Marine Biology
Ghent University

ILVO
Institute for Agricultural and Fisheries Research

Lies VANSTEENBRUGGE

THE NON-INDIGENOUS CTENOPHORE *MNEMIOPSIS LEIDYI* IN THE SOUTHERN NORTH SEA

Ecological and socio-economic effects related to its trophic position and the current distribution of gelatinous zooplankton

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Ecological and socio-economic effects related to its trophic position and the current distribution of gelatinous zooplankton

DE NIET-INHEEMSE KAMKWAL *MNEMIOPSIS LEIDYI* IN DE ZUIDELIJKE NOORDZEE:

Ecologische en socio-economische effecten gerelateerd aan de trofische positie van de soort en de huidige verspreiding van gelatineus zoöplankton

Lies VANSTEENBRUGGE

Promotors:

Prof. Dr. Marleen De Troch

Dr. Kris Hostens

Members of the reading committee

Dr. Aino Hosia (University of Bergen, Norway)

Dr. Gilles Lepoint (University of Liège, Belgium)

Prof. Dr. Tom Moens (Ghent University, Belgium)

Members of the examination committee

Prof. Dr. Dominique Adriaens (Ghent University, Belgium) – chairman

Prof. Dr. Marleen De Troch (Ghent University, Belgium) - promotor

Dr. Kris Hostens (ILVO, Belgium) - promotor

Dr. Aino Hosia (University of Bergen, Norway)

Dr. Gilles Lepoint (University of Liège, Belgium)

Prof. Dr. Tom Moens (Ghent University, Belgium)

Dr. Carl Van Colen (Ghent University, Belgium)

Dr. Sofie Vandendriessche (ILVO, Belgium)

Prof. Dr. Magda Vincx (Ghent University, Belgium)

Jellies don't need
bones, blood or brains

For 500 million years, their
seemingly simple systems
have helped them thrive

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Dankwoord

Kwallen… wie had ooit gedacht dat ik zo gefascineerd kon zijn door deze wezentjes. Het begon allemaal in de vroege zomer van 2010: een schemerige bureau en een man met een vlechtje. Na dat eerste gesprek met Kris, lieten de kwallen me niet meer los. In het bijzonder die Amerikaanse soort die sinds kort was opgedoken in onze wateren. Hoewel de meeste mensen kwallen kennen als de hoopjes slijm die langzaam verteren op het strand, zijn het – levend, in het water - prachtige, intrigerende dieren. En het gekke is, we weten er eigenlijk niet zo veel over… Dit is waar deze studie probeert verandering in te brengen. De eerste Belgische studie specifiek over kwallen… lees verder en laat je meevoeren!

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Lies

Oktober 2015

And the arms of the ocean are carrying me
And all this devotion was rushing out of me
And the crashes are heaven for a sinner like me
But the arms of the ocean delivered me

Florence + the machine – never let me go

Summary

In recent years, abundances of gelatinous zooplankton are thought to have increased all over the world, resulting in jellyfish-dominated ecosystems and food webs. This trend, also referred to as 'jellification', is mainly based on a few local case studies and media-driven public perception, because long-term jellyfish datasets are scarce. Sudden increases in jellyfish densities (blooms) are a common life cycle characteristic, mainly driven by natural fluctuations in the climate. However, certain anthropogenic pressures, such as overfishing, eutrophication, non-deliberate transport of non-indigenous species can be correlated with varying jellyfish abundances. Moreover, gelatinous zooplankton blooms can directly interfere with anthropogenic activities, for example by clogging and rupturing of commercial fishing nets, by stinging beach tourists or by killing fish in sea farms. To be able to manage and reduce the potential problems and economic costs related to jellyfish outbreaks, understanding the mechanisms driving these outbreaks is imperative.

In the 1980s, the ctenophore *Mnemiopsis leidyi* A. Agassiz 1865, indigenous to the Atlantic coast of North and South America, was introduced in the Black Sea through ballast water of ships. The densities of this invasive species rapidly increased in the Black Sea, and in addition to overfishing and eutrophication led to a collapse of the major fisheries, causing vast ecological and socio-economic losses. Consequently, the first observations of *M. leidyi* in northern European marine waters in 2005, and the subsequent sightings of this non-indigenous ctenophore in Belgian marine waters in 2007 caused the appropriate concern. **This PhD thesis, as part of the Interreg Iva 2 Seas MEMO project, aimed to assess the structural and functional role of the non-indigenous ctenophore *Mnemiopsis leidyi* in the southern North Sea. More specifically, we focussed on (1) the current distribution of *M. leidyi* in Belgian marine waters, the adjacent ports and the Westerschelde estuary, related to other gelatinous zooplankton, (2) the trophic ecology and interactions of *M. leidyi* in the planktonic food web, (3) the potential ecological and socio-economic effects of the presence of *M. leidyi* and other gelatinous zooplankton in these waters, and (4) the overall threat of *M. leidyi* and the implications for non-indigenous species's management.**

After a general introduction (**Chapter 1**) on jellyfish and gelatinous zooplankton, the potential introductory pathways of invasive species and the life cycle characteristics of *M. leidyi*, the data and results are presented in seven chapters. Different approaches were used to tackle the research questions and objectives, including field studies, experimental and laboratory work, database analyses and public questionnaires. In this PhD study, the term 'gelatinous zooplankton' was narrowed down to the planktonic medusa phase (jellyfish) of the phylum Cnidaria (classes Hydrozoa and Scypohozoa) and the phylum Ctenophora.

In the southern North Sea, long-term data on gelatinous zooplankton are scarce, which is partly driven by the difficulties to sample and preserve these often 'fragile' organisms, *M.*

leidyi in particular. Therefore, in **Chapter 2**, methodological issues concerning sampling and preservation were investigated. We focused on two different types of plankton nets: a WP2 net (mesh size 200 µm) and ring trawl net (mesh size 1000 µm). Based on their different mesh size and way of deployment (vertical versus undulating trawl), we evaluated whether they can be compared in terms of *M. leidyi* density and size distribution. *Mnemiopsis leidyi* densities from 245 sampling events were analysed according to net type and revealed that WP2 nets do not provide a good estimate of its presence compared to ring trawl nets. Moreover, when *M. leidyi* was present in both nets, much larger density estimates were found by the WP2 net (45.2 ± 114.0 ind.m^{-3} for WP2 net versus 12.8 ± 28.5 ind.m^{-3} for ring trawl net). The ring trawl net gave a good overview of adult population structure, but may underestimate some of the small ctenophores. Consequently, both the filtered volume and the mesh size largely determine the catch. We also tested different preservation solutions and methods with respect to morphological and genetic identification of *M. leidyi* and in function of stable isotope analyses. From our experiments it became clear that unpreserved samples are preferred for any type of analysis. However, in many situations, direct identification in the field is not possible and preserving the sample is inevitable. Then, short-term preservation in Lugol's solution or RCL2® may provide a good alternative, but shrinkage was observed in both preservatives. For stable isotope analyses, different preservation methods resulted in significant differences in both $\delta^{13}C$ and $\delta^{15}N$, which should be considered when comparing different isotopic compositions. The findings and recommendations formulated in this study should be considered in future *M. leidyi* monitoring. Zooplankton samples collected during this PhD were analysed on board and *M. leidyi* was morphologically identified and measured alive (except for Tentaculata larvae which were genetically identified).

The discovery of *M. leidyi* in Belgian waters in 2007 created a unique opportunity to enhance our knowledge on the distribution of gelatinous zooplankton in the southern North Sea (**Chapter 3**). Monthly and seasonal CalCOFI ring trawl plankton net samples (mesh size 1000 µm, undulating trawl) from the Belgian part of the North Sea and the adjacent Westerschelde estuary were gathered between March 2011 and February 2012. The gelatinous zooplankton in these samples consisted of three Scyphozoa, three Ctenophora and 27 Hydrozoa taxa, including three non-indigenous species: *M. leidyi*, *Nemopsis bachei* and *Lovenella assimilis*. In an addendum (Addendum II), we also described the re-discovery of larval mantis shrimp (*Rissoides desmaresti*), which has not been observed in the Belgian part of the North Sea since 1913. Average gelatinous zooplankton densities reached up to 18 ind.m^{-3} near the coast, gradually declining towards the open sea, while in the brackish Westerschelde, average densities remained below 3 ind.m^{-3}. Gelatinous zooplankton densities were highest in summer and autumn, and the ctenophore *Pleurobrachia pileus* and the hydromedusa *Clytia* sp. were present year-round and at every location. Gelatinous zooplankton densities never outnumbered the non-gelatinous zooplankton densities from

the CalCOFI net. Due to the larger mesh size (1000 µm) of this net, only the larger fraction of the zooplankton is captured. The spatial and temporal distribution patterns seemed to be mainly driven by temperature (season) and salinity (location). In terms of population dynamics, the predatory ctenophore *Beroe* sp. followed the three reproductive cycles of its prey *P. pileus*, but may profit from the high abundances of *M. leidyi* in summer and autumn by reaching higher densities. This study provides a firm baseline to evaluate potential gelatinous zooplankton increases in the Belgian part of the North Sea and the Westerschelde estuary.

The analysis of the spatio-temporal distribution and population dynamics of *M. leidyi* in the southern North Sea and its main coastal ports (**Chapter 4**) revealed that *M. leidyi* occurred from August to December, but was never found more than 30 km offshore. Densities were generally low (average 0.8 ± 2.8 ind.m^{-3}) compared to other invaded ecosystems in Europe (densities up to 867 ind.m^{-3}). Highest densities of *M. leidyi* were found in the semi-enclosed basin in the port of Oostende (18.4 ind.m^{-3}) and the Westerschelde estuary (1.9 ind.m^{-3}). The presence of larvae and the sudden appearance of high numbers across the size distribution in August indicated that ports and estuaries may act as sources, populating the adjacent coastal (sink) areas. By means of a zero-inflated negative binomial regression model, the observed variation in *M. leidyi* densities was related to temperature (highest densities when temperature starts to decrease), wave height (higher densities in low energetic systems) and dissolved oxygen concentrations (higher densities at low oxygen concentrations). Although *M. leidyi* densities remained relatively low since its first appearance in Belgian waters in 2007, a permanent *M. leidyi* population has established in the southern North Sea. As outbreaks may happen with only small changes in environmental parameters, further monitoring of this notorious non-indigenous species is recommended.

Knowledge on the diet of *M. leidyi* and its interactions with other components of the pelagic food web will largely contribute to assess the impact of this non-indigenous species on the ecosystem. Using both stable isotope (SI; $\delta^{13}C$ and $\delta^{15}N$) and fatty acid (FA) analyses, we revealed spatial and temporal variation in the trophic ecology of *M. leidyi* in different ecosystems in the southern North Sea (**Chapter 5**). Based on the isotopic composition, we found that spatial differences were largely driven by variation at the base of the food web rather than diet changes of *M. leidyi* in the different ecosystems. Temporal variation in *M. leidyi* SI composition was also influenced by shifting baseline values and driven by seasonal changes in the associated plankton communities. In this chapter, we provide the first data on the FA composition of *M. leidyi* as compared to FA concentrations of two other (indigenous) ctenophores. The total FA concentration of *M. leidyi* was three to four times lower compared to *Pleurobrachia pileus* and *Beroe* sp., categorising this non-indigenous ctenophore as a lipid-poor organism. Trophic interactions between *M. leidyi* and the two co-occuring ctenophores (*P. pileus* and *Beroe* sp.) showed considerable resource differentiation, which could be the result of competition or both ctenophores could have different diets. A

mixture of zooplankton was identified as potential food source for *M. leidyi*. FA markers supported the carnivorous diet of *Beroe* sp., but its SI composition did not confirm it as a predator of *M. leidyi*.

The feeding ecology of *M. leidyi* was further investigated as its invasive success is partly related to a broad and flexible planktivorous diet. In **Chapter 6**, we investigated the feeding rates and carbon assimilation of *M. leidyi* by means of grazing experiments with a pelagic diatom species *Phaeodactylum tricornutum* and three potential mesozooplankton prey species: nauplii from brine shrimp *Artemia salina*, a copepod *Acartia tonsa,* and eggs and larvae of European sea bass *Dicentrarchus labrax*. Clearance rates were on average 0.2 ± 0.1 $L.mL_{M.leidyi}^{-1}.h^{-1}$, with no significant differences in clearance rates between prey type or prey size. The assimilation of carbon by *M. leidyi* for these different prey types was determined using ^{13}C tracer experiments. Highest carbon assimilation was observed for *Acartia* and sea bass larvae (most efficiently assimilated), and lowest for the pelagic diatom *P. tricornutum*. To further elucidate the prey-dependent variation in carbon uptake, we investigated the effect of each prey type in terms of fatty acids as a proxy for food quality. The consumption of sea bass larvae, characterised by higher levels of DHA (an essential fatty acid), resulted in significantly higher FA concentrations in *M. leidyi*. As *M. leidyi* does not convert excess food into storage lipids, survival, growth and reproduction are likely enhanced by the higher food quality, which might contribute to its invasive success. As global warming may result in an earlier appearance of *M. leidyi* and thus temporal overlap with high quality prey such as fish larvae, a substantial impact on the ichthyoplankton community in the southern North Sea might be expected.

The third aim of this PhD study was to determine the potential ecological and socio-economic effects of the presence of *M. leidyi* in Belgian waters. For the latter, we needed to broaden our scope by focusing on the impact of all prevailing jellyfish on beach tourism in Belgium, as *M. leidyi* is too small and fragile, and thus rarely noticed by tourists (except for divers). In **Chapter 7**, we examined to what extent the main jellyfish messages in the Flemish media corresponded with the knowledge and perception in the tourism sector along the Belgian coast. We searched Flemish newspapers for jellyfish-related articles issued between January 2000 and September 2012 and executed questionnaires at the Belgian coast in the summer of 2012. The number of Flemish newspaper articles increased from less than 5 in 2000 to 27 articles in 2010. Almost 75 % of these articles reported on the causes and economic consequences of jellyfish blooms, and many articles mentioned the dramatic consequences of stinging, poisonous and invasive species. The analysis of the questionnaires showed that the perception of beach tourists on jellyfish is only partly driven by the general media (mainly related to the causes of jellyfish blooms), while personal experience (e.g. stinging, slimy organisms) was at least an equally important driver. As public perception is a key driver for certain policy decisions, integrated coastal zone managers should consider the provision of simple and good information concerning jellyfish (e.g. billboards or aquaria) at

the beach. The results of this socio-economic study might serve as a baseline for future citizen science programs.

Finally, the main results of this PhD study were discussed in a broader perspective to manage the introduction and presence of non-indigenous or invasive species (**Chapter 8**). We performed a risk assessment for *M. leidyi* in the southern North Sea by means of the online Harmonia+ tool, which generates exposure (introduction, establishment and spread) and impact (on the environment and human activities) scores. An overall risk of 0.286 was calculated for the non-indigenous ctenophore *M. leidyi* in the southern North Sea, which is considered to be low. Up to 2012, *M. leidyi* showed a clear seasonal outbreak between summer and autumn. Relatively high densities (up to 18 ind.m^{-3}) were only observed in the ports, which are considered to be of lower ecological value compared to the 'richer' coastal zone or Westerschelde estuary, where *M. leidyi* densities mostly remained below 1 ind.m^{-3}. The combination of periods with unfavourable environmental conditions, the diverse gelatinous zooplankton community (potential competition) and the presence of predators such as *Beroe* sp., probably has limited the success of *M. leidyi* in the southern North Sea. However, this PhD study (including the more recent observations in 2014) showed that the population of *M. leidyi* is fully established in the different southern North Sea ecosystems. Therefore, *M. leidyi* should remain under close observation by means of regular monitoring surveys, and management actions should not be postponed in order to safeguard the current ecosystem services. As such, trying to keep the population under control is most probably the only way forward for a proper management of this non-indigenous species. Ratification of the Ballast Water Convention by all countries around the world is another key issue to at least prevent the introduction of new species and to avoid potential re-introduction of *M. leidyi* in our waters. In light of the Marine Strategy Framework Directive, the early detection (and subsequent eradication) of non-indigenous species (many of them appearing in the water column as some sort of zooplankton stage), for example by means of automated sampling tools, needs to be promoted. This PhD study is a baseline for future (gelatinous) zooplankton monitoring in the Belgian part of the North Sea and Westerschelde estuary (and by extention the southern North Sea).

Samenvatting

De laatste jaren wordt gedacht dat abundanties van gelatineus zooplankton wereldwijd toenemen, met als gevolg ecosystemen en voedselwebben gedomineerd door kwallen. Deze trend, ook 'verkwalling' genoemd, is vooral gebaseerd op enkele lokale case studies en publieke perceptie onder invloed van de media, want lange-termijn datasets zijn schaars. Plotse toenames in kwallendensiteiten (bloeien) maken een normaal deel van de levenscyclus uit en worden vooral gedreven door natuurlijke fluctuaties in het klimaat. Echter, de druk van verschillende menselijke activiteiten (vb. overbevissing, eutrofiëring, onopzettelijk transport van niet-inheemse soorten) kan bijdragen tot variatie in kwallenabundanties. Bovendien kunnen bloeien van gelatineus zoöplankton direct interfereren met menselijke activiteiten, bijvoorbeeld door het opstoppen en scheuren van commerciële visnetten, het netelen van badgasten of het doden van vis in open zeekwekerijen. Voor een goed beheer en om potentiële problemen en kosten gerelateerd aan kwallen bloeien te reduceren, is het noodzakelijk om de mechanismen achter deze bloeien te begrijpen.

In de jaren 80 werd de kamkwal *Mnemiopsis leidyi* A. Agassiz 1865, die oorspronkelijk voorkomt langs de Atlantische kusten van Noord- en Zuid-Amerika, geïntroduceerd via ballastwater in de Zwarte Zee. De densiteiten van deze invasieve soort namen snel toe en samen met overbevissing en eutrofiëring, droeg deze soort bij aan de ineenstorting van de belangrijkste visserij in dat gebied, wat enorme ecologische en socio-economische verliezen met zich meebracht. De eerste waarnemingen van deze niet-inheemse kamkwal in Noord-Europese mariene wateren in 2005 en de daaropvolgende observaties van *M. leidyi* in Belgische mariene wateren in 2007 zorgde bijgevolg voor de nodige bezorgdheid. **Deze doctoraatsthesis, kaderend in het Interreg IVa 2 Zeeën MEMO project, heeft als doel de structurele en functionele rol van de niet-inheemse kamkwal *Mnemiopsis leidyi* in de Zuidelijke Noordzee te bepalen. Deze studie zal in het bijzonder focussen op (1) de huidige verspreiding van *M. leidyi* in Belgische mariene wateren, de aangrenzende havens en het Westerschelde estuarium gerelateerd aan ander gelatineus zoöplankton, (2) de trofische ecologie en interacties van *M. leidyi* in het zoöplankton voedselweb, (3) de potentiële ecologische en socio-economische effecten van de aanwezigheid van deze soort in deze wateren, en (4) de algemene bedreiging van *M. leidyi* en de implicaties voor beheer van niet-inheemse soorten.**

Na een algemene inleiding (**hoofdstuk 1**) over kwallen en gelatineus zoöplankton, de potentiële introductieroutes van invasieve soorten en de karakteristieken van de levenscyclus van *M. leidyi* worden de data en resultaten gepresenteerd in zeven hoofdstukken. Er werden verschillende benaderingen gebruikt om de onderzoeksvragen en doelstellingen aan te pakken, waaronder veldonderzoek, experimenten en

laboratoriumwerk, databankanalyses en enquêtes. In deze doctoraatsthesis betekent de term 'gelatineus zoöplankton' de planktonische kwallenstadia (kwallen) van het fylum Cnidaria (classen Hydrozoa en Scyphozoa) en het fylum Ctenophora.

In de Zuidelijke Noordzee zijn lange-termijn data voor gelatineus zooplankton schaars, wat deels het gevolg is van de moeilijkheden bij het nemen van stalen en bij het bewaren van deze vaak 'fragiele' organismen, en *M. leidyi* in het bijzonder. In **hoofdstuk 2** worden daarom methodologische aspecten omtrent staalname en bewaring onderzocht. We focusten op twee verschillende planktonnettypes: een WP2 net (maaswijdte 200 µm) en een ring trawl net (maaswijdte 1000 µm). Gebaseerd op hun verschillende maaswijdte en gebruik (verticale versus undulerende sleep) evalueerden we of deze netten konden worden vergeleken in zake densiteit en grootteverdeling van *M. leidyi*. *Mnemiopsis leidyi* densiteiten van 245 staalname evenementen werden geanalyseerd volgens nettype en hieruit bleek dat WP2 netten geen goede inschatting geven van de aanwezigheid van *M. leidyi* in vergelijking met de ring trawl netten. Als *M. leidyi* aanwezig was in beide netten, dan werden veel hogere densiteitsschattingen gevonden voor het WP2 net (45.2 ± 114.0 ind.m^{-3} voor het WP2 net versus 12.8 ± 28.5 ind.m^{-3} voor het ring trawl net). Het ring trawl net gaf een goed overzicht van de adulte populatiestructuur, maar kan mogelijks de kleine kamkwallen onderschatten. Zowel het gefilterde volume als de maaswijdte bepalen dus grotendeels de vangst. We testten ook verschillende bewaringsmiddelen en –methodes met betrekking tot morfologische en genetische identificatie van *M. leidyi* en in functie van stabiele isotopen analyses. Uit onze experimenten konden we afleiden dat onbewaarde stalen worden verkozen voor elk type analyse. In verschillende situaties is onmiddellijke identificatie in het veld echter niet mogelijk en het staal bewaren is dan onvermijdelijk. In dit geval kunnen Lugoloplossing of RCL2® voor kortetermijnbewaring een goed alternatief bieden, hoewel het krimpen van specimens werd geobserveerd voor beide bewaringsmiddelen. Voor stabiele isotopen analyses leidden de verschillende bewaringsmethodes tot significante verschillen in zowel $\delta^{13}C$ als $\delta^{15}N$. Hiermee dient rekening te worden gehouden wanneer verschillende isotopen samenstellingen worden vergeleken. De bevindingen en aanbevelingen die geformuleerd worden in deze studie moeten worden overwogen in toekomstige monitoring van *M. leidyi*. Zooplanktonstalen verzameld tijdens dit doctoraatsonderzoek werden aan boord geanalyseerd en *M. leidyi* werd levend geïdentificeerd en gemeten (behalve voor Tentaculata larven, deze werden genetische geïdentificeerd).

De ontdekking van *M. leidyi* in Belgische wateren in 2007 vormde een unieke opportuniteit om onze kennis omtrent de verspreiding van gelatineus zoöplankton in de Zuidelijke Noordzee te verbeteren (**hoofdstuk 3**). Maandelijkse en seizoenale CalCOFI ring trawl stalen van het Belgische deel van de Noordzee en het aangrenzende Westerschelde estuarium werden verzameld tussen maart 2011 en februari 2012. Deze stalen onthulden de aanwezigheid van drie Scyphozoa, drie Ctenophora en 27 Hydrozoa taxa, inclusief drie niet-inheemse soorten: *M. leidyi*, *Nemopsis bachei* en *Lovenella assimilis*. In een addendum

(Addendum II) beschrijven we de herontdekking van larvale bidsprinkhaankreeften (*Rissoides desmaresti*) die niet meer waargenomen waren sinds 1913. Gemiddelde densiteiten van gelatineus zoöplankton waren hoogst nabij de kust (tot 18 ind.m^{-3}), en namen gradueel af naar de open zee toe, terwijl in de brakke Westerschelde, gemiddelde densiteiten onder 3 ind.m^{-3} bleven. Densiteiten van gelatineus zoöplankton waren hoogst in de zomer en herfst, maar de kamkwal *P. pileus* en de hydromeduse *Clytia* sp. waren jaarrond en op elke locatie vertegenwoordigd. De ruimtelijke en temporele verspreidingspatronen lijken vooral gedreven door temperatuur (seizoen) en saliniteit (locatie). Qua populatiedynamiek lijkt de kamkwal *Beroe* sp. de drie reproductieve cycli van zijn prooi *P. pileus* te volgen; maar deze soort kon ook profiteren van de hoge abundanties aan *M. leidyi* in de zomer en herfst. Deze studie biedt een stevige basis om potentiële gelatineuze zoöplankton toenames in het Belgisch deel van de Noordzee en de Westerschelde te evalueren.

Een analyse van de ruimtelijke en temporele verspreiding en populatie dynamiek van *M. leidyi* in de Zuidelijke Noordzee en de belangrijkste havens (**hoofdstuk 4**) toonde aan dat *M. leidyi* voorkomt van augustus tot december, en dat de soort nooit verder dan 30 km uit de kust werd teruggevonden. Densiteiten waren algemeen laag (gemiddeld 0.75 ± 2.84 ind.m^{-3}) vergeleken met andere gebieden in Europa waar de soort voorkomt. De hoogste densiteiten van *M. leidyi* werden in het half-ingesloten bekken in de haven van Oostende (18.4 ind.m^{-3}) en het Westerschelde estuarium (1.9 ind.m^{-3}) vastgesteld. De aanwezigheid van larven en de plotse verschijning van hoge aantallen over de hele lengteverdeling in augustus tonen aan dat havens en estuaria kunnen optreden als bronnen (sources) die de aangrenzende kustgebieden kunnen bevolken. Met behulp van een zero-inflated negatief binomiaal regressiemodel, werd aangetoond dat de geobserveerde variatie in *M. leidyi* densiteiten vooral gerelateerd is aan temperatuur (hoogste densiteiten als temperatuur begint te dalen), golfhoogte (hoogste densiteiten in laag energetische systemen) en zuurstofconcentraties (hogere densiteiten bij lage zuurstofconcentraties). Hoewel *M. leidyi* densiteiten relatief laag bleven sinds de eerste waarneming in Belgische wateren in 2007, konden we aantonen dat er een permanente populatie is gevestigd in de Zuidelijke Noordzee. Gezien 'bloeien' kunnen optreden bij slechts kleine veranderingen in omgevingsparameters, wordt verdere monitoring van deze beruchte niet-inheemse soort aanbevolen.

Kennis van het dieet van *M. leidyi* and zijn interacties met andere componenten van het pelagisch voedselweb zullen grotendeels bijdragen aan het bepalen van de impact van deze niet-inheemse soort op het ecosysteem. Door gebruik te maken van stabiele isotopen (SI; $\delta^{13}C$ and $\delta^{15}N$) en vetzuuranalyses (FA) konden we ruimtelijke en temporele variatie bepalen in de trofische ecologie van *M. leidyi* in verschillende ecosystemen in de Zuidelijke Noordzee (**hoofdstuk 5**). Gebaseerd op de isotopische samenstelling vonden we dat ruimtelijke verschillen eerder worden bepaald door variatie aan de basis van het voedselweb dan

veranderingen in het dieet van *M. leidyi* in de verschillende ecosystemen. Temporele variatie in de stabiele isotopen samenstelling van *M. leidyi* werd eveneens beïnvloed door het verschuiven van de baseline en gedreven door seizoenale veranderingen in de geassocieerde planktongemeenschappen. In dit hoofdstuk leveren we de eerste data aan betreft de vetzuursamenstelling van *M. leidyi* vergeleken met vetzuurconcentraties van twee andere (inheemse) kamkwallen. De totale vetzuurconcentratie van *M. leidyi* was drie tot vier keer lager dan die van *Pleurobrachia pileus* en *Beroe* sp. Hierdoor wordt deze niet-inheemse soort gecategoriseerd as vetarm organisme. Trofische interacties tussen *M. leidyi* en de twee daarmee samenvoorkomende kamkwallen (*P. pileus* en *Beroe* sp.) toonden bron differentiatie aan, wat het resultaat kan zijn van competitie of van het feit dat beide kamkwallen verschillende diëten hebben. Een mengeling van zoöplankton werd geïdentificeerd als potentiële voedselbron van *M. leidyi*. Vetzuurmerkers ondersteunden het carnivore dieet van *Beroe* sp., maar zijn stabiele isotopensamenstelling bevestigde niet dat het zich voedt met *M. leidyi*.

De voedingsecologie van *M. leidyi* werd verder onderzocht gezien zijn invasief succes deels gerelateerd is aan een breed en flexibel planktivoor dieet. In **hoofdstuk 6** onderzoeken we de voedingssnelheden en assimilatie van koolstof door *M. leidyi* d.m.v. voedingsexperimenten met een pelagische diatomeeënsoort *Phaeodactylum tricornutum* en drie potentiële mesozooplankton prooisoorten: nauplii van pekelgarnalen *Artemia salina*, de copepode soort *Acartia tonsa* en eieren en larven van Europese zeebaars *Dicentrarchus labrax*. 'Clearance rates' waren gemiddeld 0.2 ± 0.1 $L.mL_{M.leidyi}^{-1}.h^{-1}$, maar er waren geen significante verschillen aanwezig in clearance rates tussen prooitype en prooigrootte. De assimilatie van koolstof door M. leidyi voor deze verschillende prooitypes werd bepaald met behulp van ^{13}C tracer experimenten. De hoogste koolstofassimilatie werd geobserveerd voor Acartia en zeebaarslarven (meest efficiënt geassimileerd), en laagst voor de pelagische diatomee *P. tricornutum*. Om verder de prooi afhankelijke variatie in koolstofopname op te helderen, werd het effect van elk prooitype uitgedrukt in vetzuren als proxy voor voedselkwaliteit onderzocht. De consumptie van zeebaarslarven, gekenmerkt door hoge niveaus van het essentiële vetzuur DHA, resulteerde in significant hogere totale vetzuurconcentraties in *M. leidyi*. Gezien *M. leidyi* overschot aan voedsel niet omzet naar vetreserves, geeft de hogere voedselkwaliteit van vislarven aanleiding tot verbeterde overleving, groei en reproductie van deze soort, wat op zich bijdraagt aan zijn invasief succes. Gezien hogere temperaturen, gerelateerd aan de opwarming van de aarde kunnen leiden tot het vroeger voorkomen van *M. leidyi*, zal de temporale overlap met vislarven groter worden en zou een substantiële impact op de ichthyoplankton gemeenschap in de Zuidelijke Noordzee kunnen worden verwacht.

De derde doelstelling van deze doctoraatsstudie was het bepalen van de potentieel ecologische en socio-economische effecten van de aanwezigheid van *M. leidyi* in Belgische wateren. Voor het bepalen van deze socio-economische effecten verbreedden we onze

scope en focusten we op de impact van alle aanwezige kwallen op het toerisme aan de Belgische kust. *Mnemiopsis leidyi* is namelijk te klein en te fragiel is en wordt dus zelden opgemerkt door toeristen (behalve door duikers). We onderzochten in welke mate de belangrijkste kwallenberichten in de Vlaamse media overeenkwamen met de kennis en perceptie in de toeristische sector langs de Belgische kust (**hoofdstuk 7**). We doorzochten Vlaamse kranten naar artikels over kwallen gepubliceerd tussen januari 2000 en september 2012 en we voerden enquêtes uit aan de Belgische kust in de zomer van 2012. Het aantal Vlaamse krantenartikels steeg van minder dan 5 in 2000 naar 27 artikels in 2010. Bijna 75% van deze artikels rapporteerde over oorzaken en economische gevolgen van kwallenbloeien en veel artikels vermeldden de dramatische gevolgen van netelende, giftige en invasieve soorten. De analyse van de enquêtes toonde dat de perceptie van badgasten omtrent kwallen slechts deels wordt bepaald door de algemene media (vooral gerelateerd met de oorzaken van kwallenbloeien), terwijl persoonlijke ervaring (bv. netelende, slijmerige organismen) op zijn minst even belangrijk was. Gezien publieke perceptie een belangrijk aspect is voor bepaalde beleidsbeslissingen, zou in het kader van geïntegreerd kustzone beleid eenvoudige en goede informatie over kwallen (vb. borden op het strand) moeten voorzien worden. De resultaten van deze socio-economische studie kunnen dienen als basis voor toekomstige burgerwetenschap (citizen science) over kwallen.

Uiteindelijk werden de belangrijkste resultaten van deze doctoraatsstudie bediscussieerd in een breder perspectief naar het beheer van niet-inheemse of invasieve soorten (**hoofdstuk 8**). We voerden een risico analyse uit voor *M. leidyi* in de Zuidelijke Noordzee met behulp van de online Harmonia+ tool. Deze tool genereert blootstellingsscores (introductie, vestiging en verspreiding) en impactscores (op de omgeving en op menselijke activiteiten). De algemene risicoscore voor de niet-inheemse kamkwal *M. leidyi* in de Zuidelijke Noordzee was 0.286, wat als laag wordt beschouwd. Tot 2012 vertoonde *M. leidyi* duidelijke seizoenale bloeien in de zomer en herfst. Relatief hoge densiteiten (tot 18 ind.m^{-3}) werden enkel vastgesteld in de havens, die worden aanzien als gebieden met een lage ecologische waarde vergeleken met de 'rijkere' kustzone en Westerschelde, waar *M. leidyi* densiteiten onder 1 ind.m^{-3} bleven. De combiantie van periodes met ongunstige omgevingscondities, de competitie voor voedsel binnen de gelatineuze zoöplankton gemeenschap en de aanwezigheid van predatoren zoals *Beroe* sp., beperken wellicht het succes van *M. leidyi* in de Zuidelijke Noordzee. Toch toont deze doctoraatsstudie (inclusief de recentere waarnemingen in 2014) dat de populatie van *M. leidyi* zich heeft gevestigd in de verschillende ecosystemen van de Zuidelijke Noordzee. Daarom moet *M. leidyi* van dichtbij gevolgd worden met behulp van regelmatige monitoringscampagnes, en acties vanuit het beleid mogen niet worden uitgesteld. Enkel zo kunnen de huidige ecosysteemdiensten worden gevrijwaard. De populatie onder controle houden is nu wellicht de enige optie met het oog op een gepast beleid van deze niet-inheemse soort. Ratificatie van de ballastwaterconventie door alle landen over de hele wereld is ook belangrijk om zo

introducties van nieuwe soorten en mogelijke herintroducties van *M. leidyi* in onze wateren te vermijden. Met het oog op de Kaderrichtlijn Mariene Strategie moeten vroege detectie en bestrijding van niet-inheemse soorten, bijvoorbeeld door middel van geautomatiseerde toestellen gepromoot worden. Tenslotte is deze doctoraatsstudie de basis voor toekomstig monitoring van gelatineus zoöplankton in het Belgisch deel van de Noordzee en het Westerschelde estuarium (en bij uitbreiding de Zuidelijke Noordzee).

Photo previous page: © Karl Van Ginderdeuren

1

GENERAL INTRODUCTION

1.1 Jellyfish and their blooms

1.1.1 Jellification: a real problem?

In recent decades, jellyfish (Text box 1) abundances are thought to have increased all over the world, resulting in jellyfish-dominated ecosystems and food webs. This trend, also referred to as 'jellification', is based on a few local case studies (e.g. in the Black Sea (Kideys, 2002) or the Sea of Japan (Uye, 2008)) and media-driven public perception (Condon *et al.,* 2012). Overall, jellyfish have been neglected in traditional ecological surveys, resulting in only a few long-term jellyfish datasets (Brotz *et al.,* 2012). The lack of research interest is likely the result of the difficulties to sample and preserve these 'fragile' organisms, and by the absence of a direct (global) economic interest (e.g. as compared to fish) (Boero *et al.,* 2008; Laakmann and Holst, 2014). However, based on the available long-term jellyfish datasets, Condon *et al.* (2013) conducted a comprehensive analysis of the global temporal trends in jellyfish populations, but found no real evidence for 'jellification' of the world's oceans. Instead, they showed that jellyfish populations worldwide undergo large oscillations with a periodicity of approximately 20 years.

The formation of blooms or sudden increases in jellyfish densities during favourable conditions are a normal part of the population dynamics of jellyfish and inherent to their life cycle (Mills, 2001; Purcell, 2005). Judging from fossilised mass strandings, this phenomenon is probably ongoing for more than 500 million years (Hagadorn *et al.,* 2002). Inter-annual oscillations in jellyfish densities are mainly driven by natural fluctuations in the climate (Purcell *et al.,* 2007; Purcell, 2012; van Walraven *et al.,* 2014). Lynam *et al.* (2004) for example found a relation between jellyfish blooms in the northern North Sea and the North Atlantic Oscillation (NAO) climatic cycle. Another example is the recurrent presence and blooms of the scyphozoan jellyfish *Pelagia noctiluca* in the western Mediterranean, which has been linked to the combined effect of specific environmental parameters, *i.e.* lack of rainfall, high temperatures and high atmospheric pressure between May and August (Goy *et al.*, 1989).

Text box 1: 'Jellyfish' and 'Gelatinous zooplankton': what's in a name?

The broad terms 'jellyfish' and 'gelatinous zooplankton' are used to describe a broad spectrum of marine planktonic organisms, which are mostly transparent, characterised by a soft, gelatinous body and a total water content of approximately 95%. The term 'jellyfish' usually refers to the bell-shaped scypho-, hydro- and cubomedusae (Purcell, 2012), while 'gelatinous zooplankton' comprises the Ctenophora, scypho-, hydro- and cubomedusae, Siphonophora, Pteropoda, Thaliacea, Appendicularia (Larvacea) and a number of meroplanktonic larvae (Hamner, 1975). As such, approximately 2000 species are lumped together (Daly et al., 2007; Mills, 2011). In this PhD study, the term 'gelatinous zooplankton' was narrowed down to the planktonic medusa phase (jellyfish) of the phylum Cnidaria (classes Hydrozoa and Scyphozoa) and the phylum Ctenophora.

Armed with cnidocysts or colloblasts, most gelatinous zooplankton are carnivores feeding on almost anything, from unicellular organisms to much larger prey (Alvariño, 1985; Purcell and Mills, 1988). In favourable conditions, they can reach high abundances, often referred to as 'blooms'. However, the ecological role of jellyfish or gelatinous zooplankton is regularly oversimplified and even misunderstood. Therefore, Brotz et al. (2011) recommended to group jellyfish based on functional diversity (e.g. based on diet, metabolic demand, reproductive strategy, life history) rather than on taxonomic diversity. In this PhD study, a combination of both taxonomic and functional approaches has been used.

Besides natural inter-annual fluctuations in jellyfish densities, some human activities may locally contribute to jellyfish increases especially in coastal waters (Mills, 2001; Purcell, 2012). Anthropogenic proliferations and pressures resulting in global warming, overfishing, eutrophication, habitat modification and transport of non-indigenous species cumulatively change the ecosystem. Although direct evidence is often lacking, these pressures, and more specifically the combination of changing environmental parameters and shifts in the food web, can be related to varying jellyfish abundances in certain areas (Mills, 2001; Purcell *et al.,* 2007; Richardson *et al.,* 2009). For example, eutrophication encourages flagellate phytoplankton blooms. These flagellates are smaller than diatoms and therefore favour jellyfish instead of fish (Richardson *et al.,* 2009). Moreover, eutrophication may result in reduced oxygen levels, which are better tolerated by jellyfish than fish. Another example are the outbreaks of the scyphozoan jellyfish *Chrysaora hysoscella* in the Benguela upwelling system. Human overexploitation of the locally dominant, small filter-feeding anchovy stock resulted in altered competition relationships, in favour of this scyphomedusa (Lynam *et al.,* 2006). Both natural fluctuations as well as human pressures work simultaneously, which makes it difficult to distinguish whether an increase in jellyfish densities is a true increase as a result of human pressures or is part of the natural fluctuation in jellyfish blooms.

