficiency was considered to be 100%; all density and biomass values should be considered as minimum estimates.

1.3. Preliminary results

Fig. 1.2a shows the temperature gradients in the three estuaries. Since the estuaries were sampled North to South (Elbe first, then Westerschelde and finally Gironde), the temperature differences between the three systems were quite pronounced: in the Elbe temperature was still quite low (7.5-9.5°C), whereas the Gironde was already characterized by late-spring temperatures (16-18°C). This has implication for the composition of the hyperbenthos and for the stage composition of the *Neomysis integer* population (see below).

In Fig. 1.2b dissolved oxygen concentration is plotted against salinity. In Gironde and Elbe the dissolved oxygen concentration decreased slightly in an upstream direction. In the Westerschelde, the dissolved oxygen concentration in the marine part is comparable to these recorded in Elbe and Gironde, but in the eastern brackish part of the estuary the it rapidly decreases to very low concentrations.

The relative species composition of the hyperbenthic community along the estuarine gradient is plotted in Fig. 1.3. Complete species lists and density and biomass of all species will be available for each station in the data base. In the brackish parts of all 3 estuaries the mysid *Neomysis integer* dominates the hyperbenthic community. In the more downstream reaches of Westerschelde and Gironde, *N. integer* is replaced by the mysid *Mesopodopsis slabberi*. This species is known to migrate to shallow coastal areas in winter (Mees *et al.* 1993). This explains the absence of the species in the Elbe. Due to the prevailing winter temperatures, the late-spring immigration into the estuary has probably not yet started. In the more downstream stations of the Elbe, still other mysid species (*Schistomysis kervillei*, *Schistomysis spiritus* and *Praunus flexuosus*) are equally well represented. In the Gironde, a range of lower salinities was sampled: the peak of abundance of *Neomysis integer* was situated more towards the sea than in the other estuaries. The community of the most oligohaline stations was dominated by amphipods: *Gammarus salinus* in pK 70 to pK 50 and *Gammarus zaddachi* in pK 40 and pK 30.

In Fig. 1.4 the density of *Neomysis integer* is plotted against salinity. In the Elbe, peak density is 3 and 4 times higher than in Westerschelde and Gironde, respectively. In Elbe and Gironde, the abundance peak is situated in salinities of 1 to 5. In the Schelde, the peak is shifted towards higher salinities (12). This is caused by the oxygen-depletion in the lower salinity zone. The decrease in density is usually observed to be steeper (e.g. Mees *et al.*, 1993). It should be noted here that the survey was conducted going upstream with the tide during an exceptionally high spring tide.

The stage composition and the length-frequency distribution of the *Neomysis integer* population is presented in Fig. 1.5. The relative importance of juveniles in the population increases from Elbe, over Westerschelde, to Gironde. The mysids in the Elbe belong to the overwintering generation (see below, part 4). The bimodal appearance of the length-frequency distribution is solely caused by sex-related size differences: adult males (8-11 mm) are significantly smaller than adult females (15-18 mm). In the Westerschelde, the 2 modes in the length-frequency distribution represent 2 different generations. The overwintering cohort constitutes the peak of large individuals (median of 16 mm). This generation has already reproduced and has released juveniles which constitute the spring cohort. The latter is characterized by smaller individuals (median length of 8 mm). In the Gironde 3 modes can be distinguished: the smallest peak (15 mm) represents the remaining individuals of the overwintering generation; the spring cohort (10 mm) is fully developed and has already produced juveniles of the second summer generation (5 mm). These differences in the composition of the populations are caused by differences in environmental temperature: latitudinal effects are amplified by the North to South sampling sequence.

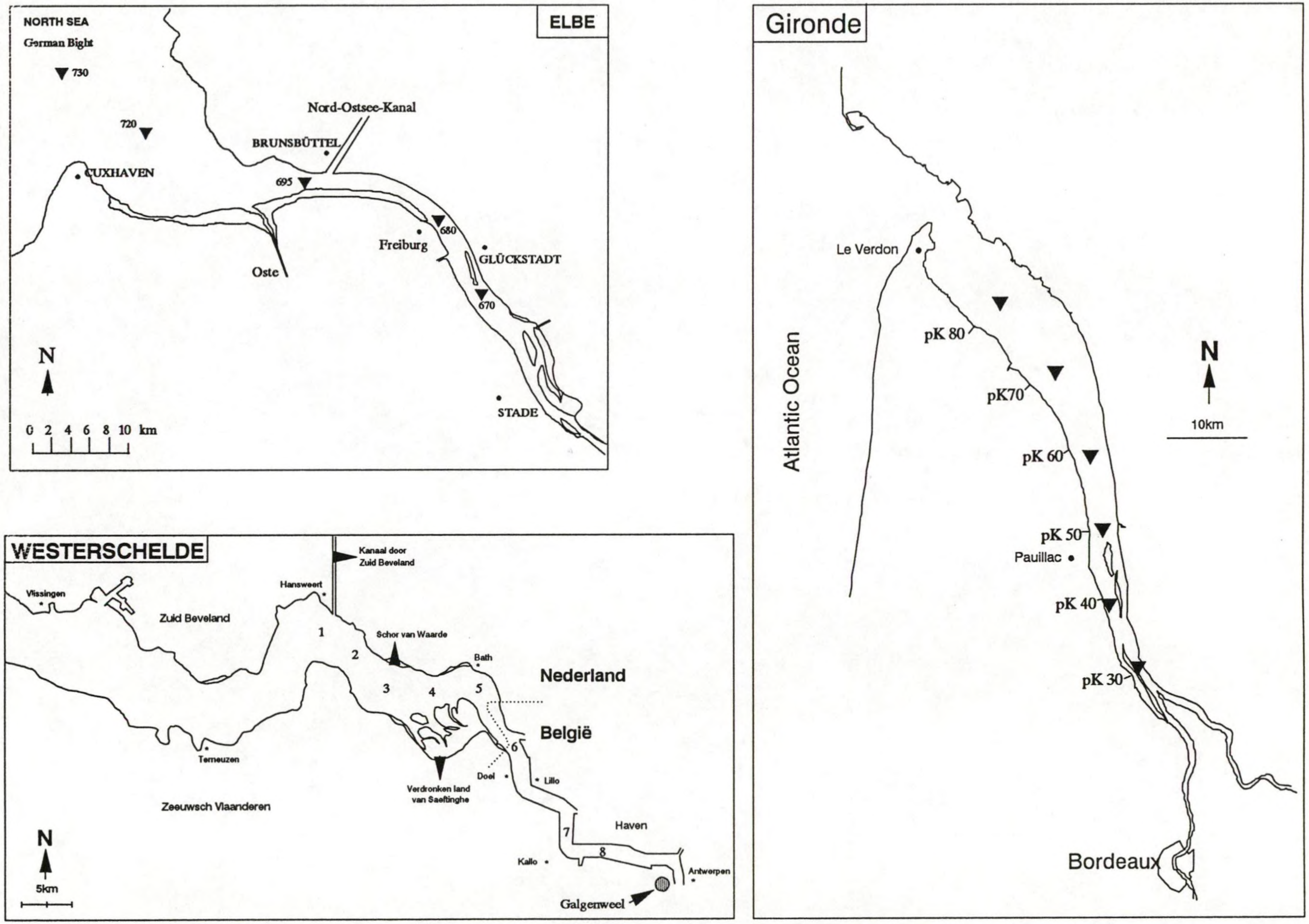


Figure 1.1. Stations sampled during the joint surveys.

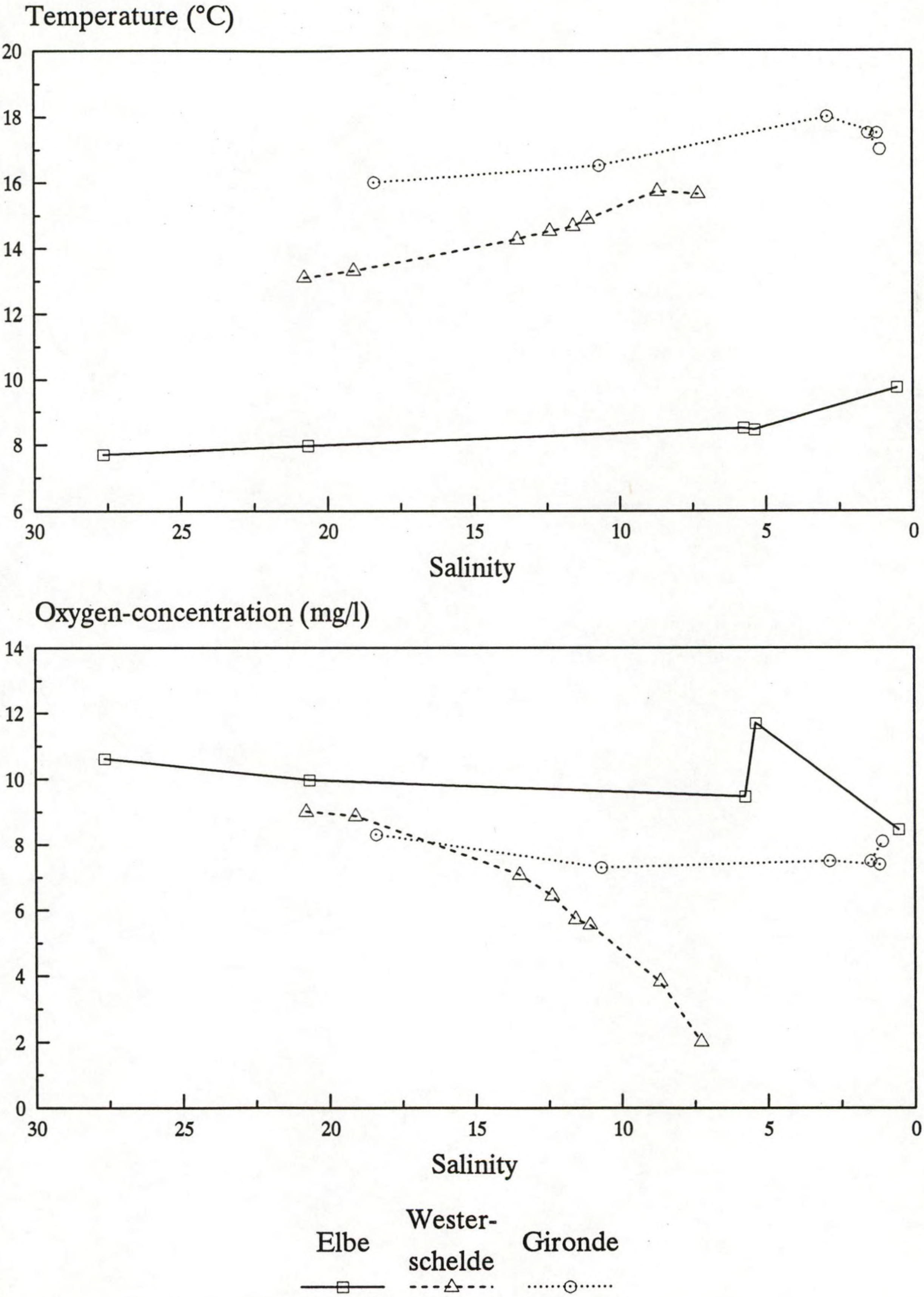


Figure 1.2. Temperature and dissolved oxygen concentration along the transects of the joint sampling campaigns.

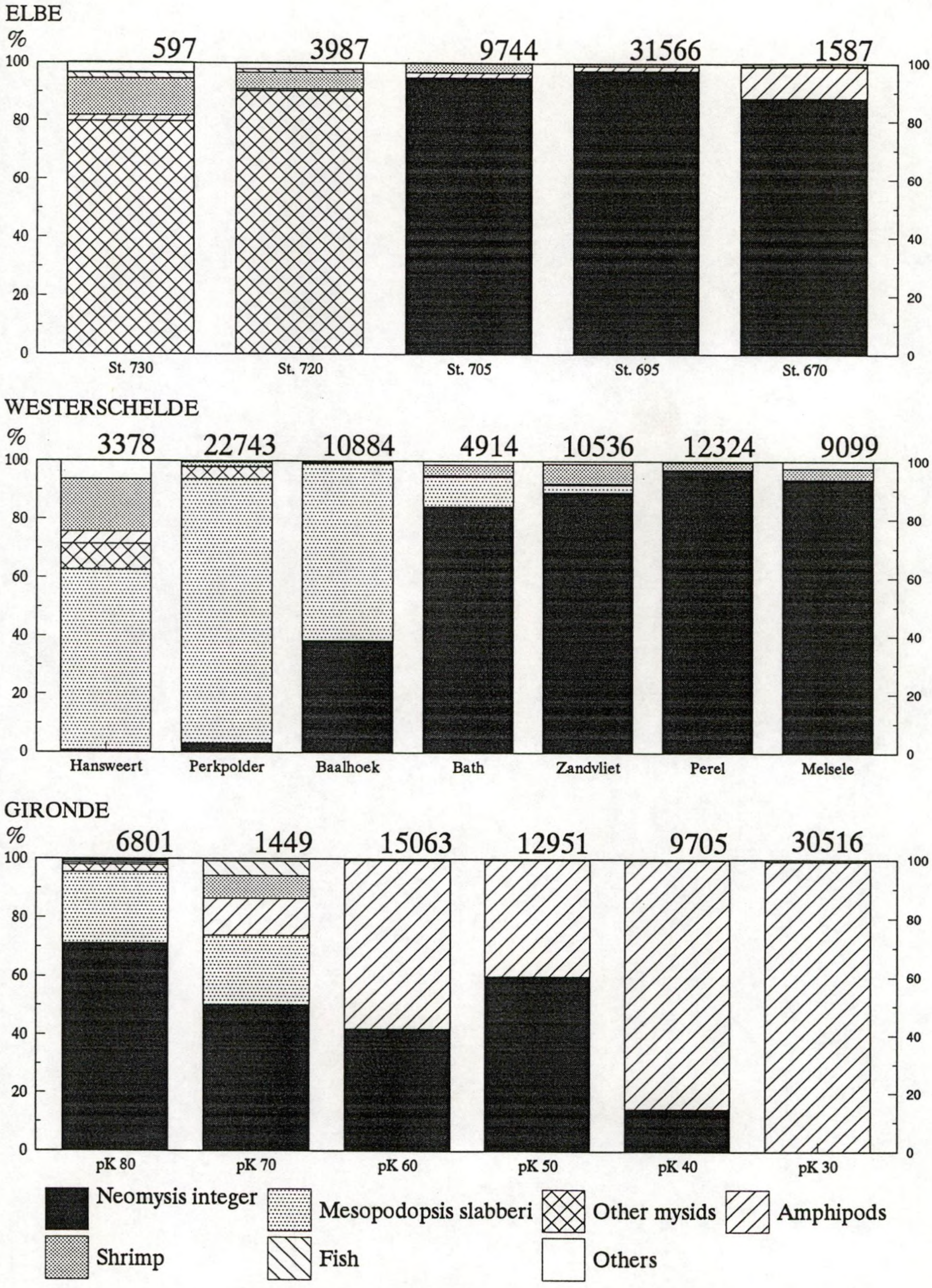


Figure 1.3. The composition of the hyperbenthic community and total hyperbenthic density (N/1000m²) along the transects of the three joint sampling campaigns.

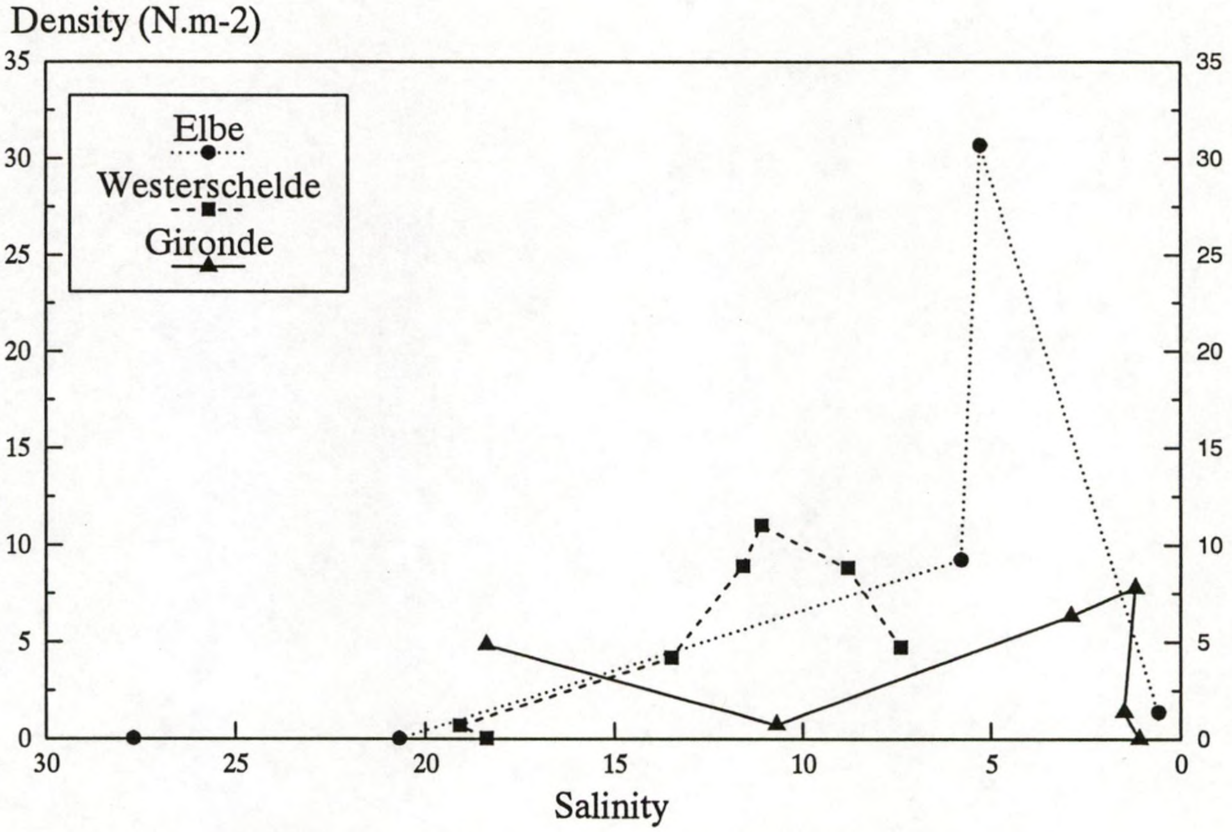


Figure 1.4. Distribution and density of *Neomysis integer* along the transects in the three estuaries.