1.1.2 Interference with human activities

In many cases, vast jellyfish blooms leave an impression on the rather narrow human frame of reference, which probably nurtured the above described jellification paradigm. Moreover,

gelatinous zooplankton blooms may directly interfere with human activities such as fisheries (e.g. net clogging), tourism (e.g. stinging swimmers), aquaculture (e.g. killing farmed fish) and coastal industries (e.g. clogging of cooling-water intake screens) causing adverse socio-economic effects (Purcell *et al.,* 2007; Brotz *et al.,* 2012; Purcell, 2012). Purcell (2012) stated that problems with jellyfish will probably continue to increase due to human population growth and development, especially in the coastal areas. Therefore, understanding the mechanisms driving jellyfish outbreaks, by distinguishing natural and human-induced variations in jellyfish densities, is imperative in order to develop management actions (Richardson *et al.,* 2009). Additionally, Condon *et al.* (2013) recommended to invest in jellyfish monitoring to prepare our society for the recurrent phases of rise and fall in jellyfish populations.

1.2 Invasive *versus* non-indigenous species

1.2.1 Definitions and invasion process

According to the EU regulation on invasive alien species (1143/2014), 'non-indigenous or alien species' are "species (…) introduced [by natural means or human actions] outside their natural range (…), which might survive and subsequently reproduce". The same EU regulation defines 'invasive species' as "non-indigenous species whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services".

Invasive species have been identified as a major threat to marine ecosystems, leading to biodiversity loss, changes in the community structure and food webs, and causing adverse environmental, economic and social consequences (Darrigran and Pastorino, 1995; Occhipinti-Ambrogi and Savini, 2003; Molnar *et al.,* 2008).

The invasion process and the succession from 'non-indigenous' to 'invasive' species is shown in Figure 1.1. A successful invasion depends on both the recipient environment (the area's "invasibility") and the species' characteristics (Leung and Mandrak, 2007). The migration pathway represents the first step in this process and certain human activities, such as cross-oceanic ballast water transport, have shown to facilitate the ability of a certain species to reach new areas ("propagule pressure") (Lockwood *et al.,* 2005). Upon arrival, invaders must persist in the new environment and persistence depends upon the match between the individual species' traits and the new environment, which may be influenced by both evolutionary and environmental changes (Facon *et al.,* 2006). Evolutionary changes entail genetic changes in the invader's behaviour or feeding strategy resulting in a better match between species and environment. Wide physiological tolerances and a wide dietary niche, but also high fecundity, rapid growth and short generation times are common features of colonising species (Sakai *et al.,* 2001). Environmental changes (biotic or abiotic) may result in a better fit between the environment and the niche requirements of an invader. When the

recipient system is already under pressure (e.g. overfishing resulting in disturbed communities and the lack of top-predators) or when the ecological niche of the invader is not occupied, the chance for a successful invasion increases (Daskalov *et al.,* 2007; Richardson *et al.,* 2009).

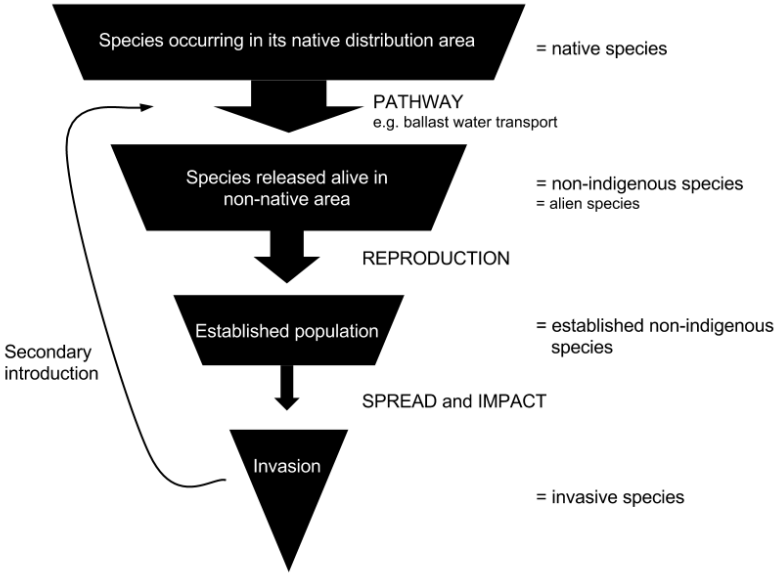


Figure 1.1 Different successive steps in the biological invasion process (based on Sakai *et al.,* 2001; Lodge *et al.,* 2006; and EU regulation on invasive alien species 1143/2014)

An overview of the non-indigenous species in the Belgian part of the North Sea up to 2012 has been described in Kerckhof *et al.* (2007) and by the VLIZ alien species consortium (Vandepitte *et al.,* 2012).

1.2.2 Ballast water as an introductory pathway

Due to increased globalisation, geographical barriers can be crossed and the numbers of marine biological invasions are rising (Carlton and Geller, 1993; Molnar *et al.,* 2008). The main introductory pathway of non-indigenous species at sea is international shipping, and more specifically by means of ballast water transport and hull fouling (Ruiz *et al.,* 1997; Streftaris *et al.,* 2005; Molnar *et al.,* 2008). Figure 1.2 illustrates that the areas frequently visited by cargo vessels and tankers are also most affected by invasive species.

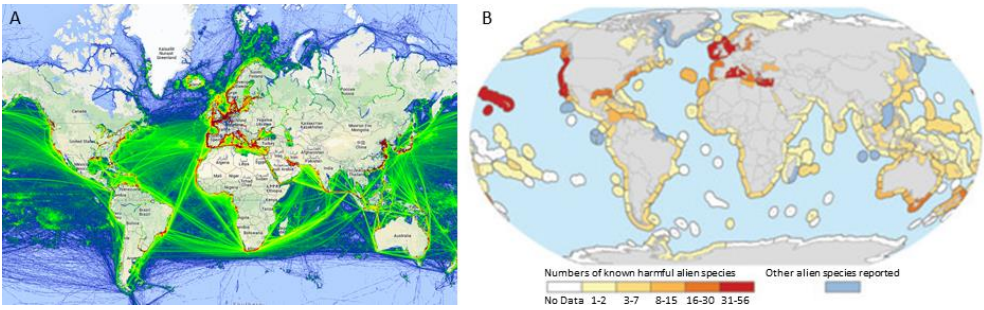


Figure 1.2 (A) map showing shipping routes of cargo vessels and tankers in 2014, colour gradient from green to red indicating increasingly crowded areas (from marinetraffic, 2015); (B) map with number of harmful alien species by coastal ecoregion, with darker shades of red indicating a greater number of species with high ecological impact; ecoregions with less harmful alien species are shown in dark blue (from Molnar *et al.,* 2008)

Ballast water is considered the most important vector for the transport of planktonic organisms. Since the 1880s, ships have used water as ballast to maintain the balance and stability of the ship when it is empty or only partially loaded with cargo (Figure 1.3). Globally, 3-5 billion tonnes of ballast water are transferred each year (GloBallast, 2015). These large volumes of ballast water contain entire coastal planktonic assemblages, which are transported from shallow, coastal waters across oceanic barriers within days or weeks to similar coastal habitats. Carlton and Geller (1993) analysed plankton samples from Japanese ballast water released in Oregon and identified 367 taxa. Of course not all transported organisms survive in the hostile environment of the ballast tanks, especially when large temperature differences are encountered, for example when crossing the equator (Zaiko *et al.,* 2015). Nevertheless, Kaluza *et al.* (2010) accounted for this limited amount of survivors in their stochastic population model, and still found that approximately 300 ports could be invaded by an invasive species from a random port within 50 years of the initial invasion.

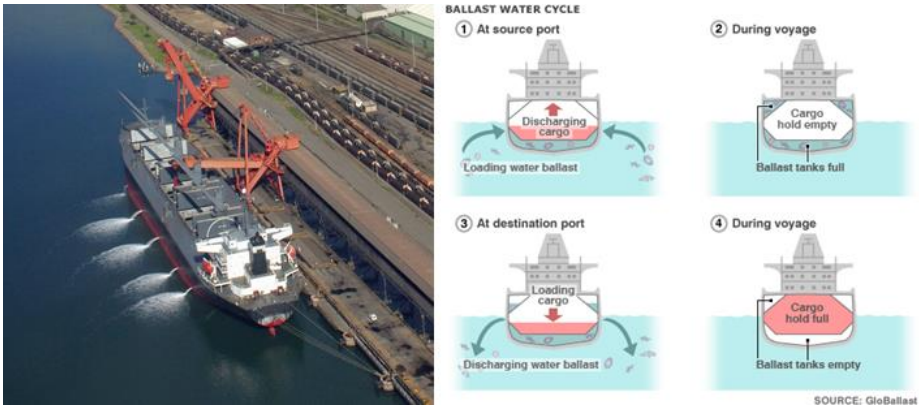


Figure 1.3 Ballast water is discharged in the port when the ship is loaded with cargo, the opposite happens at the port of destination when cargo is discharged (From Globallast, 2015)

Once non-indigenous species have established populations in the invaded habitat, it is nearly impossible to eliminate them (Thresher and Kuris, 2004). Therefore, a precautionary approach is highly recommended entailing the interception or removal of pathways (Carlton and Ruiz, 2005; Ojaveer *et al.,* 2015). In 2004, the international convention on the control and management of ships's ballast water and sediments (BWM Convention) was adopted, which aims to prevent the adverse effects of biological invasions by ballast water and sediments (GloBallast, 2015; IMO, 2015). It requires all ships to implement a ballast water management plan. Two main approaches have been developed to meet the Ballast Water Performance Standard: 1) ballast water exchange and 2) ballast water treatment (David and Gollasch, 2008; IMO, 2015). The BWM convention is a crucial step towards the reduction of the spread of non-indigenous species regionally and worldwide. The convention will enter into force 12 months after ratification by 30 states, representing 35% of world merchant shipping tonnage, which is currently not yet the case (dd. 22 September 2015 last update on www.imo.org).

1.3 The ctenophore *Mnemiopsis leidyi*

1.3.1 Invasion history

One of the most notorious marine invasive species is the ctenophore *Mnemiopsis leidyi* A. Agassiz 1865. From its native range along the temperate and subtropical Atlantic coasts of North and South America, this species has been transported several times across the Atlantic most likely in ballast waters (Figure 1.4; Purcell *et al.,* 2001).

Figure 1.4 Worldwide distribution of *Mnemiopsis leidyi* in its native area (green) and invaded areas (red) based on Costello *et al.,* 2012 and Javidpour *et al.,* 2006; Van Ginderdeuren *et al.,* 2012b; van Walraven *et al.,* 2013; Antajan *et al.,* 2014; Hosia and Falkenhaug, 2015

Especially the invasion of *M. leidyi* in the Black Sea in the 1980s did not remain unnoticed. The population expansion of the non-indigenous ctenophore coincided with a collapse in

commercial fisheries, causing large socio-economic and ecological losses (Vinogradov *et al.,* 1989; GESAMP, 1997; Kideys, 2002; Knowler, 2005; Oguz *et al.,* 2008). Research showed that concurrent environmental problems, including overfishing and eutrophication, contributed to both the collapsing fish stocks and the invasive success of *M. leidyi* (Bilio and Niermann, 2004; Daskalov *et al.,* 2007). In the 1990s, *M. leidyi* densities had reached up to 304 ind.m^{-3} (Vinogradov *et al.,* 1989) and the ctenophore had spread to adjacent seas (secondary introduction), including the Sea of Azov and Marmara, the Caspian Sea and Eastern Mediterranean 'Aegean' Sea (Studenikina *et al.,* 1991; Ivanov *et al.,* 2000; Shiganova *et al.,* 2001). Roohi *et al.* (2010) showed that the predatory impact of *M. leidyi* also had substantial effects on the Caspian Sea ecosystem causing trophic cascades (Text box 2). More recently, *M. leidyi* has spread in the Mediterranean Sea and has been reported since 2005 in the northern Adriatic Sea and the southern coast of France (Shiganova and Malej, 2009), and since 2009 from coastal waters in Israel (Galil *et al.,* 2009), Italy (Boero *et al.,* 2009) and Spain (Fuentes *et al.,* 2010).

Text box 2: "Massacre in the Caspian Sea"

Stone (2005) described the invasion of M. leidyi *in the Caspian Sea rather theatrically. Nevertheless, the seriousness of the situation and its devastating consequences are clear.*

"Bandar-E Anzali, Iran – The invasion began 6 years ago, when an advance force slipped into the Caspian Sea. A massacre followed. Three-quarters of the zooplankton species in the southern Caspian were annihilated, sending a shock wave through the food chain that dealt the biggest blow to kilka, a favourite of Iran's fishing industry. The aggressor – one of the most feared and reviled invasive species, the comb jelly Mnemiopsis leidyi *– had transformed the world's largest lake into a killing field."*

In northern Europe, *M. leidyi* was first observed in autumn 2005, more specifically in the Norwegian Oslo fjord (Oliveira, 2007). Furthermore, in several estuaries in northern France and in some important ports along the English Channel area (Calais, Gravelines and Dunkerque), *M. leidyi* was present from 2005 onwards (Antajan *et al.,* 2014). One year later, in 2006, *M. leidyi* was observed in the Baltic and North Sea, including the southern Dutch Zeeland estuaries (Faasse and Bayha, 2006; Javidpour *et al.,* 2006; Boersma *et al.,* 2007). The first record of *M. leidyi* in Belgium was reported by Dumoulin (2007) in the port of Zeebrugge in summer 2007. The species remained unnoticed at sea until 2009 (Van Ginderdeuren *et al.,* 2012b), most probably due to the lack of regular zooplankton monitoring in the Belgian part of the North Sea.

Both Reusch *et al.* (2010) and Ghabooli *et al.* (2011) found evidence of multiple introductions in different European areas, based on microsatellites and sequence variation in the ITS (Internal Transcribed Spacer) region, respectively. They concluded that the Black Sea was colonised from Central American populations (Florida, USA; Gulf of Mexico) while in

northern Europe organisms originated from North American populations (Narragansett Bay, Woods Hole, USA).

The most recent and detailed update on the distribution of *M. leidyi* in Europe is shown in Figure 1.5 (Jaspers *et al.,* 2014a). A recent modelling study based on temperature, salinity and food requirements for *M. leidyi* survival and reproduction showed that certain areas along the southern North Sea, which are currently not yet invaded, also serve as a potential habitat (Collingridge *et al.,* 2014): for example the Thames estuary (Bandura, 2013; Antajan *et al.,* in prep.). Furthermore, van der Molen *et al.* (2015; Addendum I) calculated that *M. leidyi* might be transported over considerable distances via currents in the North Sea and that colonisation from estuaries to other potential habitats is possible.

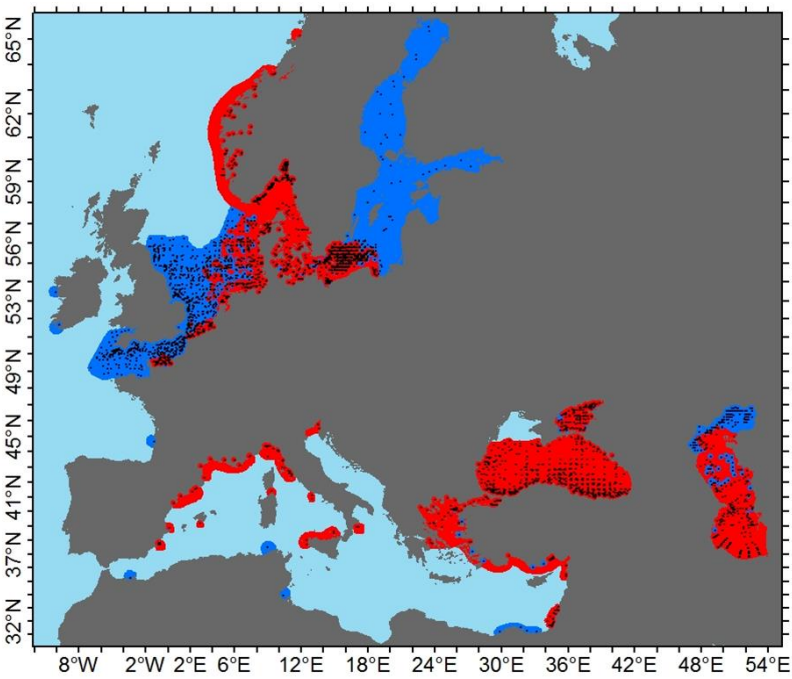


Figure 1.5 Recent and detailed overview of the distribution of *M. leidyi* in Europe with red = presence and blue = absence based on sampling locations (black dots) (adapted from Jaspers *et al.,* 2014)

1.3.2 Study area: The southern North Sea ecosystem

In this PhD study, we focus on the southern North Sea and in particular on the Belgian Part of the North Sea (BPNS) and the Westerschelde estuary (Figure 1.6). The BPNS has a surface of nearly 3500 km² and is bound by a 67 km sandy coastline. The Westerschelde (The Netherlands) covers 310 km² and stretches from Vlissingen (lower estuary) to Bath (upper estuary) over 58 km. It is characterised by a macro-tidal current regime, which keeps the water column (average depth 30 m) well mixed (Meire *et al.,* 2005). The estuary connects

important ports in Terneuzen and Antwerp with the North Sea through busy shipping lanes. Atlantic water is transported in a north-easterly direction through the Channel towards the BPNS, where the currents meet the south-westerly oriented Westerschelde estuarine outflow near the Dutch-Belgian border (Vlaeminck *et al.*, 1989; Lacroix *et al.*, 2004).

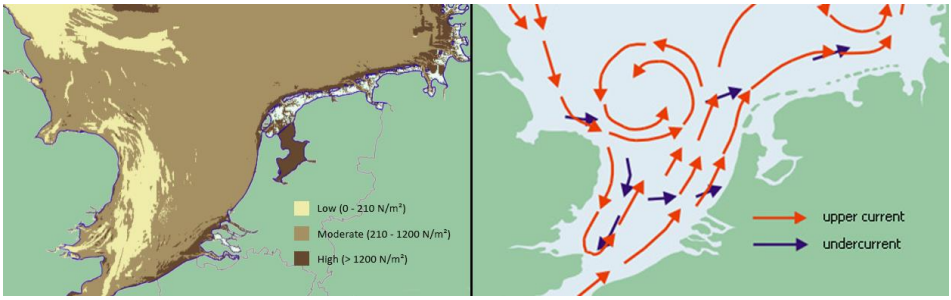


Figure 1.6 Study area southern North Sea with indication of wave power (left) and dominant currents (right)

The North Sea and English Channel follow the classic seasonal pattern for temperate regions. In winter, the water is well-mixed, nutrients are replenished and light is limited. Spring is characterised by a strong phytoplankton bloom (diatoms and flagellates) followed by a zooplankton bloom (Daro *et al.,* 2006; Van Ginderdeuren *et al.,* 2014), often becoming nutrient-limited when summer stratification sets in. Finally, in autumn, a smaller secondary bloom occurs, as increased mixing breaks down the thermocline and nutrients are released again (Hay *et al.,* 2011). In the shallower southern North Sea (depth rarely exceeds 80 m), locally-strong tidal and shelf fronts interfere with this general Atlantic pattern, leading to permanently mixed water columns.

The zooplankton community of the BPNS is a mixture of coastal species, combined with oceanic plankton species, that are occasionally imported with the inflow of oceanic water masses via the English Channel. The recent study of Van Ginderdeuren (2013a) gives a detailed overview of the species composition and its dynamics in the BPNS. Four species of scyphomedusae, 11 taxa of hydromedusae and three species of ctenophores (*i.e. Beroe gracilis*, *Pleurobrachia pileus* and *M. leidyi*) were described. Finally, the Belgian Register of Marine Species (BERMS, Vandepitte *et al.,* 2010) provides a list of all (zooplankton) species recorded in the BPNS up until 2010. Data from this PhD study will be included in the online version of this list (www.marinespecies.org/berms).

1.3.3 Identification

Mnemiopsis leidyi is a lobate ctenophore (Ctenophora: Tentaculata order Lobata), characterised by the presence of eight comb rows (*i.e.* cilia used for locomotion) and two large lobes. Costello *et al.* (2012) reviewed the taxonomic debate on whether or not there are two species in the genus *Mnemiopsis*: *M. leidyi* and *M. mccradyi*, the latter having papillate warts as only morphological difference. Based on molecular analysis, they

concluded that there was more evidence for the monospecific identity of *Mnemiopsis*. Any morphological differences observed can thus be attributed to phenotypic plasticity in respons to changes in the environment.

Mnemiopsis leidyi resembles *Bolinopsis infundibulum*, another lobate ctenophore, which is a native species in the North Sea, but prefers colder waters such as the northern North Sea, Baltic Sea and Arctic waters (Greve, 1975). During the first years of its introduction in northern Europe, *M. leidyi* might have been misidentified (morphologically) as *B. infundibulum* (Faasse and Bayha, 2006). The termination of the oral lobes is the main morphological feature that distinguishes adults of these two species. In *M. leidyi*, lobes terminate near the statocyst, whereas in *B. infundibulum* they end half-way between the mouth and the statocyst (Faasse and Bayha, 2006) (Figure 1.7).

Gorokhova and Lehtiniemi (2009) pointed to the importance of a correct identification and encourage genetic identification to support the morphological identification. Van Ginderdeuren *et al.* (2012b) used the internal transcribed spacer (ITS) DNA sequence as a genetic biomarker with primers KN8-9-11 (based on Fuentes *et al.,* 2010; Ghabooli *et al.,* 2011) to identify *M. leidyi* in Belgian waters. In this PhD study, these molecular techniques are used in particular to identify ctenophore larvae, because the cydippid stage of *M. leidyi* resembles that of any other tentaculate (Tentaculata), for example *Pleurobrachia pileus* larvae, which hampers morphological identification.

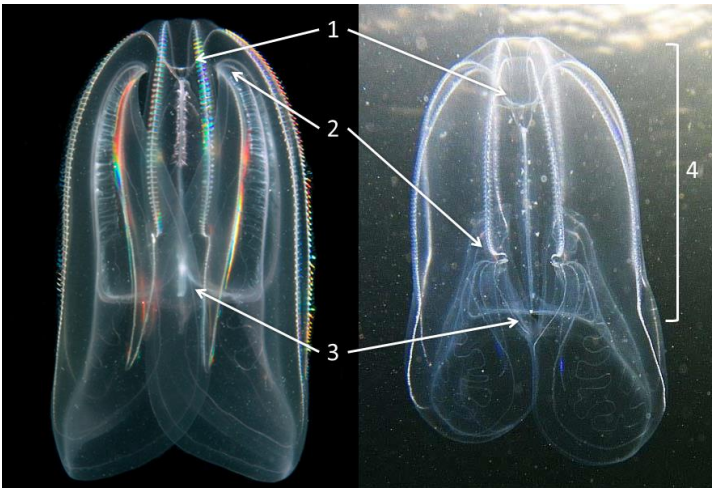


Figure 1.7 Morphological identification of *Mnemiopsis leidyi* (left) and *Bolinopsis infundibulum* (right) focussing on the termination of the oral lobes (2) relative to the position of the statocyst (1) and the mouth (3) (oral-aboral length (4) ~50 mm) © Bram Conings and Gordon Lang

1.3.4 Life cycle characteristics

Mnemiopsis leidyi is a holoplanktonic organism, which means that all four stages in the life cycle occur in the water column (Figure 1.8). Being a simultaneous, self-fertile

hermaphrodite, one adult can produce more than 11000 fertilised eggs per day (Baker and Reeve, 1974; Jaspers *et al.,* 2014b). Jaspers *et al.* (2015) showed that *M. leidyi* adults continue to produce eggs, even when starved for up to 12 days. The eggs (± 0.5 mm) hatch into tentaculate cydippid larvae. Ontogeny from cydippid to lobate stages entails the development of rudimentary oral lobes during the transitional stage. This generally begins when larvae are about 5 mm in total length, although some variability in size exists (Rapoza *et al.,* 2005). Additionally, Martindale (1987) described dissogony (*i.e.* the sexual maturity of larvae) at this stage. The transformation into a lobate adult is accomplished when tentacle regression is complete (± 15 mm; Rapoza *et al.,* 2005). Generation times can be short: at favourable temperatures (15-30 °C) and food levels (>24 µg $C.L^{-1}$), eggs can hatch and develop into reproducing adults within 13 days (Baker and Reeve, 1974; Kremer and Reeve, 1989).

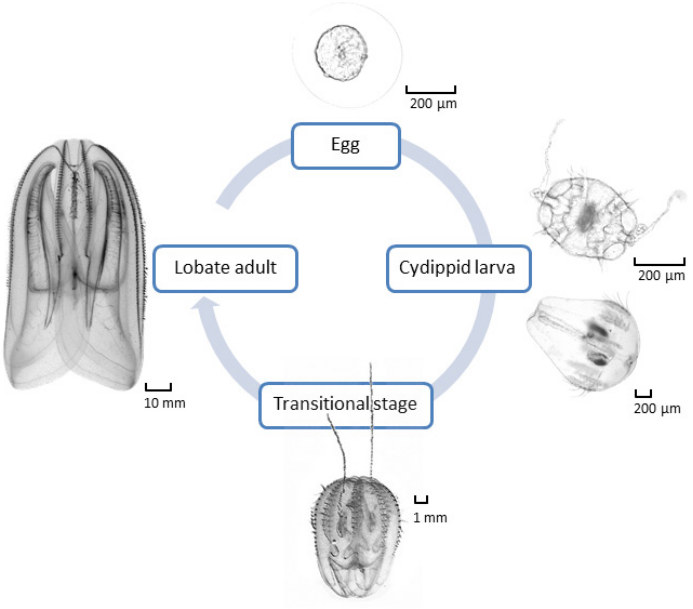


Figure 1.8 Four stages in the *M. leidyi* life cycle; note the different scale bars; cydippid larva is shown in both lateral and aboral view © ILVO and Bram Conings

The feeding mechanisms of *M. leidyi* vary depending on its ontogenetic stage. Cydippid larvae are ambush predators and use their tentacles to catch microplankton (Rapoza *et al.,* 2005; Sullivan and Gifford, 2007; Sullivan 2010). From the transitional stage onwards, a diet of microplankton alone is not sufficient to maintain growth (Sullivan and Gifford, 2007) and with the lobes expanding and the tentacles reducing, two other feeding mechanisms develop. The first involves creating a feeding current through the continuous beating of the cilia lining the four auricles (Waggett and Costello, 1999). This current entrains prey between the oral lobes without generating mechanical disturbances detectable by the prey

(Colin *et al.,* 2010). Prey with reduced mobility (e.g. larvae of crustaceans or fish eggs) are collected by the colloblasts on the tentillae and subsequently transported to the mouth (Waggett and Costello, 1999). Larger prey that can actively escape (e.g. copepods and fish larvae) are unresponsive to this current until they are surrounded by the lobes. When these prey collide with the inner surfaces of the lobes, the second feeding mechanism is triggered: contraction of the lobes. The prey are trapped, which reduces their chances to escape (Costello and Coverdale, 1998). Both prey capture mechanisms function simultaneously, and allow *M. leidyi* to feed on a broad range of prey, including micro-, meso- and ichtyoplankton (Javidpour *et al.,* 2009b; Granhag *et al.,* 2011; Costello *et al.,* 2012). Consequently, when *M. leidyi* densities are high, a considerable impact on the mesozooplankton populations may be measured (Deason and Smayda, 1982).

Other than its broad and flexible planktivorous diet, the invasive success of *M. leidyi* can be attributed to its broad tolerance to different environmental parameters, such as salinity (0-40), temperature (0-32 °C) or oxygen levels (as low as 1.0 mg L^{-1}) (Table 1.1; Purcell *et al.,* 2001; Decker *et al.,* 2004; Fuentes *et al.,* 2010; overview in Haraldsson *et al.,* 2012). However, the survival threshold of *M. leidyi* depends upon a combination of these parameters. For example, in the sea of Azov with surface salinities ranging between 0 and 14, *M. leidyi* does not survive temperatures <4 °C (Shiganova and Malej, 2009).

Table 1.1 (A)biotic survival and reproduction thresholds of *Mnemiopsis leidyi*

	Survival	Reproduction	References
Temperature (°C)	0-32	>12; 15-30	Kremer and Reeve, 1989; Purcell *et al.,* 2001; Lehtiniemi *et al.,* 2011
Salinity	0.1-40	≥6?	Shiganova *et al.,* 2004; Fuentes *et al.,* 2010; van Walraven *et al.,* in prep.
Oxygen (mg.L^{-1})	≥1.0		Decker *et al.,* 2004; Greve and Breitburg, 2005
Food (µg C.L^{-1})	>3	>24	Reeve *et al.,* 1989; Kremer, 1994; Jaspers *et al.,* 2015

The combination of high feeding, growth and reproduction rates and a broad environmental tolerance enables *M. leidyi* populations to thrive when conditions are favourable, but also gives it a competitive advantage when conditions are less favourable in a variety of ecosystems.

1.3.5 Dynamics of *M. leidyi* populations in native and exotic habitats

The native range of *M. leidyi* spreads over a large geographical area from temperate to subtropical latitudes (Purcell *et al.,* 2001; Figure 1.4). To elucidate the dynamics of *M. leidyi* in its native habitat, we focus on the northern populations (Atlantic coasts of North America) and more specifically on Narragansett Bay (Rhode Island, USA) and Chesapeake Bay (Virginia, USA), which harbour well-studied populations (reviewed in Purcell *et al.,* 2001). Differences with more southern populations are mainly situated in the size and timing of the peak abundances, being smaller and later in the year (Purcell *et al.,* 2001). In the northern populations, the ctenophore occurs year-round in the coastal waters, but shows clear seasonal dynamics. Three factors determine the abundance of *M. leidyi* with temperature being the most important, followed by food availability and mortality through predation (Kremer, 1994). During the warm (late) summer period, *M. leidyi* is capable of supporting extensive biomass and may dominate the planktonic biomass and planktonic community structure (Deason and Smayda, 1982; Condon and Steinberg, 2008). During the colder winter periods, *M. leidyi* is unable to reproduce and densities are reduced (Purcell *et al.,* 2001; see §1.3.4). However, also predation was considered as an important driver of the distribution and abundance of *M. leidyi*. Three predators co-occur in the native habitat of *M. leidyi* (Purcell *et al.,* 2001; Condon and Steinberg, 2008). In Narragansett Bay, *Cyanea capillata* scyphomedusae feed on the ctenophores in spring, *Chrysaora quinquecirrha* scyphomedusae excert predation pressure in summer, and *Beroe ovata* was shown to reduce *M. leidyi* populations in September (Kremer and Nixon, 1976; Purcell *et al.,* 2001). In addition, a variety of fishes are known to consume gelatinous species. Especially harvestfish, *Peprilus alepidotus* and butterfish *P. triacanthus* are known predators of *M. leidyi*, but predation is often inhibited due to their poor tolerance for low salinities in contrast to *M. leidyi* (GESAMP, 1997).

A successful invasion of *M. leidyi* in the Black Sea (exotic habitat) was facilitated by changes in the ecosystem due to several anthropogenic pressures (reviewed in Purcell *et al.,* 2001). Similar to its native habitat, *M. leidyi* prefers the inshore waters, where two density peaks (instead of one in the native area) were measured in spring and autumn respectively. Provided that inter-annual variation occurred, these two peaks yearly persisted until the arrival of the ctenophore *Beroe ovata* in the late 1990s (Finenko *et al.,* 2003). This new predator was present from spring until late summer and substantially reduced *M. leidyi's* population outbreaks. Besides predation pressure, also temperature and salinity influence survival especially in winter (e.g. Shiganova and Malej, 2009; §1.3.4).

1.4 Research framework and objectives

1.4.1 The MEMO project

This PhD study was part of the Interreg IVa 2 Seas project 'MEMO' (*Mnemiopsis* ecology and modelling: Observation of an invasive comb jelly in the southern North Sea). The motivation

to start this EU-project was driven by three facts: 1) a non-indigenous ctenophore (*M. leidyi*) was present in the southern North Sea, 2) the same species had caused dramatic changes in the Black Sea ecosystem and 3) very little was known on the distribution, ecology and potential impact of the species in the southern North Sea area. This caused concern and lead to a partnership between five scientific research institutes from France (Ifremer – Institut français de recherche pour l'exploitation de la mer and ULCO-LOG – Université du Littoral Côte d'Opale – Laboratoire d'Océanology et de Géosciences), the UK (CEFAS – Centre for Environment, Fisheries and Aquaculture Science), Belgium (ILVO – Institute for Agricultural and Fisheries Research) and The Netherlands (Deltares). Between January 2011 and December 2013, the following activities were investigated in the MEMO project:

In activity 1, we explored the spatial and temporal distribution of *M. leidyi* based on existing and dedicated sampling surveys by all partners using the same protocol for sampling and preservation (Van Ginderdeuren *et al.,* 2012b; Bandura, 2013; Antajan *et al.,* 2014; Vansteenbrugge *et al.,* 2015b; Antajan *et al.,* in prep; Chapter 2 (Vansteenbrugge *et al.,* in prep.a)). The obtained information was compiled into an integrated database and used to develop several models regarding reproduction, survival and dispersal of *M. leidyi* (Collingridge *et al.,* 2014; van der Molen *et al.,* 2015; David *et al.,* 2015).

In activity 2, the physiology and feeding ecology of *M. leidyi* were studied through laboratory experiments and field measurements. More specifically, grazing experiments were executed, field samples were analysed using biochemical markers and a Dynamic Energy Budget (DEB) model was developed (Augustine *et al.,* 2014; Chapter 5 (Vansteenbrugge *et al.,* in revision); Chapter 6 (Vansteenbrugge *et al.,* in prep.b)).

In activity 3, socio-economic studies were conducted in Belgium, the UK and France by means of questionnaires. These results were used to estimate the (potential) impact of *M. leidyi* (and jellyfish in general) on several groups of stakeholders, *i.e.* tourists, fishermen, local officials of seaside communities and coastal industries with cooling-water intake screens (Schaafsma *et al.,* 2013; Vandendriessche *et al.,* in revision).

The different chapters of this PhD thesis contributed to each of the three activities investigated in the MEMO project.

1.4.2 Research objectives

For the Belgian part of the North Sea, only few historic zooplankton studies are available and they sporadically mention gelatinous species (Van Meel, 1975; Rappé, 1989; Gilson collection (1898–1939) as presented in Van Loen and Houziaux, 2002; De Blauwe, 2003). In addition, these studies mostly present qualitative (e.g. beach findings) rather than quantitative data. The discovery of *M. leidyi* in our waters created a unique opportunity to enhance our knowledge on gelatinous zooplankton as an often 'forgotten' ecosystem component.

This PhD thesis aimed to assess the structural and functional role of the non-indigenous ctenophore *Mnemiopsis leidyi* in the southern North Sea. More specifically, we focused on (1) the current distribution of *M. leidyi* in Belgian marine waters, the adjacent ports and the Westerschelde estuary, related to other gelatinous zooplankton, (2) the trophic ecology and interactions of *M. leidyi* in the planktonic food web, (3) the potential ecological and socio-economic effects of the presence of *M. leidyi* and other gelatinous zooplankton in these waters, and (4) the overall threat of *M. leidyi* and the implications for non-indigenous species's management.

The following research questions were investigated:

- What are the effects of using different net types for quantitative sampling of *M. leidyi* and how do different preservation techniques influence morphological and genetic identification of this fragile species?
- What is the current spatial and temporal distribution of *M. leidyi* in relation to the associated zooplankton community in the Belgian part of the North Sea (BPNS) and the adjacent Westerschelde estuary?
    - How is the gelatinous zooplankton community distributed in terms of density and diversity?
    - Which environmental drivers govern the current spatial and temporal distribution of *M. leidyi* and what is the course of the population dynamics?
- How does *M. leidyi* behave in the food web of the southern North Sea (including the BPNS and Westerschelde estuary)?
    - How can the variation in the trophic ecology of *M. leidyi* be explained and what are the trophic interactions of this species with co-occurring (native) ctenophores and potential food sources in the southern North Sea food web?
    - What is the effect of different prey types and their quality in terms of fatty acids on the feeding rates and carbon assimilation of *M. leidyi*?
- What is the perception of the tourism sector at the Belgian coast on jellyfish (blooms), compared to newspaper articles on this matter?
- What is the overall threat of *M. leidyi* to the southern North Sea ecosystem and what are the implications for non-indigenous species' management?

The knowledge and experience obtained in this PhD study is crucial for current and future management decisions considering *M. leidyi* as a non-indigenous species in the southern North Sea. It highlights the need to evaluate already-established non-indigenous species and to take adequate management actions to prevent new introductions in order to preserve current ecosystem services (in particular provisioning services). Finally, it illustrates the need to invest in basic zooplankton (including gelatinous zooplankton) monitoring, with the presented data forming the baseline against which a potential increase in gelatinous

zooplankton in the Belgian part of the North Sea and Westerschelde estuary can be measured.

1.5 Outline of the PhD thesis

The current chapter (**Chapter 1**) provides a general background on *M. leidyi* (and jellyfish in general) and summarises the objectives and outline of this PhD thesis. The main findings are presented in the following seven chapters (Chapters 2-8) organised in five parts. The interrelations between the different parts are presented in Figure 1.9. Different approaches have been used to tackle the research questions and objectives, including field studies, experimental and laboratory work, database analyses and public questionnaires.

In **Part I** (**Chapter 2;** Vansteenbrugge *et al.,* in prep. a), sampling and preservation of *M. leidyi* was discussed. Considering the potential threat of *M. leidyi* as an invasive species, monitoring is imperative, but it is hampered by the species' fragility. Different net types were compared in terms of *M. leidyi* densities and population structure and different preservation solutions and methods were tested regarding morphological and genetic identification of *M. leidyi*. Additionally, the effect of several pre-treatment methods was determined with respect to the outcome of stable isotope analyses. Several recommendations were formulated for monitoring of *M. leidyi*.

In **Part II** the current distribution of *M. leidyi* in relation to the associated zooplankton community was investigated. More specifically, **Chapter 3** (Vansteenbrugge *et al.,* 2015a) assembled detailed information on the current spatio-temporal distribution and population dynamics of the gelatinous zooplankton community in the Belgian part of the North Sea and the adjacent Westerschelde estuary. Both diversity and densities of Hydrozoa, Scyphozoa and Ctenophora were discussed with respect to the non-gelatinous zooplankton community and some environmental parameters. **Chapter 4** (Vansteenbrugge *et al.,* 2015b) zoomed in on the spatio-temporal distribution of *M. leidyi* over a 2-year period. Source-sink dynamics were identified for the study area and a zero-inflated logistic regression model was developed to link the most important environmental drivers to the observed population dynamics.

Part III focused on *M. leidyi* in the food web of the southern North Sea. In **Chapter 5** (Vansteenbrugge *et al.,* in revision), the trophic ecology of *M. leidyi* was investigated in three different systems: Belgian and Dutch coastal waters, major ports in northern France and Belgium, and three estuarine systems (Westerschelde, Oosterschelde and Grevelingen). Biochemical tracer analyses (stable isotopes and fatty acids) were performed, providing an analysis of the diet integrated over time. The contributions from different food sources were elucidated, based on the 'you are what you eat' principle. Spatial, temporal and ontogenetic variation in the diet were investigated and the interactions with the associated planktonic food web was examined. In **Chapter 6** (Vansteenbrugge *et al.,* in prep.b), lab experiments

were performed to investigate the effects of different prey types and their quality in terms of fatty acids on the feeding rates and carbon assimilation of *M. leidyi* using stable isotope and fatty acid biomarkers.

In **Part IV** (**Chapter 7**; Vandendriessche *et al.,* in revision), the perception of different stakeholders from the tourism sector on jellyfish (blooms) was described. A questionnaire survey (**Addendum III**) was performed in the summer of 2012 among tourists, recreational users, professionals and local officials of seaside communities along the Belgian coast. The answers were compared to what was presented on jellyfish in newspaper articles in the general media in Flanders. Results were discussed with respect to integrated coastal zone management.

Finally, in **Part V (Chapter 8)**, the main results of this PhD study were discussed in the broader perspective of non-indigenous species management. First, a risk assessment of *M. leidyi* in the southern North Sea was performed (**Addendum IV**), based on expert judgement, using the Harmonia+ protocol (D'hondt *et al.,* 2015). The overall threat (risk) was estimated based on the exposure (introduction, establishment and spread) and impact (on the environment and human activities) scores. Then, implications of our results were translated to policy and management recommendations. Finally, the main conclusions of this PhD study were summarised and remaining challenges concerning *M. leidyi* and other non-indigenous species were put forward.

Four addenda were added:

Addendum I is related to **Chapters 1 and 8**. A modelling study is presented focusing on connectivity between different *M. leidyi* habitats in de southern North Sea and considering the species' characteristics in terms of survival and reproduction (van der Molen *et al.,* 2015).

Addendum II is related to research performed in **Chapter 3** and describes the re-discovery of larval mantis shrimp (*Rissoides desmaresti*) in Belgian waters since 1913 (Gilson collection, RBINS) (Vansteenbrugge *et al.,* 2012).

Addendum III is related to **Chapter 7** and provides the questionnaire which was used to evaluate the perception of the tourism sector concerning jellyfish (blooms) (Vandendriessche *et al.,* in revision).

Addendum IV is related to **Chapter 8** and presents the risk assessment of *M. leidyi* in the southern North Sea using the Harmonia+ protocol.

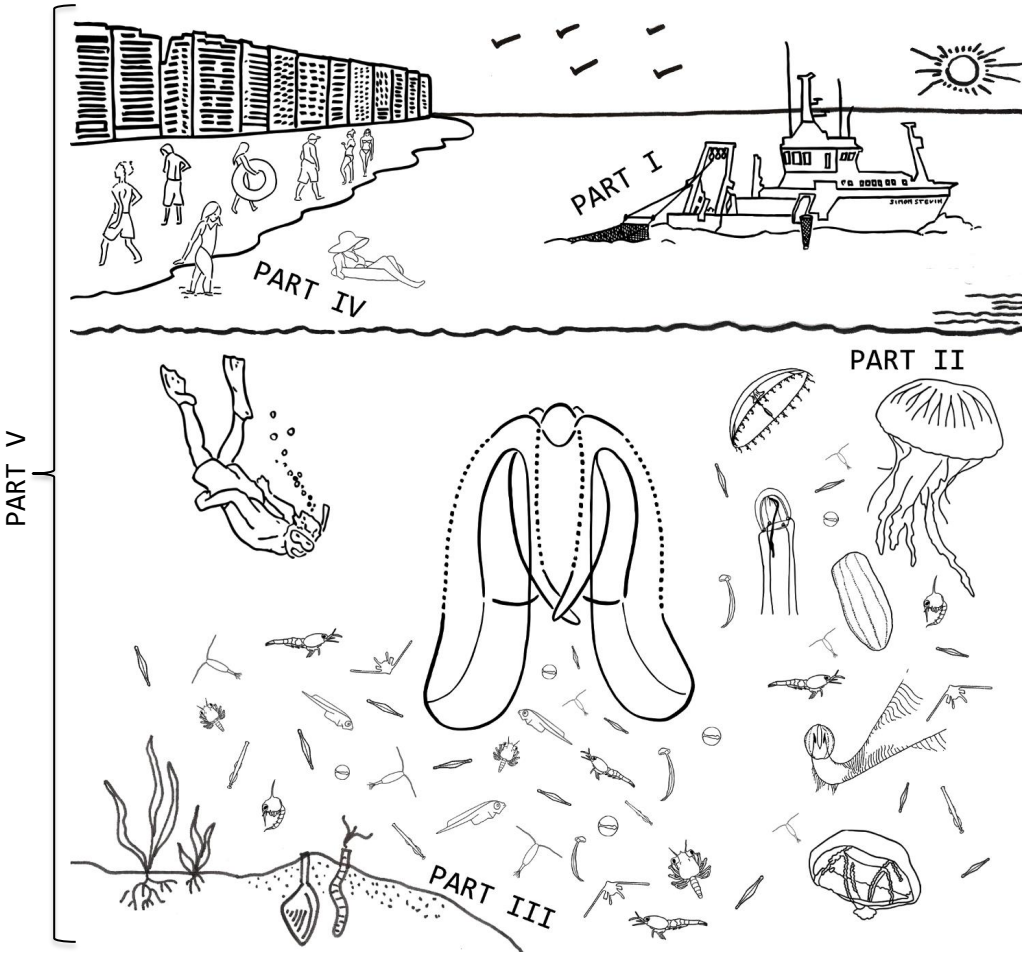
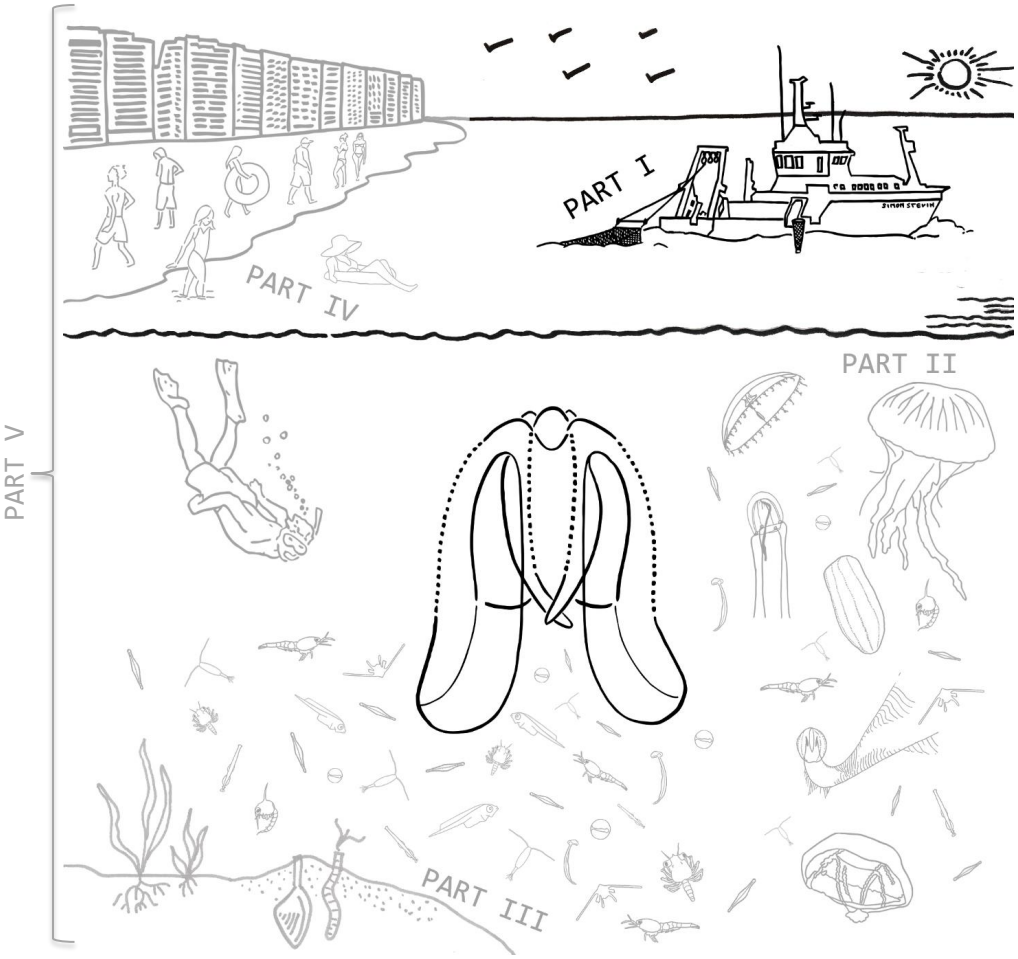


Figure 1.9 Overview of the different parts covered by this PhD thesis and their interrelations

PART I



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2

EVALUATING DIFFERENT SAMPLING AND PRESERVATION TECHNIQUES FOR THE FRAGILE CTENOPHORE *MNEMIOPSIS LEIDYI* (CTENOPHORA, LOBATA)

Manuscript in preparation:

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ABSTRACT

Gelatinous planktonic organisms are often excluded from zooplankton studies partly due to their fragility. Especially the ctenophore *Mnemiopsis leidyi* frequently gets damaged during sampling and standard preservatives hinder both morphological and genetic identification. Considering its potential threat as an invasive species in the southern North Sea, monitoring its distribution and abundance is imperative. Therefore, some methodological issues needed to be solved. In this study, we focused on two different types of plankton nets: a WP2 net (mesh size 200 µm) and ring trawl net (mesh size 1000 µm). Based on their different mesh size and way of deployment (vertical versus undulating trawl), we evaluated whether they can be compared in terms of *M. leidyi* density and size distribution. *Mnemiopsis leidyi* densities from 245 sampling events were analysed according to net type and revealed that WP2 nets do not provide a good estimate of its presence compared to ring trawl nets. Moreover, when *M. leidyi* was present in both nets, much larger density estimates were found by the WP2 net (45.2 ± 114.0 ind.m^{-3} for WP2 net versus 12.8 ± 28.5 ind.m^{-3} for ring trawl net). The ring trawl net gave a good overview of adult population structure, but may underestimate some of the small ctenophores. Consequently, both the filtered volume and the mesh size largely determine the catch. We also tested different preservation solutions and methods with respect to morphological and genetic identification of *M. leidyi* and in function of stable isotope analyses. From our experiments it became clear that unpreserved samples are preferred for any type of analysis. However, short-term preservation in Lugol's solution or RCL2® may provide a good alternative, but shrinkage was observed in both preservatives. For stable isotope analyses, different preservation methods resulted in significant differences in both $\delta^{13}C$ and $\delta^{15}N$, which should be considered when comparing different isotopic compositions. The findings and recommendations formulated in this study should be considered in future *M. leidyi* monitoring.

2.1 Introduction

Gelatinous planktonic organisms are often excluded from zooplankton studies, partly due to their fragility (Boero *et al.,* 2008). These organisms frequently get damaged during sampling, and preservatives may hinder both morphological and genetic identification (Laakmann and Holst, 2014). Recently, the adverse effects of jellyfish blooms on fisheries, industries and tourism, gave rise to a growing interest in gelatinous zooplankton (Richardson *et al.,* 2009; Brotz *et al.,* 2012; Purcell, 2012). Consequently, some methodological issues needed to be solved (partly reviewed in Purcell, 2009). In this study, we focus on the ctenophore *Mnemiopsis leidyi* A. Agassiz 1865. Considering its potential threat as an invasive species in the southern North Sea, monitoring its distribution and abundance is imperative. However, in comparison with native ctenophores, such as *Pleurobrachia pileus* and *Beroe gracilis*, *M. leidyi* easily gets damaged when squeezed against the mesh of a net, when handled outside the water and when preserved in standard preservation solutions (e.g. formaldehyde solution) (personal observation).

Several sampling techniques have been used to study *M. leidyi*, from standard plankton nets (e.g. MIK, Isaac-kid, Bongo, WP2, WP3, CalCOFI, MOCNESS and Multinet; as described in UNESCO, 1968; Harris *et al.,* 2000; Wiebe and Benfield, 2003) to handheld dip-nets, buckets and simple home-made devices (Figure 2.1). Although the latter allow to obtain undamaged specimens (Raskoff *et al.,* 2003), these simple techniques are mainly appropriate for qualitative sampling. For quantitative sampling, plankton nets are most frequently used and the choice of a particular net mainly depends on the sampling environment. A multinet for example allows to sample at different depths and has been succesfully used to study *M. leidyi* in deep fjords (Haraldson *et al.,* 2012). Closing the cod-end mesh in plankton nets (*i.e.* a non-filtering cod end) and using a larger cod-end generally limits the damage to the gelatinous organisms (Hosia and Pagès, 2007). The mesh size is an essential net feature since it determines the composition of the catch (Tseng *et al.,* 2011). In fact, large-mesh-sized plankton nets underestimate the smaller fraction of the zooplankton. Other factors to consider are the capacity of the research vessel and the appropriate deployment of the plankton nets in relation to water depth and current speed (overview in Wiebe and Benfield, 2003). There is a choice between vertical or oblique tows and also the tow speed and duration can be adjusted (Wiebe *et al.,* 2014). All these factors influence the filtered volume, which is an important value to determine density. When standardised plankton nets cannot be used for example due to logistic constraints, handheld dip-nets may still provide an alternative for quantitative estimates by using the towed distance to determine the filtered volume and density.

Other then plankton nets, scuba divers can gather information on the presence/absence of *M. leidyi* and can visually estimate the abundance or collect qualitative samples (e.g. Costello and Mianzan, 2003; Antajan *et al.,* 2014; Chapter 4). Optical devices have also been used to

investigate for example diel vertical migration of *M. leidyi*. Haraldson *et al.* (2014) operated a video-plankton-net (a plankton net with a camera in the open cod end), which allowed to quantify and measure the specimens passing through the net from the video footage (Figure 2.1E). Other optical tools include an *In Situ Ichthyoplankton Imaging System* (ISIIS; Luo *et al.,* 2014), *Video Plankton Recorder* (VPR; Davis *et al.,* 1996), *Shadowed Image Particle Profiling and Evaluation Recorder* (SIPPER; Remsen *et al.,* 2004) and *Zooplankton Visualisation and Imaging System* (ZOOVIS; Bi *et al.,* 2012). As handling errors can be avoided, the popularity of these optical tools is rapidly increasing, especially when focusing on small and fragile animals.

All these sampling techniques have their advantages and disadvantages. However, it is unclear to what extent results (e.g. in terms of densities and size distribution) can be compared, especially for fragile gelatinous zooplankton.

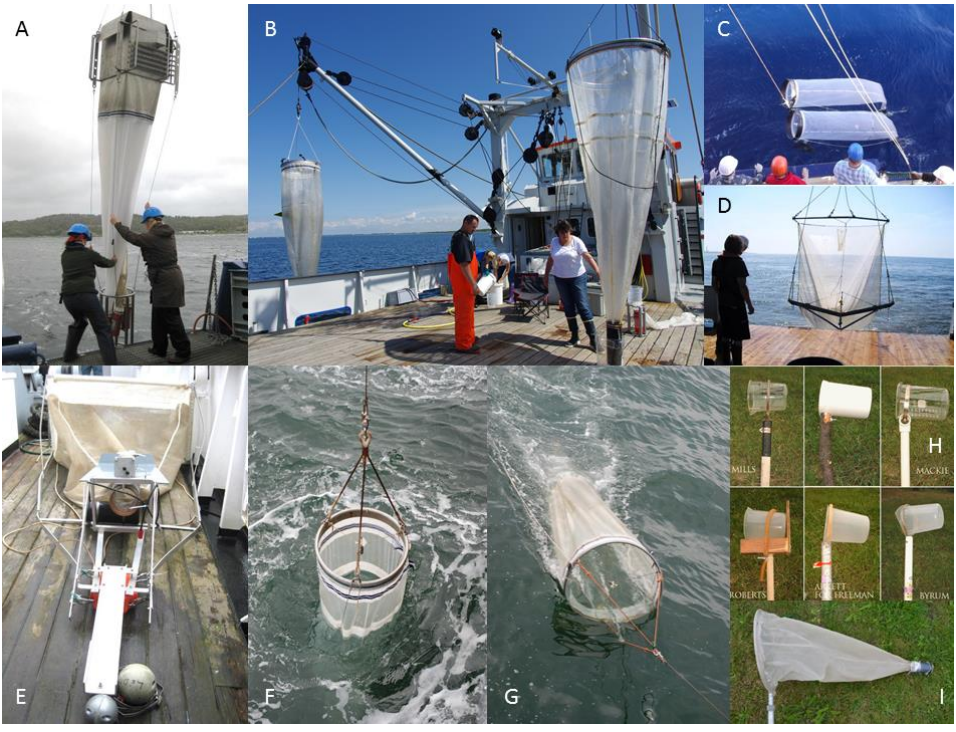

Figure 2.1 Sampling jellyfish with plankton nets: (A) Multinet; (B) CalCOFI net (left) and WP3 net (right); (C) Bongo net; (D) Isaac-kid mid water trawl; (E) video-plankton-net. Difference between vertical tow with WP2 net (F) and oblique haul with CalCOFI net (G). (H) Different versions of 'home-made' devices (© C.E. Mills) for qualitative jellyfish sampling and (I) handheld dip-net

Besides damage caused by sampling, fixation or preservation also hampers the morphological and genetic identification of gelatinous zooplankton, and *M. leidyi* in particular. Schuchert (2012) recommended to examine *living* gelatinous zooplankton

specimens for morphological identification, because diagnostic features, such as pigmentation, are often unrecognisable after preservation. Although most hydrozoan and scyphozoan medusae are well-preserved in standard formaldehyde solution, ctenophores, such as *M. leidyi*, are notably difficult to preserve in this solution (exception *Pleurobrachia* sp.; personal observation; Sullivan and Gifford, 2009 and references therein). Purcell (1988) described the disintegration of *M. leidyi* in formaldehyde solution, but argued that its remaining tentacle bulbs still allow to determine its abundance in the sample. However, during algal blooms (e.g. *Phaeocystis* sp.), this is rather difficult. To overcome the preservation difficulties, Adams *et al.* (1976) suggested a protocol which targets histological preparations, using Trichloroacetic acid (TCA), propylene phenoxetol, propylene glycol and formaldehyde solution. This is a labour intensive technique and therefore rarely used in monitoring studies (but see van Walraven *et al.,* 2013 appendix).

Another commonly used preservative in monitoring studies is ethanol. Depending on the concentrations, ethanol causes shrinkage, contraction and distortion of diagnostic features, hampering proper morphological identification (Russell, 1953; Schuchert, 2012). However, absolute ethanol (>99%) is recommended as the better preservative for subsequent DNA analyses (Schuchert, 2005). Genetic identification is essential when morphological identification is hampered or to identify ctenophore larvae, because the cydippid stage of *M. leidyi* resembles that of any other Tentaculata. Therefore, complementary (preferably time-saving and budget friendly) alternatives are needed, which allow for later analyses in the laboratory.

In non-native habitats, *M. leidyi* may have an impact on the overall functioning of an ecosystem, particularly on the food web (GESAMP, 1997; Thompson *et al.,* 2012). Biomarkers, such as stable isotopes (SI), are useful tools to elucidate food web relationships (e.g. Ying *et al.,* 2012; Nagata *et al.,* 2015). However, several studies have shown that the outcome of SI analysis may depend on the preparation and preservation of the samples prior to analysis (e.g. freezing, freeze drying, ethanol-preserved; Pitt *et al.,* 2009; Fleming *et al.,* 2011; D'Ambra *et al.,* 2014). More specifically, $\delta^{13}C$ values and ^{15}N values may be significantly more enriched in frozen or ethanol-preserved specimens compared to fresh ones (Feuchtmayr and Grey, 2003; Fleming *et al.,* 2011).

Considering the fragility of *M. leidyi* and existing methodological pitfalls, sampling and preservation protocols of *M. leidyi* needed further investigation. In this study, we focused on two different types of plankton nets: a WP2 net (mesh size 200 µm) and ring trawl net (mesh size 1000 µm). Based on their different mesh size and way of deployment, we evaluated whether they can be compared in terms of *M. leidyi* density and size distribution. We also tested different preservation solutions and methods with respect to morphological and genetic identification of *M. leidyi* and in function of stable isotope analyses. Several recommendations are formulated for future monitoring of *M. leidyi*.

Table 2.1 Overview of the 32 locations sampled over a 4-year period using both WP2 and ring trawl nets (coordinates in WGS84)

Area	Station	Location	Lat (N)	Long (E)	2011	2012	2013	2014
German part of the North Sea	R0061	intermediate	53,91	7,16			J	
	R0062	intermediate	53,93	7,42			J	
	R0063b	coastal	54,12	8,21			J	
	R0069	intermediate	54,84	7,60			J	
	R0073	coastal	54,62	8,09			J	
Dutch part of the North Sea	Q0091	intermediate	53,65	4,76		J		
	S0061	open sea	54,21	4,16				J
Dutch estuaries	GR1	Grevelingen	51,75	3,83		S	J F M J J A O N D	
	GR2		51,75	3,88			J F M J J A O N D	
	GR3		51,74	3,95			J F M J J A O N D	
	GR4		51,72	3,99			J F M J J A O N	
	GR5		51,69	4,04			J F M J J A O N	
	GR6		51,69	4,13			J F M J J A O N	
	OS1	Oosterschelde	51,60	3,74		S	J F M J J A O N D	
	OS2		51,61	3,84		S	J F M J J A O N D	
	OS3		51,57	3,90		S	J F M J J A O N D	
	OS4		51,53	3,98		S	J F M J J A O N D	
	OS5		51,52	4,08		S	J F M J J A O N D	
	OS6		51,48	4,18		S	J F M J J A O N D	
	OS7		51,63	3,88			J F M J J A O N	
	OS8		51,60	3,95			J F M J J A O N	
	OS9		51,61	4,03		S	J F M J J A O	
	OS10		51,64	4,09		S	J F M J J A O	
	WS2	Westerschelde	51,39	3,78	M J S D	S		
	WS3		51,42	4,04	M J S D	S		
	WS4		51,35	4,24	M J S D	S		
Belgian part of the North Sea	BE2	coastal	51,45	3,24	J F M A M J J A S O N D	J F J A S O N D		
	BE5	intermediate	51,53	2,87	J F M A M J J A S O N D	J F J A S O N		
	BE6	open sea	51,75	2,70	J F M A J J A S O N D	J F		
French port	DK1	Port Dunkerque	51,04	2,17			S O D	
	DK2		51,02	2,17			S O D	
	DK3		51,02	2,19			O D	

2.2 Material and Methods

2.2.1 Comparison of two plankton net types

Quantitative zooplankton samples were collected using two types of plankton nets during several sampling surveys in different areas in the North Sea organised within the INTERREG IVa 2 Seas MEMO project (06-008-BE-MEMO) (Figure 2.2; Table 2.1). During the same sampling event (245 occasions in total, spread over 32 locations and 4 years), a WP2 plankton net (mesh size 200 µm) and a ring trawl plankton net (mesh size 1000 µm) were deployed. The WP2 net (diameter 0.57 m; Fraser, 1966; UNESCO, 1968) was deployed in a vertical tow (±1 $m.s^{-1}$; Wiebe *et al.,* 2014). The ring trawl net (a WP3 or CalCOFI net with diameter of 1 m and 1.13 m respectively; UNESCO, 1968; Wiebe and Benfield, 2003) was towed through the water column, undulating three times from sea surface to bottom at a speed of 3 knots relative to the bottom (single net towyo as described in Wiebe *et al.,* 2014). Considering their same mesh size and way of deployement, the WP3 net and CalCOFI net were evaluated as one net type: the ring trawl net.

The obtained zooplankton samples were analysed on board and *M. leidyi* densities ($ind.m^{-3}$) were determined based on morphological and genetic identification. Samples for genetic identification (larvae) were preserved in 99.97% ethanol and analysed as described in paragraph 2.2.2. All *M. leidyi* individuals from the BPNS and the Westerschelde estuary were measured alive (oral-aboral length; ± 1 mm).

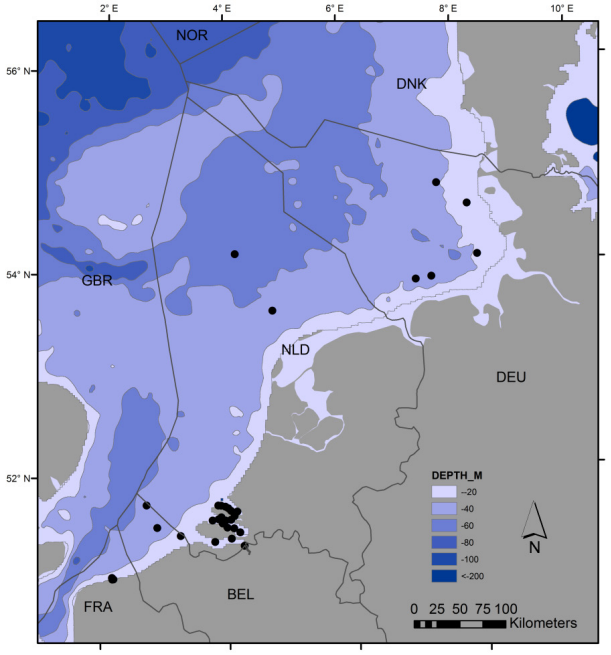

Figure 2.2 Map of the southern North Sea with indication of locations where WP2 and ring trawl nets were deployed during the same sampling event

2.2.2 Testing preservation solutions and methods

With regard to morphological identification

A handheld dip-net (mesh size 200 µm; diameter 0.20 m) and 1 L-beakers were used to qualitatively collect *M. leidyi* in the port of Oostende (sluice dock, Belgium; 51.23°N 2.95°E). Undamaged individuals were selected to test different preservation solutions and methods with regard to (long-term) morphological identification. Fifteen *M. leidyi* individuals of approximately the same size (31 ± 4 mm oral-aboral length; no significant difference MWU + Bonferroni correction p >0.05) were transferred into petri-dishes for each treatment and kept at room temperature (± 20 °C). The different test solutions were: (1) absolute ethanol (99.97%; VWR Chemicals), (2) 4% formaldehyde solution (buffered with sea water; salinity 33; VWR Chemicals), (3) Battaglia sauce, another formaldehyde solution (Lelièvre *et al.,* 2012), (4) acid Lugol's solution (1, 2, 5, 8 and 10% concentration mixed with seawater; salinity 33; Edler, 1979 as referred to in Engell-Sørensen *et al.,* 2009), (5) RNAlater™ (patent US 8178296 B2, Ambion Inc.), and (6) RCL2®, a formaldehyde-free fixative (patent WO 2004/083369, Alphelys, Plaisir, France). The six preservation solutions were selected based on their frequent use in zooplankton (2 and 3) and phytoplankton (4) monitoring (Harris *et al.,* 2000). Furthermore, ethanol is generally recommended for genetic analysis (e.g. Schuchert, 2012), RNAlater™ stabilises the nucleic acids (Gorokhova, 2005), and RCL2® is often chosen as an alternative for formaldehyde solution in pathology and histological studies (Delfour *et al.,* 2006; Masir *et al.,* 2012). At specific times (after 5h, 10h, 24h, 48h, 3d, 4d, 7d, 10d, 17d, 24d, 31d, 38d, 52d, 66d and 80d), the condition of each individual was scored, with 3 = undamaged, 2 = damaged but morphological identification still possible, and 1 = completely damaged and morphological identification no longer possible. Petri-dishes were removed from the experiment when the animal reached condition 1. Individuals in condition 2 or 3 were also measured (without removing it from the sealed petri-dish) to determine shrinkage.

With regard to genetic identification

In addition to the effects on morphological quality, we tested the effect of different preservatives (1-17 individuals per treatment stored at room temperature) on the quality of the DNA and the DNA concentration to determine the success of the DNA extraction using the Invisorb® Spin Tissue Mini Kit (Invitek, Isogen Life Sciences), and on the amplification strength of the ITS1 marker (1082 bp) with primers KN8-9 (Fuentes *et al.,* 2010; Ghabooli *et al.,* 2011). For these analyses, samples preserved in ethanol (70% and 99.97%), Battaglia sauce, RCL 2®, RNAlater™ and Lugol's solution (1, 2, 5, 8 and 10%) were used. Additionally, the effect of freezing at -20 °C and freezing at -80°C with subsequent freeze-drying on *M. leidyi* DNA extraction and ITS1 amplification was verified.

For the DNA extraction, *M. leidyi* tissue was obtained from the samples using tweezers (max. 0.75 mL) and subsequently dried. Frozen samples were thawed first and then a pipet was

used to retain 0.5 mL. The Invisorb® Spin Tissue Mini Kit protocol for 0.5-40 mg tissue was followed (Invisorb® Spin Tissue Mini Kit, 2012). Crosslinking between DNA, RNA and proteins can be facilitated by certain preservatives. In formaldehyde-based solutions, such as Battaglia sauce for example, crosslinking is often encountered. Therefore, some of the Battaglia sauce samples were treated according to the formaldehyde-preserved-tissue protocol to check whether better results for DNA extraction could be obtained (instructions from Invisorb® Spin Tissue Mini Kit, 2012). This protocol entails incubation with dithiothreitol (DTT), a substance which reduces protein bonds. More specifically, samples were incubated for 20 min at 99 °C in an Eppendorf Thermomixer® with DTT and phosphate buffered saline (PBS) prior to DNA extraction. Subsequently, the rest of the Invisorb® DNA extraction protocol was executed, but with DTT and PBS solution also added to the lysis solution for incubation overnight at 52 °C. The DNA concentration ($ng.\mu L^{-1}$) was determined by using the NanoDrop 2000 Spectrophotometer (Thermo Scientific) based on the absorbance at 260 nm. The DNA purity was assessed from the ratio of the absorbance at 260 nm (A260) and 280 nm (A280). A ratio of approximately 1.8-2.0 is an indication for a 'pure' DNA extraction (Thermo Fisher Scientific-NanoDrop Products, 2011).

Amplification of the ITS1 fragment was performed in a 40 µL PCR mix volume using the VWR Red Taq DNA Polymerase Master Mix (2x; 1.5 mM $MgCl_2$), the KN-8 and KN-9 forward and reverse primers in a final concentration of 0.5 µM with 2µL of DNA template (Fuentes *et al.,* 2010). The PCR protocol consisted of 3 steps. First, an initial DNA denaturation and polymerase enzyme activation step was run (1 cycle of 10 minutes at 95 °C), followed by a second step involving the formation of the fragment (38 cycles of 45 s at 95 °C, 45 s at 47 °C and 60 s at 72 °C). Finally, elongation was enhanced during 1 cycle for 5 minutes at 72°C. The PCR product was preserved at 16°C until further processing. The PCR product length was verified on a 1% agarose gel (LE, analytical grade, Promega), stained with Gelred™ nucleic acid stain (Biotium, USA) and visualised and photographed under UV light (UVtransilluminator 265 nm TFX20M).

With regard to stable isotope analyses

Finally, the effect of the preservation method was determined with respect to the stable isotope composition of *M. leidyi*. A handheld dip-net (mesh size 200 µm; diameter 0.20 m) and 1 L-beakers were used to qualitatively collect *M. leidyi* individuals in the port of Oostende (sluice dock, Belgium; 51.23°N 2.95°E) on 12 September 2012. Undamaged individuals were selected, measured (size class 35-55 mm was retained) and the gastro-intestinal canal was removed with a scalpel to avoid measuring the signal from the ingested prey items (Feuchtmayr and Grey, 2003; D'Ambra *et al.,* 2014). The remaining tissue was stored in 10 mL tubes. Eighteen samples were frozen at -20 °C, 18 samples were preserved at -80 °C and subsequently freeze-dried before analysis (cfr. Fleming *et al.,* 2011) and 17 samples were not preserved but immeadiately prepared for SI analysis. Prior to SI analysis,

all samples, irrespective of their preservation method, were rinsed with deionized water to reduce the salt and transferred to tin capsules (8x5 mm; Elemental Microanalysis). The tin capsules were dried overnight (60 °C), folded, weighed and placed in a sterile and sealed 96-multiwell. Dual stable isotope analyses (C, N) using a continuous flow isotope ratio mass spectrometer (Europe Integra) was performed at the UC Davis Stable Isotope Facility (USA) (see Chapter 5). Significant differences (p <0.05) between the three pre-treatments were explored and tested in R v 3.1.3 (R Core Team, 2015) using a non-parametric Kruskal-Wallis test (parametric assumptions were not met) and several Mann-Whitney U tests (with Bonferroni correction) for $\delta^{13}C$ and $\delta^{15}N$ values separately.

2.3 Results

2.3.1 Comparison of two plankton net types for *M. leidyi* sampling

Density estimates

Mnemiopsis leidyi was caught in both WP2 and ring trawl (WP3 or CalCOFI) nets. Some specimens were damaged but morphological identification of adults was in all cases still possible. From the 245 sampling events where both ring trawl and WP2 nets were deployed, 105 did not contain *M. leidyi*. In 56 sampling events, *M. leidyi* was present in the ring trawl, but absent in the WP2 net samples. In contrast, only at three occasions *M. leidyi* was found in the WP2 and not in the ring trawl net. Due to the different deployment, the average filtered volume of the ring trawl nets (389.0 ± 176.6 m^3) was much larger than that of the WP2 net (7.0 ± 19.0 m^3). Consequently, there is a higher chance to catch *M. leidyi* with the ring trawl nets, but higher average densities were found for the WP2 net (over all 245 sampling events: 15.0 ± 68.1 $ind.m^{-3}$ for WP2 net versus 4.3 ± 17.4 $ind.m^{-3}$ for ring trawl nets; over all 81 sampling events where *M. leidyi* was present in both nets, 45.2 ± 114.0 $ind.m^{-3}$ for WP2 net versus 12.8 ± 28.5 $ind.m^{-3}$ for ring trawl net). This may indicate an under- or overestimate by either net (Figure 2.3).

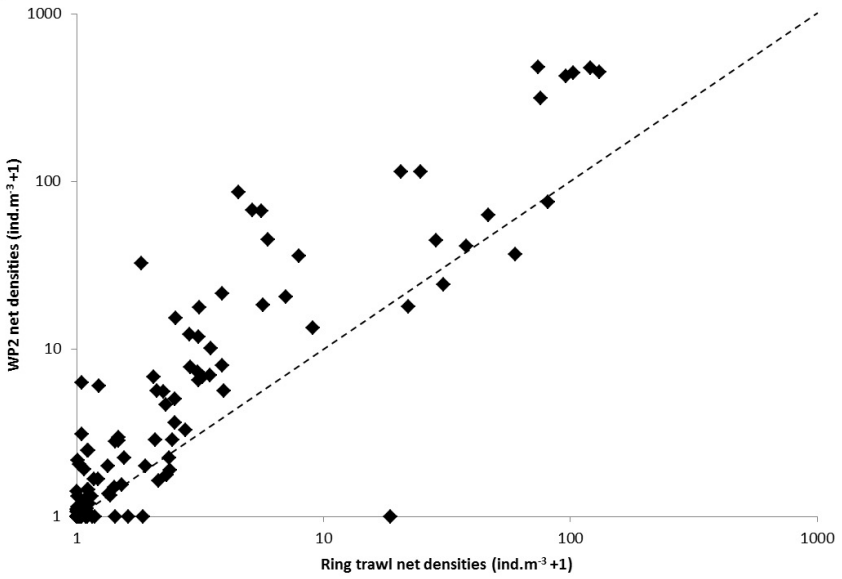

Figure 2.3 Densities (+1) of *M. leidyi* measured by the WP2 net relative to the ring trawl nets (WP3 and CalCOFI) for all 245 sampling events (note logarithmic scale on both axes); dotted line represents equal densities in both net types

Size distribution estimates

We also compared the sizes of *M. leidyi* (oral-aboral length, mm) in both net types, to evaluate which net gave the best population structure (size distribution) estimate. For this comparison, we focused on sampling events from the Belgian coastal location (BE2) and the Westerschelde estuary (WS2-4) in September and December 2011 and 2012 (Figure 2.2; Table 2.1) when *M. leidyi* was present in both nets, *i.e.* 8 sampling events with 403 individuals caught in the ring trawl (CalCOFI) net and 77 individuals in the WP2 net. Due to the smaller mesh size of the WP2 net (200 µm), relatively more small individuals (≤10 mm) were captured (60% compared to 9% in the CalCOFI net) (Figure 2.4). The 1000 µm-mesh of the CalCOFI net predominantly sampled intermediate (11-40 mm) and large individuals (note outlier density in length class 71-75 mm).

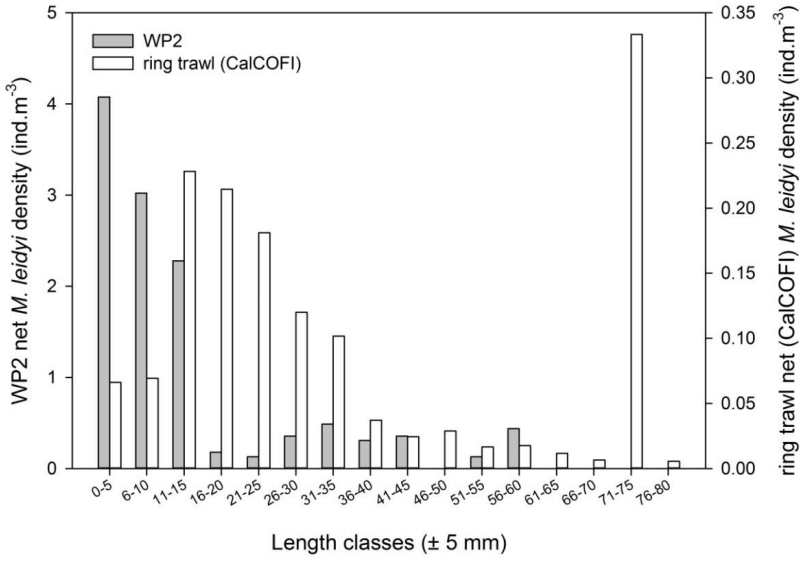

Figure 2.4 *Mnemiopsis leidyi* densities (ind.m^{-3}) distributed over the different length classes (oral-aboral length, ± 5 mm) as sampled by the WP2 net and CalCOFI ring trawl in the Belgian coastal location (BE2) and the Westerschelde estuary (WS2-4)

When comparing densities for these samples and removing the small ctenophores (≤10 mm) from the analysis, the WP2 net still measured higher densities compared to the CalCOFI net (Figure 2.5).