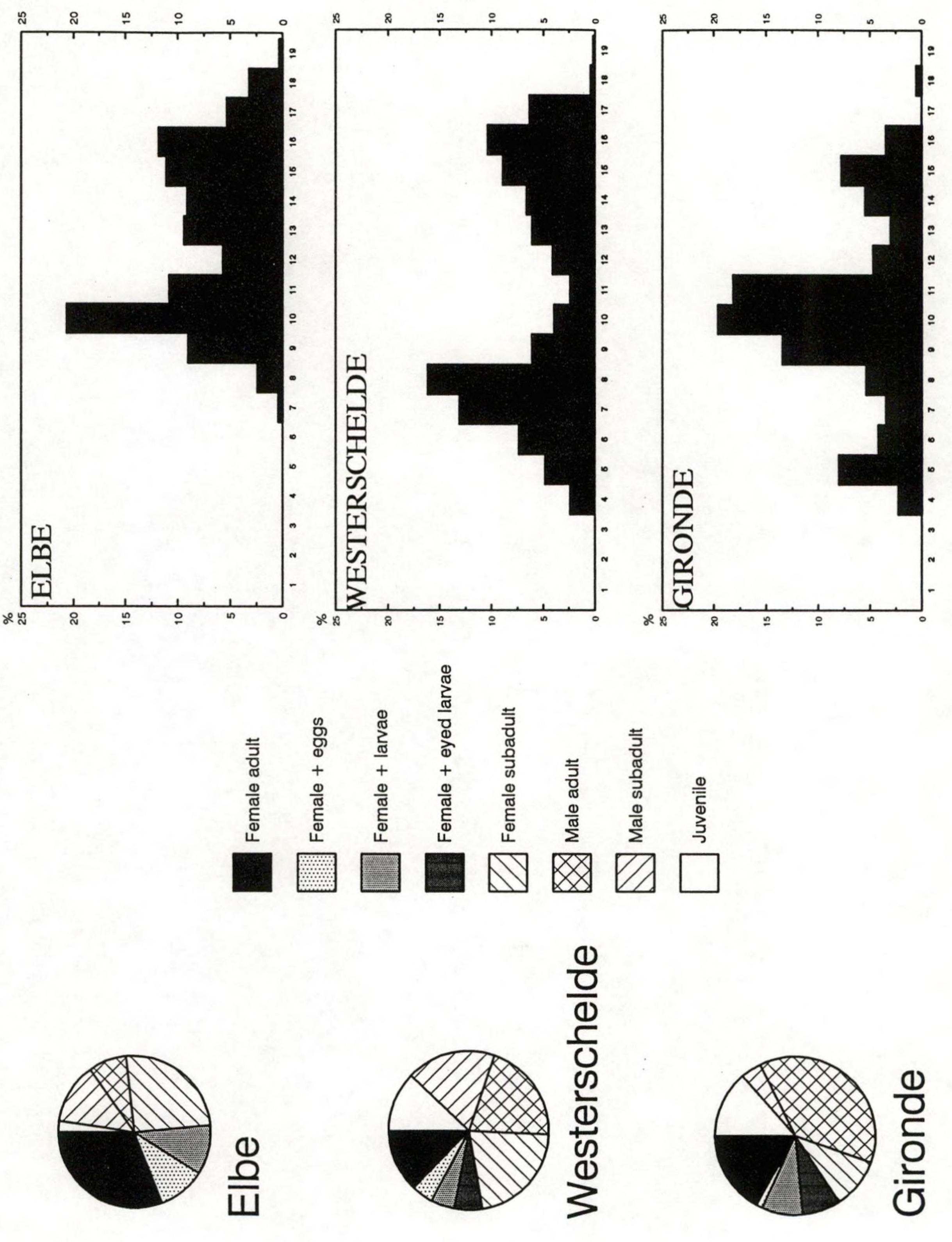


Figure 1.5. Length-frequency distribution and stage-composition of the *Neomysis integer* population in the three estuaries.

2. Feeding ecology of *Neomysis integer*

2.1. Introduction

The hyperbenthos in the vicinity of the MTZ was dominated by a single species: the mysid *Neomysis integer*. It constituted up to 90% of total hyperbenthic density and biomass in the low salinity-zone of the estuaries (Mees *et al.*, submitted; section 1). The species is a major prey item for higher trophic levels in the estuary, many of which are commercially important fish and shrimp. Mysids are usually described as omnivores feeding on zooplankton, phytoplankton and detritus. Still, information on the diet of estuarine mysids is generally lacking. No detailed information on the diet of *Neomysis integer* is available.

2.2. Methodology

The diet of the mysids is studied both quantitatively and qualitatively. The amount of food present in the stomach at a given time is expressed as a fullness index (F.I.):

$$F.I. = \frac{Dry\ weight\ (stomach\ content)}{dry\ weight\ (mysid)}$$

The mysids are carefully dissected. The stomach and its content and the mysid are dried (60°C for 4 days) and weighed with a microbalance. This is done for 20 adult males per sample. A method for microscopic stomach content analyses of mysids was developed. The mysids are dehydrated and imbedded in glycerine. Fixed slides of the stomach content are prepared. The recognisable phyto- and zooplankters are identified, counted and measured. Identifiable organisms encountered so far include copepods (identified to species), rotifers, unicellular and filamentous green algae, diatoms, mites,... Macrophyte detritus and other, unidentifiable detritus particles are divided in 5 different size classes. Sediment particles and pollen, when present, are also recorded. Results are based on 15 males and 15 females per sample.

2.3. Samples

The following samples are available for stomach analyses:

The samples collected during the joint sampling campaigns will allow for a comparison of the diet in the MTZ of Elbe, Gironde and Westerschelde. Diurnal (day-night and/or tidal) feeding rhythms in Gironde and Elbe can be assessed from the 24 hour cycle samples from the same campaigns. A 24 hour sampling campaign in the Westerschelde is available from September 1991.

The Westerschelde population was sampled monthly in 1991, both in the main estuarine channel and in the intertidal creeks of the major adjacent tidal marsh.

2.4. Preliminary results

The evolution of the fullness-index of *Neomysis integer* during a 24h-cycle in the Westerschelde is presented in Fig. 2.1 top. The index does not vary significantly over the 24 hour period. Exceptions are the significantly higher FI measured at 7.45 h. and 13.45 h., samples coinciding with the turning of the tide during daytime.

The fullness-indices of individuals caught near the bottom seem to be consistently higher than these of individuals caught in the surface layers (Fig. 2.1 bottom).

Fig. 2.2 shows the evolution of the fullness-indices of individuals caught during a tidal cycle in the marsh-creek. Though a feeding pattern is evident from the graph, more data will have to be collected before this signal can be interpreted.

Qualitative stomach analysis of *Neomysis integer* collected in 3 seasons in the Westerschelde are presented in Fig. 2.3. The relative importance of detritus decreases from winter to summer, while the relative importance of zooplankton and phytoplankton increases. In February the zooplankton consumed is mainly *Eurytemora* spec., in August *Acartia* spec.

In Elbe, Westerschelde and Gironde (Fig. 2.4) the stomach contents mainly consist of the copepod *Eurytemora affinis*. In the Elbe, rotifers belonging to the genus *Keratella* are also important. In the Gironde a high number of pollen (*Pinus spec.*) were found in the stomachs.

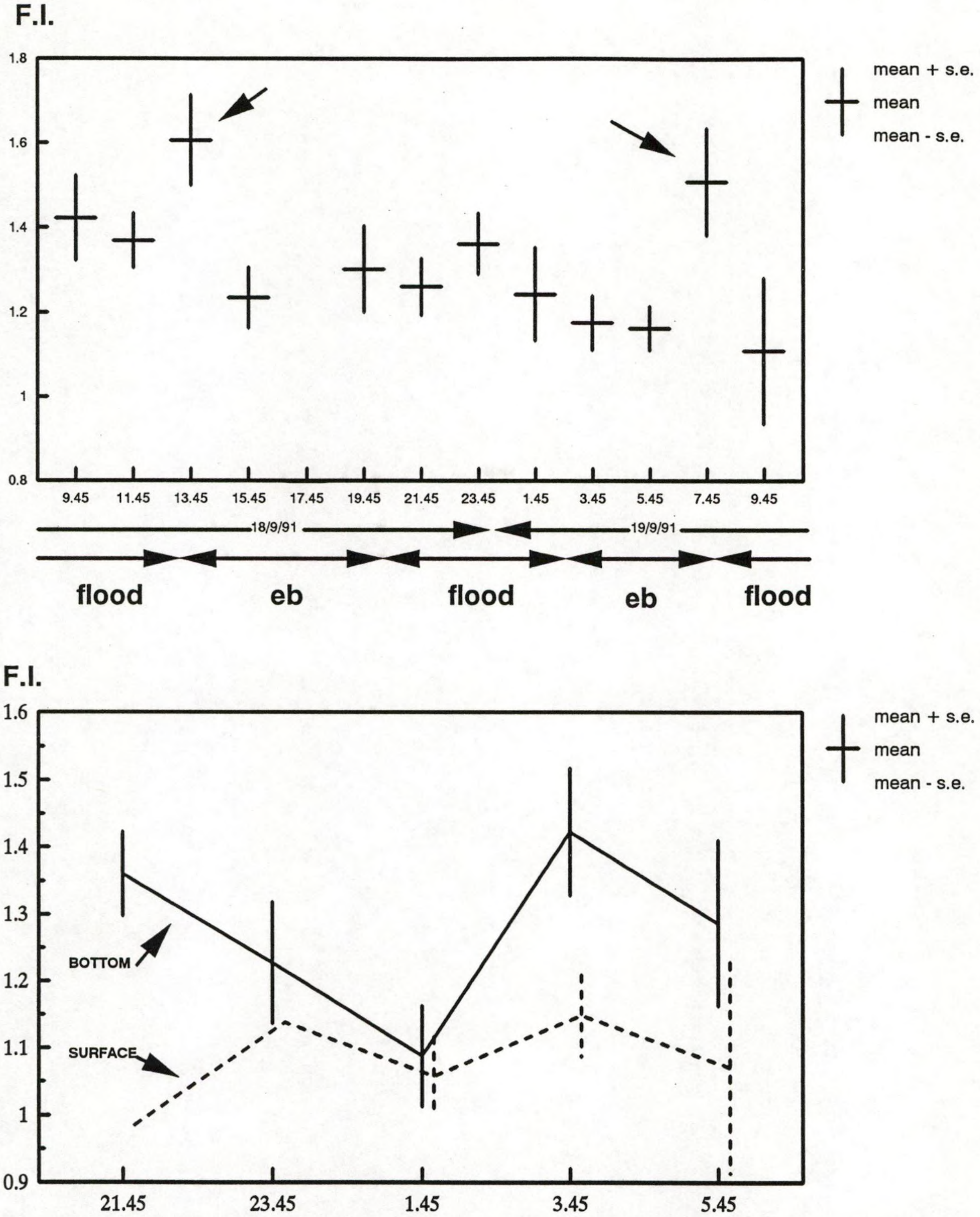


Figure 2.1. Diurnal patterns in the fullness index of *Neomysis integer* in the Westerschelde (top) and comparison of the fullness index in upper and lower water layers (bottom).

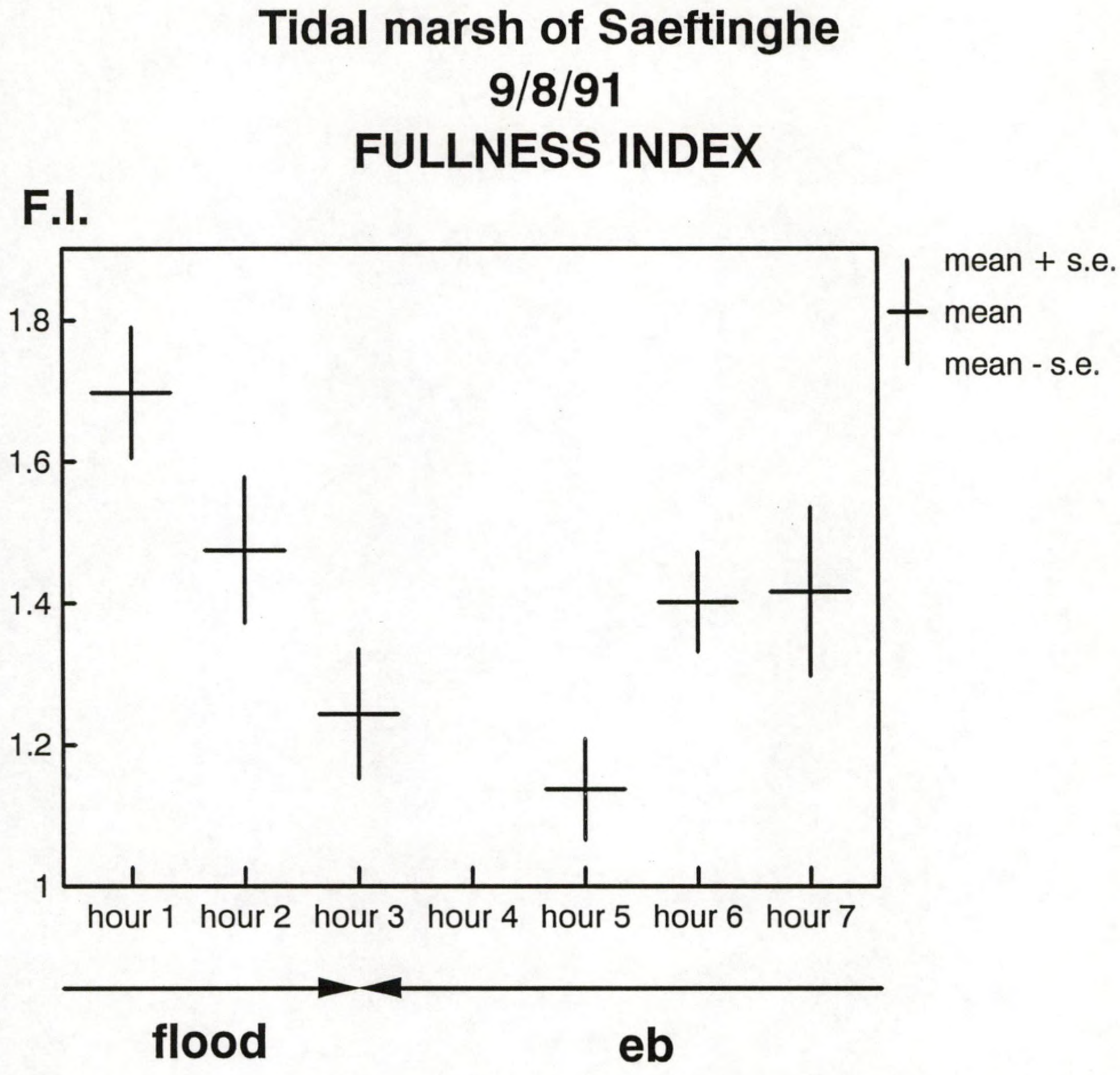


Figure 2.2. The fullness index of *Neomysis integer* entering and leaving the tidal marsh of Saeftinghe.

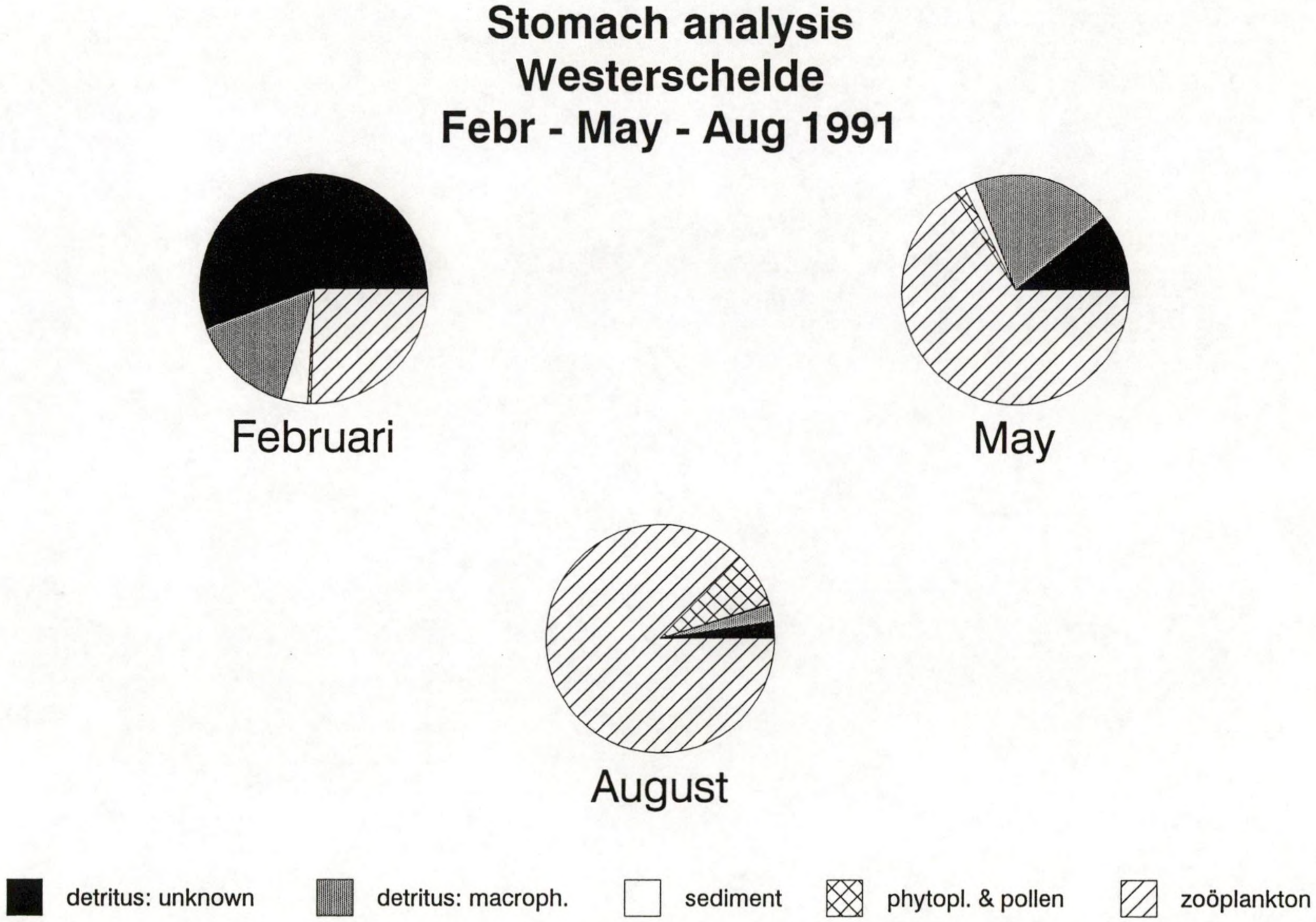


Figure 2.3. Seasonal patterns in the diet composition of *Neomysis integer* in the Westerschelde.