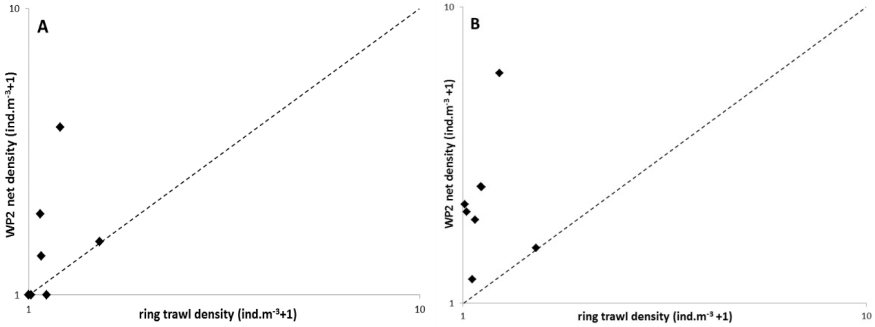

Figure 2.5 Densities (+1) of *M. leidyi* measured by the WP2 net relative to the ring trawl net (CalCOFI) for the 8 sampling events in BE2 and WS2-4 where *M. leidyi* was measured and present in both nets; A) densities for the large length classes only (>10 mm) and B) densities for all length classes for comparison (note logaritmic scale on both axes); dotted line represents equal densities in both net types

2.3.2 The effect of preservatives on *M. leidyi*

With regard to morphological identification

The experiments showed that ethanol (99.97%), buffered formaldehyde solution (4%) and RNAlater™ resulted in immediate disintegration (within minutes) and deformation of the *M.*

leidyi individuals (condition 1). Similarly, Battaglia sauce impaired morphological identification in all 15 replicates after only 5 h. The formaldehyde-free RCL2® is a better preservative, with still 29% of the samples left at condition 2 after 24 h (71% in condition 1) (Figure 2.6A). However, individuals shrank from 31.3 ± 3.4 mm to 17.3 ± 0.5 mm (45% shrinkage) within 24 h (Figure 2.6B). The effect of Lugol's solution (dissolved in seawater at salinity 33) varied considerably with concentration. Nevertheless, Lugol's solution was the only preservative that allowed *M. leidyi* preservation and identification beyond 96 h (4 days; Figure 2.6A). The preservation efficiency (*i.e.* proportion of the initial 15 individuals remaining intact after preservation) substantially decreased over time for all Lugol's solution concentrations. Less than 50% of the samples was left after 10 h for the 1, 2 and 8% concentrations, after 24 h for the 10% concentration and after 48 h for the 5% concentration. However, after 10 days (240 h), most samples were left for the 10% concentration (33%), followed by the 2% concentration (13%) and the 1 and 8% concentration (7%). None of the samples were left for the 5% concentration.

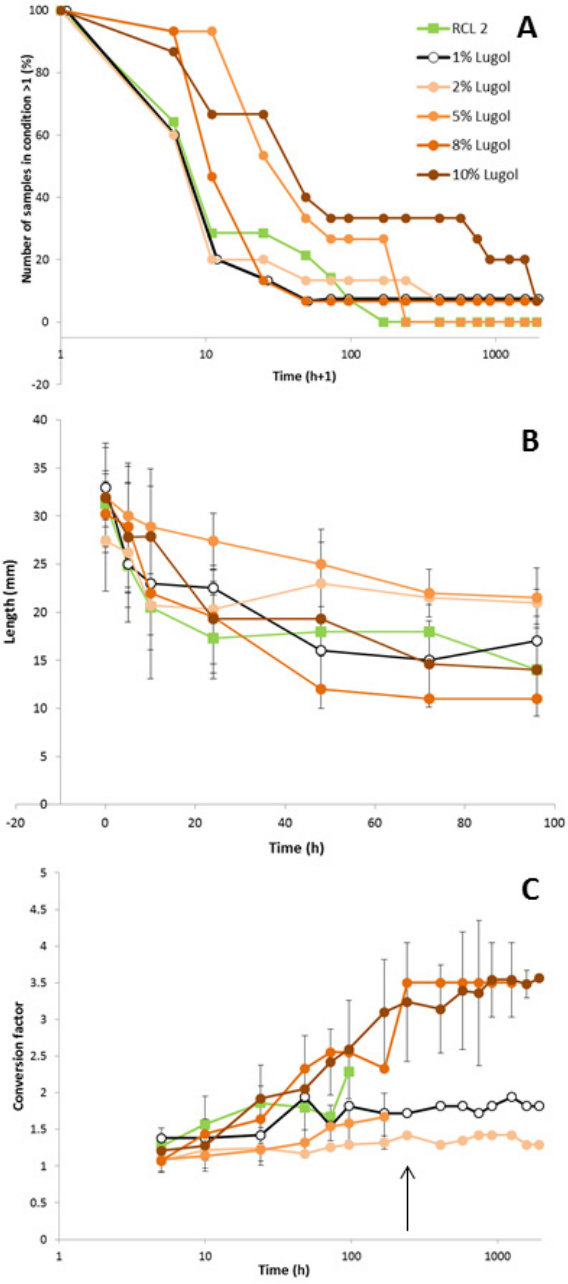

Figure 2.6 A) Number of samples in condition >1 (%), B) shrinkage during the first 96 h (expressed as length, mm) and C) conversionfactors varying over time for 6 preservation solutions and concentrations; each point represents the percentage (A), the mean (± SD) length (B) or the mean (± SD) conversionfactor (C) of the remaining samples (condition >1) (note that data points for 1% Lugol were slightly shifted in A for clarity; x-axis on logarithmic scale for A and C; arrow in C indicates 240 h)

After 24 h, most shrinkage was observed in the highest Lugol's solution concentrations (8 and 10%; Figure 2.6B), and ctenophores had a raisin-like structure (Figure 2.7). Overall, the least shrinkage was noted for the 1, 2 and 5% concentrations of Lugol's solution.

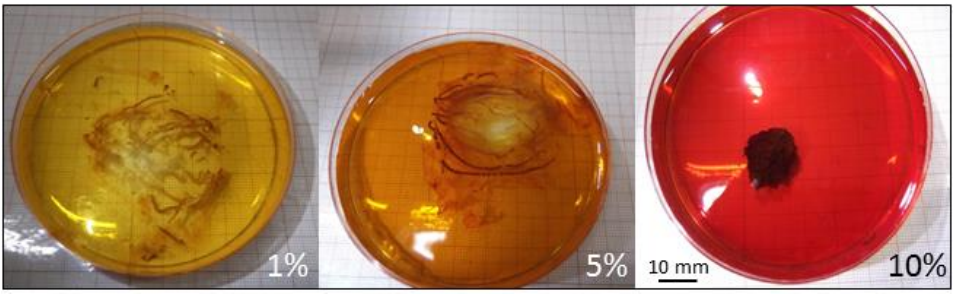

Figure 2.7 The effect of 1%, 5% and 10% concentrations of Lugol's solution after 24 h on some of the *M. leidyi* specimens

Due to the considerable amount of shrinkage, we calculated conversion factors (length T_0/ length T_1). In the case of RCL2®, a conversion factor of 1.8 ± 0.1 is needed to get an indication of the original length of the specimens after 48 h (Figure 2.6C). For Lugol's solution, shrinkage and length conversion factors exponentially increased up to 10 days (*i.e.* 240 h) after the start of the preservation experiment, and then remained constant (Figure 2.6C indicated by the arrow).

With regard to genetic identification

Good results in terms of DNA extraction were obtained for *M. leidyi* preserved in absolute ethanol (99.97%), RCL2®, Lugol's solution (8% and 10%) and freeze-dried (Table 2.2). For these preservatives, a high DNA concentration (>100ng.µL^{-1}) and DNA purity (1.8-2.0) were obtained (Thermo Fisher Scientific-NanoDrop Products, 2011). However, only ethanol (99,97%), RCL2® and freeze-dried samples gave also good results in terms of ITS1 amplification (visual inspection of band on agarose gel). Surprisingly, ITS1 amplification was also possible for samples preserved in ethanol (70%), Battaglia sauce+DTT, RNAlater, Lugol's solution (1,2 and 5%) and frozen samples at -20 °C, which were characterised by low DNA concentrations and/or DNA purity (poor DNA extraction results). The use of DTT in the DNA extraction protocol of the Battaglia sauce samples clearly influenced both the DNA concentration and the DNA purity and resulted in a successful ITS1 amplification (Table 2.2).

Table 2.2 Quality control of the DNA extractions and ITS1 amplification using different preservatives, with DNA concentration as indication for DNA yield, A260/A280 as indication for DNA purity and + and – for indication of successful or unsuccessful amplification respectively

Preservative	DNA concentration (ng.µL^{-1})	A260/A280	ITS1
Absolute ethanol (99.97%)	354.2 ± 332.8	1.99 ± 0.22	+
Absolute ethanol (70%)	306.0	2.1	+
Battaglia sauce	2.1 ± 0.7	1.8 ± 0.8	-
Battaglia sauce + DTT	23.3 ± 18.3	2.0 ± 0.0	+
RCL2®	227.0 ± 146.2	2.0 ± 0.1	+
RNAlater™	6.0 ± 5.6	2.7 ± 1.1	+
Lugol's solution (1%)	72.7 ± 1.6	1.6 ± 0.0	+
Lugol's solution (2%)	35.4	1.6	+
Lugol's solution (5%)	934.2	1.5	+
Lugol's solution (8%)	168.0	1.8	-
Lugol's solution (10%)	184.1	1.8	-
Frozen (-20°C)	63.6 ± 2.8	2.2 ± 0.0	+
Frozen (-80°C) + freezedried	255.3	1.9	+

With regard to stable isotope analyses

SI analyses showed considerable variation in the isotopic composition of *M. leidyi* related to the pre-treatment of the samples (Figure 2.8). The unpreserved samples were most depleted in both ^{13}C (av. $\delta^{13}C$ = -19.0 ± 0.4) and ^{15}N (av. $\delta^{15}N$ = 15.0 ± 1.1), compared to the frozen samples (av. $\delta^{13}C$ = -18.5 ± 0.3; $\delta^{15}N$ = 16.5 ± 0.6) and freeze-dried samples (av. $\delta^{13}C$ = -18.4 ± 0.9; $\delta^{15}N$ = 15.5 ± 1.4). The latter showed the most within-group variation. Although samples from the same location, date and length class were selected, the average weight of the samples varied according to the preservation treatment. The freeze-dried samples had the highest average weight (6.6 ± 10.8 mg), followed by the unpreserved (av. 4.5 ± 0.8 mg) and frozen samples (av. 2.9 ± 1.2 mg).

Significant difference were found between the treatments for $\delta^{13}C$ (K-W chi² = 10.7; df = 2; p = 0.005) and more specifically between frozen and unpreserved samples (MWU p = 0.001). For $\delta^{15}N$, significant differences were identified (KW chi² = 17.24; df = 2; p = 0.0002) between frozen and unpreserved (MWU p < 0.001) and freeze-dried samples (p = 0.04).

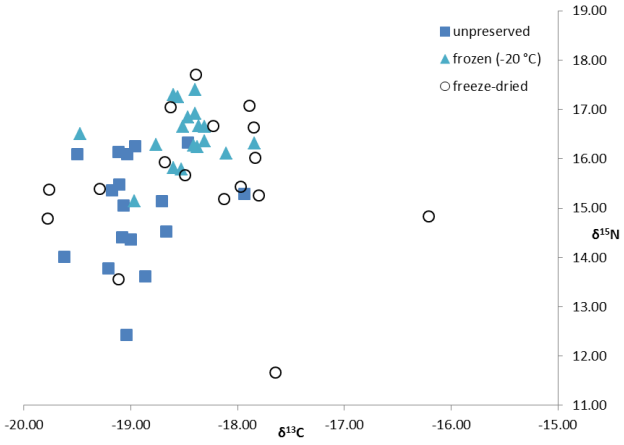

Figure 2.8 Stable isotopes bi-plot with indication of the pre-treatment of the samples

2.4 Discussion

2.4.1 Using plankton nets for *M. leidyi* sampling

The comparison of the two net types (WP2 and ring trawl net) revealed that both filtered volume and mesh size largely determine the catch in terms of *M. leidyi* densities and population structure. A WP2 net was shown to be unreliable in determining presence or absence of *M. leidyi*, as in 56 sampling events the species was present but only retained in the ring trawl net and not in the WP2 net. Furthermore, when *M. leidyi* was present in both nets, much larger density estimates were found by the WP2 net. As densities remained higher when removing the small ctenophores ($\leq$10 mm), the filtered volume (directly related to the deployment of this WP2 net) rather than the mesh size explained both of these findings. To sample larger plankton, such as *M. leidyi*, it is important to filter a large water volume, because these larger organisms are relatively more scarce (UNESCO, 1968).

The larger filtered volume and mesh size of the ring trawl net resulted in a good overview of the adult population structure, but may underestimate some of the small *M. leidyi* ctenophores. Together with the towing speed of 3 knots relatively to the bottom, the 1 mm mesh size probably allowed that some of the small ctenophores were squeezed through the meshes. This should be less the case for the WP2 net which is deployed in a vertical haul and has a smaller mesh size.

Unfortunately, this study cannot assess the efficiency of either net relative to what is actually present in the water and can thus not determine which net over- or underestimates the actual *M. leidyi* density. Remsen *et al.* (2004) deployed a platform containing plankton nets and optical tools to determine and compare zooplankton abundance estimates by these different tools. They found that net estimates largely underestimated (approximately 7 times) the zooplankton abundance compared to the optical tools, especially in surface

waters (<40 m depth). Moreover, nets were shown to underestimate cnidarian/ctenophore abundance by 1200%. This was somewhat surprising as larger medusae may not be able to enter the 92 cm² sampling tube mouth, which was used by Remsen *et al.* (2004). Nevertheless, optical tools may provide a way to assess the efficiency of the nets. The use of optical tools in shallow coastal waters such as the BPNS has hitherto been hampered by turbidity and should be optimized first (personal communication André Cattrijsse, VLIZ). For now, a ring trawl net, gives the most realistic impression of *M. leidyi* density and size distribution compared to the WP2 net. Towing a ring trawl net at a lower speed with a smaller mesh size (e.g. 500 µm) could compensate for the loss of the small fraction of the *M. leidyi* population. However, the catch efficiency of the net should then be re-evaluated, especially during phytoplankton blooms when clogging of the net may form a real sampling problem (Harris *et al.,* 2000; Wiebe and Benfield, 2003). Additionally, we suggest the deployment of a hyperbenthic sledge to quantitatively assess the abundance of *M. leidyi* close to the sea bed. Although we did not test this, several publications suggest that when conditions are unfavourable, *M. leidyi* migrates downward in the water column and remains close to the sea bed (Costello *et al.,* 2006; Mianzan *et al.,* 2010; Chapter 4). During sampling, plankton nets typically remain at least 1 m from the sea bed due to technical constraints and in order to avoid damage to the nets. Consequently, these individuals are most probably not caught by a WP2 or ring trawl net.

2.4.2 The effect of preservatives on *M. leidyi*

All tested preservatives had a considerable effect on morphological and genetic identification and stable isotope analyses of *M. leidyi*. Especially preservatives containing formaldehyde (formaldehyde 4% and Battaglia sauce) did not allow any identification (which confirmed findings of Purcell (1988) on morphological identification). However, the formaldehyde-preserved-tissue protocol using DTT provides a good alternative to at least genetically identify the specimens (Invisorb® Spin Tissue Mini Kit, 2012). More specifically, the yield of the DNA extract in the Battaglia sauce+DTT samples slightly improved after the application of this protocol, facilitating ITS 1 amplification. This is the result of reducing the number of protein bonds and reversing the effects of crosslinking, which are normally formed after preservation in formaldehyde solution. Hitherto several studies have demonstrated the extraction of DNA from formaldehyde preserved samples, which may allow the investigation of historic samples, as these were traditionally preserved in formaldehyde (Shedlock *et al.,* 1997; Duval *et al.,* 2010; Palero *et al.,* 2010; Paireder *et al.,* 2013; Sengüven *et al.,* 2014).

Although morphological identification after preservation in absolute ethanol (99.97 %) is impossible due to the distortion of diagnostic features, this preservative gave, together with freeze-dried samples, the best results for DNA extraction and ITS1 amplification of *M. leidyi* (which confirmed findings of Schuchert (2012)). The differences between frozen (-20 °C) and

freeze-dried samples were situated at the DNA extraction level. Rather poor results were obtained for the frozen samples, probably as a result of thawing causing degradation of the DNA (Thermo Fisher Scientific, 2015). Nevertheless, amplification was successful. Similarly, RNAlater™ samples were characterised by poor extraction results, but successful amplification. Although morphological identification is not possible for RNAlater™ samples, this preservative is frequently used because it immediately stabilises the RNA/DNA and provides reliable gene expression data (Gorokhova, 2005; Michaud *et al.,* 2011). However, the temperature in which the sample is preserved prior to analysis may affect the genetic identification on the long term (Straube and Juen, 2013; Riehl *et al.,* 2014). As the samples used in this study were all preserved at room temperature (instead of freezer), we suspect this may have adversely affected the outcome of the extraction.

The best results in terms of morphological and genetic identification were obtained for the RCL2® and Lugol's solution samples. For morphological identification, the preservation efficiency and the amount of shrinkage are important factors to consider. For neither RCL2® nor Lugol's solution (1-10% concentration) the preservation efficiency allowed long-term storage of *M. leidyi* for e.g. in museums. However, the best results were obtained for the 10% Lugol's solution concentration, leaving 33% of the samples in a good condition for morphological identification after 10 days (240 h). Probably, the other concentrations were too diluted resulting in faster deterioration. Although this 10% concentration resulted in most shrinkage, the length remained more or less constant after 10 days, which allowed to calculate a conversion factor. Before using this conversion factor, we recommend a thorough validation process to estimate their accuracy and determine whether they can be used in the field (prediction interval). Unfortunately, genetic identification of the 10% Lugol's solution concentration samples was unsuccessful. Good DNA extracts were found, but the amplification of the ITS1 sequence failed (also for the 8% concentration). Three possible explanations are suggested: 1) traces of Lugol's solution may have resulted in binding inhibition during the amplification process; 2) enough DNA was available, but it consisted of small fragments and 3) the NanoDrop provided data on the concentration of double-stranded and single-stranded DNA, RNA and proteins (based on spectrophotometry), which was an overestimation. Newer technologies, such as the Quantus™ fluorometer could give a more precise result, e.g. measure concentration of only double-stranded DNA (Promega Quantus™ Fluorometer, 2015). For the samples preserved in 1, 2 and 5% Lugol's solution the opposite was observed with rather poor DNA extracts and successful amplification. Especially the low A260/A280 ratio (<1.8) points towards samples contaminated by residual phenols or other reagents associated with the extraction protocol (Thermo Fisher Scientific-NanoDrop Products, 2011).

On the other hand, samples preserved with the non-crosslinking fixative RCL2® revealed good DNA extracts and a successful amplification of the ITS1 sequence. RCL2® is frequently used in clinical research for histological purposes and specifically for molecular analyses

(Delfour *et al.,* 2006; Masir *et al.,* 2012). For short-term preservation (less than 5 h), this preservative could also provide a solution with respect to morphological identification.

In contrast to the samples used for morphological identification, the number of replicates for genetic identification were rather limited, sometimes resulting in large variances within treatments (e.g. DNA concentration of absolute ethanol 99.94%). We therefore recommend a more thorough analysis in the future with more replicates and using a more specific tool to investigate the DNA concentration (e.g. Quantus™ fluorometer).

Freezing is often the preferred method to preserve samples for stable isotope analysis, as it was shown that freezing does not affect the isotopic values for several marine species (Bosley and Wainwright, 1999; Carabel *et al.,* 2009). However, similar to the findings of Fleming *et al.* (2011) for *Aurelia aurita*, our results showed enrichment in ^{15}N after freezing of *M. leidyi* samples (1.5‰ in this study; 2.0‰ for *A. aurita* in Fleming *et al.,* 2011). We also observed enrichment in ^{13}C (0.5‰), which was similar to Feuchtmayr and Grey (2003) for several freshwater zooplankton species. Freeze-dried samples showed less enrichment in ^{13}C and ^{15}N compared to frozen samples. The mechanical processes associated with these preservation methods may invoke changes at the cellular level, causing this isotopic enrichment. Freezing for example can cause the breakdown of cells, resulting in the loss of compounds with higher or lower $\delta^{13}C$ values, via leaching during the thawing process. Higher $\delta^{15}N$ values may be the result of the denaturation of proteins caused by freezing (Wroblowski *et al.,* 1996; Paredi *et al.,* 2010). Additionally, the large variation within freeze-dried samples may be a consequence of the presence of salts. Some specimens contained more than others, which was reflected in the large variation in sample weight. Caution is needed when comparing *M. leidyi* SI composition between different studies. As $\delta^{13}C$ and $\delta^{15}N$ help to elucidate the trophic interactions in the food web, these shifts are important to consider (De Niro and Epstein, 1976; Post, 2002).

Table 2.3 gives an overview of all tested and commonly-used preservation solutions and methods with respect to identification, long-term preservation (several years) and stable isotope analysis (as food web biomarker). It is clear that using no preservatives, *i.e.* unpreserved samples of *M. leidyi*, yields the best overall results.

Table 2.3 Overview of all tested and commonly-used preservation solutions and methods with respect to identification, long-term preservation and stable isotope analysis; *using the formaldehyde preserved tissue protocol (+DTT); ** see van Walraven *et al.* (2013); $alive; £ long-term preservation affected by storage temperature

Preservation solution or method	Identification		Preservation	Food web
	morphological	genetic	long-term	biomarker: SI
Absolute ethanol (99.97%)	-	+	+	?
Buffered formaldehyde solution (4%)	-	-	+	-
Battaglia sauce*	-	+	-	?
RCL 2®	+	+	-	?
RNAlater™	-	+	-£	?
TCA-based protocol**	+	-	+	-
Lugol's solution	+	+	-	?
Unpreserved	+	+	+$	+
Frozen (-20°C)	-	+	+	+
Frozen (-80°C) + freezedried	-	+	+	+

However, in certain conditions, preservatives are indispensable. The choice of which preservative to use not only depends on what tissue quality is needed for the objectives of the study, but also on the available budget, handling time and toxicity. Some preservatives such as RCL2® and RNAlater™ are patented and more expensive. The recipe of the latter is available and can be prepared in the laboratory. However, this will barely reduce the costs, because staff costs will increase. Similarly for Battaglia sauce, the time needed for the preparation of the solution might form a constraining factor. Moreover, some preservatives involve time-consuming protocols. For example, the TCA-based preservation protocol described by Adams *et al.* (1976) and adapted by van Walraven *et al.* (2013, appendix), takes up to seven days to complete. Next to budget and time, the toxicity may play a role. Some chemicals are more toxic to humans than others, and the speed of degradation in the environment may vary.

The most suitable preservative, tested in our study, with respect to morphological and genetic identification would be Lugol's solution. Lugol's solution is regarded as a soft and cheap (compared to RCL2®) fixative and is frequently used for phytoplankton and fragile organisms (without calcium carbonate) (Edler, 1979 as referred to in Engell-Sørensen *et al.,* 2009). It provided the best results in terms of preservation efficiency compared to the other tested preservatives and is known to be less harmful to humans compared to aldehyde-based fixatives (Engell-Sørensen *et al.*, 2009). The 5% concentration was recommended by

Sullivan and Gifford (2009) for preservation of *M. leidyi* larvae. This concentration also gave good results in this study for short-term preservation. However, using this solution to preserve bulk zooplankton samples may cause problems. All species colour brown-yellow, which might masks diagnostic features to identify certain taxa, such as for example the melanophores of fish larvae. On the other hand, Jaspers and Carstensen (2009) confirmed the use of Lugol's solution for copepods and larvaceans, while Engell-Sørensen *et al.* (2009) suggest adding sodium thiosulphate to remove some of the tanning. Finally, the salinity of the Lugol's solution also affects the shrinkage of the ctenophores (this study: salinity 33, compared to Engell-Sørensen *et al.* (2009): salinity 14).

For SI analyses, using unpreserved samples is also recommended. However, when this is not possible, we suggest freezing as an alternative. However, it should be condisered that variability in the isotopic composition is reduced compared to unpreserved samples. Furthermore, calibration experiments should be conducted first in order to derive correction factors. Another option is that researchers are consistent in the use of the pre-treatement method throughout the same study (*cfr.* Chapter 5).

2.5 Conclusions

For quantitative sampling of *M. leidyi*, a WP3 or CalCOFI ring trawl net (mesh 1 mm) gave a realistic impression of *M. leidyi* density and size distribution. However, the smaller fraction of the *M. leidyi* population could be underestimated. Therefore, a ring trawl net, towed at a slower speed (<3 knots) with a smaller mesh size (<1000 µm) could compensate for the loss of smaller ctenophores. A WP2 net underestimated the presence of *M. leidyi* and found higher density estimates when *M. leidyi* was abundant due to the lower filtered volume.

Considering preservation of *M. leidyi*, it is clear that unpreserved samples are preferred for any type of analyses. However, when there is no time to analyse the samples on board, or when further analyses in the laboratory are desirable (e.g. morphological or genetic identification), short-term preservation in Lugol's solution or RCL2® provides a good alternative. For length measurements after preservation, shrinking should be considered to avoid underestimating the sizes of the individuals. For food web studies, using unpreserved *M. leidyi* samples is recommended. However, when this is not possible, the shifts in isotopic composition should be considered when interpreting the results.

2.6 Acknowledgements

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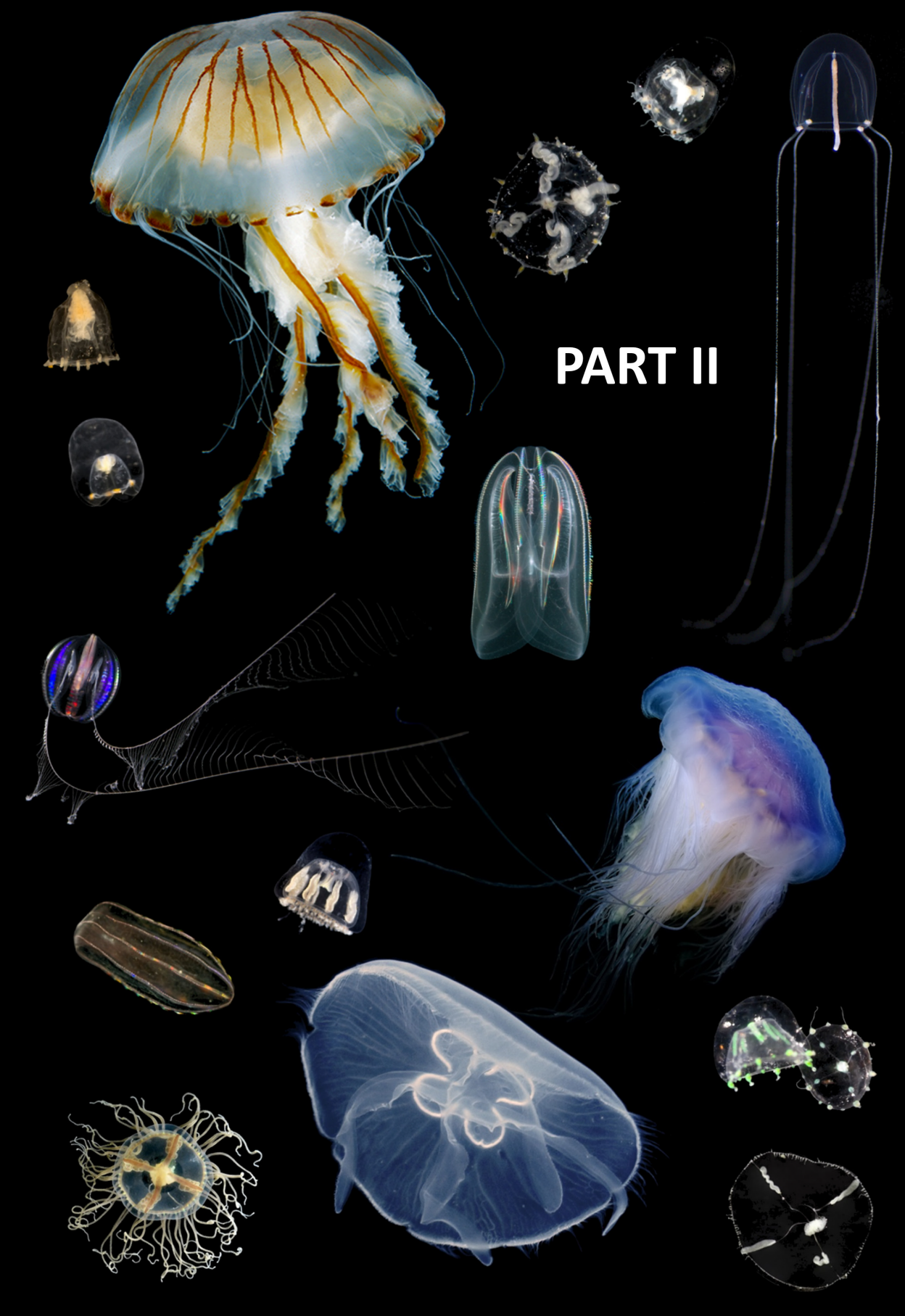
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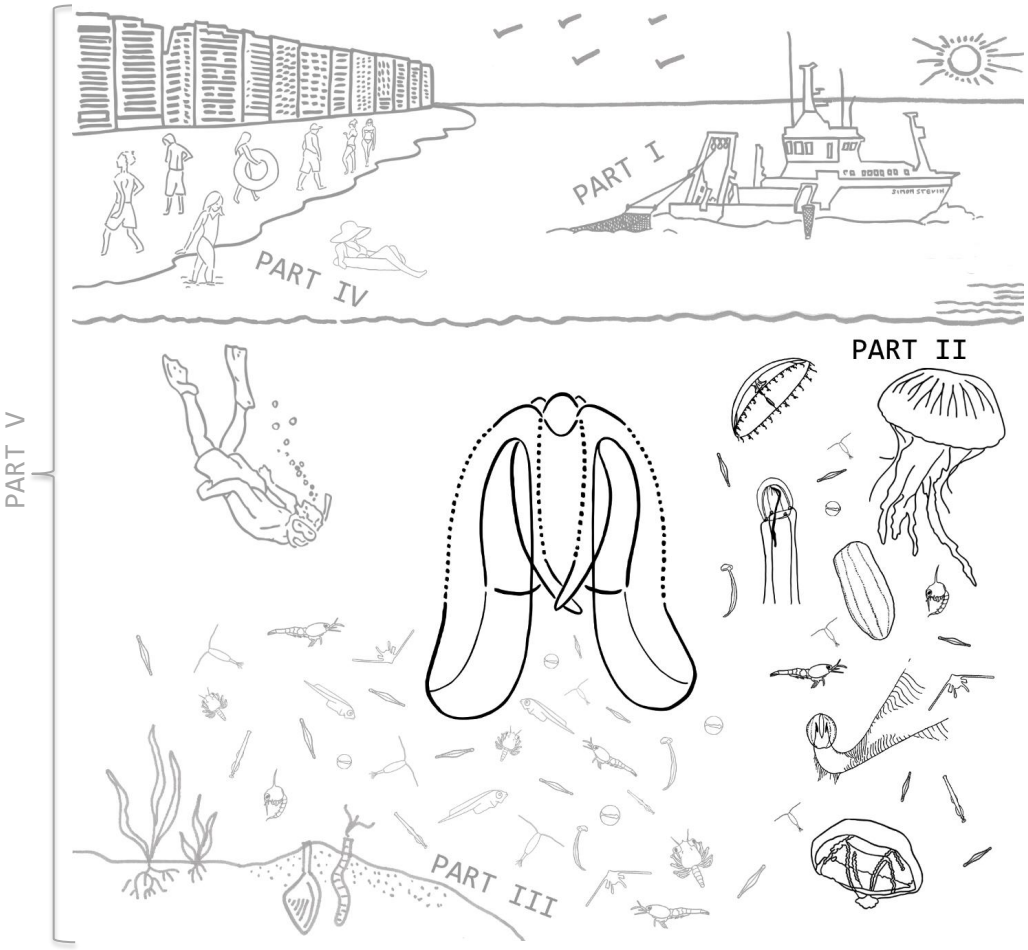

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3

GELATINOUS ZOOPLANKTON IN THE BELGIAN PART OF THE NORTH SEA AND THE ADJACENT WESTERSCHELDE ESTUARY

Spatio-temporal distribution patterns and population dynamics

Modified from:

Vansteenbrugge, L., Van Regenmortel, T., De Troch, M., Vincx, M., Hostens, K., 2015. Gelatinous zooplankton in the Belgian part of the North Sea and the adjacent Schelde estuary: Spatio-temporal distribution patterns and population dynamics. Journal of Sea Research 97, 28-39.

ABSTRACT

Many ocean ecosystems are thought to be heading towards a dominance of gelatinous organisms. However, gelatinous zooplankton has been largely understudied and the absence of quantitative long-term data for the studied area impedes drawing conclusions on potential increasing densities. This study gives a comprehensive overview of the spatio-temporal distribution patterns of gelatinous zooplankton in terms of diversity and density between March 2011 and February 2012 in the Belgian part of the North Sea and the adjacent Westerschelde estuary, based on monthly and seasonal samples respectively. Three Scyphozoa, three Ctenophora and 27 Hydrozoa taxa were identified, including three non-indigenous species: *Mnemiopsis leidyi*, *Nemopsis bachei* and *Lovenella assimilis*. In general, one gelatinous zooplankton assemblage was found across locations and seasons. Average gelatinous zooplankton densities reached up to 18 ind.m^{-3} near the coast, gradually declining towards the open sea. In the brackish Westerschelde estuary, average densities remained below 3 ind.m^{-3}. Highest gelatinous zooplankton densities were recorded in summer and autumn. Overall, Hydromedusae were the most important group both in terms of diversity and density. The ctenophore *Pleurobrachia pileus* and the hydromedusa *Clytia* sp. were present in every season and at every location. Gelatinous zooplankton densities never outnumbered the non-gelatinous zooplankton densities from the CalCOFI net. Due to the larger mesh size (1000 µm) of this net, only the larger fraction of the zooplankton is captured. The spatial and temporal distribution patterns seemed to be mainly driven by temperature (season) and salinity (location). Other environmental parameters including the non-gelatinous zooplankton densities (as a potential food source) were not retained in the most parsimonious DistLM model.

In terms of population dynamics, *Beroe* sp.[1] seemed to follow the three reproductive cycles of its prey *P. pileus* and the presence of *M. leidyi*, which was abundant in a broad size spectrum in summer and autumn. This study provides a baseline against which a potential increase in gelatinous zooplankton in the Belgian part of the North Sea and the Westerschelde estuary can be measured.

3.1 Introduction

Global ocean ecosystems are thought to be heading towards a dominance of gelatinous organisms since the past decade (Condon *et al.*, 2013). This public perception is strengthened by an increased reporting of problems caused by jellyfish, both in the public and scientific media, often related to bloom formations (Condon *et al.*, 2012; Chapter 7). Jellyfish blooms are characteristic in the life cycle of many gelatinous zooplankton species (Mills, 2001; Purcell, 2005), and often affected by changes in the environment. Lynam *et al.* (2004) for example found that jellyfish populations in the northern North Sea are related to the North Atlantic Oscillation (NAO) climate cycle. Goy *et al.* (1989) linked the recurrent presence and blooms of *Pelagia noctiluca* in the western Mediterranean to the combined effect of specific environmental parameters, *i.e.* lack of rainfall, high temperatures and high atmospheric pressure between May and August. However, jellyfish densities also show large inter-annual fluctuations (Purcell, 2012; van Walraven *et al.,* 2014), which makes it difficult to distinguish natural fluctuations from changes caused by anthropogenic perturbations (Mills, 2001). Global warming, overfishing, eutrophication, habitat modification and transport of non-indigenous species cumulatively affect the ecosystem and intensify the natural fluctuations in jellyfish abundance (Mills, 2001; Purcell *et al.*, 2007; Richardson *et al.*, 2009). Moreover, ecosystems under high anthropogenic pressure are vulnerable to regime shifts, which may tilt the balance towards a jellyfish dominated ecosystem (Daskalov *et al.,* 2007; Richardson *et al.,* 2009).

In this study, the broad term “gelatinous zooplankton” encompasses the planktonic medusa phase (jellyfish) of the phylum Cnidaria (classes Hydrozoa and Scyphozoa) and the phylum Ctenophora. Although being different phyla, both groups share certain life-history characteristics. Armed with cnidocysts or colloblasts, most gelatinous zooplankton are carnivores feeding on almost anything, from unicellular organisms to much larger prey (Alvariño, 1985; Purcell and Mills, 1988). Consequently, the predation and competition pressure on the co-occurring plankton community and on higher trophic levels (e.g. fish) can be considerable during blooms (Alldredge, 1984; Schneider and Behrends, 1998; Purcell and Arai, 2001). Moreover, gelatinous zooplankton blooms sometimes interfere with human

[1] *Beroe* specimens were not identified to species level. Most likely, all specimens belonged to the species *Beroe gracilis* (Greve, 1975). However, the lateral canals were not checked for each specimen and there is a slight possibility that juveniles of *Beroe cucumis* were present. From here onwards, *Beroe* specimens are referred to as ‘*Beroe* sp.’.

activities such as fisheries (e.g. clogging nets), tourism (e.g. stinging swimmers) and industries (e.g. clogging cooling-water intake screens) (Purcell *et al.,* 2007; Brotz *et al.,* 2012). These findings contributed to the growing interest in this ecosystem component, but unfortunately, quantitative, long-term abundance data on gelatinous zooplankton are scarce in many regions (Brotz *et al.*, 2012; Condon *et al.*, 2013). Zooplankton research traditionally focusses on small crustaceans, especially copepods (e.g. Fransz *et al.*, 1991; Haddock, 2004). Gelatinous zooplankton is often excluded from these studies, as these organisms get damaged during sampling and identification problems arise related with preservation (Boero *et al.*, 2008; Laakmann and Holst, 2014).

For the Belgian part of the North Sea (BPNS), only few historic studies on zooplankton are available, providing qualitative rather than quantitative data, and they only sporadically mention gelatinous species (Van Meel, 1975; Gilson collection (1898-1939) as presented in Van Loen and Houziaux, 2002). During the past decades, scattered data on gelatinous zooplankton have been published in local Belgian journals, most of them concerning beach findings (e.g. Rappé, 1989; Dumoulin, 1997; De Blauwe, 2003). The recent zooplankton studies by Van Ginderdeuren *et al.* (2012a; 2014) hint the presence of a diverse gelatinous zooplankton community in the BPNS. However, the overview was probably incomplete because the studies focused on other groups and because the used net type (vertical WP2 net hauls) filters less water resulting in a lower chance to catch larger (gelatinous) zooplankton (Chapter 2; UNESCO, 1968). In the adjacent Westerschelde estuary, several zooplankton studies have been executed in relation to eutrophication, focussing on copepods, rotifers and ciliates (Soetaert and Van Rijswijk, 1993; Appeltans *et al.*, 2003; Azémar *et al.*, 2010). To our knowledge, no data on gelatinous zooplankton have been published for the Westerschelde estuary. This study aims to close the gap of knowledge for both areas by characterising the spatial and temporal distribution patterns of the gelatinous zooplankton community in terms of diversity and density, and in relation to a number of environmental parameters, such as temperature, salinity, chlorophyll *a*, turbidity, oxygen concentration, water current and the non-gelatinous zooplankton fraction. Secondly, we investigated the population dynamics for the most important gelatinous species in this area. This study provides a baseline against which a potential increase in gelatinous zooplankton in the BPNS and the Westerschelde estuary can be measured.

3.2 Method

3.2.1 Study area

The Belgian Part of the North Sea (BPNS) is situated in the Southern Bight of the North Sea (Figure 3.1). The main water currents transport Atlantic water in a north-easterly direction through the English Channel towards the BPNS, where they meet the south-westerly oriented Westerschelde estuary outflow in the east (Vlaeminck *et al*., 1989; Lacroix *et al*., 2004). The Westerschelde estuary is characterised by a macro-tidal current regime, which

keeps the water column (average depth 30 m) well mixed (Meire *et al.*, 2005). Moreover, the estuary connects the North Sea with important ports e.g. in Terneuzen and Antwerp through busy shipping lanes.

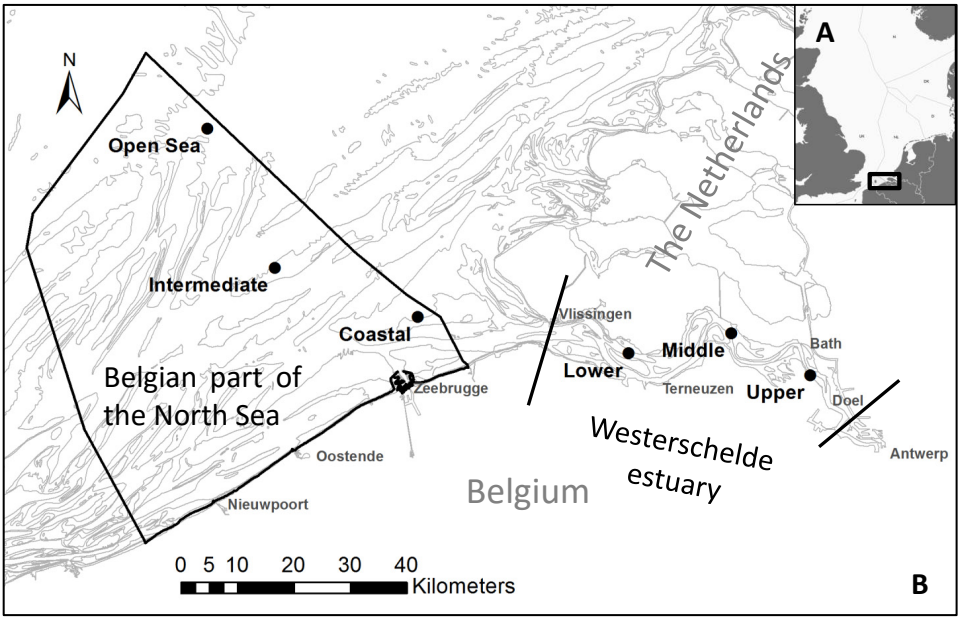

Figure 3.1 A) North Sea exclusive economic zones with indication of the study area. B) Position of the six sampling locations in the Belgian part of the North Sea and adjacent Westerschelde estuary.

3.2.2 Data collection

Gelatinous zooplankton samples were collected on board the RV Zeeleeuw using a 1 m-diameter, 1000 µm-mesh CalCOFI plankton net, undulated three times through the water column (sine wave from sea surface to bottom) at a speed of 3 knots (Wiebe and Benfield, 2003; Wiebe *et al.,* 2014). Three replicates were taken at each station as recommended in literature (Kramer *et al.,* 1994), but only one was included in this study, mainly due to time constraints. In another study, we compared replicates of three stations and four months and found an average similarity of 79.1 ± 9.8% (Van Regenmortel, 2012). Average depths and flowmeter values per station (digital flowmeter placed in the circular opening of the net; 1 rotation = 1 m distance) are summarised in Table 3.1. In the BPNS, three locations on a coastal – intermediate – open sea transect were monthly sampled from March 2011 until February 2012 (Figure 3.1; Table 3.1; the open sea location was not sampled in May 2011 due to bad weather). Additionally, seasonal samples (March, June, September and December 2011, reflecting the beginning of the four seasons) were taken at three locations in the Westerschelde estuary from the lower over the middle to the upper estuary (Figure 3.1; Table 3.1).

Table 3.1: Overview of sampling locations in the Belgian part of the North Sea and the Westerschelde estuary with information on average depth, number of analysed samples and average flow meter values. For the Westerschelde, 'distance to shore' was measured from the mouth of the estuary (*i.e.* from Vlissingen).

Area	Location	Coordinates		Distance to shore (km)	Depth (m) ± SD	# samples	Av. flowmeter value ± SD
		Latitude	Longitude				
Belgian Part of the North Sea	open sea	51.75°N	2.70°E	57	34.8 ± 4.2	11	369.2 ± 181.0
	intermediate	51.53°N	2.87°E	30	20.9 ± 2.1	12	186.9 ± 87.2
	coastal	51.45°N	3.24°E	10	7.6 ± 1.1	12	83.9 ± 67.7
Westerschelde estuary	lower	51.39°N	3.78°E	16	17.2 ± 2.7	4	311.3 ± 135.9
	middle	51.42°N	4.04°E	33	12.1 ± 1.0	4	237.3 ± 152.2
	upper	51.35°N	4.24°E	48	14.1 ± 0.0	4	272.5 ± 122.2

Most Ctenophora and Scyphozoa were isolated from the CalCOFI samples on board the research vessel and immediately identified (morphologically), counted and measured (oral-aboral length and disc diameter, respectively; mm). This prevented that some ctenophore species would dissolve in the 4% formaldehyde preservation solution buffered with sea water (salinity ± 33) (personal observation), which was used to preserve the rest of the samples. Ctenophore larvae (tentaculata) were preserved in 99.97% ethanol for later genetic identification as described in Chapter 2. The formaldehyde fixed samples were further analysed in the laboratory, where the hydromedusae and the remaining small ctenophores and scyphomedusae were isolated, counted and identified to species or higher taxon level, using a stereomicroscope and the identification guides of Russell (1953) and Schuchert (2010). Species names were verified and updated through the World Register of Marine Organisms (WORMS; www.marinespecies.org). The same samples were used to count and identify the non-gelatinous zooplankton to a higher taxonomic level (e.g. Copepoda, Amphipoda, Mysida or Mollusca). Subsampling was applied according to van Guelpen *et al.* (1982). Densities (ind.m^{-3}) were calculated using filtered volume (m³) estimated by the flow meter and the surface of the net opening.

A CTD (Seabird 19plusV2) instrument package measured salinity, temperature, oxygen and turbidity at each sampling location. Due to the absence of *in situ* chlorophyll *a* measurements, reduced resolution (1.2 km) data from the Medium Resolution Spectroradiometer (MERIS; images available for 285 days) were used to estimate concentrations at the three locations in the BPNS, based on the algal_2 product algorithm used for coastal waters (MERIS Quality Working Group, 2005; Doerffer and Schiller, 2007). Vanhellemont (2012) showed that the annual cycle for chlorophyll *a* in Belgian waters is reproduced well, with clear spring algal blooms and winter minima. Based on Vanhellemont and Ruddick (2011) the mean values from a 5 by 5 pixel box around the sampling location were extracted. If less than 13 out of 25 pixels were valid (*i.e.* in cloudy conditions), we chose the value closest in time. Chlorophyll *a* measurements from the Westerschelde estuary were extracted from the waterbase database of the Dutch Ministry of Infrastructure and the Environment (Rijkswaterstaat). Water currents were calculated for each location using the three-dimensional hydrodynamic model OPTOS-BCZ (resolution 750 m x 750 m,

COHERENS V2.4.1, Royal Belgian Institute of Natural Sciences (RBINS) – OD Nature). The few missing (a)biotic values for different parameters (< 30%, mainly for oxygen and turbidity) were complemented from other databases, such as the RV Belgica ODAS database (RBINS – OD Nature) and the 'Flemish banks' monitoring network (Agency for Maritime and Coastal Services, supported by Flanders Marine Institute, VLIZ).

3.2.3 Statistical analyses

Diversity was expressed as species richness (S) and the Shannon-Wiener index (H', calculated with natural logarithm). Significant differences (p< 0.05) between locations (coastal, intermediate, open sea, lower, middle and upper estuary) and seasons (spring, summer, autumn, winter) were tested using a non-parametric Kruskal-Wallis test and several Mann-Whitney U tests, applying the Bonferroni correction for multiple pair-wise tests (assumptions for parametric tests were not met). A two-way crossed SIMPER analysis (similarity percentages, contribution of variables to similarity) was performed to identify the relative importance of certain species to the similarity within seasonal and spatial groups.

The variation in the gelatinous zooplankton dataset (47 samples) was examined using a Bray-Curtis similarity matrix after square root transformation of the density data. Spatial and temporal differences in the species composition of the samples were investigated using PERMANOVA (Permutational ANOVAs) to test for the factors season and location. Both factors and their combination were tested (p<0.05; PERMANOVA main test) followed by pair-wise testing. A PERMDISP test was performed to test the homogeneity of multivariate dispersion for each factor. A Principal Coordinates analysis (PCO) visualises the differences among seasons and locations detected by PERMANOVA, using the same Bray-Curtis resemblance matrix. Gelatinous zooplankton species showing a correlation >0.3 (multiple correlations), explaining most of the observed multivariate pattern, were plotted as vectors to visualise the potential linear relationships of these species with the ordination axes.

Finally, the environmental variables (temperature, salinity, oxygen concentration, turbidity, chlorophyll *a* concentration) and non-gelatinous zooplankton densities were related to the spatio-temporal patterns in gelatinous species composition and abundance via distance-based linear models (DistLM). To avoid multi-collinearity, draftsman plots were made for all environmental variables and a cut-off value of 0.8 was used to remove variables from the analysis based on intercorrelation (Clarke and Gorley, 2006). Salinity was reversely transformed to correct for a left-skewed distribution using log(c-y), where c is a value larger than the maximum salinity measured (y) (Clarke and Gorley, 2006). Subsequently, the most parsimonious model (based on the 'Best' procedure and the AICc and BIC selection criterion) was plotted using a distance-based Redundancy Analysis (dbRDA) (multiple correlations).

All univariate and multivariate analyses were performed using Primer version 6, Permanova+ software (Clarke and Gorley, 2006; Anderson *et al.*, 2008) and Statistica version 10 (StatSoft Inc.).

3.3 Results

3.3.1 Diversity and abundance

Three Ctenophora, three Scyphozoa and 27 Hydrozoa taxa were identified in 47 samples (Table 3.2). Species richness (S) of the gelatinous zooplankton ranged from zero to 15 and species diversity (Shannon-Wiener, H') from 0 to 1.5. Overall, hydromedusae (Hydrozoa) were most abundant (80% of the total gelatinous zooplankton density), followed by Ctenophora (18%) and Scyphozoa (2%). The four most abundant species, *Clytia* sp. (average 4.8 ± SD 11.6 ind.m^{-3}), *Eucheilota maculata* (1.7 ± 10.1 ind.m^{-3}), *Pleurobrachia pileus* (1.3 ± 3.7 ind.m^{-3}) and *Beroe* sp. (0.3 ± 1.5 ind.m^{-3}), occurred respectively in 70, 40, 77 and 34% of the samples. *Leuckartiara octona* was the rarest species. Three non-indigenous species were also present: *Mnemiopsis leidyi* (average density 0.03 ± 0.13 ind.m^{-3}, frequency of occurrence 19%), *Nemopsis bachei* (0.1 ± 0.4 ind.m^{-3}, 17%) and *Lovenella assimilis* (0.01 ± 0.02 ind.m^{-3}, 13%;Text box 3).

The most abundant taxa of non-gelatinous zooplankton were Copepoda sp. (57% of the total non-gelatinous zooplankton, 38 ± SD 103 ind.m^{-3}), Mysida sp. (17%, 11 ± 31 ind.m^{-3}) and Decapoda sp. (12%, 8 ± 17 ind.m^{-3}). Other taxa like Cirripedia, Cladocera, and Euphausiacea were only found in very low densities and classified as rare taxa (Table 3.2).

Text box 3: **Lovenella assimilis *or* Eucheilota menoni**

Data on L. assimilis *in our study area contributed to a publication of Brylinski et al. (in press). They reported hydromedusae, morphologically resembling the Indo-Pacific leptomedusa* Lovenella assimilis *(Browne, 1905) (Cnidaria: Hydrozoa: Lovenellidae), for the first time in both the eastern English Channel and the Southern Bight of the North Sea. Analyses of past zooplankton samples from a French long-term monitoring program suggest that this non-indigenous species has been present in the eastern English Channel at least since 2007. Genetic analyses identified the specimens as* Eucheilota menoni *based on nearly identical 18S ribosomal RNA gene, mitochondrial cytochrome oxydase subunit gene I (COI) sequences, and 16S Ribosomal RNA gene. Consequently, Brylinski et al. (in press) compared published morphological descriptions of* L. assimilis *and* E. menoni *and discussed their species status with regard to morphological and genetic evidence. They concluded that these two species are indistinguishable and should be merged.*

3.3.2 Spatio-temporal distribution patterns

No significant differences in gelatinous zooplankton diversity (S and H') were detected between locations (Kruskal-Wallis *p*-values = 0.29 for S and 0.10 for H'). Average gelatinous

zooplankton densities were highest in coastal waters (18 ind.m^{-3}, 28% of the total zooplankton density) and lowest in the upper estuary (0.3 ind.m^{-3}) (Figure 3.2; Table 3.2; K-W p = 0.1). Ctenophora densities in the coastal locations differed significantly from the intermediate (Mann-Whitney U p = 0.0009) and open sea locations (M-WU p = 0.0003). The ctenophore *P. pileus* was present at all six locations, with highest densities at the coastal and lower estuary locations (Figure 3.2). Similarly, the hydromedusa *Clytia* sp. was omnipresent, although most specimens were found along the coastal – open sea transect and were only occasionally noted in the estuary (Figure 3.2). Species such as *L. octona*, *Podocoryna carnea* and *Ectopleura dumortieri* were found at one of the sea locations, while the scyphomedusa *Cyanea lamarckii* was present at all sea locations, but was never found in the Westerschelde estuary. The hydromedusae *Bougainvillia* sp. and *N. bachei*, on the other hand, occurred in all three Westerschelde locations in similar or higher densities compared to the locations at sea (Table 3.2, Figure 3.2). Gelatinous zooplankton densities never exceeded the non-gelatinous zooplankton at any of the sampling locations. No significant differences in non-gelatinous zooplankton densities were detected over all six locations (K-W p = 0.39). Non-gelatinous zooplankton was most abundant in the upper estuary (approximately 134 ind.m^{-3}) and least abundant in the lower estuary (approximately 23 ind.m^{-3}). In the upper estuary, the non-gelatinous zooplankton community consisted mainly of Mysida and Copepoda, while Echinodermata, Cumacea and Tunicata were absent in this low salinity zone.

Gelatinous species richness (S) in spring differed significantly from summer (M-W U p = 0.004), autumn (M-W U p = 0.0003) and winter (M-W U p = 0.002). Only nine taxa were present in spring compared to 23 taxa in summer, 30 in autumn and 18 in winter (Table 3.2). However, no significant differences (after Bonferroni correction) in diversity were found between seasons for the Shannon-Wiener diversity index (H'). Four taxa were present in all seasons: *P. pileus*, *Beroe* sp., Corynidae sp. and *Clytia* sp. The highest average density of gelatinous zooplankton was observed in autumn (16 ind.m^{-3}; 46% of the total zooplankton density), which was significantly different from the low average density in spring (1 ind.m^{-3}; M-W U p = 0.0008; Figure 3.2; Table 3.2). Only the Hydrozoa densities differed significantly according to season (K-W p = 0.0002) and more specifically spring versus autumn (M-W U p = 0.0001) and winter (M-W U p = 0.003). The scyphomedusa *C. lamarckii*, however, reached highest densities in spring (0.3 ind.m^{-3}), thereby highly contributing to the differences between spring and the other seasons, as confirmed by the SIMPER analysis (*C. lamarckii* contributing 70% to average similarity within spring samples across all locations). Several Hydrozoa taxa bloomed in summer or autumn, e.g. *Clytia* sp. (15 ind.m^{-3}), *E. maculata* (6 ind.m^{-3}) and *Bougainvillia* sp. (0.4 ind.m^{-3}) (Figure 3.2). Non-gelatinous zooplankton densities never outnumbered the gelatinous zooplankton densities over all seasons (Figure 3.2). Average densities (ind.m^{-3}) of non-gelatinous zooplankton differed significantly between summer versus spring (M-W U p = 0.003) and autumn (M-W U p = 0.006), with lowest

average densities in spring (11 ind.m^{-3}) and highest average densities in winter (175 ind.m^{-3}; Table 3.2).

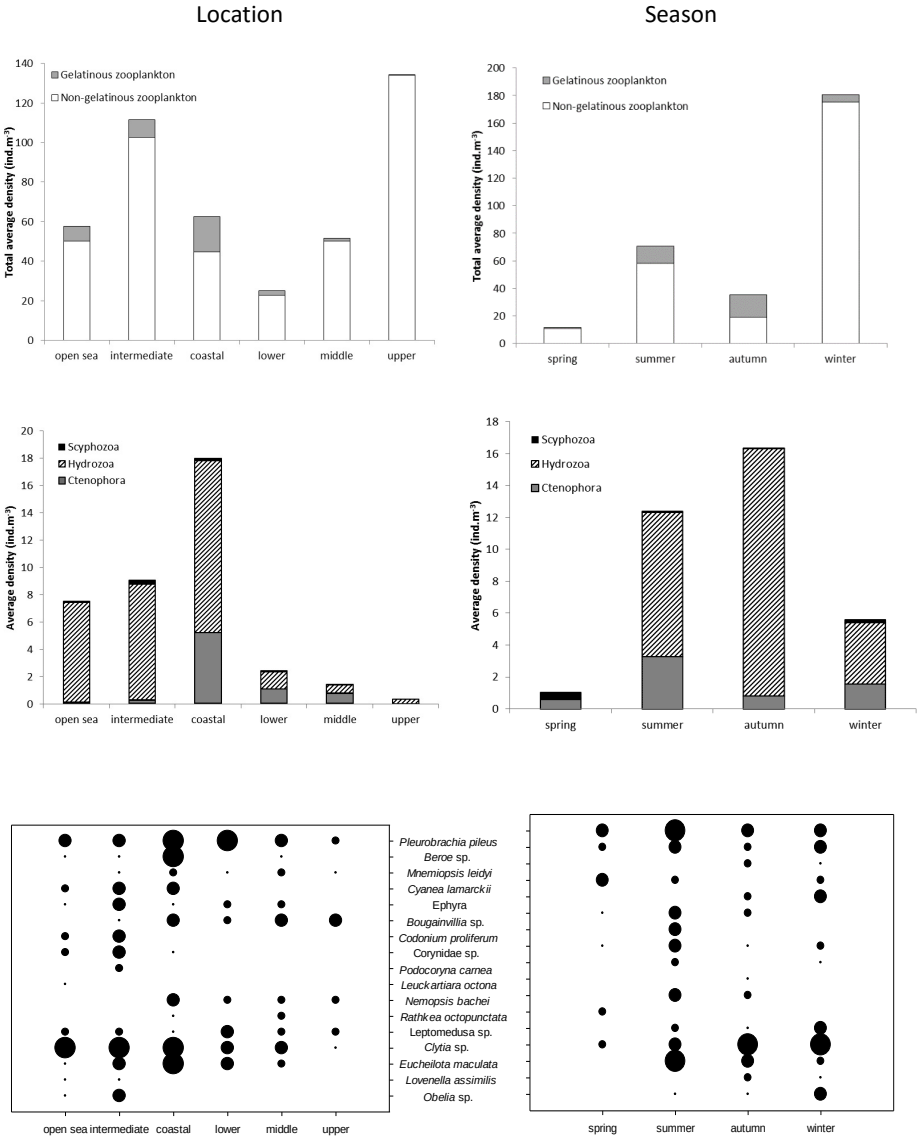

Figure 3.2 Average densities (ind.m^{-3}) for gelatinous and non-gelatinous zooplankton (upper graph), for the three gelatinous zooplankton groups (middle graph), and bubble plot for 17 abundant gelatinous zooplankton taxa (lower graph; 0 < • < 0.01 ind.m^{-3}; 0.01 ≤ ● < 0.1 ind.m^{-3}; 0.1 ≤ ● < 1 ind.m^{-3}; ● > 1 ind.m^{-3}). Left panel shows information per location and right panel per season.

Table 3.2: Average densities (ind.m^{-3}) per season and per location for gelatinous and non-gelatinous zooplankton taxa from CalCOFI net samples (1000 µm mesh size), and average values for the measured environmental variables

			Location						Season			
			open sea	intermediate	coastal	lower	middle	upper	spring	summer	autumn	winter
Gelatinous zooplankton	Ctenophora	*Pleurobrachia pileus*	0.11	0.29	3.96	1.08	0.75	0.04	0.58	3.08	0.67	0.69
		Beroe sp.	<0.01	<0.01	1.18	-	0.01	-	0.06	0.22	0.04	0.87
		Mnemiopsis leidyi	-	<0.01	0.09	<0.01	0.02	<0.01	-	-	0.09	0.01
		Total average	**0.11**	**0.29**	**5.23**	**1.08**	**0.78**	**0.04**	**0.64**	**3.30**	**0.81**	**1.56**
	Scyphozoa	*Aurelia aurita*	<0.01	<0.01	<0.01	<0.01	-	-	<0.01	<0.01	-	<0.01
		Chrysaora hysoscella	-	-	<0.01	-	-	-	-	<0.01	<0.01	-
		Cyanea lamarckii	0.07	0.15	0.15	-	-	-	0.33	0.06	-	0.01
		Ephyra unidentified	0.01	0.15	<0.01	0.06	0.03	-	-	-	0.02	0.18
		Total average	**0.08**	**0.31**	**0.16**	**0.06**	**0.03**	**-**	**0.33**	**0.06**	**0.02**	**0.19**
	Hydrozoa	Hydromedusa sp.	<0.01	0.02	-	-	0.03	-	-	0.01	<0.01	0.02
		Anthomedusa sp.	<0.01	0.01	<0.01	-	0.01	-	<0.01	0.01	<0.01	-
		Amphinema dinema	<0.01	<0.01	<0.01	-	-	-	-	-	0.01	<0.01
		Bougainvillia sp.	-	<0.01	0.32	0.09	0.15	0.18	<0.01	0.38	0.09	-
		Codonium proliferum	0.04	0.88	-	-	-	-	-	0.92	-	-
		Corynidae sp.	0.03	0.11	<0.01	-	-	-	<0.01	0.12	<0.01	0.02
		Ectopleura dumortieri	-	<0.01	-	-	-	-	-	-	<0.01	-
		Euphysa sp.	-	<0.01	<0.01	-	-	-	-	<0.01	<0.01	-
		Podocoryna carnea	-	0.02	-	-	-	-	-	0.02	-	<0.01
		Leuckartiara octona	<0.01	-	-	-	-	-	-	-	<0.01	-
		Margelopsis haeckelii	-	<0.01	<0.01	-	-	-	-	0.01	<0.01	-
		Nemopsis bachei	-	-	0.28	0.08	0.03	0.06	-	0.31	0.03	-
		Rathkea octopunctata	-	-	<0.01	-	0.09	-	0.04	-	-	-
		Leptomedusa sp.	0.01	0.02	0.01	0.33	0.04	0.06	-	0.03	<0.01	0.14
		Aequorea sp.	-	0.01	<0.01	-	-	-	-	0.01	<0.01	<0.01
		Campanulariidae sp.	-	0.01	-	-	-	-	-	-	0.01	-
		Clytia sp.	7.16	6.53	5.65	0.14	0.15	<0.01	0.01	0.80	14.91	3.12
		Eirenidae sp.	-	<0.01	-	-	-	-	-	-	<0.01	-
		Eucheilota maculata	0.01	0.28	6.28	0.61	0.10	-	-	6.37	0.40	0.04
		Lovenella assimilis	0.01	0.01	-	-	-	-	-	-	0.01	<0.01
		Eutonina indicans	<0.01	<0.01	-	-	-	-	-	<0.01	<0.01	-
		Eutima gegenbauri	-	<0.01	0.01	-	<0.01	-	-	0.01	<0.01	-
		Eutima gracilis	0.01	0.02	<0.01	-	-	-	-	0.02	0.01	<0.01
		Eutima sp.	<0.01	-	-	-	-	-	-	-	<0.01	-
		Laodicea undulata	-	0.02	-	-	-	-	-	0.02	-	-
		Obelia sp.	<0.01	0.49	-	-	-	-	-	<0.01	<0.01	0.49
		Gossea corynetes	-	-	<0.01	-	-	-	-	-	<0.01	-
		Total average	**7.29**	**8.45**	**12.58**	**1.26**	**0.59**	**0.29**	**0.05**	**9.04**	**15.50**	**3.84**
	Gel. zoopl. average density		**7.48**	**9.05**	**17.97**	**2.40**	**1.40**	**0.33**	**1.03**	**12.40**	**16.33**	**5.59**
non-gelatinous zooplankton		Amphipoda sp.	0.84	0.42	0.80	0.47	0.21	0.29	0.14	0.60	0.33	1.25
		Chaetognatha sp.	0.63	4.02	1.16	0.48	0.26	0.55	<0.01	0.62	1.48	4.08
		Copepoda sp.	35.57	62.60	26.30	8.14	30.33	47.63	0.94	1.10	0.59	147.66
		Cumacea sp.	0.23	1.10	0.05	<0.01	-	-	0.12	0.07	0.01	1.17
		Decapoda sp.	7.57	16.69	7.02	2.57	0.60	0.96	1.24	27.34	2.62	0.93
		Echinodermata sp.	0.41	1.39	0.73	-	-	-	0.78	0.12	<0.01	1.65
		Isopoda sp.	0.05	0.10	0.04	0.02	0.13	0.61	0.01	0.06	0.16	0.21
		Mollusca sp.	0.25	0.19	0.10	0.07	0.58	0.66	0.02	0.20	0.01	0.73
		Mysida sp.	0.17	0.18	6.87	10.44	17.80	82.99	5.73	21.87	13.31	3.85
		Pisces sp.	0.94	1.15	0.60	0.40	0.09	0.24	0.22	1.83	0.12	0.71
		Pisces sp. egg	1.39	0.57	0.17	0.01	<0.01	-	0.33	1.41	<0.01	0.31
		Polychaeta sp.	0.46	4.21	0.52	0.01	0.05	<0.01	0.15	0.25	0.34	4.44
		Tunicata sp.	1.59	9.87	0.33	-	0.03	-	1.02	2.61	<0.01	8.11
		Rare taxa	0.01	<0.01	0.01	0.03	0.03	0.05	<0.01	0.02	<0.01	0.03
	Non-gel. zoopl. average density		**50.11**	**102.49**	**44.69**	**22.65**	**50.11**	**133.98**	**10.70**	**58.09**	**18.98**	**175.15**
All zooplankton average density			**57.6**	**111.5**	**62.7**	**25.1**	**51.5**	**134.3**	**11.7**	**70.5**	**35.3**	**180.7**
Environmental vars		Temperature (°C)	12.7	12.2	12.0	12.9	13.2	14.2	7.9	17.0	16.7	8.5
		Salinity (‰)	35.0	34.2	32.4	26.7	22.1	14.0	28.8	30.8	30.7	31.7
		Chlorophyll *a* (mg.m^{-3})	2.9	4.2	6.4	2.9	3.2	8.2	6.3	6.5	3.4	2.4
		Turbidity (NTU)	3.7	4.3	33.6	70.0	49.0	90.0	33.3	16.9	22.6	41.0
		Oxygen concentration (mg.L^{-1})	9.7	9.6	9.0	8.3	8.8	6.9	10.8	9.7	7.2	8.6
		Water current (m.s^{-1})	0.6	0.6	0.5	0.7	0.5	<0.1	0.6	0.5	0.6	0.4

Multivariate PERMANOVA analyses confirmed the presence of seasonal and spatial differences in the gelatinous zooplankton dataset (Main test: p = 0.0008, pseudo-F = 3.25 and p = 0.001, pseudo-F = 2.59 respectively), but an interaction between season and location was not detected (p = 0.79 pseudo-F = 0.85). PERMDISP tests for both season and location were significant (F = 5.14 p = 0.008 and F = 5.59 p = 0.01 respectively), indicating that the significant PERMANOVA results could also be explained by the dispersion of the samples within season or location. Significant temporal differences were present between spring and all other seasons and between autumn versus winter (Table 3.3A). Seasonal differences, such as the clustering of spring samples (average similarity based on two-way crossed SIMPER: 39.3%), correlated to the first PCO axis, explaining 41% of the total variation (Figure 3.3). Spatial differences between the lower, middle and upper estuary locations could not be tested due to the low number of samples. However, significant differences were present between the sea locations in correlation to the second PCO axis, explaining 19% of the total variation (Table 3.3B, Figure 3.3). The vector of the newly observed non-indigenous hydromedusa in Belgian waters, *L. assimilis*, pointed in the opposite direction of the spring cluster, implying absence in that season. The species seemed mainly present at the open sea and the intermediate locations rather than the coastal or estuarine locations (also see Table 3.2, Figure 3.2; Brylinski *et al.,* in press). The vector of the hydromedusa *Clytia* sp. was strongly related to the sea locations in autumn, winter and summer. The vector of the ctenophore *P. pileus* pointed towards the coastal station and a less clear relation with the spring season was noted. The scyphomedusa *C. lamarckii*, although contributing 70% to the average similarity within spring samples (SIMPER analysis across all locations), did not appear as a vector on this PCO plot (correlation <0.3).

Table 3.3 PERMANOVA pair-wise testing significance levels (*p*-values) for temporal (a) and spatial (b) differences in gelatinous zooplankton distribution patterns.

A	spring	summer	autumn	winter
Spring				
Summer	**0.02**			
Autumn	**<0.01**	0.23		
Winter	**0.02**	0.21	**0.04**	

B	open sea	intermediate	coastal	lower	middle	upper
open sea						
intermediate	0.35					
Coastal	**<0.01**	**0.02**				
Lower	**0.03**	0.13	0.14			
Middle	**0.02**	0.13	0.24	/		
Upper	**0.04**	**0.03**	**<0.01**	/	/	

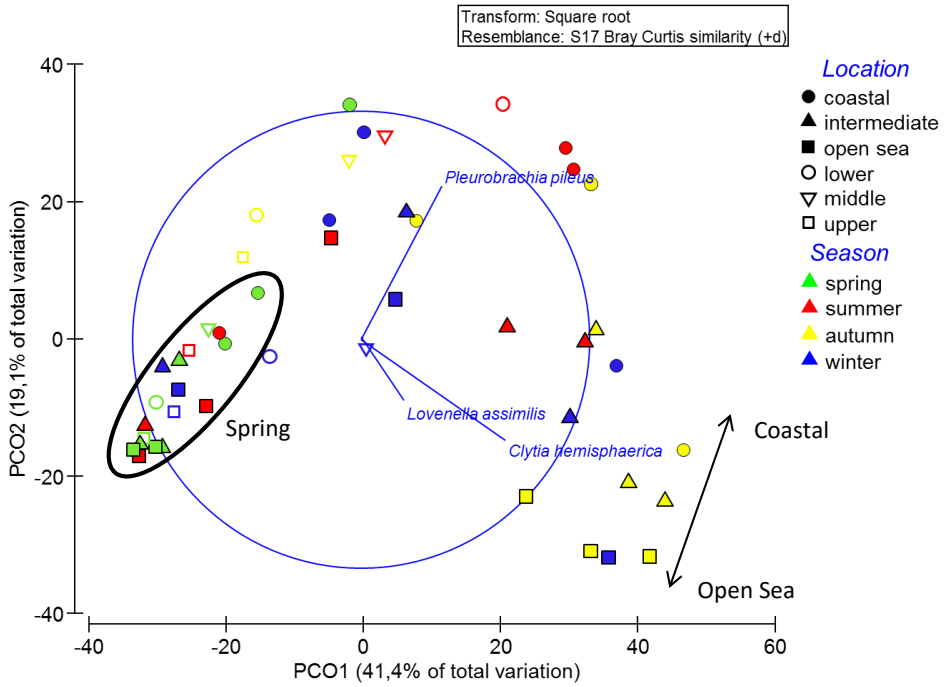

Figure 3.3 Multivariate PCO analysis of gelatinous zooplankton densities related to the factors location and season. Vector overlay showing species vectors with a correlation of more than 0.3 with both PCO axes and circle radius representing maximum correlation (=1).

The most parsimonious DistLM model, both based on AICc and BIC, included the variables temperature, salinity (transformed as log(40-salinity)) and oxygen concentration, together explaining 30% of the variation (Table 3.4). The marginal tests show the proportion of the variation that would be explained by each variable separately (Table 3.4). Temperature varied little over the locations, with a slightly higher temperature near the upper estuary compared to the locations at sea (Table 3.2). The seasonal variation in temperature was higher, with lowest temperatures measured in spring and winter compared to the warmer summer and autumn. Seasonal variation in salinity was absent, but there was a clear downward trend from the high saline open sea to the upper estuary location. Oxygen concentrations were lower in the estuary and in autumn, but never measured below 6.9 $mg.L^{-1}$. The relation between the gelatinous zooplankton composition and the environmental drivers as calculated by the DistLM is visualised in the constrained dbRDA plot, with the first two axes explaining 29% of the total variation (Figure 3.4). The first axis (23% of total variation) was related to season (temperature and inversely related to oxygen concentration), and clustered the colder seasons (spring and winter) against summer and autumn. The second axis (6% of total variation) was related to salinity, which clearly discriminated the estuarine and sea samples. The non-gelatinous zooplankton explained a

small part of the total variation in the gelatinous zooplankton dataset (Table 3.4), but this variable was not included in the 'most parsimonious' model.

Table 3.4 The most parsimonious DistLM model, based on AICc and BIC, is shown for environmental variables explaining best the variation in the gelatinous zooplankton dataset (density ind.m^{-3}). Marginal tests show the proportion of the variation that would be explained by each environmental variable separately.

Model	AICc	BIC	R^2
temp; log(40-sal); oxy	341.90	348.35	0.30
MARGINAL TESTS			
Variable	Pseudo-F	*p*	Explained proportion
Temperature (°C)	5.53	0.001	0.11
log(40-salinity) (‰)	4.53	0.003	0.09
Chlorophyll a (mg m^{-3})	1.68	0.122	0.04
Turbidity (NTU)	1.87	0.097	0.04
Oxygen (mg L^{-1})	5.59	0.001	0.11
Water current (m s^{-1})	0.48	0.837	0.01
Total non gelat zoopl CalCOFI (ind.m^{-3})	1.97	0.069	0.04

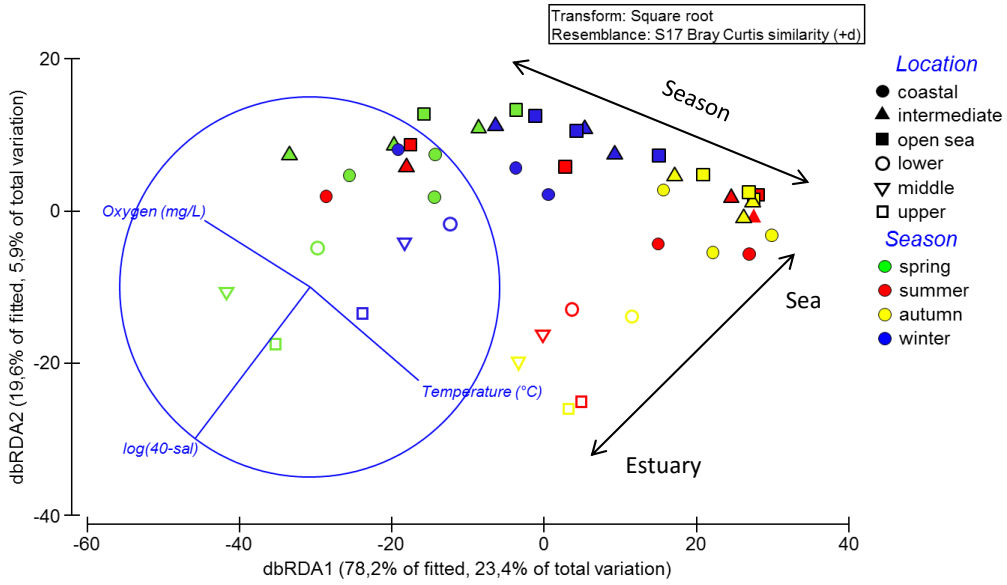

Figure 3.4 Visualisation of the DistLM most parsimonious model in a dbRDA plot including the environmental variables temperature, salinity (log(40-salinity)) and oxygen concentration (circle radius representing maximum correlation of the environmental variables), with an indication of the factors season and location.

3.3.3 Population dynamics

The size of the ctenophore *Pleurobrachia pileus* ranged from 1 to 40 mm in oral-aboral length (Figure 3.5). This species was year-round present at the coastal location and small individuals were observed three times a year. Its predator, the ctenophore *Beroe* sp. followed a similar pattern. During autumn the non-indigenous ctenophore *Mnemiopsis leidyi* appeared in a broad size spectrum (7 to 55 mm) at the coastal location, whereas only few individuals ranging between 7 and 20 mm were observed at the middle estuary location. The scyphomedusae *Cyanea lamarckii* occurred at small sizes close to the coast, while a wider size spectrum was observed in stable densities further offshore.