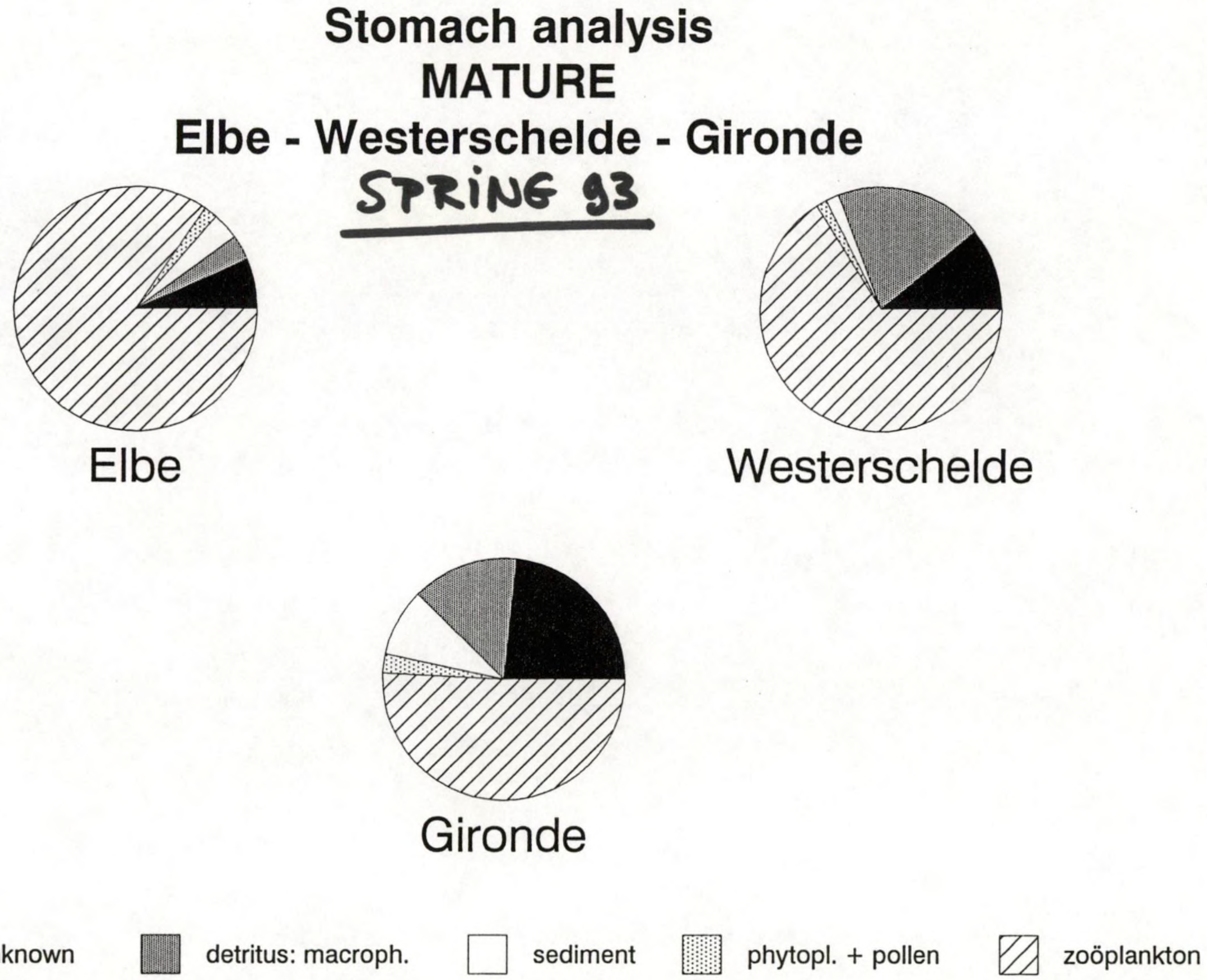


Figure 2.4. The diet of *Neomysis integer* in the three estuaries.

3. Experimental studies

3.1. Introduction

A culturing technique for the mysid *Neomysis integer* was developed and refined. Handling techniques were perfected and a first experiment to assess the effect of temperature on growth was conducted.

3.1. Methodology

The stock population of *Neomysis integer* for the culture was taken from a brackish pond near Antwerpen (Galgenweel, Fig. 1.1). The mysids are sampled with a handnet and are transported to the laboratory in aerated plastic containers. In the laboratory, the culture is kept in an aquarium of 200 l equipped with a under-gravel filter. The filter consists of layers of cotton wadding, sand, cotton wadding and an upper layer of broken oystershells. The culture medium is natural seawater diluted with aerated tap water (salinity of 5). The stock-culture is kept at 20 ± 2°C and a 12h dark : 12h light fotoperiod. The mysids are fed *ad libitum* with freshly hatched *Artemia* nauplii (1-2 days) twice per day. The density of the mysids is approximately 5 individuals per liter.

Gravid females are isolated in 2 l containers. These aquaria are checked daily for the presence of juveniles, which are used in the experiments.

For the experiments, the juveniles (never older than 1 day) were kept individually in 400 ml containers in artificial brackish water with a salinity of 5. Experiments were conducted at 3 temperatures: 15°C, 20°C and 25°C. These temperatures were chosen as to represent typical spring and summer situations in Westerschelde and Gironde. Every day, half of the culture medium was replaced by new aerated artificial water and the mysids were fed freshly hatched *Artemia* nauplii (300 nauplii in the first 2 weeks, 500 nauplii for older individuals).

The growth of *Neomysis integer* in the experiments was followed indirectly: direct measuring of the length of the individuals causes too much stress. The containers were checked daily for moults. The rigid parts of the moults were measured. Standard length was calculated from allometric regressions. Length-dry weight and length-ashfree dryweight regressions were also determined.

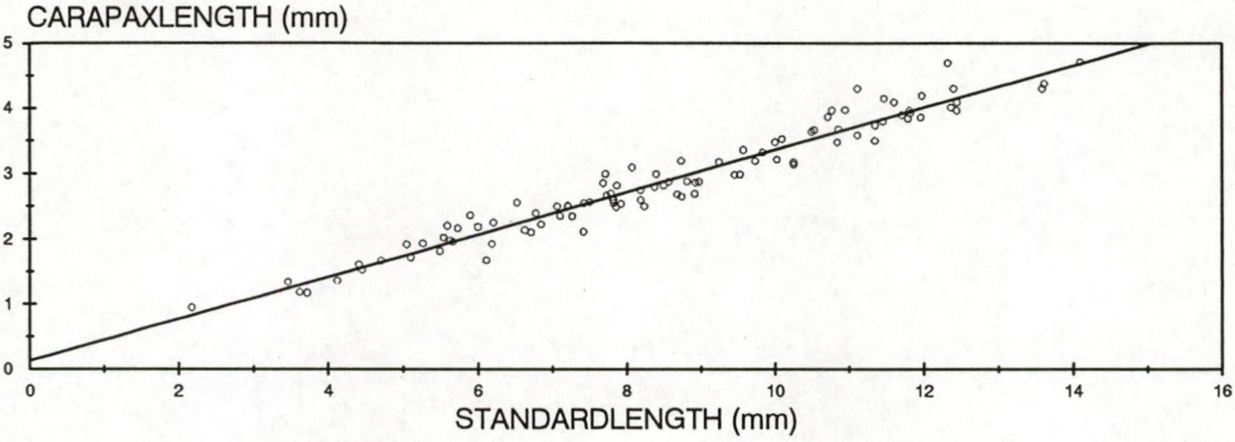
3.2. Preliminary results

The allometric regressions and the length-weight regressions are presented in Figs. 3.1 and 3.2. The growth curves resulting from the experiments are exemplified in Fig. 3.3.

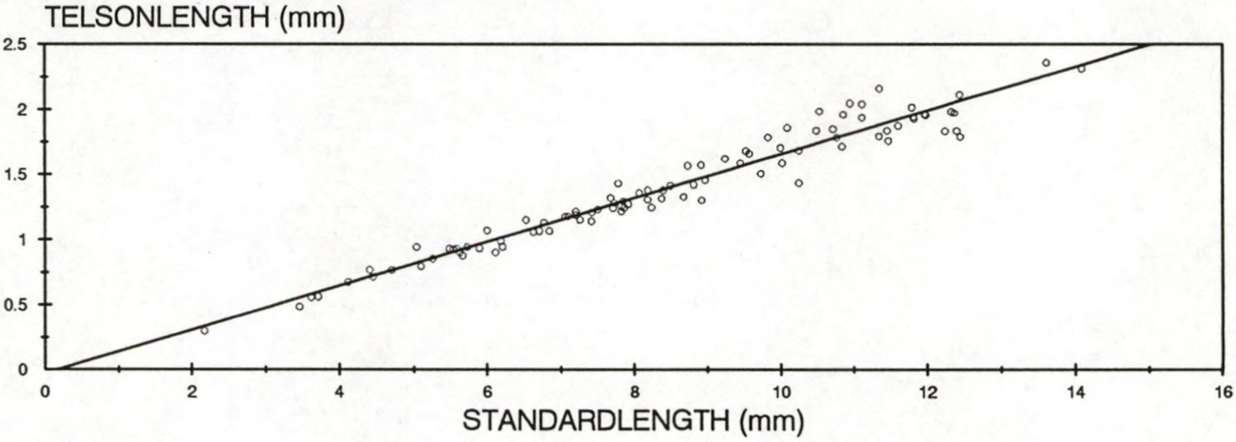
The mean growth rate (in mm.day^{-1}) is the growth rate over the total life cycle of an individual (Fig. 3.4). There seems to be no difference in growth rate for the different temperatures. The growth rate of juveniles and subadults (<9mm) is higher than the growth rate of adults (>9mm).

The mean moulting frequency (in N.day^{-1}) is plotted for the different temperatures in Fig. 3.5. The frequency of moulting is higher for juveniles and subadults than for adults. There is a higher mean moulting frequency at higher temperatures.

SL = 0.081227 + 2.92823 CL
r=9741; N=99; p<0



SL = 0.5589542 + 5.66626 TELL
r=0.9763; N=95; p<0



SL = 0.8362833 + 3.36970 A.PL. TOTAAL
r=0.9770; N=100; p<0

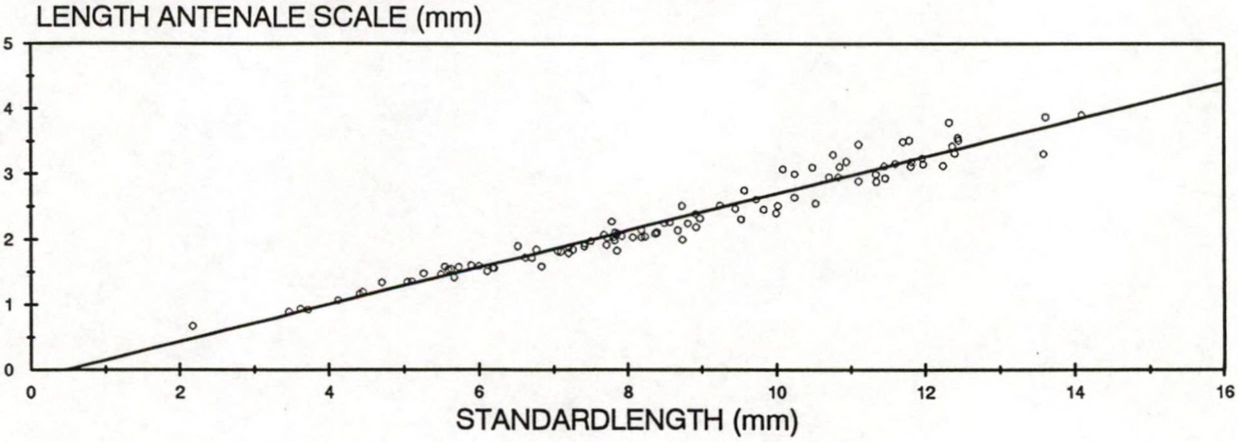


Figure 3.1. Allometric regressions of *Neomysis integer*.

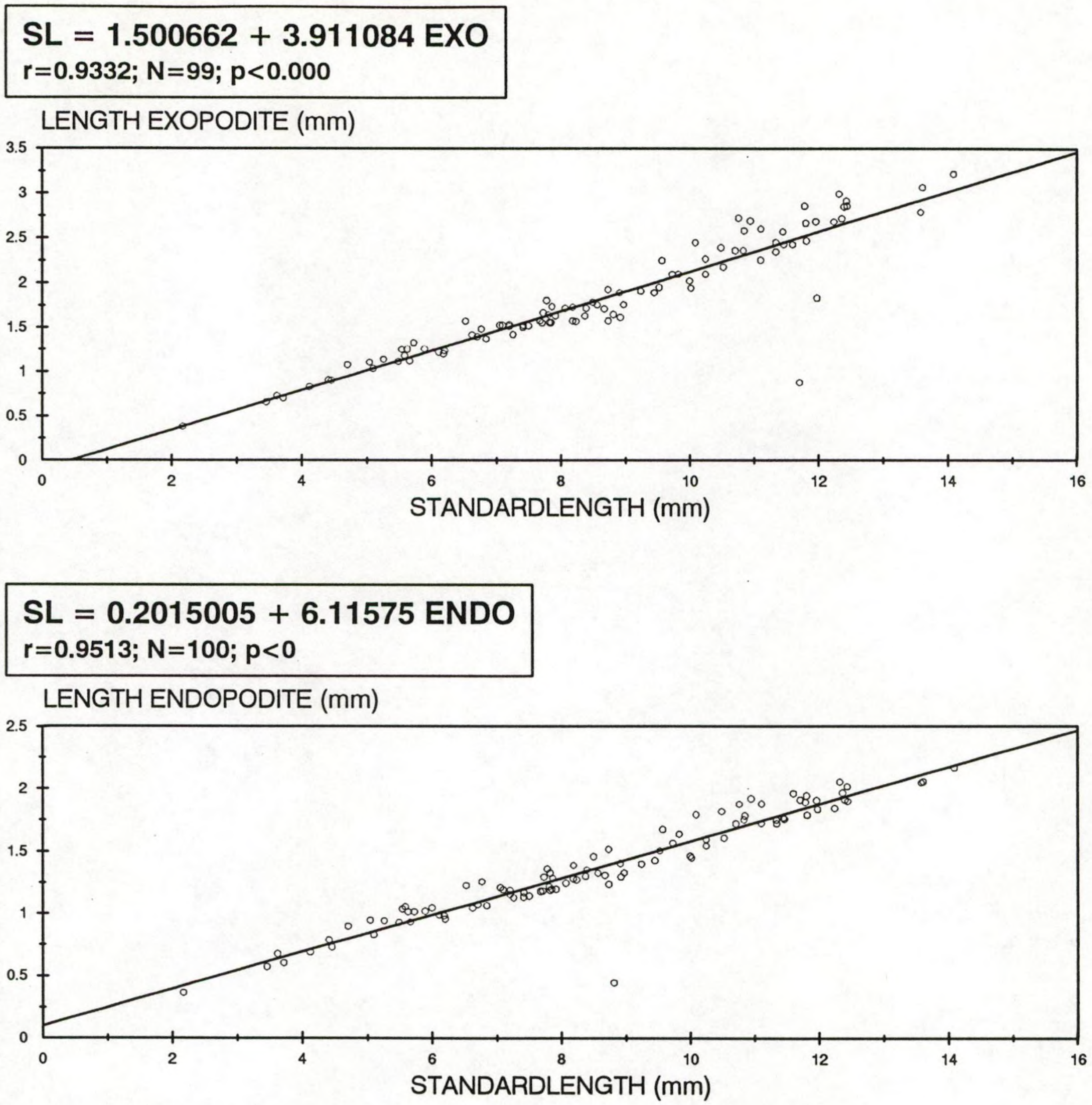
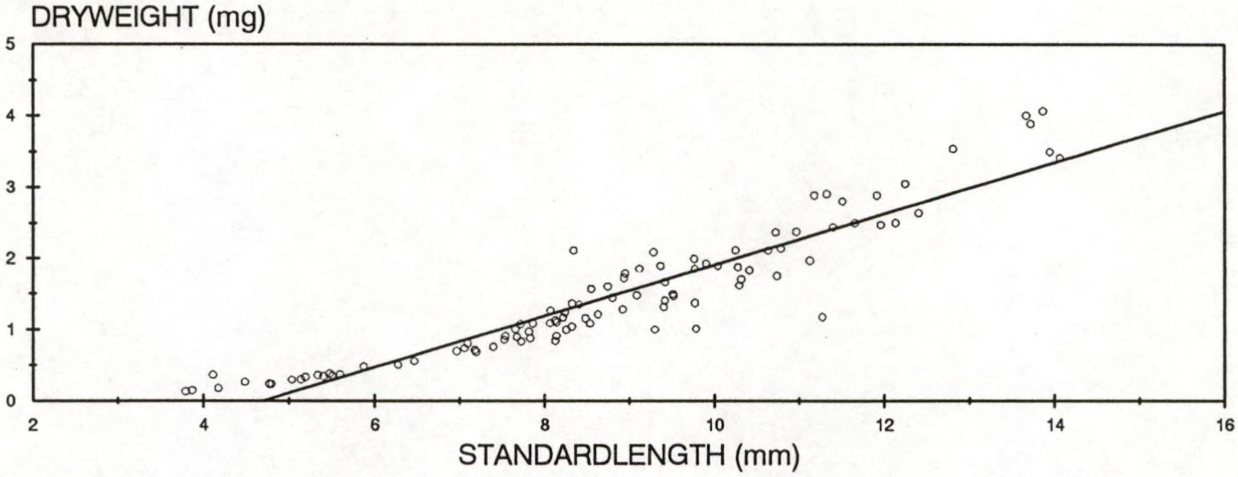


Figure 3.1. Allometric regressions of *Neomysis integer*.