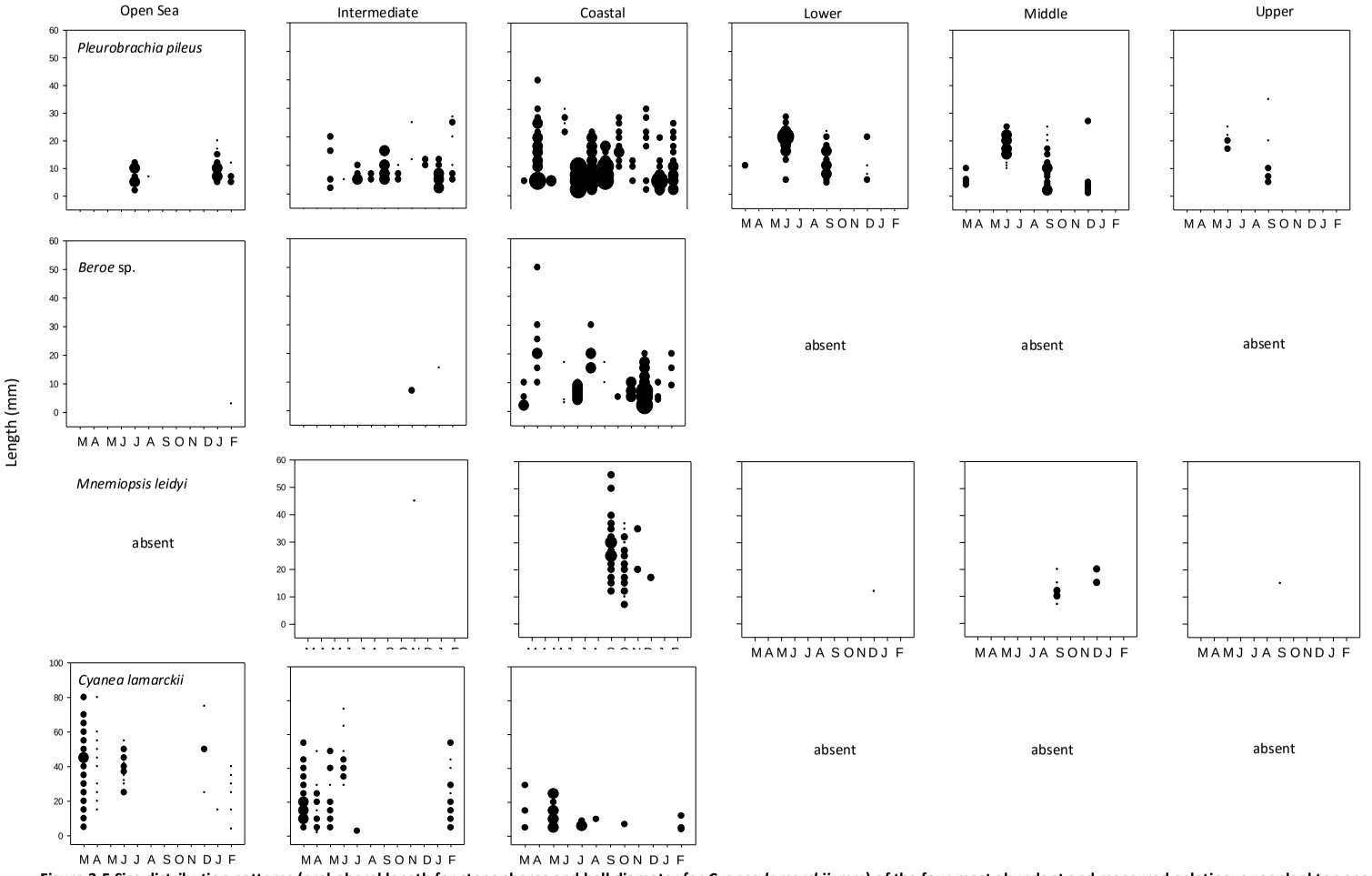

Figure 3.5 Size distribution patterns (oral-aboral length for ctenophores and bell diameter for *Cyanea lamarckii*, mm) of the four most abundant and measured gelatinous zooplankton species (*Pleurobrachia pileus*, *Beroe* sp, *Mnemiopsis leidyi* and *Cyanea lamarckii*) per location and per month (BPNS, 3 left panels) or season (Westerschelde estuary, 3 right panels; only data available from March, June, September and December) between March 2011 and February 2012. Legend: 0 < • < 0.01 ind.m^{-3}; 0.01 ≤ ● < 0.1 ind.m^{-3}; 0.1 ≤ ● < 1 ind.m^{-3}; ⬤ > 1 ind.m^{-3}).

3.4 Discussion

3.4.1 Diversity and abundance

This study presents a comprehensive overview of the gelatinous zooplankton diversity and density in the BPNS and the Westerschelde estuary. Thirty-three gelatinous zooplankton taxa were identified in 47 samples, some not yet included in the Belgian Register of Marine Species (Vandepitte *et al.*, 2010). Particularly in autumn and at the coastal location, gelatinous zooplankton contributed considerably to the overall zooplankton abundance (46% and 28% of the total zooplankton density in the CalCOFI samples; but see §3.4.4). Most species are typical for the North Sea and North-western Atlantic (Russell, 1953; Kramp, 1959; Schuchert, 2010).

Studying gelatinous zooplankton comes with certain challenges. The chosen sampling method may result in an over- or underestimation of the gelatinous zooplankton diversity and density (e.g. Remsen *et al.,* 2004; Barz and Hirche, 2007; Chapter 2). For example Frost *et al*. (2012) found 11 taxa at the Dogger Bank (Central North Sea) and Daan (1989) collected 7 taxa in Dutch coastal waters, which is lower compared to the diversity in the BPNS and Westerschelde estuary. Van Ginderdeuren *et al.* (2012a) collected 18 species in the BPNS with vertical WP2 net hauls (mesh size 200 µm), which is only half the number of taxa caught in the undulating CalCOFI net hauls used in our study. However, these studies used a different sampling methodology, which makes comparison difficult. Likewise, comparing density estimates between different studies (e.g. Daan, 1989; Lucas *et al.*, 1995; Wang *et al.*, 1995; Barz and Hirche, 2007; Hosia *et al*., 2008; de Wolf, 2012) requires cautiousness. The filtered volume largely contributes to different estimates of the total gelatinous zooplankton density and the chance to catch more species is likely to increase when more water is filtered. Additionally, the mesh sizes of the plankton net affects the catch. The 1000 µm mesh size used in our study probably underestimated the presence and density of small species and larval stages of both gelatinous and non-gelatinous zooplankton. Also larger scyphozoans might be underestimated. Scyphomedusae form aggregations (Graham *et al.,* 2001) and are more adequately sampled with larger net types, such as pelagic trawl nets. Moreover, certain gelatinous zooplankton density peaks may remain unnoticed related to temporal (sampling frequency; see Chapter 4) and spatial resolution.

Another challenge is the morphological identification of gelatinous zooplankton. The fragile bodies of gelatinous species are often damaged during sampling, which makes the identification to species level impossible (Bouillon *et al.,* 2006). In addition, phenotypic variation in hydromedusae is large and young individuals do not exhibit all adult characteristics (Cornelius, 1990). By using genetic tools, Laakmann and Holst (2014) proved the presence of (at least) two species of *Clytia* in the German Bight area. Additionally, preservation affects the identification characteristics. Although a 4% formaldehyde solution is an appropriate preservative for many gelatinous species (Cornelius, 1995; Holst and

Laakmann, 2014; Laakmann and Holst, 2014), Schuchert (2001) argued that, for example, Corynidae medusae are best studied alive. The same is true for some fragile Ctenophora species, like *Mnemiopsis leidyi* (Chapter 2). In our study, all three factors hampered the identification of some specimens, e.g. *Eutima* missing tentacles, only the umbrella present in the samples and damaged ephyrae due to preservation. In those cases, we identified to genus or family level. Only ctenophore larvae (Tentaculata) were genetically identified (Chapter 2).

3.4.2 Spatio-temporal distribution patterns

In general, the same zooplankton species assemblage (both gelatinous and non-gelatinous taxa) was present across locations and seasons in the BPNS and the Westerschelde estuary. According to the PCO and DistLM analyses, the little variation we noted in terms of density and species composition seems to be driven by salinity, temperature and/or oxygen concentration.

The main parameter explaining the spatial patterns in gelatinous zooplankton distribution was salinity. Although the Westerschelde estuary was sampled less frequently, gelatinous zooplankton species composition and densities were considerably lower compared to the locations at sea, especially in the upper Westerschelde estuary (salinity between 10 and 19 ‰). Similar patterns have been observed in other estuarine areas (Arai, 1992; Padmavati and Goswami, 1996; Holst and Jarms, 2010 and references therein). The absence of the scyphomedusa *C. lamarckii* in the Westerschelde estuary corresponds with the findings of Gröndahl (1988), who observed higher abundances of *C. lamarckii* medusae at higher salinities. Differently, the non-indigenous hydromedusa *Nemopsis bachei* was present at different salinities throughout the Westerschelde estuary. Moore (1962) demonstrated a high tolerance towards a broad salinity range in its native distribution area in the northern Gulf of Mexico. Since its introduction, *N. bachei* has permanently established populations in the brackish and marine waters of the Dutch and German Wadden Sea, the Elbe estuary and the German Bight (Laakmann and Holst, 2014 and references therein). The population found in the Westerschelde estuary might have been introduced through ballast water discharge (Carlton, 1985; Globallast, 2015) in the port of Antwerp. Also, some non-gelatinous zooplankton taxa are found at high densities in the upper Westerschelde estuary. Especially Mysida and Copepoda are known to be favoured by the higher turbidity at this location, related to high detritus concentrations (Irigoien and Castel, 1995; Fockedey and Mees, 1999).

Still, highest densities and diversity of gelatinous zooplankton were found in the coastal and lower estuary locations. High nutrient inputs (related to anthropogenic activities) in the coastal zone and lower estuary may result in a higher primary production and more food (Howarth, 1988; Lohrenz *et al.*, 1999), leading to better conditions for both gelatinous and non-gelatinous plankton. Furthermore, the higher water temperature at these locations may

enhance reproduction of temperate gelatinous zooplankton taxa (Purcell *et al*., 2007; Holst, 2012a). The ctenophore *P. pileus* and the hydromedusa *Clytia* sp. were very common in the coastal zone in our study, similar to the coastal zones of north-western Europe (van der Baan, 1980; Wang *et al.,* 1995; Greve *et al.*, 2004). *Pleurobrachia pileus* relies on tidal currents for its resuspension (de Wolf, 2012), which seem to be optimal in the coastal zone and lower estuary. Additionally, medusae of *Clytia* sp. were also present in high densities at the intermediate and open sea locations. A plausible explanation is the presence of offshore wind turbines near our study locations (Brabant *et al.*, 2009). These recently-introduced hard structures form excellent sites for the affixation of Hydrozoa and Scyphozoa polyps (Degraer *et al.*, 2012; Duarte *et al.,* 2012). As such, the construction of wind farms at sea may contribute to a higher diversity and higher abundances of gelatinous zooplankton (including non-indigenous species).

Temperature plays an important role in the seasonal structure of the gelatinous zooplankton community. Species composition and densities were lower in spring, characterised by low average temperatures (7.9 °C), and tend to increase with temperature. Most species occurred in two or three seasons and some species, e.g. *P. pileus*, were present year round. De Wolf (2012) showed that temperature enhances the reproduction of zooplankton organisms. In our study, *Clytia* sp. reached highest densities in autumn (15 ind.m^{-3}) and were least represented in spring (0.6 ind.m^{-3}). A similar trend was shown by van der Baan (1980) and Daan (1989) in Dutch coastal waters, although both studies reported much higher densities for *Clytia* sp. Hosia and Båmstedt (2007) observed two reproductive cycles for *Clytia* sp. in Norwegian fjords, with highest *Clytia* sp. densities in spring/summer and a second smaller peak in autumn. According to Lucas *et al.* (1995), *Clytia* sp. could have a continuous reproduction or produce distinct cohorts in Southampton Water, depending on the year. Our results do not allow drawing conclusions on population dynamics of *Clytia* sp., as they were not measured and the density of small medusae was probably underestimated with the 1000 µm-mesh-sized CalCOFI net. Temperature also influences the budding and strobilation process of Hydrozoa and Scyphozoa (Loeb, 1972; Arai, 1992; Purcell *et al.,* 1999; Ma and Purcell, 2005; Holst, 2012a), with characteristic temperature ranges for each species (Verwey, 1942; De Blauwe, 2003). Temperatures between 5 and 15 °C are optimal for the production of *C. lamarckii* ephyrae (Holst, 2012b). As winter and spring temperatures in our study area lie within this range, extensive strobilation might have occurred, explaining why *C. lamarckii*, known as one of the early-occurring gelatinous species in the southern North Sea (Gröndahl, 1988; Barz and Hirche, 2007), was mainly found in spring.

Next to temperature and salinity, also oxygen concentration was retained in the most parsimonious DistLM model as explaining parameter, related to the downward trend from the open sea to the upper estuary location. Most gelatinous zooplankton taxa require an adequate supply of oxygen, but some taxa can survive hypoxic water (Arai, 1992). The hydromedusa *Rathkea octopunctata* has been observed in areas depleted of oxygen (Beyer,

1968). Also the non-indigenous ctenophore *M. leidyi* tolerates low oxygen concentrations (Decker *et al.*, 2004). Both species were observed towards the upper Westerschelde estuary, where the lowest oxygen concentration (6.9 $mg.L^{-1}$) was measured. However, this is still a rather high value, and as such we suppose oxygen is not a critical environmental driver behind the gelatinous zooplankton distribution patterns.

3.4.3 Population dynamics

Knowledge on the size distribution provided insight in the population dynamics of some ctenophore and scyphozoan species in the BPNS and the Westerschelde estuary. The ctenophore *P. pileus* seemed to have three reproductive cycles at the coastal location. Its gelatinous predator, *Beroe* sp. followed this reproduction pattern, with the exception of autumn when it occurred earlier. The other ctenophore *Mnemiopsis leidyi*, which is present from late summer onwards, probably served as an alternative (non-indigenous) food source for *Beroe* sp. Also, small *Cyanea lamarckii* scyphozoans reached highest densities at the coastal location, whereas the open sea location was characterised by stable densities of a broad size spectrum of *C. lamarckii* in spring. Duarte *et al.* (2012) illustrated that coastal protection enhances the presence of polyps, which might explain the higher number of small individuals in the coastal location.

3.4.4 Relation with non-gelatinous plankton

Many gelatinous zooplankton taxa are carnivorous and feed on non-gelatinous zooplankton (Alvariño, 1985; Purcell and Mills, 1988), while some species feed on other gelatinous species, e.g. *Beroe* sp. and *Cyanea* sp. (e.g. Greve *et al.,* 2004; Hosia *et al.,* 2010; Hosia and Titelman, 2011). Copepods form an important part of the diet of gelatinous zooplankton (e.g. Daan, 1989; Matsakis and Nival, 1989; Matsakis, 1993). In our study, two peaks of non-gelatinous zooplankton were observed, one in summer and one in winter, mainly related to the dominance of decapods and copepods. This observation seems to contradict the generally expected pattern in zooplankton densities in temperate regions, *i.e.* a strong spring bloom and a potential secondary bloom during autumn (O'Brien *et al.*, 2011). This 'mismatch' can be explained by the underestimation of smaller copepod species in our study as mainly 'larger' individuals were collected with the CalCOFI net (an order of magniture less than when sampled with more appropriate gear for copepods such as the WP2 net, mesh size 200 µm; Van Ginderdeuren, 2013a). From the study by Van Ginderdeuren *et al.* (2014) we know that small copepods are actually very abundant in spring (and to a lesser extent in autumn) in the BPNS. Moreover, highest chlorophyll *a* concentration were observed in spring and summer, supporting bottom-up control (Greve *et al.,* 2004) and the structuring role of phytoplankton blooms on the presence of herbivorous zooplankton (Bode *et al.*, 2005; Freund *et al.*, 2006).

The gelatinous zooplankton summer peak and persistent high densities in autumn may be related to the high densities of larger non-gelatinous zooplankton in summer, whereas the higher density of non-gelatinous zooplankton in winter was not followed by higher gelatinous zooplankton densities. Most probably the low winter temperatures inhibited the survival and reproduction of gelatinous zooplankton (Purcell *et al.,* 2007; Holst, 2012a). Reproduction in the following year seems to be ensured through the production of polyps, but probably also a few medusae and ctenophores survive the cold winter in our study area (e.g. Kramp, 1937 as referred in Hosia and Båmstedt, 2007; Costello *et al.,* 2012).

3.5 Conclusion

This study presents a comprehensive overview of the gelatinous zooplankton diversity and density in the Belgian part of the North Sea (BPNS) and the Westerschelde estuary. Both areas were year-round dominated by more or less the same, highly diverse species assemblage. However, the densities of the prevalent species varied over seasons and locations and most gelatinous species seem to prefer the coastal area over large parts of the Westerschelde estuary. This study confirmed that salinity, temperature and oxygen concentration are the main structuring variables explaining the spatial and temporal distribution of gelatinous zooplankton.

Several studies attempted to explain the main drivers of jellyfish blooms (Arai, 1992; Mills, 2001; Purcell, 2012). Due to our limited knowledge on diversity, abundance and distribution of gelatinous zooplankton in Belgian waters from the previous decennia no solid conclusions can be drawn regarding the so-called jellification paradigm. The perception that jellyfish densities have been increasing over the past years in Belgian waters seems largely influenced by personal experience, e.g. fishermen who are confronted with clogged fishing gear or tourists observing 'high' jellyfish numbers while swimming (Chapter 7). So far, our data did not demonstrate higher densities compared to other areas in the southern North Sea, the English Channel or the Wadden Sea (Lucas *et al.,* 1995; Wang *et al.,* 1995; Barz and Hirche, 2007; de Wolf, 2012). The relatively short period covered by this study is not sufficient to identify inter-annual differences or to link changes in temperature to changes in gelatinous zooplankton densities over consecutive years. However, our data show that at least the summer and autumn blooms need precautionary attention. Increased anthropogenic pressure and increasing seawater temperatures related to climate change (Purcell *et al.,* 2007), can lead to earlier appearance of gelatinous zooplankton (van Walraven *et al.,* 2014) and even better conditions for non-indigenous species. As such, the present study provides the necessary scientific baseline to further investigate the potential increase of gelatinous zooplankton in the BPNS and the Westerschelde estuary.

3.6 Acknowledgements

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4

ON THE DISTRIBUTION AND POPULATION DYNAMICS OF THE CTENOPHORE *MNEMIOPSIS LEIDYI* IN THE BELGIAN PART OF THE NORTH SEA AND WESTERSCHELDE ESTUARY

Modified from:

Vansteenbrugge, L., Ampe, B., De Troch, M., Vincx, M., Hostens, K., 2015. On the distribution and population dynamics of the ctenophore *Mnemiopsis leidyi* in the Belgian part of the North Sea and Westerschelde estuary. Marine Environmental Research 110, 33-44.

ABSTRACT

The spatio-temporal distribution and population dynamics of the non-indigenous ctenophore *Mnemiopsis leidyi* A. Agassiz 1865 were investigated through monthly and quarterly surveys in 2011-2012 at several locations in the Belgian part of the North Sea, the main coastal ports and the adjacent Westerschelde estuary. *Mnemiopsis leidyi* occurred from August to December, but was never found more than 30 km offshore. Densities were generally low (average 0.8 ± SD 2.8 ind.m^{-3}) compared to other invaded European systems. Highest densities of *M. leidyi* were found in the semi-enclosed basin of the port of Oostende (18.4 ind.m^{-3}) and in the Westerschelde estuary (1.9 ind.m^{-3}). The presence of larvae and the sudden appearance of high numbers across the size distribution in August indicated that ports and estuaries may act as sources, populating the adjacent coastal area. The zero-inflated logistic regression model showed that there is a higher chance of finding *M. leidyi* (presence) when temperature declines from late summer onwards. Combined with a negative binomial regression, our model suggests that increasing *M. leidyi* densities are associated with decreasing autumn temperatures, low wave height (low energetic systems) and low dissolved oxygen concentrations. Although densities remained relatively low since its first appearance in 2007, a permanent population seems to be established in Belgian waters. As population outbursts may occur with only a small change in environmental parameters, further monitoring of this notorious invasive species is recommended.

4.1 Introduction

Invasions of non-indigenous species in coastal waters and inland seas are common worldwide (Streftaris *et al.,* 2005; Richardson *et al.,* 2009). Invasive species have been identified as a major threat to marine ecosystems, leading to biodiversity loss and adverse environmental, economic and social impacts (Darrigran and Pastorino, 1995; GESAMP, 1997; Occhipinti-Ambrogi and Savini, 2003; Marine Strategy Framework Directive, 2008/56/EC). A

notorious example is the invasion of the ctenophore *Mnemiopsis leidyi* A. Agassiz 1865 in the Black Sea (e.g. Shiganova *et al.,* 2001; Gucu, 2002). High densities of this non-indigenous species (up to 304 ind.m^{-3}), in combination with overfishing and eutrophication caused the local fisheries and economy to collapse (Vinogradov *et al.,* 1989; Kideys, 1994; Gucu, 2002).The success of invasive species is partly attributed to their broad tolerance for different environmental parameters (Sakai *et al.,* 2001). *Mnemiopsis leidyi*, for example, has a high tolerance to salinity (0-40), temperature (0-32 °C) and low oxygen concentrations (as low as 1.0 mg L^{-1}) (Purcell *et al.,* 2001; Decker *et al.,* 2004; Fuentes *et al.,* 2010). However, the survival threshold may depend upon a combination of these parameters. For example, in the sea of Azov with surface salinities ranging between 0 and 14, *M. leidyi* does not survive temperatures less than 4 °C (Shiganova and Malej, 2009).

Furthermore, *M. leidyi* is a simultaneous, self-fertile hermaphrodite, capable of rapid reproduction (Costello *et al.,* 2012), potentially producing more than 11 000 eggs ind^{-1} daily (Baker and Reeve, 1974; Jaspers *et al.,* 2014b). It also has a broad zooplanktivorous diet, including different copepod species, barnacle nauplii, bivalve veligers and cladocerans, but also fish eggs and larvae (Purcell and Arai, 2001; Purcell *et al.,* 2001 and references therein; Costello *et al.,* 2006; Javidpour *et al.,* 2009b; Granhag *et al.,* 2011). The larvae feed on the smaller fraction of the pelagic food web, *i.e.* microplankton (Sullivan and Gifford, 2007). The combination of high feeding, growth and reproduction rates enables *M. leidyi* populations to rapidly increase when conditions are favourable (Purcell *et al.,* 2001).

Mnemiopsis leidyi invaded northern Europe around 2005 (Javidpour *et al.,* 2006; Oliveira, 2007). Since then, high densities have been noted in Limfjorden (Denmark, 867 ind.m^{-3}; Riisgård *et al.,* 2007) and the Wadden Sea (The Netherlands, 610 ind.m^{-3}; van Walraven *et al.,* 2013). Hitherto, *M. leidyi* has been observed from the English Channel (Antajan *et al.,* 2014) and the southern North Sea (Van Ginderdeuren *et al.,* 2012; van Walraven *et al.,* 2013) up to the German Bight, Danish territorial waters and mid-Norway (Boersma *et al.,* 2007; Javidpour *et al.,* 2009a; Riisgård *et al.,* 2012; Hosia and Falkenhaug, 2015). The species has not yet been encountered along the UK coasts (Antajan *et al.,* unpublished data; Collingridge *et al.,* 2014). Several estuarine systems such as the Seine estuary (Antajan *et al.,* 2014), the Westerschelde and other estuaries along the Dutch coast (Faasse and Bayha, 2006; van Walraven *et al.,* unpublished data) have already been colonised by *M. leidyi*. The rich spawning, nursery and feeding grounds situated in the coastal areas and estuaries of the southern North Sea (Beyst *et al.,* 2001; Ellis *et al.,* 2011; Van Ginderdeuren *et al.,* 2013b) may all suffer from the predatory and competitive impact of this non-indigenous ctenophore. Moreover, due to high anthropogenic pressure and depleted fish stocks, the (southern) North Sea ecosystem might be equally vulnerable to *M. leidyi* outbreaks as the Black Sea (e.g. ICES, 2005; 2006; De Backer *et al.,* 2014). However, the native ctenophore *Beroe* sp. and scyphomedusa *Chrysaora hysoscella* have been described as predators of *M. leidyi* and

may consequently hinder potential outbreaks (Hosia *et al.,* 2010; Hosia and Titelman, 2011; Chapter 3).

For the Belgian Part of the North Sea (BPNS), observations of *M. leidyi* up to 2010 have been summarized by Van Ginderdeuren *et al.* (2012b) in a semi-quantitative study. However, it is unclear how the distribution of this non-indigenous ctenophore has evolved since 2010. In the present study, we provide quantitative data on the spatial and temporal distribution patterns and population dynamics of *M. leidyi* in the BPNS, three Belgian ports and the Westerschelde estuary. The environmental variables that are potentially driving the current presence, abundance, demography and distribution of *M. leidyi* are investigated. This information will enable prediction of potential outbreaks of this invasive species.

4.2 Materials and Methods

4.2.1 Study area

The Belgian Part of the North Sea (BPNS), with a surface of nearly 3500 km², is situated in the Southern Bight of the North Sea and bounded by a 67 km sandy coastline (Figure 4.1). The main coastal ports are Nieuwpoort on the west coast (P3), Oostende (P2), and Zeebrugge on the east coast (P1). The port of Nieuwpoort is the smallest and mainly visited by small yachts. Oostende also has a marina, but is also home to 22 registered fishing vessels (*d.d.* 9 October 2014, FPS Mobility and Transport, Marine Fisheries Services). For this study, we focused specifically on the semi-enclosed basin 'Spuikom' (sluice dock), which is connected to the marina of Oostende and the North Sea through sluices. This system is mainly used for recreational activities and eel fisheries. Zeebrugge has the largest port along the Belgian coastline, with 42 registered fishing vessels (*d.d.* 9 October 2014, FPS Mobility and Transport, Marine Fisheries Services). It is home to the Belgian navy fleet, but also daily visited by a large number of container vessels from all over the world.

Atlantic water is transported in a north-easterly direction through the Channel towards the BPNS, where the currents meet the south-westerly oriented Westerschelde estuarine outflow near the Dutch-Belgian border (Vlaeminck *et al.,* 1989; Lacroix *et al.,* 2004). The Westerschelde (The Netherlands) covers 310 km² and stretches from Vlissingen (lower estuary) to Bath (upper estuary) over 58 km. It is characterised by a macro-tidal current regime, which keeps the water column (average depth 30 m) well mixed (Meire *et al.,* 2005). The estuary connects important ports in Terneuzen and Antwerp with the North Sea through busy shipping lanes.

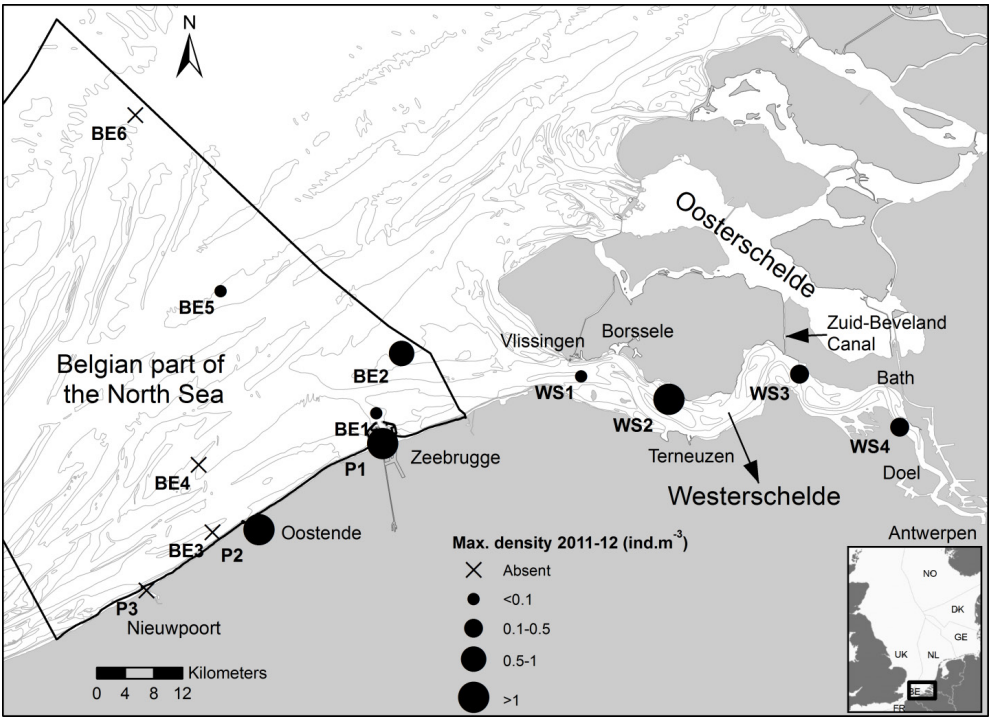

Figure 4.1 Map with sampling locations showing spatial distribution of *M. leidyi* based on the maximum densities at each location in the Belgian part of the North Sea (BE), coastal ports (P) and Westerschelde estuary (WS) in 2011 and 2012. Inset: North Sea exclusive economic zones with the square (▭) representing the study area

4.2.2 Gelatinous zooplankton data

Gelatinous zooplankton were sampled in 2011 and 2012 in the three different systems: the BPNS, the 3 coastal ports and the Westerschelde estuary. In 2011, three locations were monthly sampled in the BPNS (BE2, BE5 and BE6), and three locations (WS2, WS3 and WS4) were quarterly sampled in the Westerschelde (Figure 4.1, Table 4.1). In 2012, samples were collected at the same locations in January and February and further on a monthly basis from July to December. The three coastal ports (P1, P2 and P3) were only sampled in 2012, monthly from July to December, except for Nieuwpoort (P3) which was only sampled in August and November. In October 2012, six additional samples were collected in the BPNS (BE1, BE3, BE4) and the Westerschelde estuary (WS1, WS2 and WS3) during a European survey on board RV Thalia as part of the INTERREG IVa 2 Seas MEMO project.

Table 4.1 Overview of sampling locations in the Belgian part of the North Sea, the ports and the Westerschelde estuary with indication of sampling periods, maximum *M. leidyi* densities and filtered volume per location.

Area	Station	Location	Coordinates (WGS84)		Distance to shore (km)	2011												2012							Max. density *M.leidyi* (ind.m^{-3})	Filtered volume (m^3) ± SD
			Lat (N)	Long (E)		J	F	M	A	M	J	J	A	S	O	N	D	J	F	M-J	J	A	S	O	N	D	
Belgian part of the North Sea	BE1	coastal	51.38°	3.19°	5																		x				0.02	427
	BE2		51.45°	3.24°	10	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	0.55	229.61 ± 143.87
	BE3		51.23°	2.86°	2																		x				0	544
	BE4		51.31°	2.83°	10																		x				0	611
	BE5	intermediate	51.53°	2.87°	30	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x		0.01	473 ± 310
	BE6	open sea	51.75°	2.70°	57	x	x	x	x		x	x	x	x	x	x	x	x	x								0	719 ± 472
Ports	P1	Zeebrugge	51.34°	3.20°	0																x	x	x	x	x	x	2.42	8 ± 1
	P2	Oostende	51.23°	2.95°	0																x	x	x	x	x	x	18.37	5 ± 2
	P3	Nieuwpoort	51.15°	2.73°	0																	x			x		0	3 ± 1
Westerschelde estuary	WS1	lower	51.42°	3.60°	3																		x				0.08	504
	WS2		51.39°	3.78°	16				x			x			x		x					x	x			x	1.85	566 ± 375
	WS3	middle	51.42°	4.04°	33				x			x			x		x					x	x			x	0.37	322 ± 160
	WS4	upper	51.35°	4.24°	48				x			x			x		x					x				x	0.11	340 ± 199

At sea and in the estuary, a 1 m-diameter, 1000 µm-mesh CalCOFI plankton net was towed through the water column (undulating three times from sea surface to bottom) at a speed of 3 knots (towyo; Wiebe *et al.,* 2014) on board RV Zeeleeuw and RV Simon Stevin (Flanders Marine Institute). This 4-meter-long net was equipped with a digital flowmeter in the circular opening of the net (1 rotation = 1 m distance), allowing calculation of the average filtered volume per location (Table 4.1). One replicate was shown to be representative for the zooplankton composition of the different stations (Van Regenmortel, 2012) and therefore replicates were reduced from 3 to 1 CalCOFI net sample per location in 2012. The replicates of the 2011 samples were averaged to interpret the spatial and temporal distribution of *M. leidyi* and to construct the statistical model. Due to logistic issues, the port locations were sampled from a pontoon towing a handheld dip-net (Ø 0.20 m, mesh size 200 µm) instead of the CalCOFI net.

All ctenophore species were isolated from the samples, morphologically identified and measured (oral-aboral length, mm). If doubt arose on the morphological identification (e.g. ctenophore larvae), genetic analysis was executed as described in Van Ginderdeuren *et al.* (2012b). Additionally, the scyphomedusa *Chrysaora hysoscella* was noted in terms of presence/absence. Densities in the CalCOFI net samples (ind.m^{-3}) were calculated using filtered volume and surface of the net opening. Density conversions for the dip-net samples (no flow meter deployed) were based on the towed distance. Subsequently, average densities were calculated per month and per location.

The life cycle of *M. leidyi* encompasses four different stages: the egg, the tentaculated cydippid larva, a transitional stage and the lobate adult (Rapoza *et al.,* 2005). To investigate the population dynamics and growth of *M. leidyi* in the study area, we considered all individuals ≤5 mm (oral-aboral length) as larvae.

4.2.3 Environmental data

A CTD (Seabird 19plusV2) instrument package was used to measure salinity, temperature (°C), oxygen ($mg.L^{-1}$) and turbidity (NTU) at each sampling location. Image data (pixels) from the Medium Resolution Spectro-radiometer (MERIS; images available for 285 days) at the three locations in the BPNS were converted into chlorophyll *a* concentrations ($mg.m^{-3}$), using the algal_2 product algorithm for coastal waters (Doerffer and Schiller, 2007; MERIS Quality Working Group, 2005). Vanhellemont (2012) showed that this algorithm reproduced the annual cycle for chlorophyll *a* in Belgian waters correctly, with clear spring algal blooms and winter minima. For the Westerschelde, Chlorophyll *a* measurements were extracted from Waterbase (Dutch Ministry of Infrastructure and the Environment, Rijkswaterstaat). Wave height (m) was obtained for each location using the WAM wave model, optimised for Belgian waters (resolution 0.022° x 0.033°, Royal Belgian Institute of Natural Sciences (RBINS); WAMDI Group, 1988; Günther *et al.,* 1992; Monbaliu *et al.,* 2000; Bolaños *et al.,* 2011) and water current speed ($m.s^{-1}$) was calculated using the three dimensional hydrodynamic model OPTOS-BCZ (resolution 750 m x 750 m, COHERENS V42.4.1; RBINS). Some missing values (mainly for oxygen and turbidity) were complemented from other data sources, such as the ODAS database (RBINS) and the 'Flemish banks' monitoring network (Agency for Maritime and Coastal Services and VLIZ). Seasonal data of the large fraction of zooplankton (>1000 µm) in terms of large taxonomic groups was available from Vansteenbrugge *et al.* (2015a; Chapter 3), but only for the locations at sea and in the estuary.

4.2.4 Zero inflated negative binomial model

In order to identify the environmental variables that are potentially driving the currently observed densities of *M. leidyi*, we first checked for multicollinearity and then explored the data using a Poisson regression model. This resulted in a poor fit, due to overdispersion (overdispersion parameter = 486). A negative binomial model also resulted in a poor fit, due to the high number of zero's present in the dataset (*i.e.* absence of *M. leidyi*). To account for the many zero's, a zero inflated negative binomial model was used, which had a smaller mean squared residual error compared to the negative binomial model (Zeileis *et al.,* 2008). In addition, this model was biologically more relevant to interpret as it combines a zero inflated part (logistic regression) to model the chance that *M. leidyi* is absent (true absence), and a count part (negative binomial regression) to model the expected number in case *M. leidyi* is present in the system.

Using a forward stepwise procedure based on the AIC of the model, 8 environmental variables (salinity, temperature, oxygen, turbidity, chlorophyll *a*, wave height, water current speed and seasonal densities of the large zooplankton fraction) and their transformations (log and quadratic function) were introduced in the model. For the parameter 'temperature', also 'temperature evolution' (defined as the average temperature increase or decrease (Δt) since the previous month) and 'temperature lag' (defined as the temperature of one month

prior), were tried as variables in the model. Correlated variables were not included in the model to avoid multicollinearity. All model runs were based on the complete dataset containing all *M. leidyi* densities, except for those runs where we included seasonal zooplankton data as food proxy. These were only based on locations at sea and in the estuary, excluding the port samples. A significance level of 5% was used to include significant variables in the final model. To validate the estimates of the zero-inflated part (true absence), the estimates were compared with the estimates of a logistic regression on the dichotomised densities (presence or absence of *M. leidyi*). A Voung test indicated that the zero-inflated negative binomial model is better than the negative binomial model (*p*-value = 0.01).

The analysis was performed using the zeroinfl-function of the pscl-package (Jackman, 2012) in R3.1.3 (R Core Team, 2015).

4.3 Results

4.3.1 Spatial and temporal distribution

In total, 1986 *M. leidyi* individuals were retrieved from 188 samples. During the different sampling campaigns in 2011 and 2012, *M. leidyi* was never found at the open sea location BE6 (57 km from the coast), nor at the coastal locations BE3 and BE4 or the port of Nieuwpoort P3 (Figure 4.1). Highest densities (18.4 ind.m^{-3}) were observed in the semi-enclosed basin in the port of Oostende (P2; sluice dock) in September 2012, followed by maxima of 1.9 and 0.6 ind.m^{-3} in the lower Westerschelde estuary (WS2, October 2012) and coastal location (BE2, September 2011), respectively (Table 4.1). For all other locations, average densities remained below 0.5 ind.m^{-3} (Figure 4.1).

Mnemiopsis leidyi was observed in January-February and from September until December in 2011, and from August onwards in 2012, with peak densities in September-October in both years (Figure 4.2). The ports with relatively high *M. leidyi* densities were only sampled in 2012, which might mask inter-annual differences. Still, higher densities were noted in the coastal location in 2011 (0.5 ind.m^{-3} in September) compared to 2012 (0.07 ind.m^{-3} in September), while the estuary showed higher densities in 2012. Based on the locations where *M. leidyi* was recorded, on average 0.75 ± 2.84 ind.m^{-3} were found from August until December in 2011 and 2012.

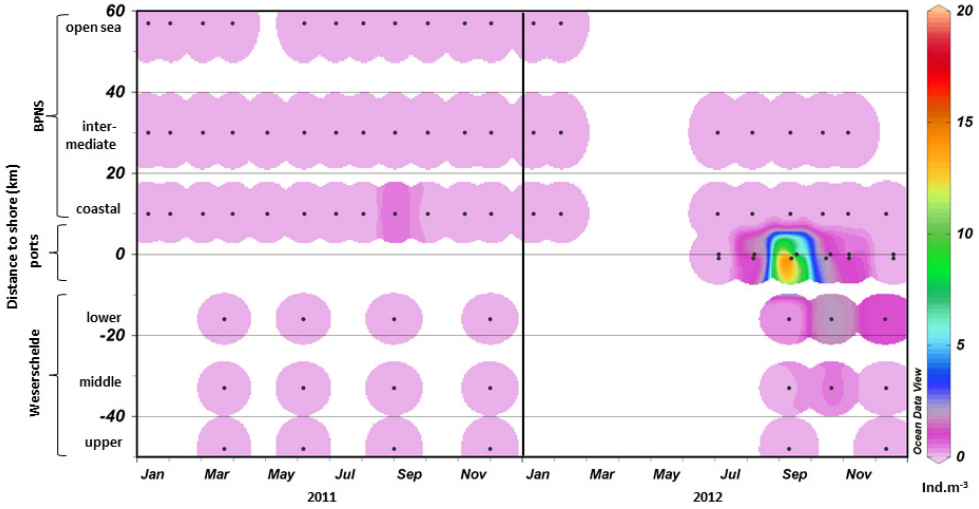

Figure 4.2 Contour plot (created with Ocean Data View; Schlitzer, 2015) showing *M. leidyi* densities (ind.m^{-3}) along a temporal x-axis (monthly) and a spatial y-axis (distance to shore in km for 8 locations: BE6, BE5, BE2, P1, P2, WS2, WS3, WS4), using weighted average gridding over all sampling events (black dots; x-scale length = 23; y-scale length = 41; white areas = no data available).

4.3.2 Relation with other gelatinous zooplankton

Pleurobrachia pileus, *Beroe* sp. and *Chrysaora hysoscella* co-occured with *M. leidyi*, but the first two taxa dominated the ctenophore community in terms of density at the coastal location (BE2), with some inter-annual variation between 2011 and 2012 in summer, autumn and winter (spring was not sampled in 2012; Figure 4.3). Similar inter-annual variation was observed in the Westerschelde estuary (WS2-4), where *P. pileus* had highest densities in 2011, while *M. leidyi* dominated in 2012. In the port of Zeebrugge (P1), the density peaks of *M. leidyi* and *P. pileus* coincided in August 2012, with slightly higher densities for *P. pileus*. A density peak of *Beroe* sp. was noted in September 2012, reaching higher densities than both of its prey. The semi-enclosed basin in the port of Oostende (P2) showed a different pattern, with high densities of *M. leidyi* (up to 18.4 ind.m^{-3}) and low densities of *P. pileus*. Moreover, the predators of *M. leidyi*, *i.e. Beroe* sp. and *Chrysaora hysoscella* were absent in this system (Figure 4.3).

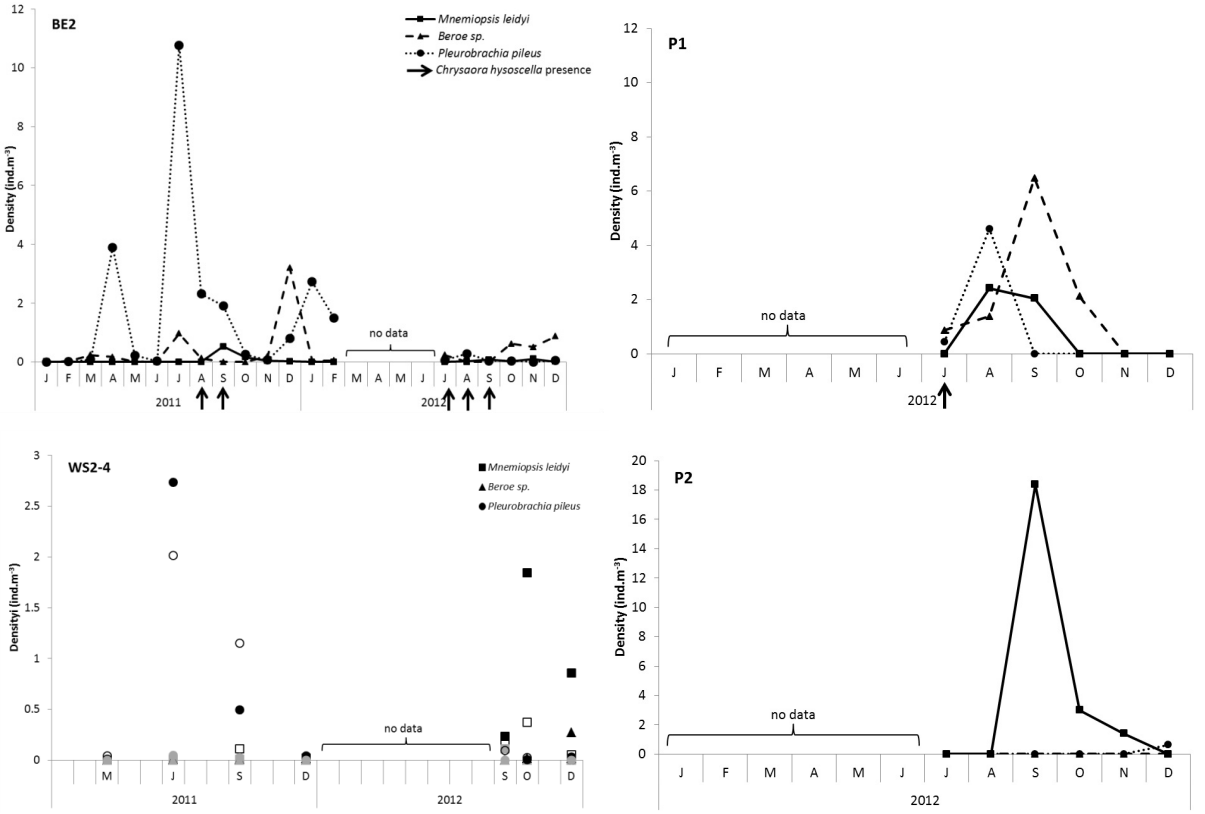

Figure 4.3 Density (ind.m^{-3}) of *M. leidyi*, *Beroe* sp. and *P. pileus*, and presence of *C. hysoscella* in the coastal location in the Belgian part of the North Sea (BE2), the ports of Zeebrugge (P1) and Oostende (P2), and three locations in the Westerschelde estuary (WS2 = black, WS3 = white, WS4 = grey). Note different y-axis scales.

4.3.3 Population dynamics

All *M. leidyi* individuals measured between 2 and 77 mm (oral-aboral length). The population outbreak started in August 2012 (no individuals caught in July 2012) and from then onwards, a medium-length population was noted in the ports and the coastal location (Figure 4.4). In September 2012, this population continued to grow, especially in the ports, where the presence of larvae (<5 mm) indicated reproduction. From September onwards, *M. leidyi* also appeared in the Westerschelde at low densities, across a broad size spectrum, ranging from 5 to 77 mm. In October, the bulk of the adult population seemed to have disappeared from the port location. In contrast, the coastal individuals grew to larger sizes, remaining at low densities, while the Westerschelde estuary population reached higher densities compared to September. Also reproduction occurred in the Westerschelde in October (presence of larvae), while in the ports, the larvae grew to larger sizes. November was characterised by low densities, which reflected the end of its seasonal occurrence in all three systems. Finally, in December, all adults disappeared, but a small reproduction event occurred, with larvae present in the coastal location and Westerschelde.

Table 4.2 Specifications of the zero inflated negative binomial model identifying significant explanatory variables (*p* <0.05) for the presence and abundance of *M. leidyi* (df = 5).

Zero-inflated model coefficients (binomial with logit link):

	Estimate	SE	*p*
Intercept	-1.31	1.19	0.270
Temperature evolution (Δt; °C)	2.30	1.16	0.048

Count model coefficients (negbin with log link)

	Estimate	SE	*p*
Intercept	5.06	1.66	0.002
Temperature evolution (Δt; °C)	-0.30	0.14	0.04
Wave height (m)	-3.53	0.65	< 0.001
Oxygen concentration (mg L^{-1})	-0.85	0.22	< 0.001

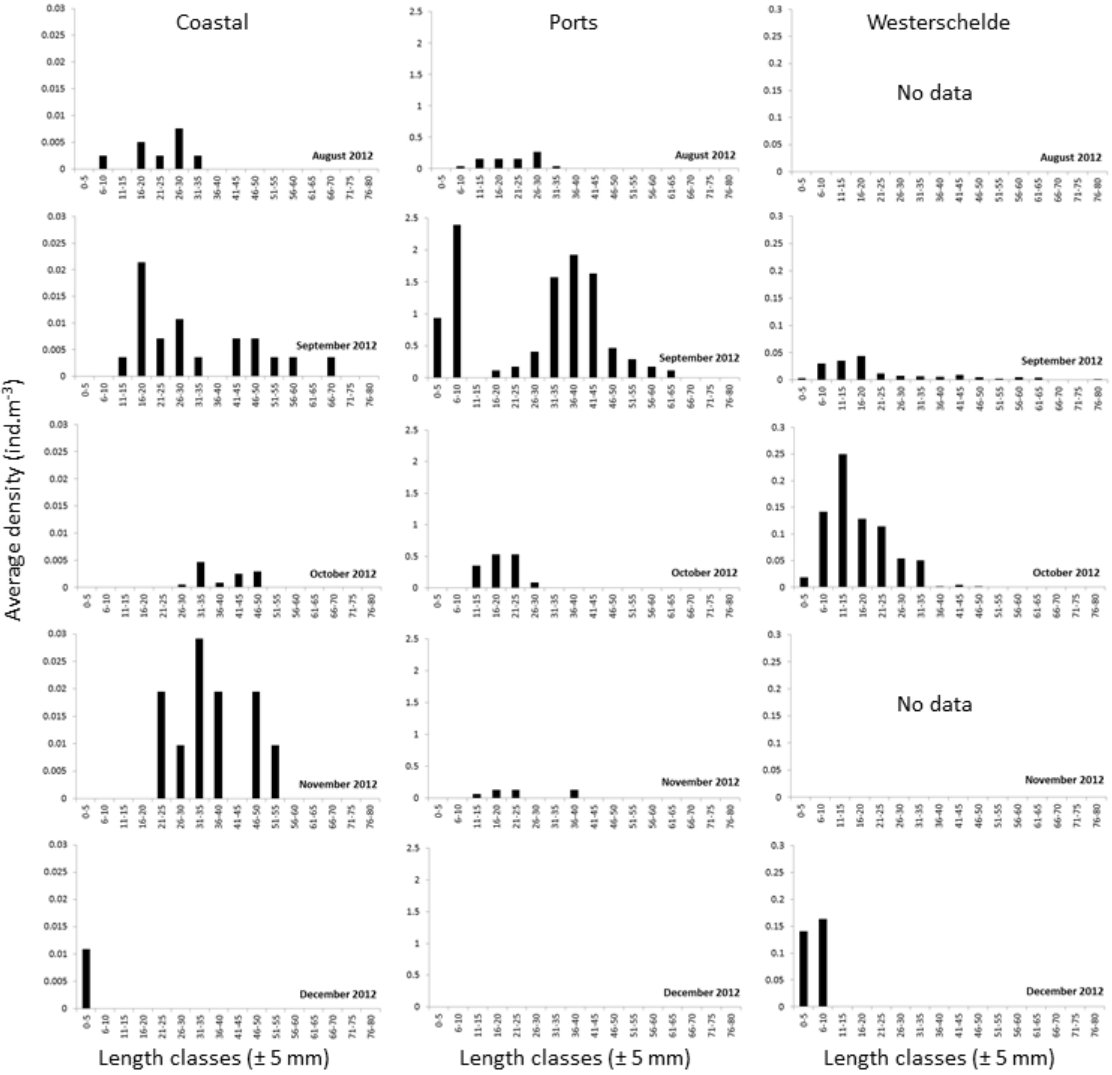

Figure 4.4 Late summer and autumn population dynamics of *M. leidyi* in the coastal Belgian part of the North Sea (BE2), the ports (P1 and P2) and Westerschelde estuary (WS2-4), from August to December 2012. Note the different y-axis scales.

4.3.4 Environmental characterisation

CTD profiles showed a vertically well-mixed water column throughout the year for temperature, salinity, turbidity and oxygen concentration (not shown). Temperature ranged from 3.4 °C in January 2011 to 18.6 °C in July 2011, with a maximum of 22 °C in July 2012 in the port of Oostende (Figure 4.5). February 2012 was exceptionally cold with average temperatures as low as 2.4 °C (compared to 4.0 °C in 2011). Salinity ranged from 30 to 35 at the coastal and port locations, showing little variation over the year. In the Westerschelde estuary, salinity varied largely depending on the location, ranging from 24.5 to 30.4 in the lower estuary, from 16.8 to 27.2 in the middle estuary, and from 6.6 to 18.1 in the upper estuary. In the upper estuary, adults were found up to a salinity of 13.6, while larvae were encountered up to a salinity of 18.1. The highest salinity where both adults and larvae were observed, was 36 at station P2. Chlorophyll *a* increased towards April, although equally high values were observed in May and July (up to 13 mg m^{-3}) at the coastal location. Dissolved oxygen concentrations measured around 8 mg L^{-1} (Figure 4.5), with highest values in spring (> 10 mg L^{-1}), coinciding with high chlorophyll *a* concentrations. Turbidity was highest in the Westerschelde, especially at the upper estuary (>120 NTU). At the coastal locations, turbidity ranged between 15 and 60 NTU, while further offshore turbidity remained below 7 NTU (with an exception of 50 NTU in February 2011). At the open sea location, wave height was highest (on average 0.9 ± 0.5 m) and gradually declined towards the coastal location. In the ports and Westerschelde wave height was much lower, with minima measured at the middle (WS3) and upper (WS4) estuary location reaching almost zero.

4.3.5 Zero-inflated negative binomial regression model

The results from the zero-inflated part (presence or absence) of the model showed that temperature evolution, defined as the average temperature increase or decrease (Δt) since the previous month, was significantly associated with the presence/absence of *M. leidyi* (*p*-value = 0.048). This part of the model showed that there is a higher chance of finding *M. leidyi* when temperature is decreasing from late summer onwards.

The negative binomial part of the model (densities) showed that temperature evolution (p = 0.04), wave height (p <0.001) and dissolved oxygen concentration (p <0.001) were significantly associated with *M. leidyi* densities in the study area (Table 2). The model showed that higher densities of *M. leidyi* can be expected when temperature is decreasing from late summer onwards, in combination with low wave height and lower oxygen levels.

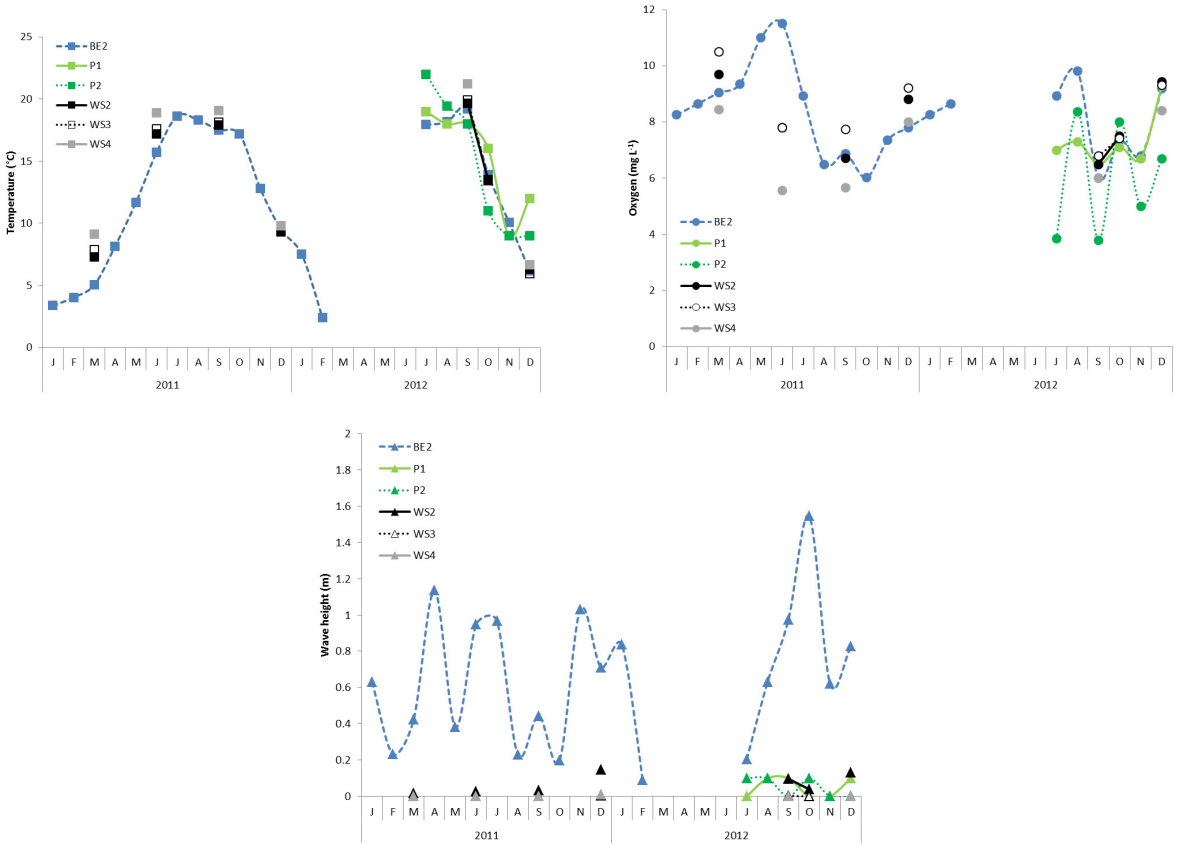

Figure 4.5 Patterns in temperature (°C), oxygen (mg L^{-1}) and wave height (m) for the stations BE2 (Belgian part of the North Sea), P1 and P2 (Ports), and WS2-4 (Westerschelde estuary).

4.4 Discussion

4.4.1 Distribution patterns and population dynamics

This study found highest *M. leidyi* densities in the ports, Westerschelde estuary and Belgian coastal zone, whereas no individuals were found further offshore[2]. Van Ginderdeuren *et al.* (2012b) also did not find any individuals offshore the Belgian coast, nor did Antajan *et al.* (2014) for the offshore northern French coast. Coastal waters, shallow bays and estuaries have been identified as preferential habitat for *M. leidyi* in the US (Kremer, 1994; Costello *et al.,* 2006) and other invaded regions throughout Europe. Fuentes *et al.* (2010) described the presence of *M. leidyi* along the Catalan coast in Spain and west coast of Italy, and reported high abundances in Jaffa port and Haifa Bay in Israel. Well-established and recurrent populations of *M. leidyi* can nowadays be found in the sheltered Wadden Sea (The Netherlands; van Walraven *et al.,* 2013) and several fjords in northern Europe (Javidpour *et al.,* 2009a; Riisgård *et al.,* 2012; Hosia and Falkenhaug, 2015). Faasse and Bayha (2006) described the first record of *M. leidyi* in the Westerschelde. Between 2007 and 2010, *M. leidyi* was occasionally observed in the coastal Belgian ports (Dumoulin, 2007; Van Ginderdeuren *et al.,* 2012b). Our study showed that, two years later, *M. leidyi* likely established a permanent population, at least in the semi-enclosed basin in the port of Oostende. Our data also indicated a further spread of this non-indigenous species throughout the Westerschelde in 2012, despite the lower salinity levels (av. 13) at the upper estuary.

Costello *et al.* (2006) found overwintering *M. leidyi* in sheltered coves in Narragansett Bay (US), while Collingridge *et al.* (2014) suggested that *M. leidyi* may retreat in restricted areas such as the Rhine estuary in winter. As such, Costello *et al.* (2006; 2012) suggested that coastal embayments, characterised by low advection and low water exchange rates, favour the retention of *M. leidyi* and might allow for overwintering populations. When conditions are favourable, such overwintering areas might serve as 'sources' to seed new populations in areas where no overwintering is possible (source-sink, meta-population theory; Hanski, 1999; Purcell *et al.,* 2001; Costello *et al.,* 2012). The requirements of a source area are: high retention, high annual production and annual persistence (Costello *et al.,* 2012). The port of Oostende especially showed reduced water exchange rates and reduced wave heights, favouring the retention of *M. leidyi* individuals. Such conditions probably also exist in the

[2] We are aware that comparison of absolute abundance data should be treated with caution due to the use of different net types. In Chapter 2, we showed that both filtered volume and mesh size of the net determined the catch. The handheld dip-net filtered less water and has a smaller mesh size compared to the CalCOFI ring trawl. Therefore, the handheld dip-net could underestimate the presence of *M. leidyi* when densities were low, while it could overestimate *M. leidyi* densities when *M. leidyi* was abundant. Furthermore, more small ctenophores may be retained by the hand-net due to the smaller mesh size and low towing speed compared to the CalCOFI ring trawl net.

port of Vlissingen, Borssele and Terneuzen, located in the lower Westerschelde estuary (van der Molen *et al.,* 2015).

Secondly, both ports (Oostende and Zeebrugge) and the Westerschelde estuary were characterised by several production and reproduction events, as documented by the high densities and the presence of larvae. According to a model presented by Collingridge *et al.* (2014), 2 to 3 reproductive cycles (life cycle length of 40 days at 15°C) can occur in the Belgian coastal system. The presence of many small cydippid larvae in the ports in September, followed by two reproduction events in the Westerschelde in October and December, confirmed this. For the coastal system, no clear reproduction event was discerned, although a few 5-mm-larvae were noted in December.

The third requirement to identify one of the monitored systems as a source was not met, as we could not confirm annual persistence. From August onwards, conditions were favourable for *M. leidyi*, leading to relatively high densities in late summer and autumn in all three systems. However, no adult individuals were found in late winter and spring. Costello *et al.* (2012) argued that winter conditions at temperate latitudes are unfavourable for *M. leidyi* and that prevailing currents might flush out complete populations. Still, *M. leidyi* might have been present in low densities. Esser *et al.* (2004) showed that ctenophores may overwinter near the bottom to save energy as a response to low temperatures. This makes it difficult to collect them with plankton nets like the CalCOFI net and handheld dip-net used in our study, as these nets stay at least 0.5 m from the sea bed. Moreover, Van Ginderdeuren *et al.* (2012b) recorded several individuals in the Belgian ports in late winter and early spring of 2009. These winter observations and the size measurements and densities observed in our study, suggest that at least the semi-enclosed basin in Oostende and the Westerschelde estuary function as a source. On the other hand, the Belgian coastal zone probably acts as a 'sink' area. The combination of length-frequency and density measurements showed that the population structure is incomplete. This could be explained by continuous outflow related to strong tidal currents, in combination with a current-driven immigration of individuals from adjacent source areas (Condon and Steinberg, 2008).

Of course, we cannot exclude potential new re-introductions of *M. leidyi* through ballast water transport in the ports and Westerschelde, as a huge number of international vessels daily visit the ports of Zeebrugge and Antwerp (Carlton, 1985; Clarke *et al.,* 2003; David and Gollash, 2008; Globallast, 2015). Furthermore, secondary introduction from nearby systems is also possible (e.g. Ghabooli *et al.,* 2011). For example, *M. leidyi* was almost absent at the lower and upper estuary in 2011, but showed high densities in the middle part of the estuary. In this area, the Zuid-Beveland canal enters the Westerschelde (Figure 4.1), connecting the estuary with the Oosterschelde, where *M. leidyi* has been present in high densities at least since 2008 (diver observations; Faasse and Bayha, 2006; van Walraven *et al.,* unpublished data).

4.4.2 Relation with other gelatinous plankton

Two native ctenophores *P. pileus* and *Beroe* sp. are present in the study area, potentially interacting with *M. leidyi* as competitor for food (Møller *et al.,* 2010; Hamer *et al.,* 2011) or as predator (Hosia *et al.,* 2010) respectively. Also, the native scyphomedusa *Chrysaora hysoscella* may predate on *M. leidyi* (Purcell *et al.,* 1994; Hosia and Titelman, 2011). These interactions may constrain *M. leidyi's* population growth.

In the port of Zeebrugge, *Beroe* sp. reached somewhat higher densities, potentially indicating that the species might benefit from the presence of *M. leidyi* as an additional prey. On the other hand, *M. leidyi* seemed to profit from the absence of *Beroe* sp. in the port of Oostende and upper Westerschelde. Purcell *et al.* (2001) indicated that low salinity habitats may serve as important refuges from less-euryhaline predators, such as *Beroe* sp. In the coastal zone, the predatory relationship between *M. leidyi* and *Beroe* sp. was less clear. However, lower densities of *M. leidyi* might be explained by competition for food with *P. pileus* due to their different feeding mechanisms. *Mnemiopsis leidyi* generates a feeding current to acquire food, which is disturbed in areas with strong tidal currents and wave action (Waggett and Costello, 1999; Colin *et al.,* 2010), while the tentaculate ambush feeding of *P. pileus* proves to be more efficient in turbulent environments (Colin *et al.,* 2010; de Wolf, 2012).

4.4.3 Environmental drivers

Several studies indicated a number of environmental variables impacting the distribution of jellyfish species (see Arai, 1992). Although *M. leidyi* has a very broad tolerance towards several parameters, alterations in the physical regime (as a combination of different parameters) may modify its distribution and abundance patterns. Purcell *et al.* (2001) for example stated that higher temperatures (>12 °C) enhance *M. leidyi* reproduction. Although we measured temperatures higher than 12 °C in June, a population outbreak of *M. leidyi* was inhibited until September, when temperatures were decreasing towards winter. This explains why temperature evolution, rather than temperature itself or temperature lag was retained in the zero-inflated negative binomial model. Considering its fast population growth potential, probably other constraining factors prevented an earlier outbreak of the *M. leidyi* population (Baker and Reeve, 1974).

It is unclear why dissolved oxygen concentration was also retained in the second part of the model, predicting higher densities with lower oxygen concentrations. The entire study area is well-mixed and well-oxygenated and *M. leidyi* has a broad oxygen tolerance (Decker *et al.,* 2004). The relation was probably influenced by the fact that highest oxygen concentrations measured some 20 km offshore, where *M. leidyi* densities were low. However, when the offshore station were excluded, dissolved oxygen concentration was still retained in the model. As similar estimates were found, *M. leidyi* is probably not particularly favoured by

low dissolved oxygen concentrations but just endures them. Furthermore, oxygen concentration might be a proxy for another, as yet unknown environmental parameter. For example, lower oxygen levels could favour *M. leidyi* in the competition with zooplanktivorous fish species. However, the latter needs to be further investigated.

Finally, low wave height was positively correlated with higher densities (highly significant), indicating that low energetic areas are preferred by *M. leidyi*. The semi-enclosed basin in the port of Oostende and most other ports are characterised by low current velocities and reduced wave height, favouring higher abundances of *M. leidyi*. As such, high wave heights further offshore also explain why we never noted *M. leidyi* at the open sea location. Other studies showed that *M. leidyi* can migrate downward in the water column in choppy sea conditions, because turbulence negatively affects their feeding efficiency and may cause physical damage (Waggett and Costello, 1999; Mianzan *et al.,* 2010). In contrast, the morphology of the larvae (tentaculate) still enables them to feed in such turbulent conditions, which could explain the absence of adults and the presence of larvae in December. This could also mean that *M. leidyi* densities were underestimated in winter when turbulent conditions are more likely. This should be taken into account when planning future *M. leidyi* monitoring.

The absence of *M. leidyi* in spring was not fully explained by the model, indicating that some important explanatory variables were not included in the model. It was surprising that food availability was not retained in the reduced model run (*i.e.* without the port locations) to explain the presence or abundance of *M. leidyi*. Although Jaspers *et al.* (2015) showed that reproduction might continue during starvation, other publications showed a clear link between food and *M. leidyi* abundance. Kremer and Reeve (1989), for example, stated that a minimum prey biomass of 24 µg $C.L^{-1}$ should be available during population growth, while Purcell *et al.* (2001) showed that *M. leidyi* outbreaks will be inhibited when food is absent. Daro *et al.* (2006) calculated that during a spring zooplankton bloom, approximately 100 µg $C\ L^{-1}$ copepods and a similar amount of microzooplankton were present in the Belgian part of the North Sea. Still, the zooplankton data we used may have partly influenced the model outcome as only the larger zooplankton fraction was included due to the larger mesh size of the CalCOFI net (Chapter 2 and 3).

4.4.4 Threat for Belgian waters

The non-indigenous *M. leidyi* is regarded as a 'pattern B' introduced species (GESAMP, 1997), meaning that the population undergoes natural fluctuations within its exotic range, which can result in both blooms and almost disappearing populations. Although *M. leidyi* has been present in Belgian waters at least since 2007 (Dumoulin, 2007), we cannot confirm that its densities have exponentially increased over the past years. Soenen *et al.* (2010) found 17 $ind.m^{-3}$ in the port of Oostende in October 2010, while 18 $ind.m^{-3}$ were noted in our study in September 2012. Van Ginderdeuren *et al.* (2012b) found a yearly average density of 0.4 ± 0.2

ind.m^{-3} in 2010, while our study reported on average 0.13 ± 0.20 ind.m^{-3} and 0.05 ± 0.04 ind.m^{-3} in coastal samples where *M. leidyi* was present in 2011 and 2012 respectively.

Still, the highest densities that were noted in Belgian waters (18 ind.m^{-3} in the semi-enclosed basin of Oostende, 0.6 ind.m^{-3} in the near coast, and 1.9 ind.m^{-3} in the lower Westerschelde estuary) remained considerably lower than those measured in other studies. In its native area (east coast of the Americas), the highest densities of *M. leidyi* were 160 ind.m^{-3} (Kremer and Nixon, 1976; McNamara *et al.,* 2010). Even higher densities were recorded in other invaded European areas, with peak densities of 867 ind.m^{-3} in Limfjorden (Denmark; Riisgård *et al.,* 2007), 510 ind.m^{-3} in Kiel Fjord (Javidpour *et al.,* 2009a,b), 610 ind.m^{-3} in the Wadden Sea (van Walraven *et al.,* 2013), and 304 ind.m^{-3} in the Black Sea (Vinogradov *et al.,* 1989).

Mnemiopsis leidyi has impacted the functioning of several ecosystems throughout the invaded area (Kideys, 2002; Roohi *et al.,* 2010). Also in Belgian waters, conditions are favourable for *M. leidyi* to reproduce and appear in the water column from summer to early winter. Well-fed ctenophores of 80 mm in size can produce 1000-3000 eggs.day^{-1} (Kremer, 1976). In the ports, *M. leidyi* reached a maximum length of 77 mm, which is larger than the 60 mm found in the low saline Kiel Fjord (Javidpour *et al.,* 2009b), but smaller than individuals from the Black Sea (120-180 mm) and Chesapeake Bay (<120 mm) (Purcell *et al.,* 2001) or Dunkirk port in northern France (90-120 mm) (Vincent *et al.,* unpublished data). Consequently, the potential for a rapid population outburst does exist. However, the presence of indigenous ctenophores, which probably serve as competitor (*P. pileus*) and predator (*Beroe* sp.), may currently help to reduce the size of the outbreaks (Hosia *et al.,* 2010; Hamer *et al.,* 2011).

A recent modelling study (van der Molen *et al.,* 2015) showed that conditions in large parts of the North Sea are favourable for *M. leidyi*, and only a limited change in the environment is needed to invoke an immediate population outburst. Several authors have shown that a temperature rise, as a result of climate change, may enhance earlier and longer periods for *M. leidyi* population growth (Costello *et al.,* 2006; Condon and Steinberg, 2008). At the end of June 2014, high *M. leidyi* densities have been observed in the port of Oostende, two months earlier than expected (personal observation, qualitative observation), probably as a result of the mild winter period. We suspect that if the data from 2014 could have been included in the model, temperature evolution (defined as the average temperature increase or decrease (Δt) since the previous month) would not have been retained in the model.

We recommend that precautionary monitoring should be further executed to identify source areas and to explore the potential of overwintering adults near the sea bottom (Costello *et al.,* 2006), for example by using divers or a hyperbenthic sledge. As gelatinous zooplankton blooms are highly dynamic, weekly rather than monthly sampling might provide even more detailed insight in the population dynamics (e.g. Javidpour *et al.,* 2009b). This could for example clarify whether the sudden appearance of *M. leidyi* specimens (10-20 mm) found in

the Westerschelde in October 2012, were transported via currents from another area or were produced *in situ* two weeks before (Baker and Reeve, 1974). Moreover, it would be opportune to expand the zero-inflated negative binomial model to allow for predicting presence or abundance of *M. leidyi*, and to test this expanded model to other invaded systems (e.g. the adjacent Oosterschelde; van Walraven, unpublished data). The combined results would allow coastal managers to take potential eradication measures (e.g. eliminate overwintering individuals by draining the semi-enclosed basin in the port of Oostende for a certain period), in order to reduce the risk of economic and ecological disasters as they were seen in other invaded areas.

4.5 Conclusion

This study showed that *M. leidyi* occurred from August to December in the Westerschelde estuary and the Belgian part of the North Sea (including the ports), but was never found more than 30 km offshore. Although densities were generally low (average 0.8 ± SD 2.8 ind.m^{-3}) compared to other invaded European systems (e.g. Riisgård *et al.,* 2012; van Walraven *et al.,* 2013), the presence of larvae and sudden appearance of high numbers across the size distribution in August indicated that ports and estuaries may act as sources, populating the adjacent coastal area. The zero-inflated negative binomial regression model related temperature, dissolved oxygen concentrations and wave height to the observed *M. leidyi* densities. A permanent population seems to be established in Belgian waters and should be monitored appropriately.

4.6 Acknowledgements

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PART III

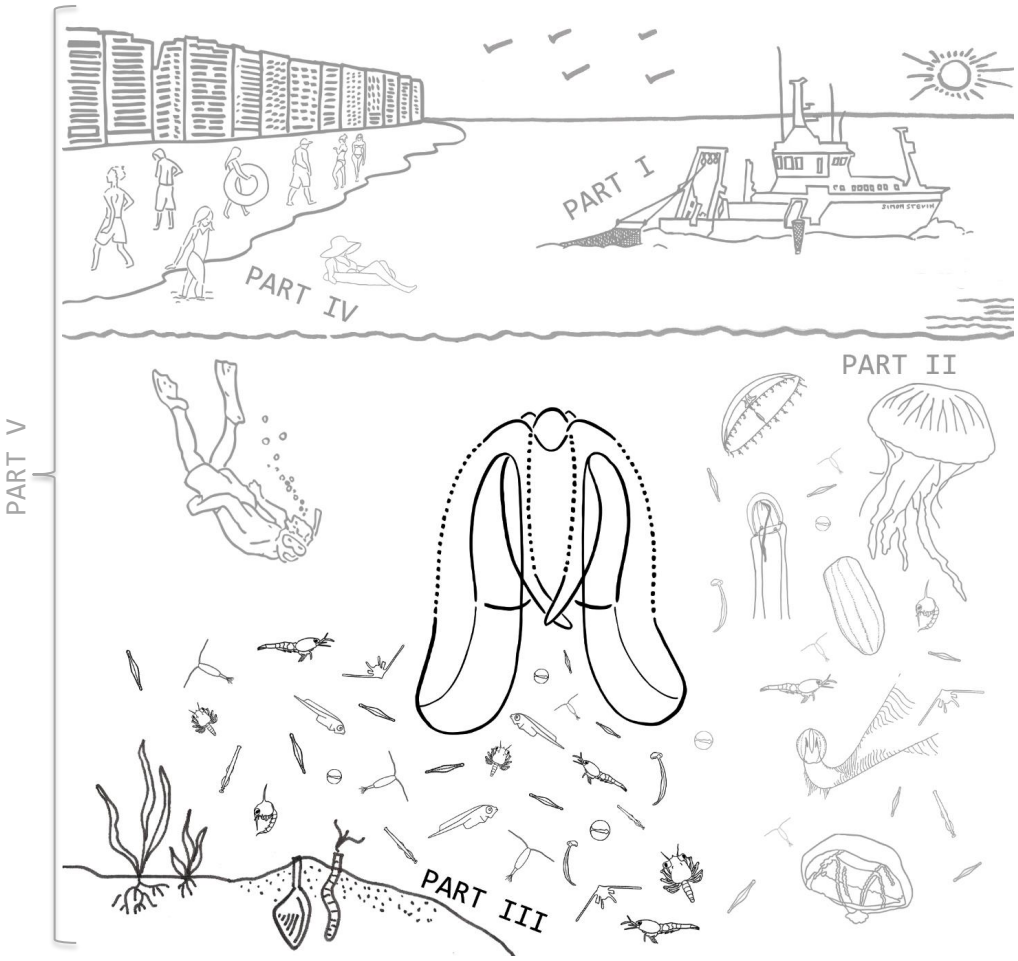

Photo previous page: © David Vuylsteke

5

TROPHIC ECOLOGY OF *MNEMIOPSIS LEIDYI* IN THE SOUTHERN NORTH SEA: A BIOMARKER APPROACH

Modified from:
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ABSTRACT

The non-indigenous ctenophore *Mnemiopsis leidyi* A. Agassiz 1865 was first observed in the southern North Sea in 2006 and has since then frequently been encountered. Knowledge on the diet of *M. leidyi* and its interactions with other components of the pelagic food web will largely contribute to assess the impact of this non-indigenous species on the ecosystem. Using both stable isotope (SI) and fatty acid (FA) analysis, this study revealed spatial and temporal variation in the trophic ecology of *M. leidyi* in different ecosystems in the southern North Sea. Based on the isotopic composition, we found that spatial differences were largely driven by variation at the base of the food web rather than diet changes of *M. leidyi* in the different ecosystems. Temporal variation in *M. leidyi* SI composition was also influenced by shifting baseline values and driven by seasonal changes in the associated plankton communities. This study provides the first data on the FA composition of *M. leidyi* as compared to FA concentrations of two other (indigenous) ctenophores. The total FA concentration of *M. leidyi* was three to four times lower compared to *Pleurobrachia pileus* and *Beroe* sp., categorising this non-indigenous ctenophore as a lipid-poor organism. Trophic interactions between *M. leidyi* and the two co-occuring ctenophores (*P. pileus* and *Beroe* sp.) showed considerable resource differentiation, which could be the result of competion or both ctenophores could have different diets. A mixture of zooplankton was identified as potential food source for *M. leidyi*. FA markers supported the carnivorous diet of *Beroe* sp., but its SI composition did not confirm it as a predator of *M. leidyi*.

5.1 Introduction

Invasions of non-indigenous species in coastal waters and inland seas are common and form a major threat to marine ecosystems worldwide (Ruiz *et al.,* 1997; Briggs, 2007; Katsanevakis *et al.,* 2013). To evaluate the impact of non-indigenous species, one can focus on their (increase in) abundance and spatial or temporal distribution patterns. However, non-indigenous species can also alter the overall functioning and balance of the ecosystem

(GESAMP, 1997; Scheffer *et al.,* 2001; Streftaris *et al.,* 2005). Food web studies offer a quantitative and integrative framework to evaluate changes in both ecosystem structure and functioning (Thompson *et al.,* 2012).

The non-indigenous ctenophore, *Mnemiopsis leidyi* A. Agassiz 1865, was observed for the first time in the southern North Sea in 2006, and has since then frequently been encountered in coastal waters, ports and estuaries in France, Belgium and The Netherlands, particularly from late summer until early winter (Faasse and Bayha, 2006; Van Ginderdeuren *et al.,* 2012b; van Walraven *et al.,* 2013; Antajan *et al.,* 2014). In addition to its distribution patterns, knowledge of the diet, trophic position, and interactions with other components of the pelagic food web will largely contribute to assess the impact of this non-indigenous species on the southern North Sea ecosystem.

Jellyfish in general (*i.e.* ctenophores and pelagic cnidarians) are usually positioned at the third trophic level in the pelagic food web, feeding on primary consumers like herbivorous crustaceans (Pauly *et al.,* 2009). Therefore, they are often pooled into one single trophic category in ecosystem models (Condon *et al.,* 2012), and can be seen as direct competitors with planktivorous fish (Sommer *et al.,* 2002; Brodeur *et al.,* 2008). However, jellyfish encompass a broad range of species, including predators of other gelatinous zooplankton (e.g. *Beroe gracilis*; Greve and Reiners, 1988). Therefore, it is essential to evaluate their trophic diversity at the species level (Nagata *et al.,* 2015). Furthermore, the diet of *M. leidyi* depends on a number of parameters such as ontogeny and food availability, both related to sampling area and period. Adult *M. leidyi* have a broad zooplanktivorous diet, including fish eggs and larvae (Purcell and Arai, 2001; Purcell, 2009), while *M. leidyi* larvae feed on the smaller microplanktonic fraction of the pelagic food web (Rapoza *et al.,* 2005; Sullivan, 2010).