SL = 5.186390 + 2.44760 DW
r=0.9380; N=99; p<0



SL = 5.161044 + 2.74825 AVDG
r=0.9374; N=99; p<0

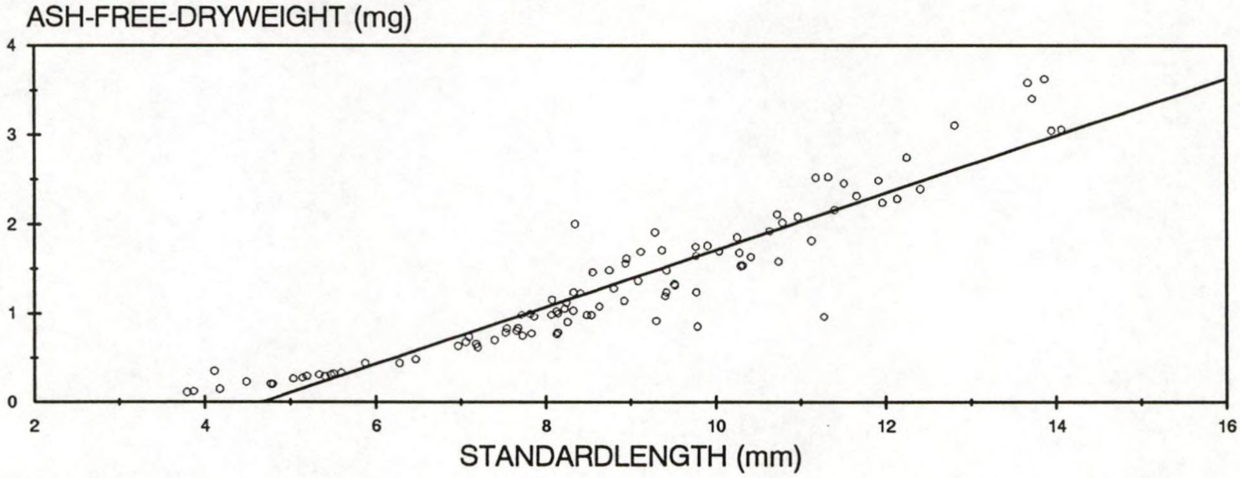


Figure 3.2. Length-weight regressions of *Neomysis integer*.

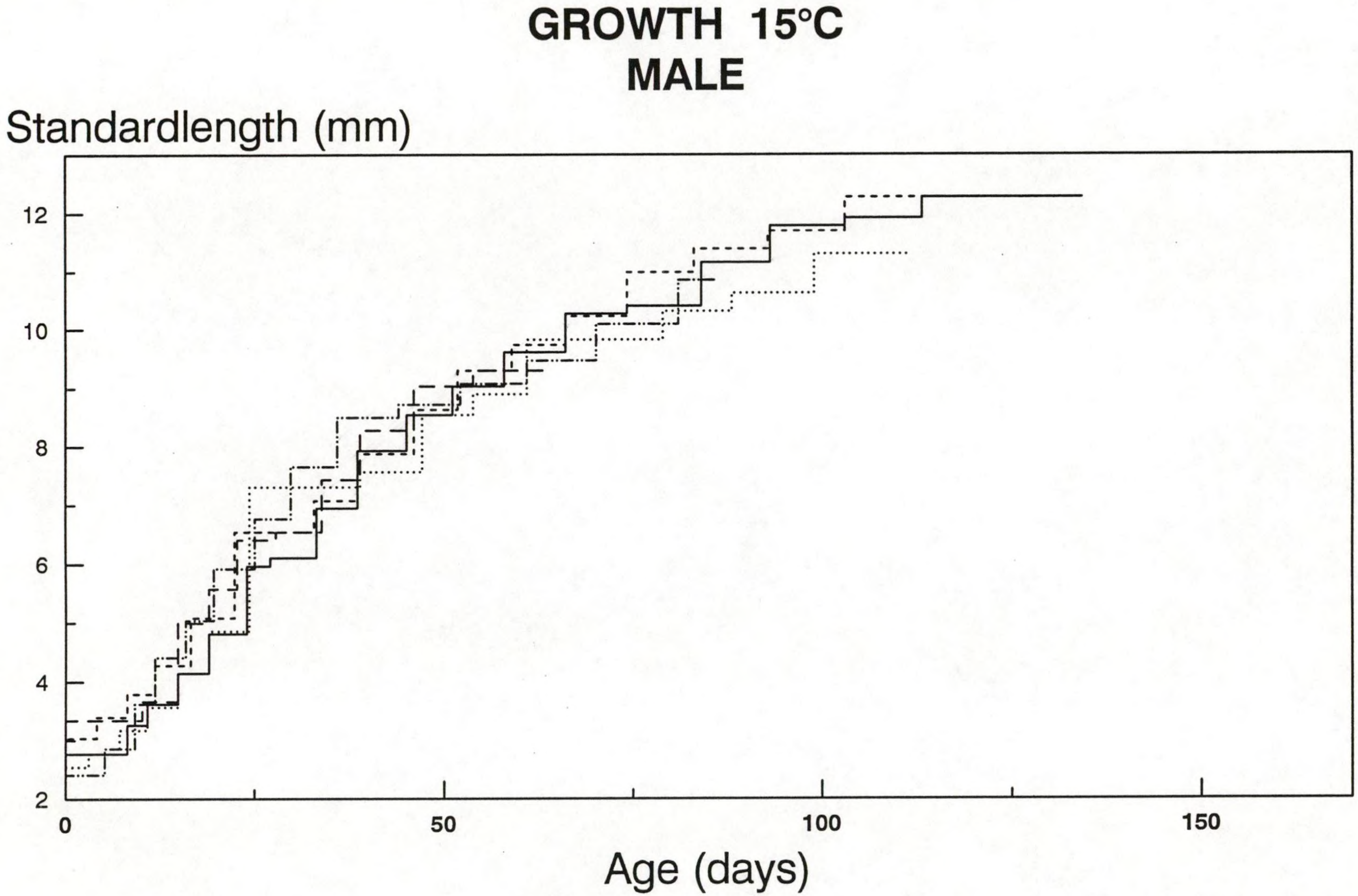


Figure 3.3. Growth of male *Neomysis integer* at 15°C.

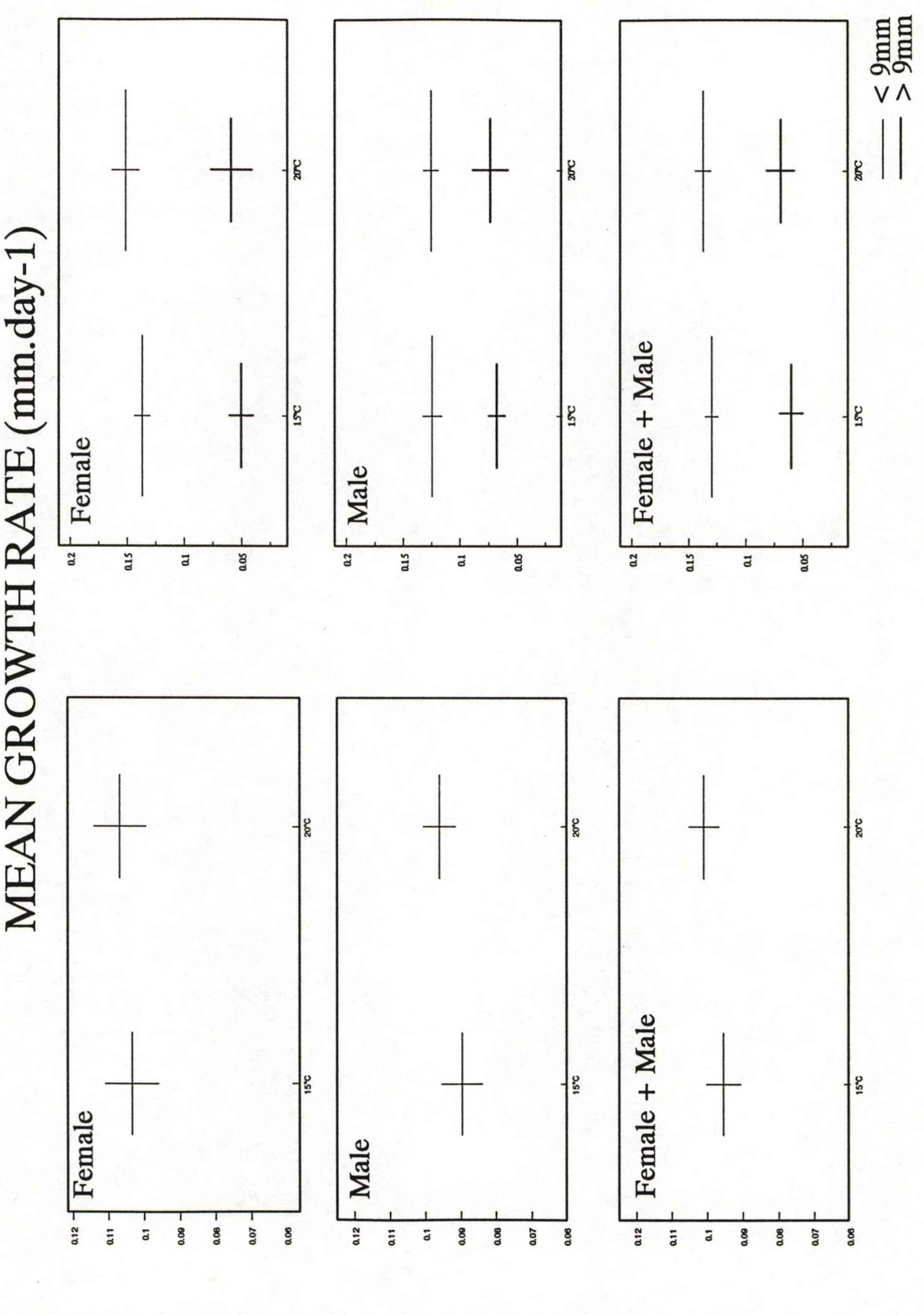


Figure 3.4. Mean growth rate of *Neomysis integer* at 15 and 20°C.

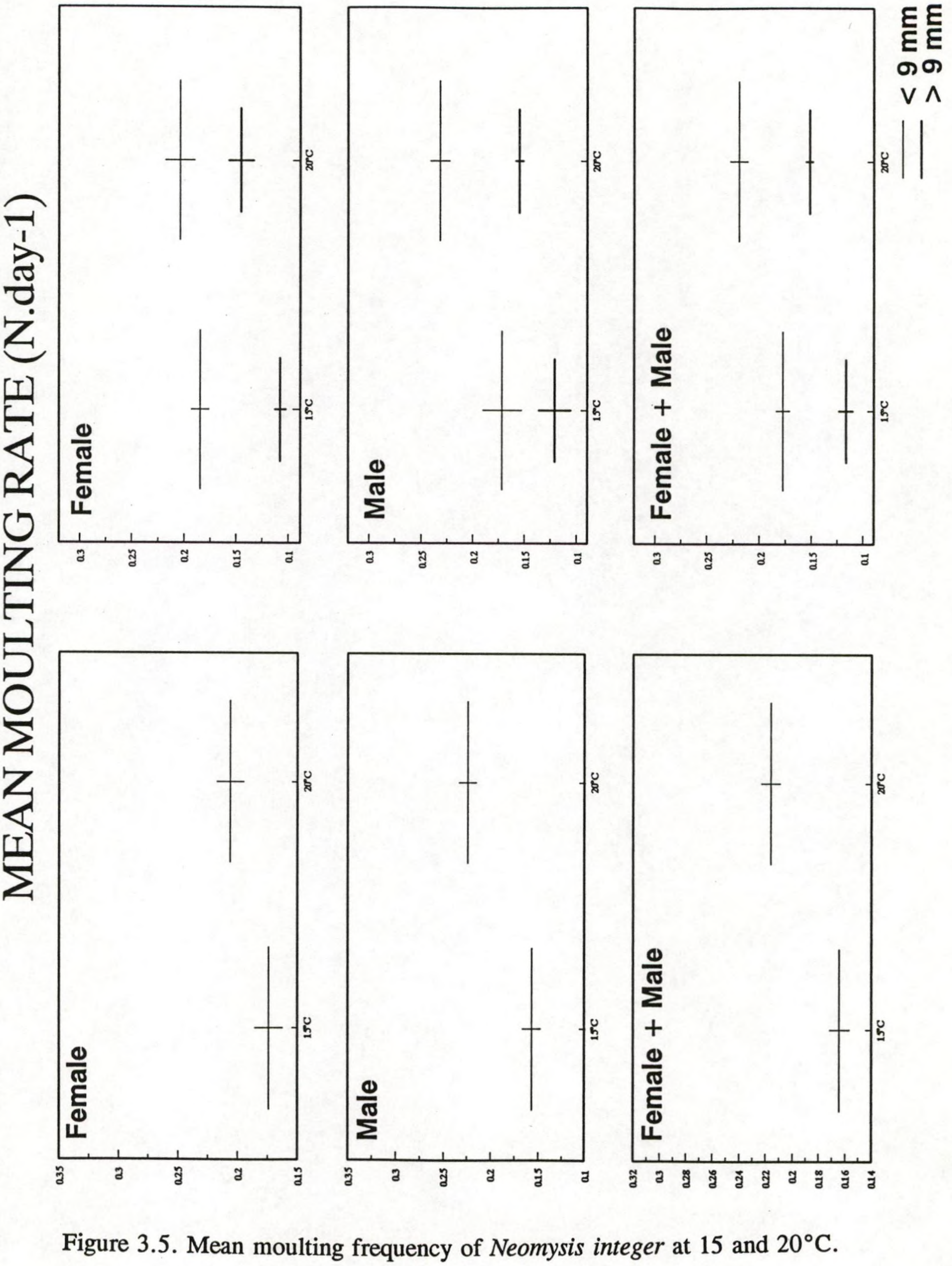


Figure 3.5. Mean moulting frequency of *Neomysis integer* at 15 and 20°C.

4. The population dynamics of *Neomysis integer* in the Westerschelde

The results of this study are summarized in the following scientific paper which is submitted for publication in open literature.

Life history, growth and production of *Neomysis integer* (Leach, 1814) in the Westerschelde estuary (S.W. Netherlands)

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Key words: *Neomysis integer*, Mysidacea, estuary, Westerschelde, life history, growth, production

Abstract

The *Neomysis integer* (Crustacea, Mysidacea) population of the brackish part of the Westerschelde estuary was sampled on a fortnightly basis from November 1990 to December 1991. Density, biomass, population structure and brood size were recorded. The Bhattacharya method was applied to the length-frequency data for the detection and separation of cohorts. Growth is described by a generalised von Bertalanffy function. Secondary production was estimated for each cohort using four approaches.

The seasonal pattern in density and biomass showed three peaks: a relatively small, yet distinct, peak in early March (30 individuals per m^2, 60 mg AFDW per m^2) and two main peaks in late spring (160 individuals per m^2, 225 mg AFDW per m^2) and in summer (140 individuals per m^2, 125 mg AFDW per m^2). Throughout winter, *Neomysis* density remained well below 30 individuals per m^2. Three periods of increased reproductive activity and subsequent input of juveniles were found. This suggests that three cohorts were produced per year. The overwintering generation lived from autumn until the following spring. The spring generation was born in early spring and lived for about three months, while the summer generation lived from summer until early winter. The three cohorts showed marked differences in their biology. The growth of the overwintering generation showed seasonal oscillations, large brood size, and a large size at maturity. Individuals belonging to the other two cohorts grew faster, produced less young per female, and attained maturity at a smaller size.

Within each cohort, both sexes exhibited different growth characteristics: the females generally lived longer, grew faster and consequently became larger than the males.

The size-frequency, growth summation and removal summation methods yielded similar production estimates. The annual production was 0.3 g AFDW.m^{-2}.yr^{-1} with an annual P/B ratio of 6. The average cohort P/B was 3. The size-frequency method gave similar results only when applied to the three cohorts and to both sexes separately. The spring cohort accounted for almost half of the annual production. Despite its longer life span, the overwintering generation accounted for only a quarter of the annual production. An independent estimate of production using the mortality rate of the different cohorts, resulted in values similar to those obtained by the other methods for the overwintering cohort, while the production of the other two cohorts was overestimated.

Introduction

Generally the lack of reliable estimates of secondary production due to poorly documented life history data for key species constitutes a major problem in understanding the functioning of aquatic ecosystems. This problem especially adheres to studies of energy flows through food webs in estuaries.

Estuaries are highly productive ecosystems. They are widely recognised as important nurseries for fish and epibenthic crustaceans. Especially the low salinity zone around the typical estuarine turbidity maximum which is characterised by high amounts of organic detritus and high densities of zooplankton (Soetaert & van Rijswijk 1993), epi- and high densities of hyperbenthos and demersal and pelagic fish (Hamerlynck *et al.* in press, Mees *et al.* in press).

The genus *Neomysis* Czerniavsky has representatives on and around each continent and detailed studies exist on the biology of other species than *N. integer* (e.g. Ishikawa & Oshima 1951, Heubach 1969, Pezzack & Corey 1979, Toda *et al.* 1982, Cooper *et al.* 1992 and references therein). *Neomysis integer* (Leach) is one of the most common mysids around the coasts of Europe. It is a hyperbenthic, euryhaline and eurythermic species, typically occurring in high numbers in estuarine, brackish water environments (Tattersall & Tattersall 1951). It also occurs in oligohaline to fresh water bodies which, in their recent geological history, were connected with the sea (Bremer & Vijverberg 1982). Tesch (1911) reported the species to be common in and characteristic for low salinity waters in the Delta area in the southwest of the Netherlands.

This study, which is part of an integrated study on major food chains in European tidal estuaries, focuses on the estimation of the production of *N. integer*.

The life history of lacustrine *N. integer* populations in the Dutch Delta has been studied by Borghouts (1978) and Platenkamp (1983). The biology of *N. integer* in lakes and pools in the Netherlands has been studied by Vorstman (1951), Beattie & de Kruijf (1978) and Bremer & Vijverberg (1982).

Other valuable information is available on populations in a Scottish loch (Mauchline 1971), an Irish loch (Parker & West 1979) and in the coastal waters in the Baltic (Jansen *et al.* 1980, Rudstam *et al.* 1986, Wiktor 1961). Life cycle studies in the Ythan (Astthorsson & Ralph 1984), the Eider-Ring (Kinne 1955) and the Gironde (Sorbe 1981) are the only estuarine studies to date. So far, no production estimates are available from estuarine populations of *N. integer*.

Baseline studies on the spatial and temporal patterns in the hyperbenthic component of the Westerschelde have been published (Mees & Hamerlynck 1992, Mees *et al.* 1993, Mees *et al.* in press). In these studies *N. integer* was shown to be the dominant species in the brackish part of the estuary, both in the main channel and the adjacent tidal marshes. The mysid was recorded in salinities ranging from 8 to 25, but it was never caught downstream from Hansweert (Fig. 4.1) and the lacustrine limit of the population lies a few kilometres upstream of the Dutch-Belgian border (Mees *et al.* 1993). As life history characteristics of a species can vary considerably from one habitat to another, basic knowledge of the biology of *N. integer* in the Westerschelde is essential for further use in ecosystem modelling, energy-flow studies and experimental work (e.g. von Oertzen *et al.* 1988).

N. integer is an omnivorous species. As a predator on zooplankton (e.g. Bremer & Vijverberg 1982) it can structure zooplankton populations and also, to a certain extent, exploit the detrital food chain. The species is also an important prey for demersal and pelagic fish and larger epibenthic crustaceans (e.g. Mauchline 1980). In the Westerschelde *N. integer* is known to be a major food source for sand goby *Pomatoschistus minutus*, Lozano's goby *P. lozanoi*, common goby *P. microps*, seabass *Dicentrarchus labrax*, bib *Trisopterus luscus*, sea snail *Liparis liparis*, pipefish *Syngnathus rostellatus* and the brown shrimp *Crangon crangon* (A. Cattrijsse and K. Hostens, pers. comm.). Other potential (and commercially important) predators on *N. integer* include sprat *Sprattus sprattus*, herring *Clupea harengus*, flounder *Pleuronectes flesus*, dab *Limanda limanda* and plaice *Pleuronectes platessa* (Mauchline 1980), all of which are common in the study area (Hamerlynck *et al.* in press). It is therefore believed to be a key species in the ecosystem of the brackish part of the Westerschelde.

Materials and Methods

Study area

The Westerschelde estuary (Fig. 4.1) is the lower part of the river Schelde. The maritime zone of the tidal system is about 70 km long from the North Sea (Vlissingen) to the Dutch-Belgian border near Bath. The Westerschelde is the last remaining true estuary of the Delta area and is characterised by a marked salinity gradient. The water is completely mixed and the residence time is rather high (about 70 days or 150 tidal cycles).

Consequently fresh water (average inflow 100 $m^3.s^{-1}$) dilution is gradual and this results in relatively stable salinity zones which are maintained in more or less the same position throughout a tidal cycle. The most important shifts occur in accordance with seasonal variations in the freshwater inflow. The abiotic environment is discussed in Heip (1988) and Van Eck *et al.* (1991).

Sampling

Samples were taken on a more or less fortnightly basis from 27 November 1990 to 16 December 1991. In December 1990, May 1991 and September 1991 the estuary could only be sampled once (Table 4.1). Each sampling day 4 stations (OV, SA, BA and LI) were covered (Fig. 4.1). Sampling was done from the R.V. Luctor. All samples were taken during daytime when hyperbenthic mysids are known to concentrate near the bottom. The samples were collected with a hyperbenthic sledge (Hamerlynck & Mees 1991) which consists of a heavy metal frame equipped with two nets one above the other. The sledge is one meter wide. Both nets are 4 m long and have a mesh size of 2x2 mm in the first 3 m and 1x1 mm in the last 1 m. The catch in both nets was pooled for this study. The total area of the nets' mouth is 0.8 m^2 and it samples the hyperbenthos from 20 to 100 cm above the bottom. The sampler was towed for 1000 m (radar readings from fixed points) at an average ship speed of 4.5 knots relative to the bottom. The total area sampled on each deployment was approximately 1000 m^2. Trawling was always done with the tide. The samples were taken in the main ebb-tidal channel of the estuary (where possible, the 10 m depth isobath was followed). The samples were immediately preserved in a buffered formaldehyde solution, 7% final concentration. Salinity, conductivity, pH, dissolved oxygen concentration, temperature (all measured at 1 m above the bottom) and Secchi disc depth were recorded at the end of each haul (Table 4.1).

Laboratory procedures

Samples were sorted and the number of *Neomysis* per sample was counted. Per sampling date the sample with the highest number of *Neomysis* was selected for further analysis (Table 4.1). The density peak was always distinctively located in one station. Data on the horizontal distribution of the population are described in Mees *et al.* (in press).

About one thousand individuals of *Neomysis integer* for each sample (500 from the upper net and 500 from the lower net) were sexed and categorized in six life cycle stages according to Mauchline (1980). Subsamples were taken after all *Neomysis* were separated from other species and detritus. The homogenized samples were emptied in a white tray which was divided in 48 squares. Randomly selected squares were then picked until a total number of 500 individuals was obtained. Six categories were distinguished in this study: adult males, adult females, subadult males, subadult females, juveniles and gravid females. Mauchline's 7th stage, post-gravid females, was not distinguished in this study.

The sex of the adult (mature) individuals is easy to determine. Adult males have elongated 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. The adult females have a fully developed marsupium between the thoracic legs. The juveniles lack either of these 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: the 4th pleopods 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 criterium is the most reliable to distinguish the immature males from the juveniles. The females were categorized as adults when their marsupia were large enough to be seen from the lateral side. The subadult females already display small oostegites between the thoracic pleopods, but these are only visible after careful examination of the ventral side of the animals. Adult females were further divided into females without larvae (fully developed but empty marsupia) and 'gravid' or ovigerous females with larvae present in the marsupium. When such gravid females were present, larval counts were made on sixty females with complete broods per sample. Bisexual individuals, i.e. animals which have elongated 4th pleopods and a marsupium, were rare (less than 30 individuals encountered in the study) and were excluded from further analyses.

For each sampling date, the standard lengths (the distance from the base of the eye stalk to the end of the last abdominal segment) of sixty individuals of *N. integer* per sex and per developmental stage were measured to the nearest 0.1 mm using a calibrated binocular microscope with drawing mirror and a digitizer (subsamples taken in a similar way as described above).

Fifty individuals (ten animals from each stage and sex, excluding gravid females) from the April and October samples were used for weight measurements. Animals covering the entire representative size range for each stage were selected for this analysis. They were dried in an oven at 60°C for five days. The dry weight of the animals was then measured using an electronic balance sensitive to $1\mu g$. The ash weight of the individuals was later measured after inceneration in a furnace at 550°C for two hours. The ash-free dry weight of the mysids was then calculated as the difference between the dry weight and ash weight. Length-weight regressions were linearised through double logarithmic transformation. Possible differences between spring and autumn animals were assessed by analysis of covariance of the corresponding linear regressions. The same 100 animals were also used to establish biometrical regressions between standard length (SL) and two other length measures frequently used in mysid research: carapace length (CL, the distance from the tip of the rostrum to the mediodorsal margin of the carapace) and total length (TL, the distance from the base of the eye stalk to the posterior end of the uropods excluding the setae).

The following allometric relationships were found:

$TL = 1.165\ SL - 0.080$ (N=112, r=0.997, p<0.001)

$CL = 0.266\ SL + 0.439$ (N=112, r=0.908, p<0.001)

Data analysis

Density of the population is expressed as number of individuals (N) per m², biomass as mg ash-free dry weight (AFDW) per m².

Per sampling date the mysids were divided into arbitrarily defined 1 mm length classes. Preliminary examination of the data revealed length and growth differences between males and females. Therefore, the length-frequency data of the two sexes were always analyzed separately, unless otherwise stated. Juveniles were divided equally over the male and female data matrices. Biomass was derived from the length-frequency distributions and the standard length-AFDW regression.

The length-frequency distributions on each sampling date were first submitted to the Bhattacharya method (Bhattacharya 1967) as interpreted by Pauly & Caddy (1985) for the detection and separation of cohorts. The method is an option in the 'complete ELEFAN' computer program package (Pauly & David 1981). It splits the composite length-frequency distributions into separate normal distributions (Sparre 1985). The means of the normal distributions for all sampling dates are then linked to trace the modal length progression of the cohorts.

Once the cohorts were separated, the resulting length-frequency distributions of each cohort and sex were submitted to ELEFAN I (Pauly & David 1981) for determination of the growth curves of the mysids.

The growth model used in ELEFAN I is a seasonally oscillating version of the generalized von Bertalanffy growth function (Gayanilo *et al.* 1989):

$$L_t = L_\infty[1-e^{(-K(t-t_0)-\frac{CK}{2\pi}(\sin 2\pi(t-t_s)-\sin 2\pi(t_0-t_s))}]$$

Where

L_t is the predicted standard length at age t

L_∞ is the asymptotic length

K is a growth constant

C is the amplitude of the seasonal growth oscillation

t_s is the starting point of that oscillation with respect to t_0; within ELEFAN t_s is replaced by the winter point WP corresponding to the time of the year with the slowest growth ($WP = t_s + 0.5$)

t_0 is the age at zero length

A detailed explanation of the different parameters is given in Pauly (1987).

Prior to fitting of the growth curves, ELEFAN restructures each distribution to emphasize peaks and intervening troughs. The following steps are involved in the restructuring process (Sparre 1985): first running averages of 5 are obtained for each frequency, then adjusted values are calculated by dividing each of the original frequencies by its corresponding running average and finally restructured frequency values are obtained by subtracting 1 from the adjusted values. The graphic outcome then displays positive values representing the cohorts, and zeroes and negative values representing the troughs between cohorts.

The program has an option that automatically searches for the optimal combination of values of the growth parameter values given a set of seeded parameters and step sizes with which the values can change. Each combination yields a curve which passes through different points in the restructured distribution. The more positive values the curve encounters, the better the fit of the curve becomes. The ratio of the explained sum of peaks accumulated from the curve (ESP) to the available sum of peaks (ASP) is a measure for the goodness of fit of the curve. This ratio is incorporated in the Rn value ($Rn=10^{ESP/ASP}/10$).

An initial estimate of the asymptotic length, which can be fixed in ELEFAN I, will make the result more accurate by reducing the number of 'free' parameters to be estimated by the program (Pauly 1987). This independent estimate of L_∞ was obtained with the Wetherall method as modified by Pauly (1986) (also available in the ELEFAN package). The length-frequency samples representing a cohort are cumulated by length class and plotted with the midlength on the X axis. Another plot represents the mean lengths computed from each class upwards minus the cutoff length versus this cutoff length. A point representing animals fully selected and recruited is then selected by the user and a regression line is fitted to the points with each point weighted by its cumulative frequency. L_∞ is then given by the intercept divided by the slope of the regression line.

Production estimates of *Neomysis integer* were obtained from the basic length-frequency data, and the length-weight regression. The first 2 sampling campaigns (27-11-1990 and 12-12-1990) were not used in the calculations. The year over which production was calculated thus started on 08-01-1991 and ended on 16-12-1991.

Three commonly used methods were applied to estimate annual production: the growth summation method, the removal summation method, and the size-frequency method. Detailed explanations on the growth summation and removal summation methods are given by Crisp (1984) and Waters (1977).

In the growth summation method production is calculated as the increment of biomass from one sampling time to the next throughout the cohort's life span. In mathematical terms this can be expressed as:

$$P=\sum N\times\Delta\overline{W}$$

where

N = the number of individuals at time t

$\Delta\overline{W}$ = the increase in weight of an average individual during the time interval

The removal summation method sums the loss (instead of growth) in weight between consecutive samples for the whole life span of the cohort and is given as:

$$P=\sum\overline{W}\times\Delta N$$

where

$\overline{W}$ = the weight of the average animal during the sampling interval

ΔN = the change in number of animals during the interval

The size-frequency method involves the calculation of an average length-frequency distribution from quantitative samples taken at evenly spaced intervals throughout the year. Production is estimated as the sum of the losses of individuals from one size class to the next and the biomass loss this represents, compensated by the increase in mean individual weight with increasing age. In this study the formulation of Menzie (1980) was used:

$$P=[i\times\sum_{j=1}^{i}(\overline{n}_j-\overline{n}_{j+1})\times(W_j\times W_{j+1})^{1/2}]\times 365/CPI$$

where

P = annual production

i = the number of size classes

$\overline{n}_j$ = the number of individuals that have developed into a particular size category (*j*) during the year

W_j = the mean weight of an individual in the (j) size category

CPI = the cohort production interval (the number of days from the date of birth of the cohort to the attainment of the largest size)

The size-frequency method was originally designed to estimate production in mixed populations of univoltine species (Hynes & Coleman 1968). However, it has usually been applied in single species production studies. In species whose life span is different from one year the cohort production is multiplied by the ratio of 365 to the cohort production interval CPI in days (365/CPI) to obtain annual production. In the present study the three cohorts differed in their life span. Hence an average CPI was obtained from the CPI's of the three cohorts. Since the three cohorts overlapped in their existence, the use of this average CPI may not yield a good estimate of production. To check on this another estimate of annual production was obtained by considering each cohort separately. Annual production according to this treatment is then the sum of the production of each of the three cohorts. Waters & Crawford (1973) suggested that separate production estimates are needed in species which show sex-related length differences. As this was clearly the case for *Neomysis integer* in the study area, production estimates were also made for the males and females separately. The results obtained from these three approaches to the size-frequency method were compared to each other and to the estimates obtained with the other two methods.

Furthermore an independent estimate of production was obtained from the mortality rate. Under certain conditions (in case of a steady state model and if individual growth is described by a von Bertalanffy function) the total mortality of a population is equal to the P/B ratio of the population (Allen 1971). If the average annual biomass of the population is known, the production can be determined as the product of the biomass and the P/B obtained from the mortality rate. Total mortality (Z) was obtained from a length converted catch curve in which the logarithm of the abundance of the mysids was plotted against relative age. In this plot abundance decreased with age (except for the very young age classes which were negatively selected by the gear). The slope of the descending arm of the curve represents the rate of mortality if the following four assumptions are met (Brey 1986):

1. mortality is equal for all age groups
2. fluctuations in subsequent recruitment are small and random in character
3. the gear used has a selection curve such that only the smaller animals are selected against and
4. the samples used represent the average population structure

Results

Location of the abundance maximum and environmental variables

The abundance maximum of the *Neomysis integer* population was usually situated in stations SA and BA (9 and 8 times, respectively). Only twice was the maximum located upstream of the Dutch-Belgian border (in station LI) and only four times in the most downstream station (OV).

Most environmental variables measured (Table 4.1) did not display clear seasonal patterns. Salinity varied between 9 and 21.4 with an average of 15.2 ± 3.12. Conductivity covaried with salinity and ranged from 15.2 to 35.1 $\mu S.cm^{-1}$ (average 25 ± 5.42 $\mu S.cm^{-1}$). Average pH was 7.66 ± 0.18. Dissolved oxygen concentration (average of 78.6 ± 18.3 % of the saturation value) only reached saturation values on 3 occasions and twice dropped below 50 %. Secchi disc depth remained low throughout winter (November through March and again from November through December). Peaks of 100 cm and more were observed in April and August-September while the annual average was about 60 cm. The temperature in the watercolumn was 8.4°C at the start of sampling in November. It decreased to a minimal value of 1.4°C in February and then gradually increased to a maximum of 22.8°C in August. From September onwards it decreased again to winter values of 4°C by the end of sampling in December.

Seasonal changes in density, biomass, length composition and population structure

The following standard length (SL) - ash free dry weight (AFDW) regression, significant at the $p<0.001$ level, was used for the calculation of biomass:

$$\ln(AFDW) = 2.267 \ln(SL) - 5.539 \qquad (N = 100;\ r = 0.997)$$

No significant differences ($p>0.5$) were found among spring and autumn animals, nor among males and females.

The seasonal pattern in density and biomass is shown in Fig. 4.2. Mean density for the study period was 28 individuals per m^2 with a minimum of 2.5 per m^2 in December 1990 and a maximum of 128 per m^2 in June. Mean annual biomass was 54 mg AFDW per m^2.

In autumn and winter (October through the end of February), densities were low (less than 25 individuals per m^2). In spring (March, April and May) densities remained quite low but biomass increased due to the growth of the animals into adult stages. From late spring onwards high densities and biomass of *N. integer* were recorded, with peak densities of more than 100 individuals per m^2 in June and August (Fig. 4.2).

The observed length-frequency distributions of the population at each sampling date show several bimodalities (Fig. 4.3a) some of which clearly referred to the recruitment of cohorts. The larvae of mysids develop in the marsupium of the females and are released as miniature mysids at a length of about 2 mm (Mauchline 1980). Recruitment of a spring cohort in April-May was obvious and an overwintering generation could be seen growing from November 1990 through May 1991 and again from October 1991 through December 1991. The situation in summer was generally too complex for visual discrimination of cohorts. Maximum length observed was 18.7 mm for a female in June.

The stage composition of the population on each sampling date is presented in Fig. 4.4. In November and December of 1990, about 40% of the population consisted of (large) juveniles. Immature males and females together accounted for another 40% of the population. Less than 20% of the population were adults. Throughout winter and in early spring juveniles appeared to be growing gradually into subadult stages, and the subadults simultaneously became adult males and females. In early March the number of adults increased sharply and some of the females carried brood. In early April nearly all mysids were adult and a substantial number of gravid females was observed. Throughout late spring and summer changes of the population structure were more difficult to follow. Still, three periods of juvenile-subadult recruitment, each preceded by an increase in the percentage of gravid females, were evident: May-June, July-August and September-November. Stage composition from November 1991 onwards was similar to the observations in the year before with a dominance of subadults.

Cohort separation

A summary of the output of the Bhattacharya method is presented in Fig. 4.5. The points in the plot are the means of the normal length-frequency distributions obtained for each sample. The lines connect the mean lengths of the normal distributions that are likely to represent the same cohort through time. Thus three cohorts can be identified. The first cohort (cohort I or overwintering cohort) was present in November 1990 at a mean length of 8 mm (so these animals were born before the start of the sampling period) and grew up to May-June when a mean length of 14 mm was reached. The cohort died out by the end of June. The corresponding cohort for the next year was found from September 1991 onwards (mean length 4 mm) and grew to a length of 9 mm by the end of the sampling program in December. The second cohort (cohort II or spring cohort) recruited in late April-May at a mean length of 3 mm. Size increased quickly to about 10 mm by the end of June. This cohort was not detectable in early August. The third cohort (cohort III or summer cohort) showed a similar increase in size from its recruitment in July to the death of the last animals in the first half of October.

Data on adult length also contributed to a reliable separation of the cohorts. The mean length of the adults of both sexes varied considerably during the course of the year (Fig. 4.6). Mean length of overwintering adult males increased steadily from about 9 mm in November to more than 12 mm in April. The decrease in mean size and the high standard deviations in June, August and November indicate co-occurrence of adult males belonging to different generations. Adult females showed similar variation in mean length, increasing from 10 to 14 mm. As in the males the occasional decrease in mean size and standard deviations indicate co-occurrence of individuals belonging to 2 generations. Bimodalities in the length-frequency distributions of gravid females and, to a lesser extent, of juveniles and subadults were also used to assign certain individuals to a specific cohort.

The resulting length-frequency distributions and the population structure of the three cohorts are presented in Figs. 4.3 (b,c,d) and 4.7, respectively. The autumn samples contained only recently released animals from cohort I, the majority of which overwintered as subadults and matured in March. Gravid females were present from April till June. Note that from May onwards Cohort I consisted almost exclusively of adult females suggesting that the males died soon after mating. The cohort I females released the young of cohort II which caused the density peak in early May. Individuals of cohort II mature very rapidly: the first gravid females were already observed in June. The last juveniles were observed in August and breeding continued until the extinction of the cohort in September. Cohort III juveniles appeared in the June samples and continued to recruit until October. This cohort also matured rapidly and bred from August until October, yielding the juveniles of the new overwintering generation. The last cohort III animals died in November. No difference in mortality between the sexes was observed for the summer cohorts.

Growth

The growth parameters of the von Bertalanffy growth curves of the three separate cohorts are presented in Table 4.2. In Fig. 4.8 these growth curves are plotted together with the corresponding modal length of the observed length-frequency distributions. Only the overwintering generation showed a seasonally oscillating growth ($C>0.5$). From September through November the animals grew at a rate of 3-4 mm per month. In December growth slowed down and ceased almost completely during the coldest winter months ($WP=t_s-0.5=0$). With the increase of water temperature in spring, the mysids regained their fast growth rate. There was no seasonal oscillation detected in the growth of the mysids of the spring and summer generations ($C=0$). In these cohorts the smaller mysids (mainly juveniles and subadults) grew fastest at a rate of 3-4 mm per month; the larger mysids (mainly adults) had a slower growth rate (1-2 mm per month).

Individuals of the overwintering generation attained a larger size than spring or summer animals (higher L_∞ for both sexes). Marked differences among the growth curves of the two sexes were evident in all cohorts: the females always had a higher L_∞ than the males. The average size at maturity for females (14 mm) and males (10 mm) of the overwintering generation was larger than for the spring and summer generations at 10 mm (females) and less than 9.5 mm (males).

Brood size

The number of larvae in the marsupium, i.e. the brood size (b) exhibited a strong positive correlation with the length (l) of the female (Fig. 4.9):

$$\ln(b)=-3.349+2.676*\ln(l)$$
$$\text{or } b=0.0365*l^{2.656}$$
$$n=420;\ r=0.866;\ p<0.001$$

Analysis of covariance showed that females of the overwintering generation had significantly larger broods than females from the spring and summer generations ($p<0.05$). The difference in brood size between the spring and summer generations was marginally significant at $p = 0.067$. The following equations were found to apply to the three cohorts (Fig. 4.10):

Cohort I	$\ln(b)=-3.720+2.828*\ln(l)$	$n=167;r=0.655;p<0.01$
Cohort II	$\ln(b)=-2.307+2.223*\ln(l)$	$n=149;r=0.562;p<0.01$
Cohort III	$\ln(b)=-0.974+1.673*\ln(l)$	$n=104;r=0.615;p<0.01$

Thus, at the same body size, a female of the overwintering generation produces a higher number of larvae per brood than a female of the spring or summer generations.

Production

The production estimates for *N. integer* obtained with the different methods and at different levels of pooling of sexes and cohorts are summarised in Tables 4.3 and 4.4. The annual production estimates obtained with the growth summation and removal summation methods are almost identical (322 mg AFDW.m^{-2}.yr^{-1}). The result of the size-frequency method is very close (2% difference) to that of the two former methods only when the calculations were performed for the three cohorts and both sexes separately. Calculations on pooled length-frequency distributions of the two sexes per cohort and of the three cohorts (average CPI of 202 days) gave estimates that were 9% and 39% higher, respectively. Annual production as estimated from the mortality rate of each cohort was 24% higher than the estimates using the growth summation, the removal summation or the maximally disaggregated size-frequency methods. Only the values for the first cohort are comparable to the ones obtained with the other methods.

Discussion

Salinity zones in the Westerschelde are relatively stable (Heip 1988). In the Westerschelde, in contrast to other European estuaries, the *N. integer* population is concentrated in a narrow zone of approximately 20 km throughout the year (Mees *et al.* in press). This is attributed to the adverse oxygen conditions upstream of the Dutch-Belgian border (Mees *et al.* 1993). The maximum population density is found around the isohaline of 15 and is therefore found at a much higher salinity than in other, less polluted, estuaries. In the Gironde, the Eems, the Elbe and the Shannon, for example, the population maximum was found at 3.5 and typically a differential distribution of the developmental stages and sexes along the salinity gradient is observed (Sorbe 1981, Hough & Naylor 1992). The restricted distribution of the species in the Westerschelde results in a homogenous mix of developmental stages. This facilitates the study of *N. integer* population dynamics and justifies the selection of a single 1000 m sample in the zone of maximal abundance for the production estimates.

The life cycle of *Neomysis integer* in the Westerschelde is similar to the life cycle previously described for this species occurring in other localities. Vorstman (1951), Mauchline (1971), Borghouts (1978), Bremer & Vijverberg (1982) and Parker & West (1979) all concluded that *N. integer* produced 3 generations per year in the IJsselmeer (former Zuiderzee), the west coast lochs of Scotland, lake Veere (south-west Netherlands), in Frisian lakes (north Netherlands) and in a loch in western Ireland, respectively. Two generations per year were found in the Ythan (Astthorsson & Ralph 1984) and Eider-Ring estuaries (Kinne 1955) and in the coastal waters in the Baltic Sea (Kinne 1955, Wiktor 1961, Jansen *et al.* 1980, Rudstam *et al.* 1986). At lower latitudes life cycles are more complex, breeding being almost continuous throughout the year (Sorbe 1981).

Reproduction of *N. integer* halted completely in winter.. It restarted in April when water temperature rose to 10°C and stopped in October when temperature dropped below the same value. Females of the overwintering generation had larger brood sizes for animals of the same length. Whether this was correlated to a higher fecundity can not be derived from the field data. Smaller brood sizes may be compensated by the production of several broods per female in the spring and summer generations. Indeed, in culture experiments conducted at 20°C individual females were found to produce upto 5 consecutive broods (C.R. Janssen unpublished). The physiological background and/or ecological adaptiveness of these different reproductive strategies remain unclear.

The results of the present study seem to corroborate the hypothesis of Bremer & Vijverberg (1982) that brood size is positively correlated with salinity: the Westerschelde population lives at a higher salinity than the other populations studied and is characterised by the largest brood sizes reported to date.

However, why this should be so and why most populations of *Neomysis integer* are found at salinity ranges that result in relatively small brood sizes remains unclear. Possible explanations may include a trade-off for suboptimal brood sizes with the advantages of living in low-salinity waters which in estuaries coincides with the zone of maximum turbidity: lower (visual) predation pressure, more available food, less competition with other mysids (e.g. *Mesopodopsis slabberi*) or benthic filter feeders.

The most frequently used method for determining growth in crustaceans involves the identification and tracing of modes (cohorts which are generated by seasonal reproduction) in the length-frequency distributions. As a result of the asynchrony of moulting between individual mysids the lengths within a cohort are assumed to be normally distributed. Growth curves can then be fitted to the mean lengths of these cohorts in relation to their age. For the analysis of growth the complete ELEFAN program package (Pauly & David 1981) proved to be very useful. The Bhattacharya method is a powerful tool for identifying cohorts, but a careful examination of the population structure and length-frequency distributions of the different developmental stages was still necessary. The growth model used was a von Bertalanffy equation which incorporates seasonal variation in growth. Differences in growth between the sexual stages had to be taken into account. Seasonally oscillating von Bertalanffy growth equations were previously only applied to the mesopelagic mysids *Meterythrops microphthalma* from the Japan Sea (Ikeda 1992) and the hyperbenthic *Antarctomysis maxima* and *Mysidetes posthon* from Antarctic waters (Siegel & Mühlenhardt-Siegel 1988).

The production estimates presented in this study are minimal estimates for the zone of maximal *N. integer* abundance. No correction was made for net efficiency which is unlikely to be 100 % as mysids are known to avoid nets to a considerable extent (Mauchline 1980). The abundance of the smallest size classes was certainly underestimated due to mesh selection. Only the water column between 0.2 m and 1 m above the bottom was sampled and, though there are indications that a large part of the population is concentrated in this zone throughout the day, some *N. integer* can be found in zooplankton samples taken at intermediate depths. Moreover, only the subtidal channel was sampled and *N.integer* makes extensive use of the salt marshes (Mees *et al.* 1993) and possibly of other shallow areas. Samples were preserved for at least four months prior to analysis and the weight loss associated with formalin preservation, estimated to be about 10% by Beattie (1982), was not taken into account. Nor was a correction made for the underestimate of weight which results from backtransformation of the linearised length-weight relationship (Baskerville 1972). Organic matter is also lost during moulting. Holmquist (1959) found that *Mysis relicta* lost 5-10% of its dry weight due to ecdysis. This bias is not necessarily relevant since use of this refractory chitinous material in the food web is probably limited. Finally no attempt was made to include intra-marsupial production in the calculations.

N.integer produced about 0.3 g AFDW.m^{-2}.yr^{-1}. This is much higher than the estimate by Bremer & Vijverberg for a lacustrine population (10 mg DW.m^{-2}.yr^{-1} with a P/B of 4.0). However, our estimate is remarkably close to the production values reported for *Neomysis mercedis* in lakes that support similar population densities (summarised in Cooper *et al.* 1992).

One of the basic assumptions of the size-frequency method is that all 'species' must have the capability of growing to the same maximum size (Hamilton 1969). Waters & Crawford (1973) noted that the method yielded estimates 15 to 26% higher than the removal summation method and postulated that this could be due to the size difference between the sexes. Although identification of cohorts is not required to obtain a production value with the size-frequency method, not doing so will lead to an important overestimate of production.

Conclusions

Biomass production of *Neomysis integer* is quite high in the brackish zone of the Westerschelde. Assessing its trophic role in the estuarine food web will require quantification of fluxes to higher trophic levels along with information on seasonal and diurnal variation in diet and consumption to estimate the species' predation pressure on zooplankton and its role in the detrital food chain.

The more or less standard method which has been developed for studying the life history and population dynamics of mysids from field data (Mauchline 1980) was successfully applied to the Westerschelde population: samples were taken at regular intervals and the temporal evolution of the population structure was assessed by dividing the animals in a number of distinct sexual and developmental stages. Length-frequency distributions per stage and per sampling date then allowed an estimate of the number of generations produced in a year. Seasonally oscillating (for the overwintering generation) or generalised (for the spring and summer generations) von Bertalanffy growth models could be fitted to the data, provided males and females were treated separately.

The growth summation and removal summation methods yielded approximately the same production estimates. The use of the size-frequency method is only recommended for populations that can easily be disaggregated into cohorts and/or sexes with more uniform life-history characteristics. Pooling of data will lead to an overestimation of production. The same conclusion applies to the method which uses the total mortality within the population to estimate production. Though not frequently used, the effort required to calculate production with this method is very low, as no abundance and mean individual weight per sampling date is necessary. Only a length-frequency distribution at each sampling date and an estimate of mean annual biomass is required.

In summary, the *Neomysis integer* population of the Westerschelde produced three generations per year.

The overwintering generation lived from autumn until spring of the following year. The spring generation was born in April and lived for around three months, while the summer generation lived from July until early winter of the same year. The spring generation produced the highest number of mysids and accounted for almost half of the annual biomass. The overwintering generation contributed least to the biomass. The biology of the mysids in the latter generation was markedly different from that of individuals belonging to the spring and summer generations: they had a lower growth rate, larger size at maturity and a larger brood size. Within any one cohort both sexes showed different growth characteristics, with the females generally living longer, growing faster and attaining a larger size than the males. The three methods for estimating secondary production yielded similar results. Annual production amounted to 0.3 g AFDW.m^{-2}.yr^{-1}. The size-frequency method only resulted in figures similar to those obtained with the growth summation and removal summation methods when it was applied to the three cohorts and both sexes separately. The spring cohort produced nearly half of the total annual production. Inspite of its longer lifespan, the overwintering cohort accounted for barely half of the total annual production.

Acknowledgements

This research was supported by the European Community (contract no. MAST-CT90-0024 and EV5V-CT92-0064) as part of the JEEP-92 and MATURE projects. We thank Wim Röber, Piet De Koeyer and Co Van Sprundel, crew of the R.V. Luctor, for their help during sampling. This is contribution No. *** of the Centre for Estuarine and Coastal Ecology.

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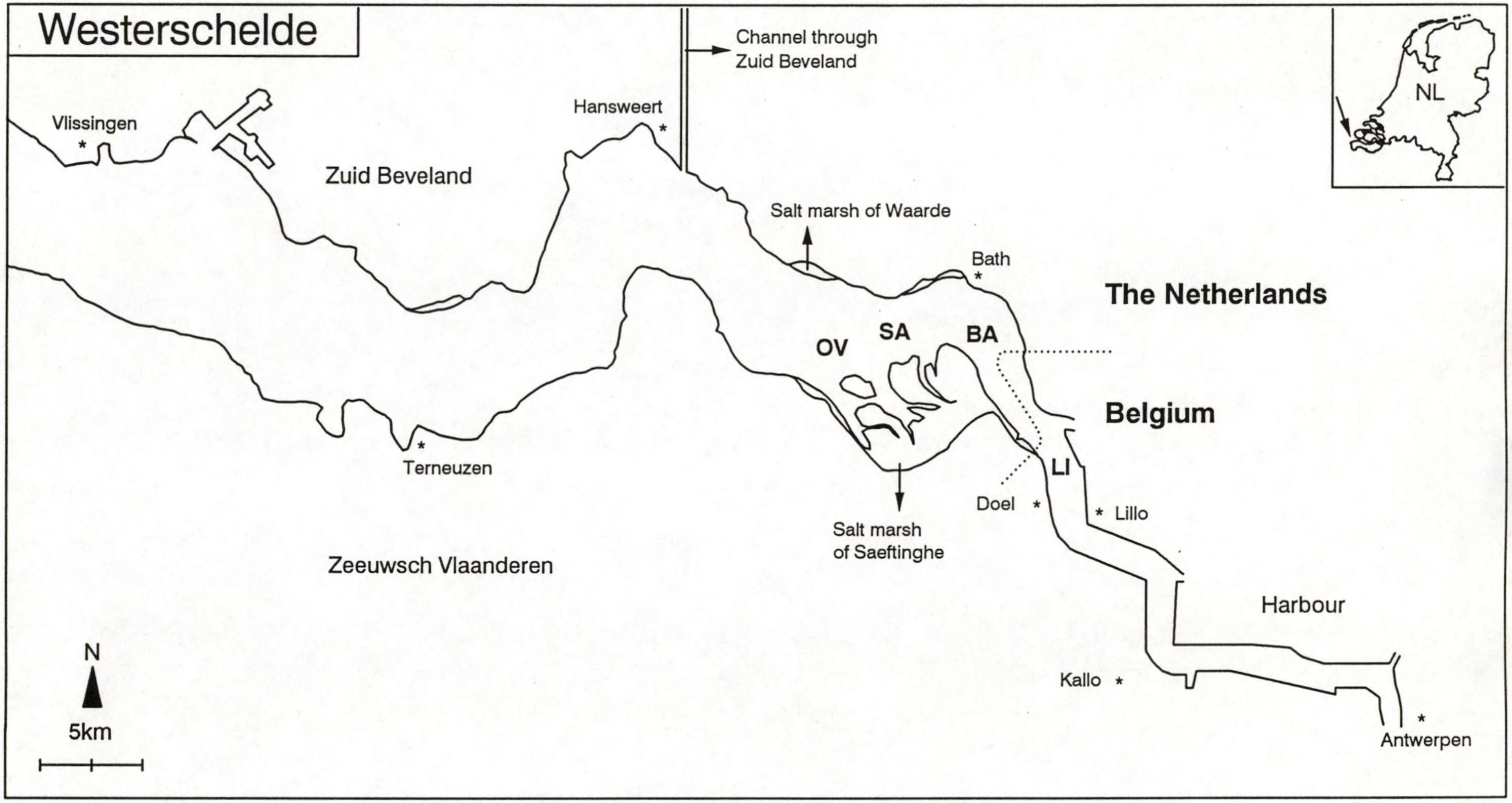

Figure 4.1. Study area and the four sampling sites

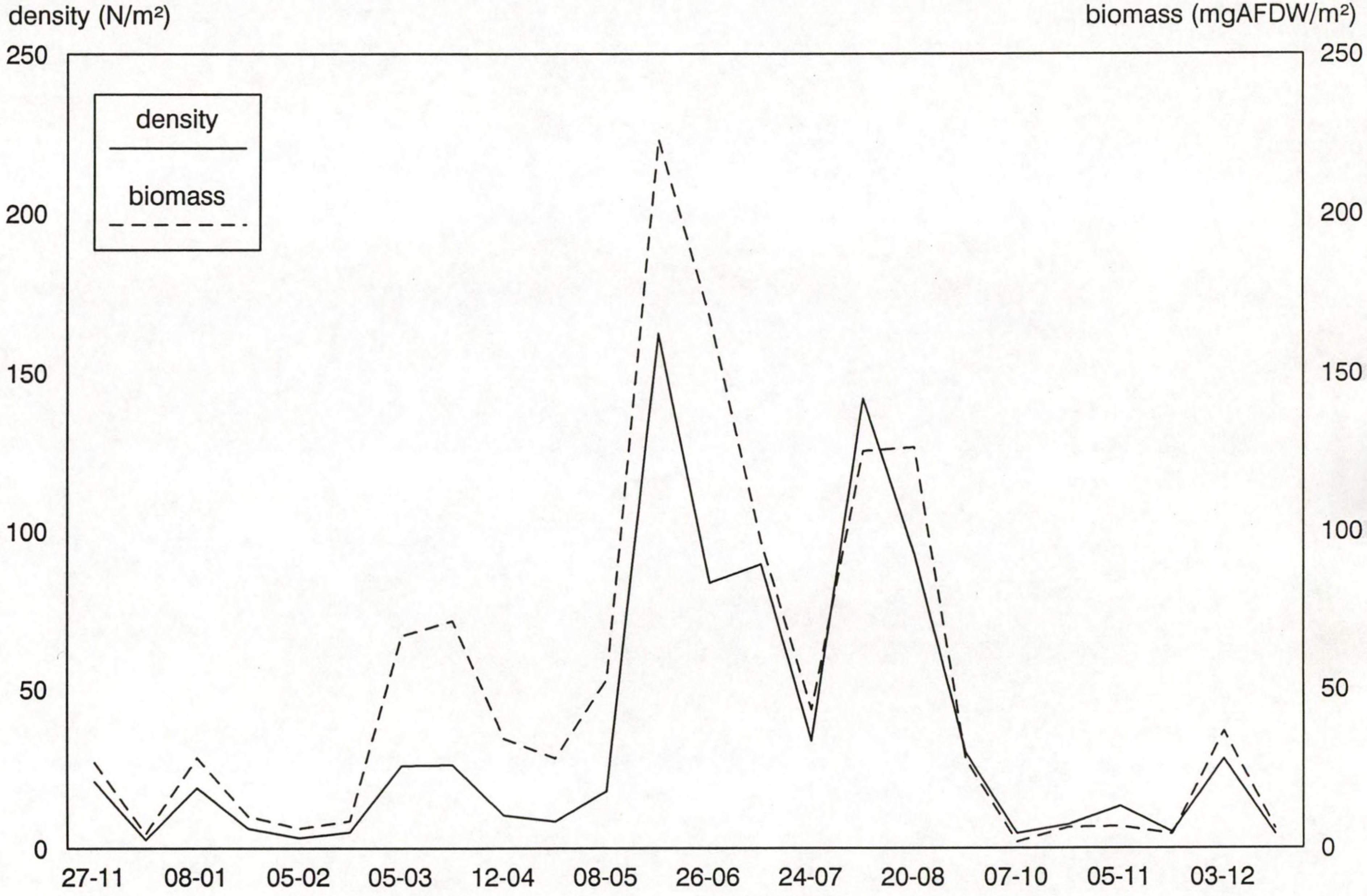

Figure 4.2. Density and biomass of *Neomysis integer* for each sampling date

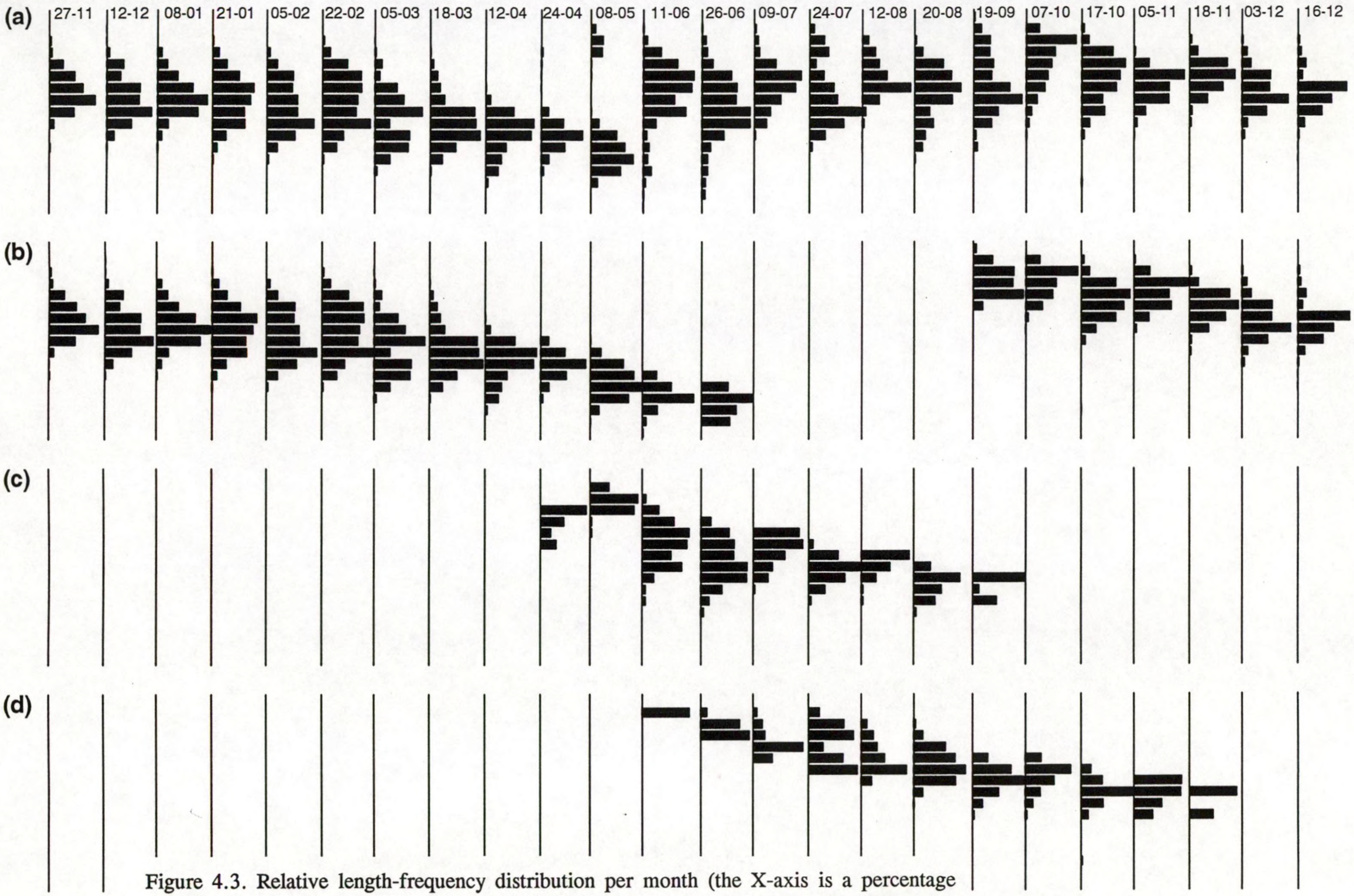

Figure 4.3. Relative length-frequency distribution per month (the X-axis is a percentage scale; the Y-axis are 1 mm classes with class marks of 1.5 to 18.5 and practical limits of 1.0-1.9, 2.0-2.9, etc.). (a) all *Neomysis*, (b) cohort I, (c) cohort II, (d) cohort III.

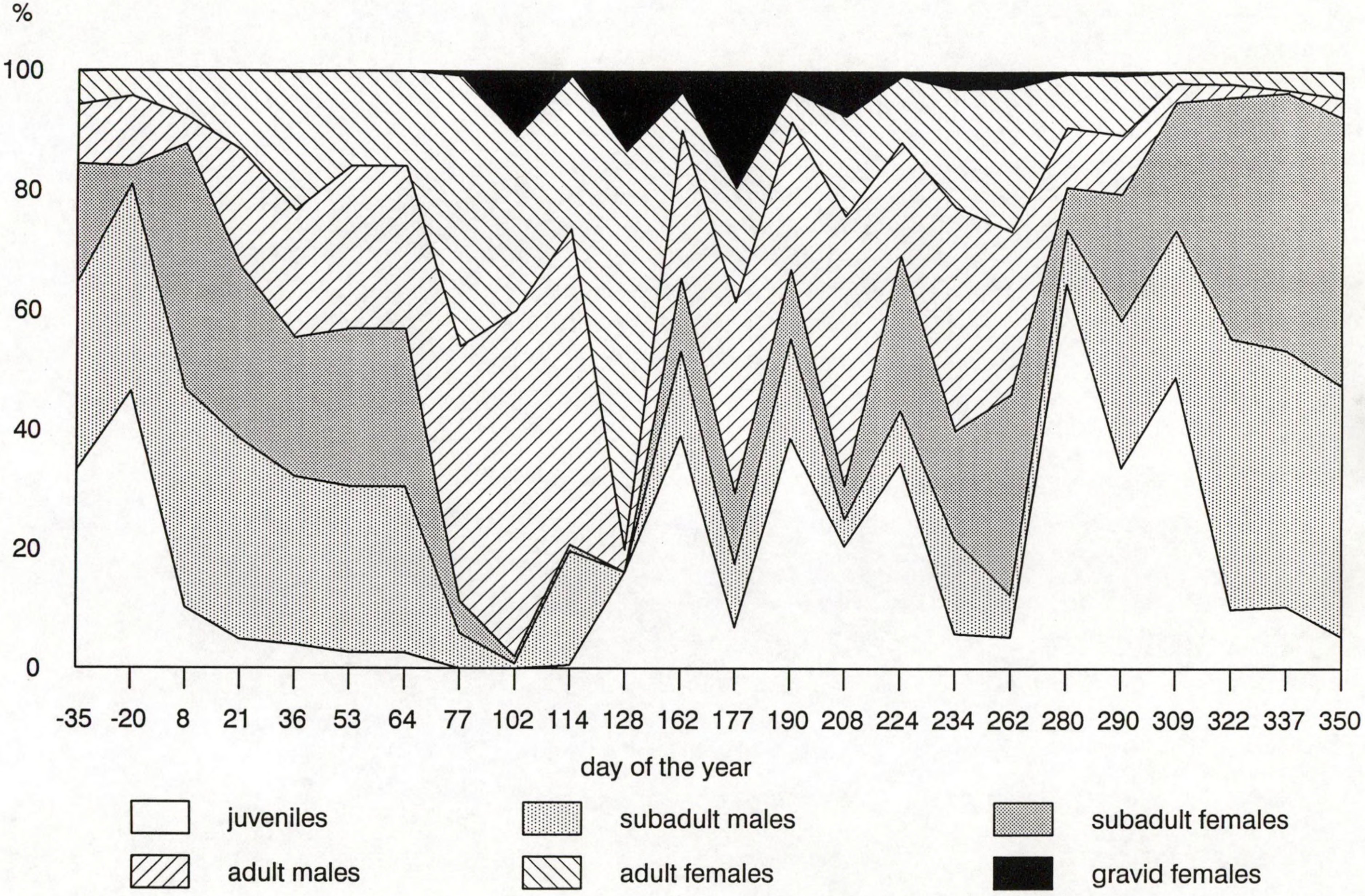

Figure 4.4. Observed population structure on each sampling date

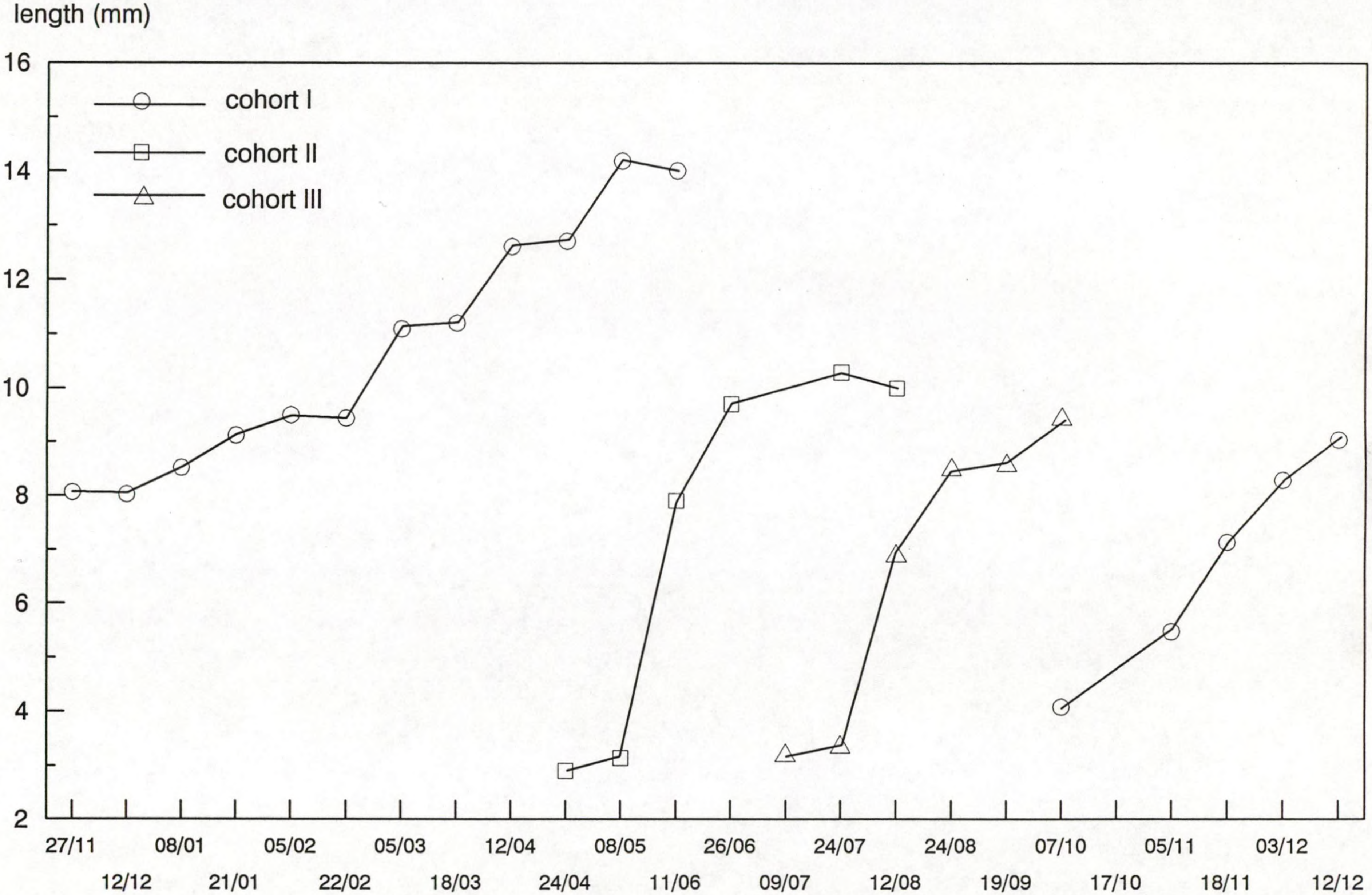

Figure 4.5. The modal length progression of the three cohorts as identified with the Bhattacharya method

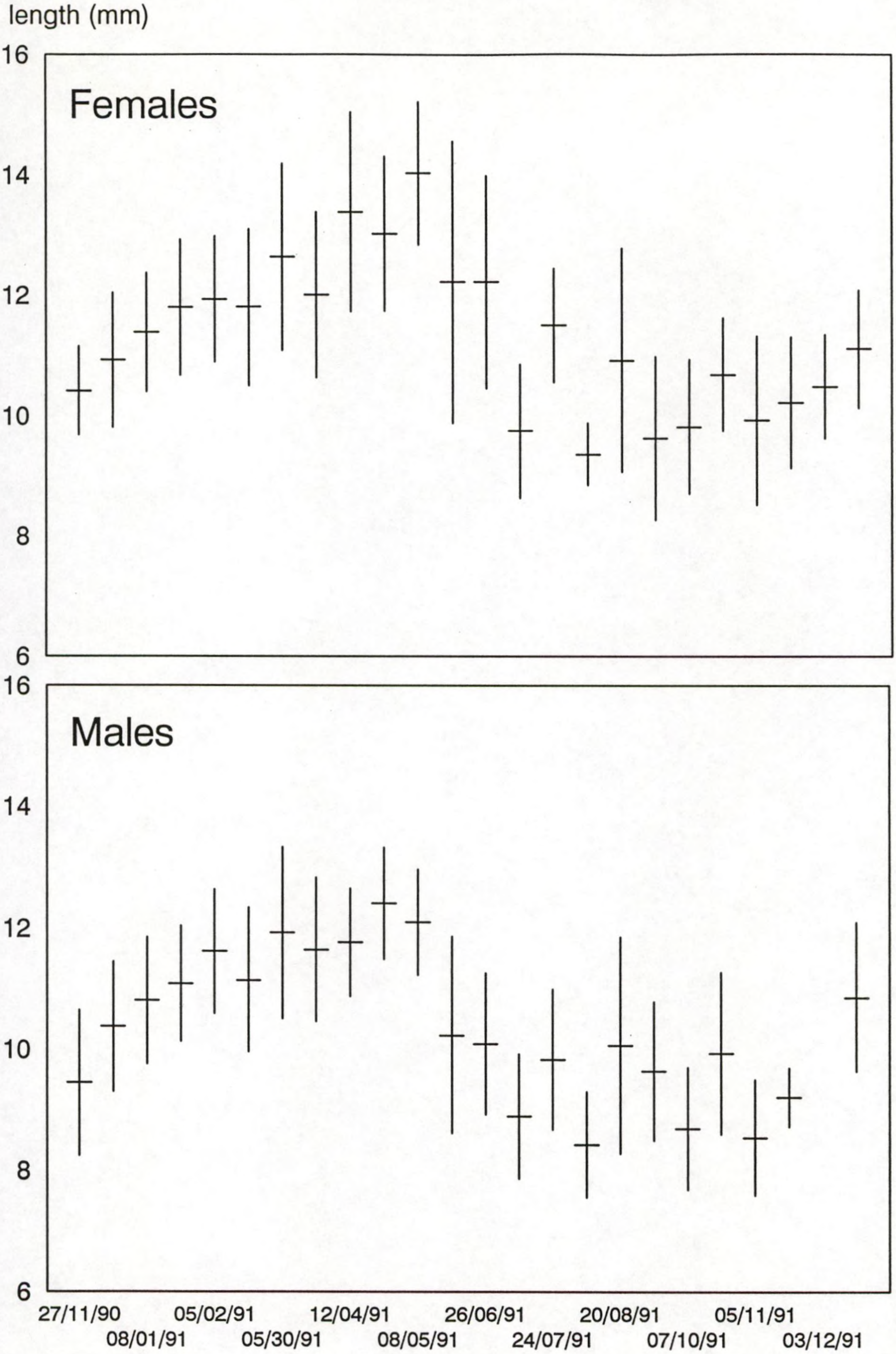

Figure 4.6. Temporal variation of the mean length and standard deviation of the adult males and females

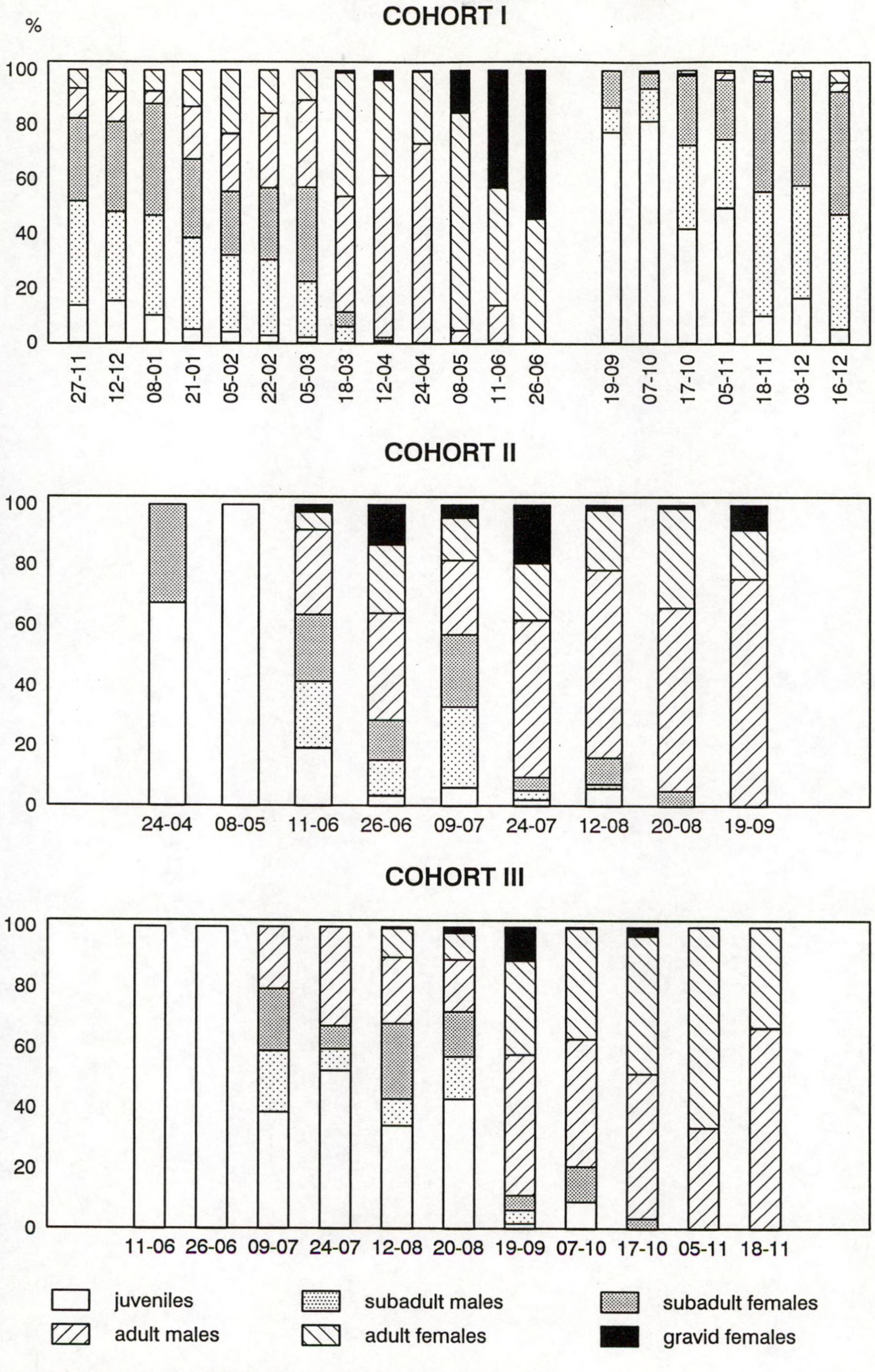

Figure 4.7. Population structure of the three cohorts

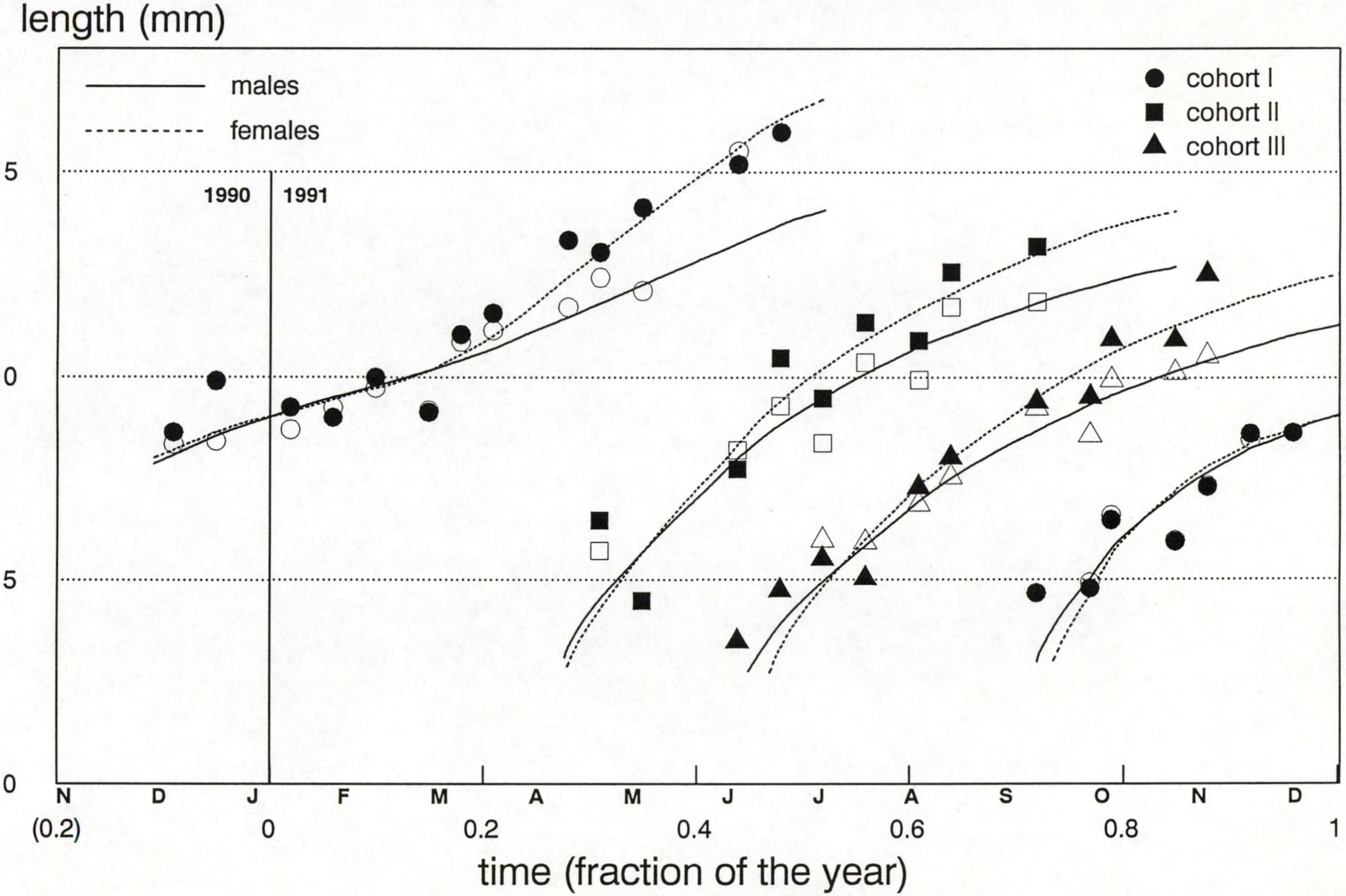

Figure 4.8. Growth curves of the three cohorts and both sexes separately. Open symbols are males; full symbols are females

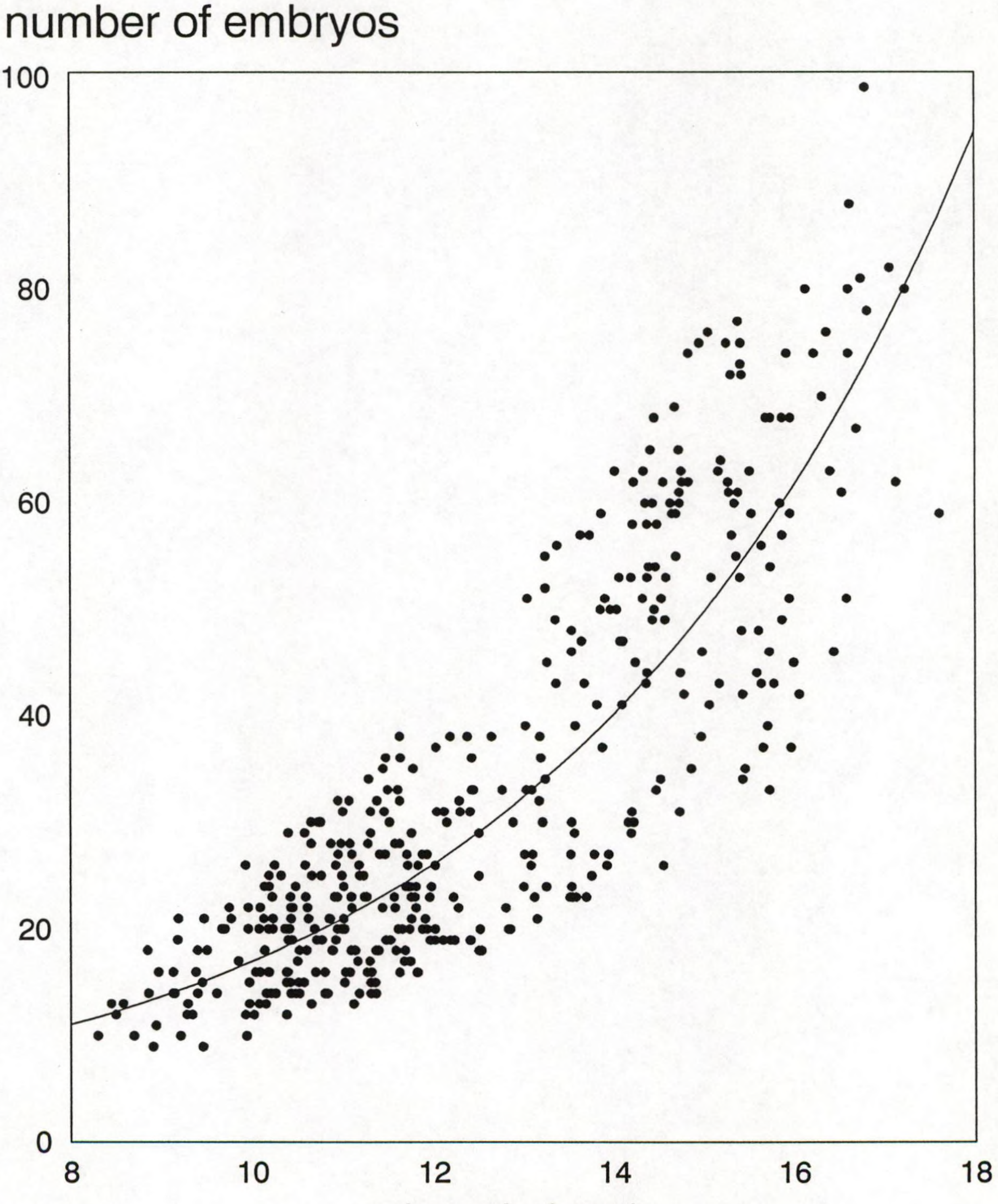

Figure 4.9. Relation between female length and brood size (all data)

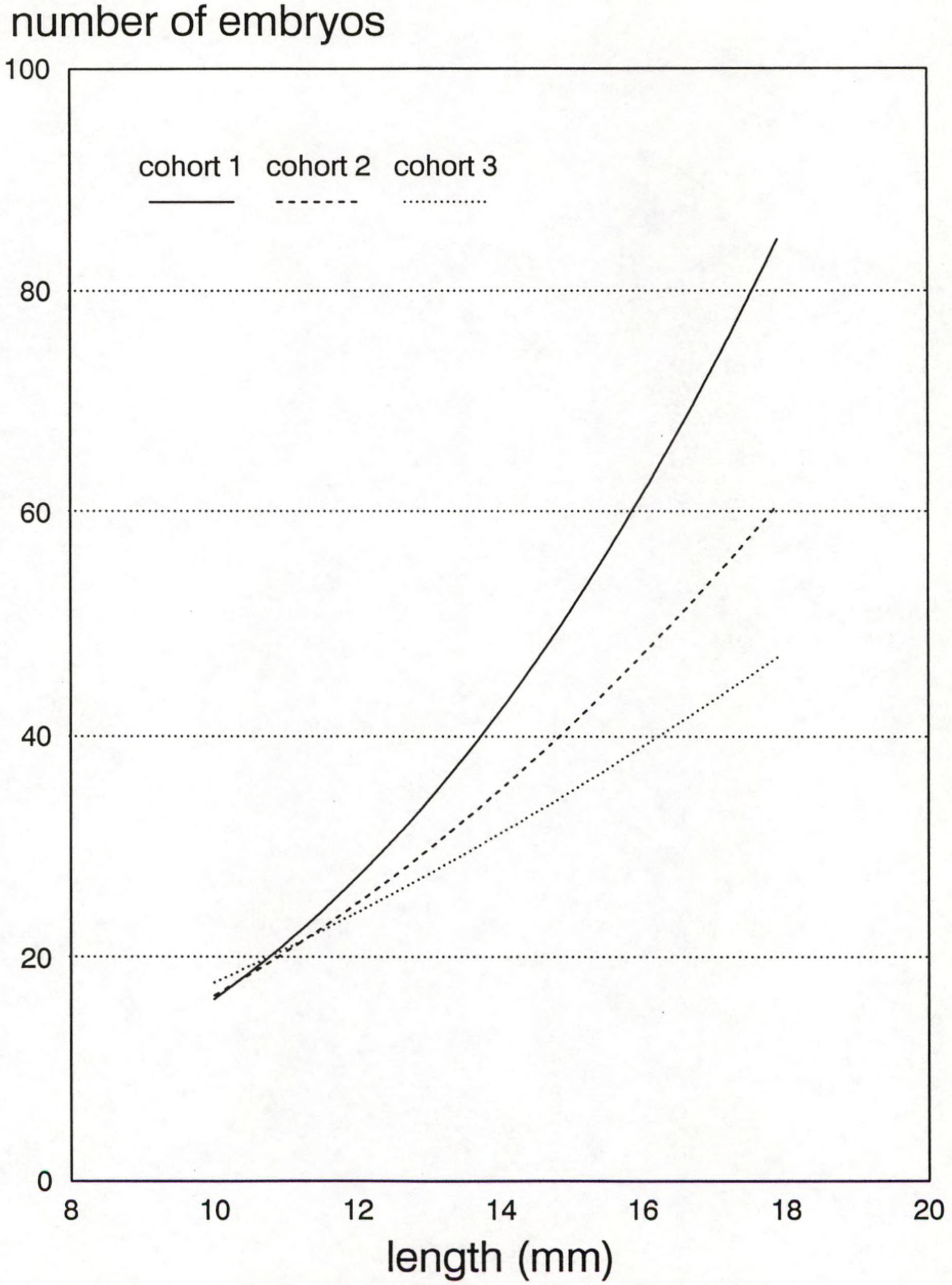

Figure 4.10. Brood size vs. standard length for the three cohorts

Table 4.1. The sampling site at which the peak abundance of *Neomysis integer* was recorded and the environmental variables measured at that site (for location of the sampling sites see Fig. 4.1).

Date	Station	Sali. (pss)	Temp. (°C)	Oxyg. (%)	Secc. (cm)	pH	Cond. (μS.cm^{-1})
27-11-90	BA	17.0	8.4	65	40	7.65	28.1
11-12-90	OV	17.1	6.0	75	45	7.60	28.3
08-01-91	SA	9.0	5.8	79	40	7.88	15.2
21-01-91	OV	14.6	4.3	90	45	7.85	25.2
05-02-91	SA	11.8	2.8	64	25	7.63	19.8
22-02-91	OV	18.2	1.4	95	40	7.88	29.9
05-03-91	BA	12.3	4.8	67	40	7.54	19.8
18-03-91	SA	13.3	8.2	47	40	7.42	21.6
12-04-91	BA	13.1	11.0	75	60	7.64	20.8
24-04-91	SA	18.7	9.7	98	100	7.86	26.5
08-05-91	LI	-	-	-	-	-	-
11-06-91	BA	15.0	15.3	88	55	7.88	24.3
26-06-91	BA	14.4	16.8	-	45	7.68	23.5
09-07-91	SA	11.7	-	-	70	7.42	16.1
24-07-91	OV	17.5	19.6	84	50	7.61	28.8
12-08-91	LI	10.3	22.8	49	50	7.45	17.6
20-08-91	SA	18.6	20.5	110	100	7.22	30.6
19-09-91	OV	18.9	21.5	103	125	7.84	34.9
07-10-91	BA	16.8	16.3	83	45	7.68	28.0
17-10-91	SA	21.4	14.8	106	80	7.85	35.1
05-11-91	BA	17.7	11.4	86	50	7.81	29.5
18-11-91	BA	13.3	9.1	70	50	7.70	22.1
03-12-91	SA	12.6	7.3	44	45	7.41	21.4
16-12-91	SA	17.3	4.0	73	55	7.67	28.9
average		15.2		79	56	7.66	25.0
standard deviation		3.1		18	23	0.18	5.4

Table 4.2. Growth parameters of the von Bertalanffy growth curves for *Neomysis integer* of the three cohorts and both sexes separately.

sex/cohort	L_∞	K	C	t_s	t_0	Rn
Males I	16.0	2.70	0.66	0.50	0.72	0.75
Females I	19.0	3.00	0.80	0.52	0.78	0.69
Males II	14.3	3.43	0.00	0.00	0.20	0.61
Females II	16.0	3.35	0.00	0.00	0.25	0.48
Males III	13.1	3.01	0.00	0.00	0.33	0.50
Females III	14.3	3.37	0.00	0.00	0.41	0.76

Table 4.3. Summary of the production estimates (mg AFDW.m^{-2}.yr^{-1}) for *Neomysis integer* obtained with different methods.

			Production	Biomass	P/B ratio
Growth summation					
		CohortI	81.65	24.20	3.4
		CohortII	164.51	51.28	3.2
		CohortIII	76.54	19.48	3.9
		Total	322.69	94.97	6.0
Removal summation					
		CohortI	81.82	24.20	3.4
		CohortII	163.82	51.28	3.2
		CohortIII	76.78	19.48	3.9
		Total	322.43	94.97	6.0
Size-frequency					
	Sexes separated				
		CohortI	91.70	25.34	3.6
		CohortII	175.41	55.93	3.1
		CohortIII	61.95	21.15	2.9
		Total	329.06	102.42	6.1
	Sexes not separated				
		CohortI	96.94	25.34	3.8
		CohortII	183.54	55.93	3.3
		CohortIII	65.63	21.15	3.1
		Total	351.82	101.85	6.5
	All Cohorts		448.52	53.73	8.3

Table 4.4. Production estimates (P in mg AFDW.m^{-2}.yr^{-1}) of *Neomysis integer* obtained from the mortality rate (Z) as compared to the estimates from the disaggregated size- frequency method (s-f).

	Z	Biomass	P (Z)	P (s-f)
Cohort I				
males	3.15	10.10	31.82	35.65
females	4.27	15.24	65.07	56.05
total		25.34	96.89	91.70
Cohort II				
males	3.27	29.37	96.04	91.31
females	4.41	26.56	117.13	84.09
total		55.93	213.17	175.41
Cohort III				
males	4.49	10.37	46.56	29.49
females	3.90	10.78	42.04	32.46
total		21.16	88.60	61.95
Annual production			398.66	329.06