Several techniques have been used to investigate the trophic ecology of jellyfish (reviewed in Pitt *et al.,* 2009). Traditionally, gut content analyses and grazing experiments are performed to study feeding ecology. However, these techniques only allow to document the food items that were recently consumed, giving a diet snapshot, rather than what is actually assimilated (Pitt *et al.,* 2009). Moreover, small or partly digested prey may be difficult to identify. Biochemical tracers, such as stable isotopes (SI) and fatty acids (FA) offer several advantages because they provide an analysis of the diet integrated over time and allow to identify contributions from different food sources based on the 'you are what you eat' principle (De Niro and Epstein, 1976; Peterson and Fry, 1987; Pitt *et al.,* 2009).

The SI composition can identify shared resources (potentially leading to competition) and predation interactions. For example, Kellnreitner *et al.* (2013) showed that juvenile herring was more enriched in ^{13}C and ^{15}N than *M. leidyi*, and concluded based on experiments that competition rather than predation by *M. leidyi* occurred. Furthermore, Hamer *et al.* (2011) also reported potential competition with the indigenous ctenophore *Pleurobrachia pileus*,

while Frost *et al.* (2012) confirmed *Beroe* sp. (an indigenous ctenophore) as a predator of *M. leidyi*, based on SI analysis.

Comparing FA concentrations and analysing the specific FA composition of organisms helps to determine whether an organism is carnivorous or omnivorous and to elucidate the main energy flow at the base of the food web (e.g. Dalsgaard *et al.,* 2003; El-Sabaawi *et al.,* 2009; Pitt *et al.,* 2009). Several studies have been conducted on the FA composition of gelatinous zooplankton (e.g. Falk-Petersen *et al.,* 2002; Nichols *et al.,* 2003; Ju *et al.,* 2004). However, for *M. leidyi* only the total lipids have been determined for the tropical Carribbean Sea by Kremer and Reeve (1989) and by Anninsky *et al.* (2005) for the Black Sea. To our knowledge, no data on the FA composition of *M. leidyi* have been published yet.

A combination of both SI and FA analyses can give an even better insight in the food web. Such a combined approach was used by Ying *et al.* (2012) to elucidate the diet and trophic position of three jellyfishes *Aurelia aurita*, *Stomolophus meleagris* and *Cyanea nozakii* in the Yellow Sea. In our study, we performed both SI and FA analyses and investigated (1) spatial, temporal and ontogenetic patterns in the trophic ecology of the non-indigenous ctenophore *M. leidyi* and examined (2) the trophic interactions of this species with co-occurring (native) ctenophores and potential food sources in the southern North Sea food web.

5.2 Material and Methods

5.2.1 Study area

The study area covers different systems in the southern North Sea, with locations in Belgian and Dutch coastal waters, major ports in northern France and Belgium, and three estuarine systems (Westerschelde, Oosterschelde and Grevelingen) in the southern part of The Netherlands (Figure 5.1, Table 5.1). The 16 locations are known to be inhabited by *M. leidyi* (Chapter 4).

5.2.2 Sample collection

Sampling occurred between July and December 2012 (always at the beginning of the month) at these 16 locations, when *M. leidyi* was most abundant (Chapter 4). This sampling strategy allowed us to evaluate both spatial and temporal patterns in the trophic ecology of *M. leidyi*. Zooplankton samples were collected using vertical WP2 net hauls (mesh size 200 µm; diameter 0.57 m) and undulating CalCOFI net tows (mesh size 1000 µm; diameter 1 m), deployed from different research vessels at sea and in the estuaries. The port locations (Zeebrugge P1, Oostende P2 and Dunkerque P4) were sampled using a handheld dip-net (mesh size 200 µm; diameter 0.20 m), deployed from moored pontoons. Phytoplankton (as basal food web component) was sampled by means of a Niskin bottle at sea and in the estuaries (closed at 3 m depth) and by means of a beaker in the ports (at the surface).

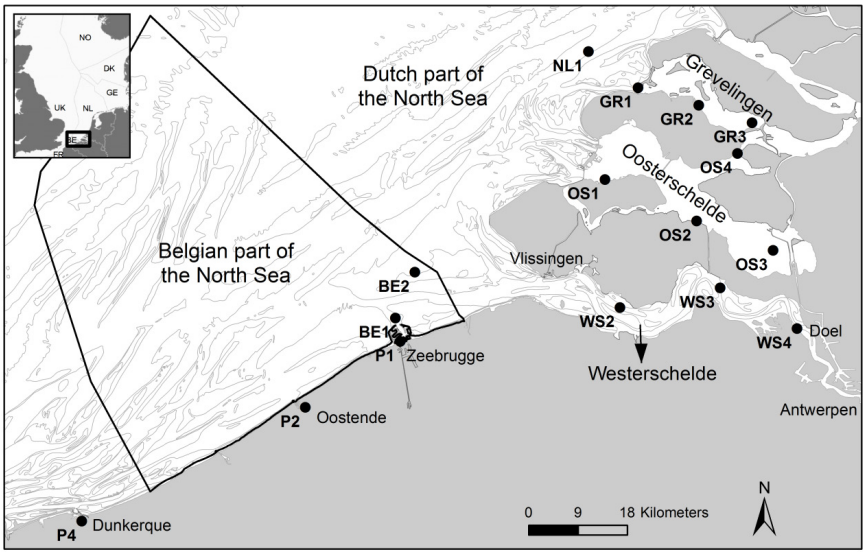

Figure 5.1 Study area with 16 locations situated in different systems (ports, estuaries and coastal waters) in the southern North Sea (see Table 5.1); inset: North Sea EEZs with indication of the study area

5.2.3 Sample processing

From the zooplankton samples, the ctenophores *M. leidyi*, *P. pileus* (as a potential competitor) and *Beroe* sp. (as a potential predator) were isolated, morphologically identified and measured (oral-aboral length, ± 1 mm). All *M. leidyi* specimens were grouped into length classes to investigate ontogenetic variation in the trophic ecology. The smallest length class of *M. leidyi* (0-10 mm) represented mainly transitional stages (Rapoza *et al.,* 2005), while the larger specimens were categorized into 4 classes (11-20 mm, 21-35 mm, 36-55 mm and >55 mm) based on the length-frequency distributions. For both *M. leidyi* and *P. pileus*, the gastro-intestinal canal was removed with a scalpel, to avoid measuring the signal from the ingested prey items (Feuchtmayr and Grey, 2003; D'Ambra *et al.,* 2014), and the remaining tissue was stored in 10 mL tubes and frozen at -20 °C for SI analysis and at -80 °C for FA analysis (Table 5.1). As no -80 °C freezer was present on board, samples were first preserved on dry ice. For *Beroe* specimens (not identified to species level) the entire individuals were stored and frozen, but no visible prey items were present in the gut.

Table 5.1 Overview of sampling locations in the southern North Sea (south to north), with shaded areas representing sampling period, letters representing sampled taxa used for SI analyses, and letters in bold representing taxa also used for FA analyses; M = *Mnemiopsis leidyi*, B = *Beroe* sp., P = *Pleurobrachia pileus*, z = zooplankton, c = copepods, m = mysids, p = phytoplankton (see §5.2.4)

Area	Station code*	Coordinates WGS84		Description	2012					
		Lat	Long		July	August	Sept.	October	Nov.	Dec.
Dunkerque port (FR)	P4	51.04°N	2.37°E	Dock connected with canal through sluice				M		
Oostende port (BE)	P2	51.23°N	2.95°E	Semi-enclosed basin			**M**,z,p	M,c,p	M,c,p	z,p
Zeebrugge port (BE)	P1	51.34°N	3.20°E	Navy dock	B,p	M,B,P,z,p	**M**,**B**,p	B,c,p	c	c,p
Belgian part of the North Sea	BE1	51.37°N	3.18°E	Coastal location				M,B,m		
	BE2	51.45°N	3.24°E	Coastal location	P,B,z,m,p	M,P,z,p	**M**,P,z,p	M,B,P,z,m,p	M,B,z,m,p	B,z,p
Westerschelde (NL/BE)	WS2	51.39°N	3.78°E	Lower estuary			M,**P**	M		M
	WS3	51.42°N	4.04°E	Middle estuary			**M**,**P**	M		
	WS4	51.35°N	4.24°E	Upper estuary			**M**			
Oosterschelde (NL)	OS1	51.6°N	3.74°E	Lower estuary, sea connected		M		M		
	OS2	51.53°N	3.98°E	Middle estuary		M		M		
	OS3	51.48°N	4.18°E	Upper estuary		M		M		
	OS4	51.64°N	4.09°E	Upper estuary, sluice connected to GR				M		
Grevelingen (NL)	GR1	51.75°N	3.83°E	Lower estuary, no connection				M		
	GR2	51.72°N	3.99°E	Middle estuary				M		
	GR3	51.69°N	4.13°E	Upper estuary, sluice connected to OS				M		
Dutch part of the North Sea	NL1	51.81°N	3.7°E	Coastal location				M,B,P,c,m		

* All locations sampled with WP2 and CalCOFI net, except the ports (P1, P2 and P4) which were sampled with a handheld dip-net

The remaining zooplankton (as potential food source) from the samples collected with both WP2 and CalCOFI nets or handheld dip-net, was washed with deionized water over a 200 µm sieve and stored at -20 °C in sealed petri dishes. Although, zooplankton samples were collected during the entire sampling period, only the samples from the Belgian part of the North Sea and the ports of Oostende and Zeebrugge were used for SI analyses (Table 5.1). For phytoplankton, up to 250 mL water per sample was filtered on pre-weighted, pre-combusted glass fibre filters (GF/F, Whatman, Ø 25 mm). These filters were stored in sealed petri dishes at -20 °C.

5.2.4 Stable isotope analyses

The use of SI analysis is based on the presence of different ratios of the common, light isotope to the heavy, rare isotope in food sources (Peterson and Fry, 1987). The most commonly used isotopic ratios are those of carbon and nitrogen. SI ratios are expressed in conventional δ notation (‰) according to the following equation: $\delta X = [(\mathrm{Rsample}/\mathrm{Rstandard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N and R is the corresponding $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio relative to the Vienna Pee Dee Belemnite standard for carbon and atmospheric nitrogen (N_2) for nitrogen. Through fractionation, $\delta^{13}C$ and $\delta^{15}N$ values generally increase through the food chain (Vander Zanden and Rasmussen, 2001; Post, 2002). $\delta^{13}C$ reflects the origin of the food source (e.g. terrestrial or marine primary production), while $\delta^{15}N$ is mainly used to infer the relative or absolute trophic position (food web complexity) (Vander Zanden and Rasmussen, 2001; Tykot, 2004).

The frozen ctenophore samples (*M. leidyi*: n=267; *P. pileus*: n=32; *Beroe* sp.: n=59; Table 5.1; Table Appendix A) were rinsed with deionized water to reduce the salt and were individually transferred to tin capsules (8x5mm; Elemental Microanalysis). For the smallest length class (0-10 mm) several individuals were pooled together per sample. After drying (60 °C, overnight) the tin capsules were folded and placed in a sterile and sealed 96-multiwell. Similarly, the most abundant zooplankton species were selected from the thawed petri dish and transferred to tin capsules. Some were filled with mysids (15 samples), others with copepods (18 samples) or a mix of zooplankton species (including chaetognaths, copepods, mysids, decapod zoea and megalopa larvae, 45 samples). The phytoplankton filters (17 in total) were treated with dilute (10%) HCl for 2h to remove the carbonates, prior to drying (4h, 60 °C), and then folded and placed into silver capsules (8x12mm, Elemental Microanalysis). Multiwell plates containing all capsules were shipped to UC Davis Stable Isotope Facility (USA) for dual SI analyses (C, N) using a continuous flow isotope ratio mass spectrometer (Europa Integra). The C:N ratio for our target species *M. leidyi* was 4:1 and average weights for carbon were 232.4 ± SD 124.7 µg and 55.8 ± SD 28.4 µg for nitrogen.

5.2.5 Fatty acid analyses

The FA trophic marker concept relies on the fact that primary producers are characterised by certain FAs in their tissues, which may be transferred with little or no modification in their structure to their consumers (Copeman and Parrish, 2003). As such they provide knowledge on prey-predator relationships but also on the base of the food web (Dalsgaard *et al.,* 2003; Pitt *et al.,* 2009). Thus, the FA profile of *M. leidyi* will reflect the FA profile of its prey and the overall composition of its diet.

Ctenophore samples (*M. leidyi*: n=45; *P. pileus*: n=9; *Beroe* sp.: n=7; Table 5.1; Table Appendix A) were freeze-dried overnight before FA extraction. Hydrolysis of total lipid extracts and methylation to FA methyl esters (FAMEs) was achieved by a modified one-step derivatisation method after Abdulkadir and Tsuchiya (2008) as in De Troch *et al.* (2012). The boron trifluoride-methanol reagent was replaced by a 2.5 % H_2SO_4-methanol solution (2.5 mL) to prevent loss of polyunsaturated fatty acids (PUFA) (Eder 1995). The fatty acid nonadecanoic acid C19:0 (20 µL, Fluka 74208) was added as an internal standard for later quantification. FAMEs were isolated through centrifuging the samples (eppendorf Centrifuge 5810R; 3 min at 1000 rpm), heating in water for 1.5 h (80 °C), adding Hexane (1.25 mL) and deionized water (1.25 mL), and centrifuging a second time. The FAMEs thus obtained, were analysed using a gaschromatograph (HP 6890N) with a mass spectrometer (HP 5973). The samples were run in splitless mode and 1 µL was injected per run at an injection temperature of 250 °C on a HP88 column (Agilent J&W, USA). The oven temperature was programmed at 50 °C for 2 min, followed by a first ramp to 175 °C at 25 °C.min^{-1} and a second ramp to 230 °C at 2 °C.min^{-1} with a 4 min hold. The FAs were identified by comparison with the retention times and mass spectra of authentic standards and a mass spectral library (WILEY275), using MSD ChemStation software (Agilent Technologies). Quantification of individual FAs was accomplished using external standards (Supelco # 47885, Sigma-Aldrich Inc., USA) through linear regression of the chromatographic peak areas and the corresponding known concentrations of the standards (ranging from 25 to 200 mg.mL^{-1}). Shorthand FA notations A:BωX were used, where A represents the number of carbon atoms, B the number of double bonds and X gives the position of the double bond closest to the terminal methyl group (Guckert *et al.,* 1985). FA concentrations were expressed as µg.g DW^{-1}.

5.2.6 Data analyses

To visualise the SI composition in bi-plots, different samples were averaged (± standard deviation) per sampling event (station and date). In case different length classes were present, they were represented separately. PERMANOVA (Permutational ANOVA, Primer version 6.1.14 with PERMANOVA add-on software version 1.0.4) was used to investigate spatial and temporal variation in the SI and FA datasets. A PERMDISP test was performed to test the homogeneity of multivariate dispersion for each factor. PERMANOVA is a good tool

to investigate variation in these unbalanced datasets, especially when using the type III partial analysis for sums of squares, which assures that the order in which terms are fit does not matter (Anderson *et al.,* 2008).

Multivariate analyses of the SI composition combined $\delta^{13}C$ and $\delta^{15}N$ data per *M. leidyi* individual (or replicate for the smallest length classes) using the Euclidean distance similarity matrix (Clarke and Gorley, 2006). To analyse spatial and temporal variation in the trophic ecology of *M. leidyi*, the smallest length class (<10 mm) was excluded to maximise the degrees of freedom. We looked for significant differences (*p*-value <0.05) based on the factors 'area', 'month' and 'area x month', and further used pair-wise tests to locate the differences either within 'area', 'month' or within 'month per area'. Monte Carlo corrections were applied when the number of permutations was too low (<100) (Anderson *et al.,* 2008). To further investigate spatial variation, we focused on the month October, as most areas (all, except for the port of Zeebrugge, P1) were represented in this month (Table 5.1). Significant differences for $\delta^{13}C$ and $\delta^{15}N$ were identified separately for the factor 'area' using one-way Anova and several pair-wise Wilcox tests, applying Bonferroni correction for multiple pair-wise tests (assumptions for parametric tests were met) in R v 3.1.3 (R Core Team, 2015). To further investigate temporal variation, we focused on the BPNS, as most months (all except for July and December) were represented in this area (Table 5.1). Similarly, significant differences for $\delta^{13}C$ were identified for the factor 'month'. To identify significant differences for $\delta^{15}N$ for the factor 'month', parametric assumptions were not met and therefore a non-parametric Kruskal-Wallis test and several Mann-Whitney U tests were performed in R, applying the Bonferroni correction for multiple pair-wise tests. To test for ontogenetic variation in the trophic ecology of *M. leidyi*, we focused on an area and month where most length classes were represented (Westerschelde, September; 5 length classes) and again performed one-way Anova and multiple pair-wise Wilcox tests (including Bonferroni correction; parametric assumptions were met) in R to identify the differences between the length classes for $\delta^{13}C$ and $\delta^{15}N$ separately.

FA data of *M. leidyi* were only available for a few locations (WS3, WS4, BE2, P1 and P2; Table 5.1), all sampled during September, and limited to three length classes (21-35 mm: n = 19, 36-55 mm: n = 17 and >55 mm: n = 9). Two-way PERMANOVA and pair-wise tests (applying Monte Carlo corrections) were used to analyse significant spatial and ontogenetic differences (*p* <0.05) within the factors 'area' (WS = locations WS3 and WS4 representing Westerschelde estuary; BE = locations BE2, P1 and P2 representing coastal and port samples), 'length class' and 'area x length class', based on a Bray-Curtis similarity matrix.

To investigate trophic interactions of *M. leidyi* with other components of the planktonic food web, we first focused on the interspecific variation of the ctenophore species in the BPNS: *M. leidyi*, *P. pileus* and *Beroe* sp. The combined $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of all three ctenophores was compared by performing multivariate analyses in PERMANOVA

(factor 'species') using the Euclidean distance similarity matrix (Clarke and Gorley, 2006). This allowed us to determine whether their position in the bi-plot/food web differed significantly (p <0.05). For the pair-wise tests, Monte Carlo corrections were applied when the number of permutations was too low (<100) (Anderson *et al.,* 2008). A PERMDISP test was performed to test the homogeneity of multivariate dispersion for the factor. Subsequently, we compared the ctenophores in terms of isotopic niche width. To define the isotopic niche space of a species in a community, convex hulls can be used (Layman *et al.,* 2007). However, this metric is sensitive to small sample sizes (Jackson *et al.,* 2001). Therefore, Jackson *et al.* (2001) suggested to calculate standard ellipse areas (SEA), using a Bayesian approach and in particular the SEAc metric as it specifically corrects for small samples sizes. Standard ellipses contain about 40% of the data and are based on a bivariate normal distribution, while the convex hulls are based on the full extent of the data (Jackson *et al.,* 2011). To compare the niche area among species, estimates of the uncertainty around the SEAc ellipses are calculated using Bayesian inference based on 100000 posterior draws (i.e. bivariate equivalents to standard deviations in univariate analysis; Jackson *et al.,* 2011). The probability that ellipses of two species are significantly different can then be determined. All these calculations were performed using the SIBER (Stable Isotope Bayesian Ellipses in R) routine in the SIAR package for R v3.1.3 (Parnell *et al.,* 2010; Jackson *et al.,* 2011; R Core Team, 2015).

Secondly, we focused on each month (July-December) separately and also considered the potential food sources of the ctenophores. Again, multivariate analyses in PERMANOVA (factor 'species') were performed and the combined $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of all species were compared (applying Monte Carlo corrections for pair-wise testing and PERMDISP). Ellipses and convex hulls were drawn on the bi-plot to visualise potential niche partitioning. Standard ellipse areas were calculated for all taxa and compared for the three ctenophore species.

Both multivariate and univariate tests were performed on the FA data. Due to the limited data (no samples with all three taxa co-occurring), one-way PERMANOVA was executed (factor 'species') for the FA composition of *Beroe* sp. (P1: n = 7) and *P. pileus* (WS2 and WS3: n = 9; Table 5.1) versus *M. leidyi* separately. Univariate non-parametric tests (KW and pair-wise MWU tests with Bonferroni correction) were performed to further compare concentrations of the specific fatty acids between the three ctenophores. Finally, the concentrations of some fatty acids were combined to calculate specific trophic and dietary FA markers for the three ctenophores: 15:0+17:0, 18:2ω6, DHA/EPA and D/F (the ratio of all diatom markers over all flagellate markers), respectively reflecting a bacterial, detritus and dinoflagellate or diatom based food web (Kaneda, 1991; Budge and Parrish, 1998; Dalsgaard *et al.*, 2003). Next to these food web markers, also the ratio of polyunsaturated over saturated FAs (PUFA/SFA), and the ratios DHA/EPA and 18:1ω9/18:1ω7 were calculated, indicating a carnivorous or an omnivorous diet (Budge and Parrish, 1998; Stevens *et al.,*

2004). Again significant differences were explored using univariate non-parametric tests (KW and pair-wise MWU tests with Bonferroni correction).

5.3 Results

5.3.1 Spatial, temporal and ontogenetic variation in *M. leidyi* stable isotope composition

The SI composition of *M. leidyi* samples collected at different stations of the same area clearly clustered together and significant differences were observed for the factors 'area', 'month' and the interaction 'area x month' (pseudo-F = 71.92, 11.31 and 5.11, respectively; p = 0.0001). Table 5.2 presents the results from the pair-wise tests for the factor 'months for area' and Figure 5.2A visualises the variation in the SI composition of *M. leidyi* with indication of the spatial differences. The samples from Dunkerque (DK) were significantly different from the rest, with the most depleted $\delta^{13}C$ and $\delta^{15}N$ values. The samples from the Westerschelde (WS) largely clustered together in the bi-plot and were characterised by low $\delta^{13}C$ values and highest $\delta^{15}N$. Another clear group consists of the Oosterschelde (OS) and Grevelingen (GR) samples (no significant differences in pair-wise tests), with high $\delta^{13}C$ values and relatively low $\delta^{15}N$ values. All other *M. leidyi* samples are situated between the WS and OS/GR samples in the bi-plot, stretching along the $\delta^{13}C$ axis, but with comparable $\delta^{15}N$ values (*i.e.* more enriched in ^{15}N than OS/GR but more depleted than WS). In this group, samples from the port of Zeebrugge (P1) were most depleted in $\delta^{13}C$, followed by the samples from the Belgian and Dutch coastal zone (BPNS and DPNS) and the port of Oostende (P2), with the latter being most enriched in ^{13}C. PERMDISP tests for both 'area' and 'month' were significant (F = 3.75 p = 0.003 and F = 19.19 p = 0.0001 respectively), indicating that the significant PERMANOVA results could also be explained by the dispersion of the samples within 'area' or 'month'. Therefore, we focused on the month October (PERMDISP F = 2.53 p = 0.06), to present only spatial variation (Figure 5.2B). Significant differences in $\delta^{13}C$ were found (one-way Anova F = 76.82 p <0.001) between samples from Dunkerque, Oostende and the Westerschelde with all other areas (p <0.05). Significant differences in $\delta^{15}N$ were found (one-way Anova F = 56.28 p <0.001) between samples from Dunkerque and all other areas (p <0.001). The Oosterschelde samples also differed significantly in $\delta^{15}N$ from all areas (p <0.03) except for Grevelingen (p = 0.99), while samples of the latter only differed with those of the BPNS and Westerschelde (p <0.001). The $\delta^{15}N$ values from the Westerschelde, BPNS, DPNS and Oostende did not differ significantly (p >0.05).

We focused on the BPNS to investigate the temporal variation (PERMDISP F = 5.29 p = 0.03), as these samples were collected with the highest temporal resolution (Table 5.1, Figure 5.2C). August was most depleted in ^{13}C and ^{15}N, while we observed gradual enrichment in both ^{13}C and ^{15}N for the other months. Significant differences were found for the factor 'month' for both $\delta^{13}C$ and $\delta^{15}N$ (one-way ANOVA F = 9.06; p <0.001 for $\delta^{13}C$; KW df = 3; p <0.001 for $\delta^{15}N$). The bi-plot confirmed the significant pair-wise differences for $\delta^{13}C$ between

samples from August with October and November (p = 0.03 and p = 0.0001 respectively) and between September and November (p = 0.007). Significant differences for $\delta^{15}N$ were found between samples from August with October and November (p = 0.01 and p = 0.007 respectively) and between samples from September with October and November (p = 0.007; p = 0.005 respectively).

Ontogenetic variation was investigated for the five length classes present in the Westerschelde samples from September (PERMDISP F = 0.39 p = 0.86). $\delta^{13}C$ values were significantly different for the factor 'length class' (F = 3.43; p = 0.02; Figure 5.3). Pair-wise testing identified significant differences between the smallest length class (0-10 mm) and length class 3 (21-35 mm; p = 0.01) and 4 (36-55 mm; p = 0.03). For $\delta^{15}N$, no significant differences were found (F = 2.16 p = 0.09). Although within-group variation was quite large, length class 1 (0-10 mm) was most depleted in ^{13}C compared to the other length classes and most enriched in ^{15}N together with length class 5 (>55 mm) compared to the length classes 2-4 (11-55 mm).

Table 5.2 Pair-wise testing showing significant differences in the stable isotope composition of *M. leidyi* (both $\delta^{13}C$ and $\delta^{15}N$) for the factor 'area x month' (p <0.05 marked in bold); A) differences within 'month' for 'area' and B) differences within 'area' for 'month' (samples of December were not included in this analysis as in this month only specimens smaller than 10 mm were collected, which were not included here to maximise the degrees of freedom (see §5.2.6)

A	August			September				October							November	
	BPNS	P1	OS	WS	BPNS	P2	P1	WS	BPNS	P2	DPNS	OS	GR	P4	BPNS	P2
Westerschelde estuary (WS)																
Belgian part North Sea (BPNS)				**0.0001**				**0.0015**								
Port Oostende (P2)				**0.0001**	**0.0001**			**0.0008**	**0.0077**						**0.0340**	
Port Zeebrugge (P1)	**0.0130**			**0.0001**	**0.049**	**0.0001**										
Dutch part North Sea (DPNS)								**0.0005**	0.1385	**0.0084**						
Oosterschelde estuary (OS)	**0.0001**	**0.0001**						**0.0001**	**0.0001**	**0.0001**	**0.0029**					
Grevelingen estuary (GR)								**0.0001**	**0.0001**	**0.0002**	**0.0054**	0.2093				
Port Dunkerque (P4)								**0.0001**	**0.0001**	**0.0005**	**0.0011**	**0.0001**	**0.0001**			

B	Westerschelde estuary		BPNS				Port Oostende			Port Zeebrugge		Oosterschelde	
	Sept	Oct	Aug	Sept	Oct	Nov	Sept	Oct	Nov	Aug	Sept	Aug	Oct
August													
September			**0.0371**							**0.0001**			
October	0.8366		**0.0009**	**0.0001**			**0.0003**					0.0006	
November			**0.0007**	**0.0002**	0.0535		0.1244	**0.009**					

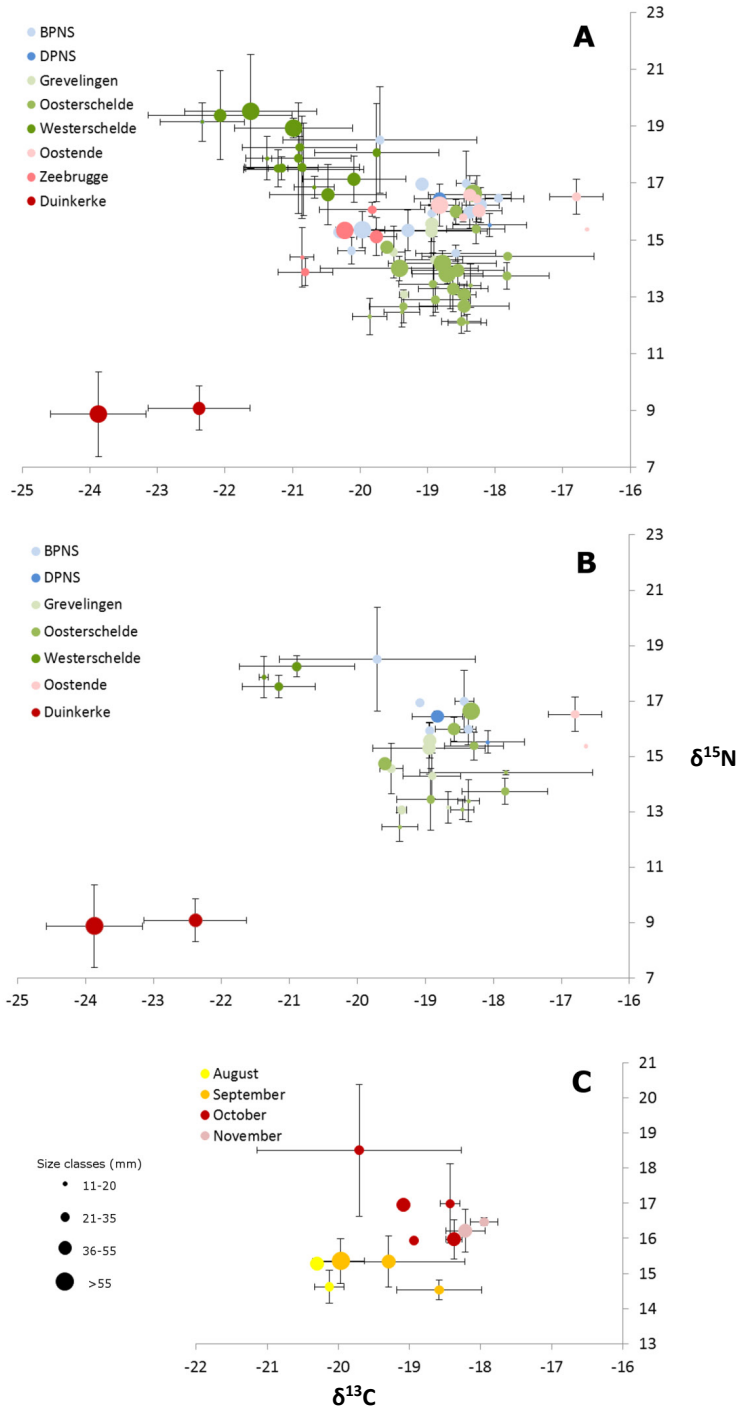

Figure 5.2 A) SI composition for all *M. leidyi* samples, indicating spatial variability; B) spatial variability in SI composition for *M. leidyi* samples from October over all sampled areas (except for Zeebrugge, where no samples were available); C) temporal variability in SI composition for *M. leidyi* samples from the Belgian part of the North Sea (BPNS) over all sampled months (except for December, when no samples were available); samples were averaged (± standard deviation) per sampling event, but with indication of different length classes (note the different scale on the y-axis and note that significant differences between these larger length classes were present for certain areas in October)

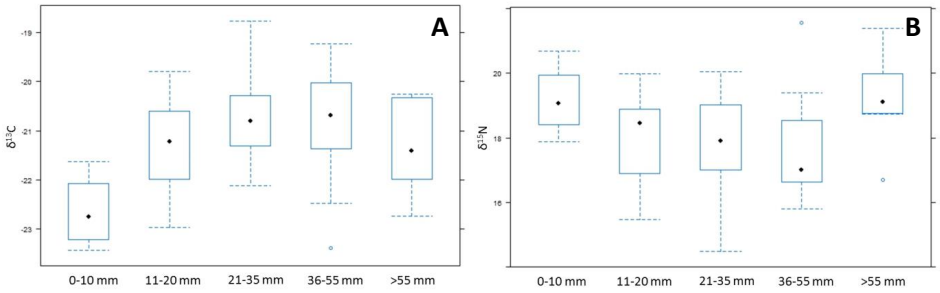

Figure 5.3 Ontogenetic variation in $\delta^{13}C$ (A) and $\delta^{15}N$ (B) for *M. leidyi* samples from the Westerschelde estuary in September with indication of the median (black dot) of the data, the lower and upper quartiles (25% and 75%) and the minimum and maximum values.

5.3.2 Spatial and ontogenetic variation in *M. leidyi* fatty acid profiles

The concentrations of 15 fatty acids were determined. The FA profiles of *M. leidyi* were not significantly different for the factor 'area' (WS vs. BE; pseudo-F = 2.50; p = 0.06) nor the interaction 'area x length class' (pseudo-F = 1.56; p = 0.16). Only some significant ontogenetic differences were noted (factor 'length class'; pseudo-F = 7.33; p = 0.0001), due to differences between medium-sized individuals (21-35 mm) and larger individuals (36-55 mm and >55 mm, p = 0.0004 and 0.0001 respectively). See Table Appendix B for detailed differences per FA.

5.3.3 Trophic interactions of *M. leidyi* with co-occuring native ctenophores and potential food sources in the planktonic food web of Belgian coastal waters based on SI and FA analyses

First, trophic interactions between *M. leidyi* and co-occurring native ctenophores in the BPNS were investigated (Figure 5.4A). Variation in $\delta^{13}C$ and $\delta^{15}N$ values as indicated by the ellipses and convex hulls demonstrated common isotopic niche areas among the three ctenophore species. However, the isotopic composition differed significantly with ctenophore species (pseudo-F = 3.76 p = 0.009), and more specifically between *Beroe* sp. and *M. leidyi* (p = 0.0001). Furthermore, based on probability estimates using Bayesian methods, the niche width of *P. pileus* (7.06‰²) was significantly larger compared to *M. leidyi* (4.47 ‰²; p = 0.04) and *Beroe* sp. (2.13 ‰²; p <0.0001) (Figure 5.4B). Additionally, the niche width of *M. leidyi* was significantly larger than *Beroe* sp. (p = 0.002).

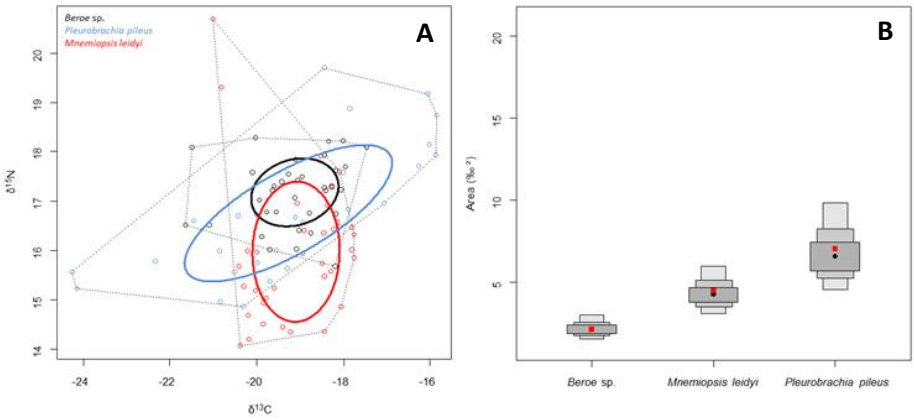

Figure 5.4 A) Variation in $\delta^{13}C$ and $\delta^{15}N$ values for *Beroe* sp., *M. leidyi* and *P. pileus* from the BPNS. Bivariate ellipses (approximately 40% credibility interval) and convex hulls, demonstrating the common isotopic niche areas of the three ctenophore species. B) Surface ellipse area (SEA) measurements per species calculated using Bayesian inference based on 100000 posterior draws. Measures of uncertainty and central tendency showing 95, 75 and 50% credibility intervals from light to dark grey respectively (black dots = mode based on SEA; red squares = mode based on SEAc (corrected for small sample size)).

As samples were collected in the BPNS over a period of six months, temporal variation could be present in the dataset (partly reflected by PERMDISP F = 13.46; p = 0.0001). To identify potential temporal differences, we analysed the data on a monthly basis and also considered the trophic interactions between these ctenophores and potential food sources. The three ctenophores clustered highest in the food web, followed by mysids and zooplankton (Figure 5.5). At the base, phytoplankton samples were present over a broad range of $\delta^{13}C$ values.

In July, resource differentiation was observed between *Beroe* sp. and *P. pileus* and their niche width did not differ significantly (p = 0.50; Figure 5.6). In fact, both species had significantly different isotopic compositions (PERMANOVA pair-wise test p = 0.003). Considering fractionation levels of 0.4-0.8‰ for $\delta^{13}C$ and 3.4‰ for $\delta^{15}N$ (Vander Zanden and Rasmussen, 2001; Post, 2002), predation interactions could be identified for *P. pileus* and mysids on zooplankton and to a lesser extend for *Beroe* sp. on *P. pileus* and zooplankton on phytoplankton. In August, samples of *P. pileus* and *M. leidyi* were positioned closely together in the bi-plot, which could be pointing to common isotopic niche areas (both feeding on zooplankton). However, their isotopic composition was significantly different (p = 0.003). Niche widths did not differ significantly between both ctenophores (p = 0.25). Furthermore, a clear shift in $\delta^{13}C$ was observed for *P. pileus* compared to July. The enrichment in ^{13}C of *P. pileus* samples continued in September. This resulted in even more resource differentiation between *P. pileus* and *M. leidyi*. Significant differences in isotopic composition for the two ctenophores were found (p = 0.001), but niche widths did not differ significantly (p = 0.34). In October, samples were available from all three ctenophore species. As in September,

resource differentiation was present between *P. pileus* and *M. leidyi*, but also with *Beroe* sp. On the other hand, *M. leidyi* and *Beroe* sp. shared an isotopic niche area, which was supported by the non-significant differences in their isotopic composition (p = 0.33). The niche width of *M. leidyi* seemed larger than in September (with extension towards more enriched $\delta^{15}N$ values) and was significantly larger than the niche width of *Beroe* sp. (p = 0.002), but not compared to the one of *P. pileus* (p = 0.70). On the other hand, the niche width of *Beroe* sp. was significantly different from *P. pileus* (p = 0.02). From the bi-plot, it is unclear what *Beroe* sp. and *M. leidyi* fed on. *Pleurobrachia pileus* probably fed on the sampled zooplankton. In November, the niche width of *M. leidyi* decreased and was not longer significantly different from the one of *Beroe* sp. (p = 0.43), but isotopic composition pointed to resource differentiation between both species (p = 0.0001). The niche area of mysids on the other hand, showed considerable overlap with that of the two ctenophore species (p = 0.17 for *Beroe* sp. and p = 0.18 for *M. leidyi*). In December, only a limited amount of samples was available. *Beroe* sp. remained at the same position in the bi-plot, while zooplankton showed a substantial decrease in its niche width.

Note that the small number of replicates for each taxon, resulted in a large range in Bayesian SEA estimates (e.g. phytoplankton samples; Figure 5.6). PERMDISP values were not significant for all months (p >0.05), except for August (F = 5.99 p = 0.02).

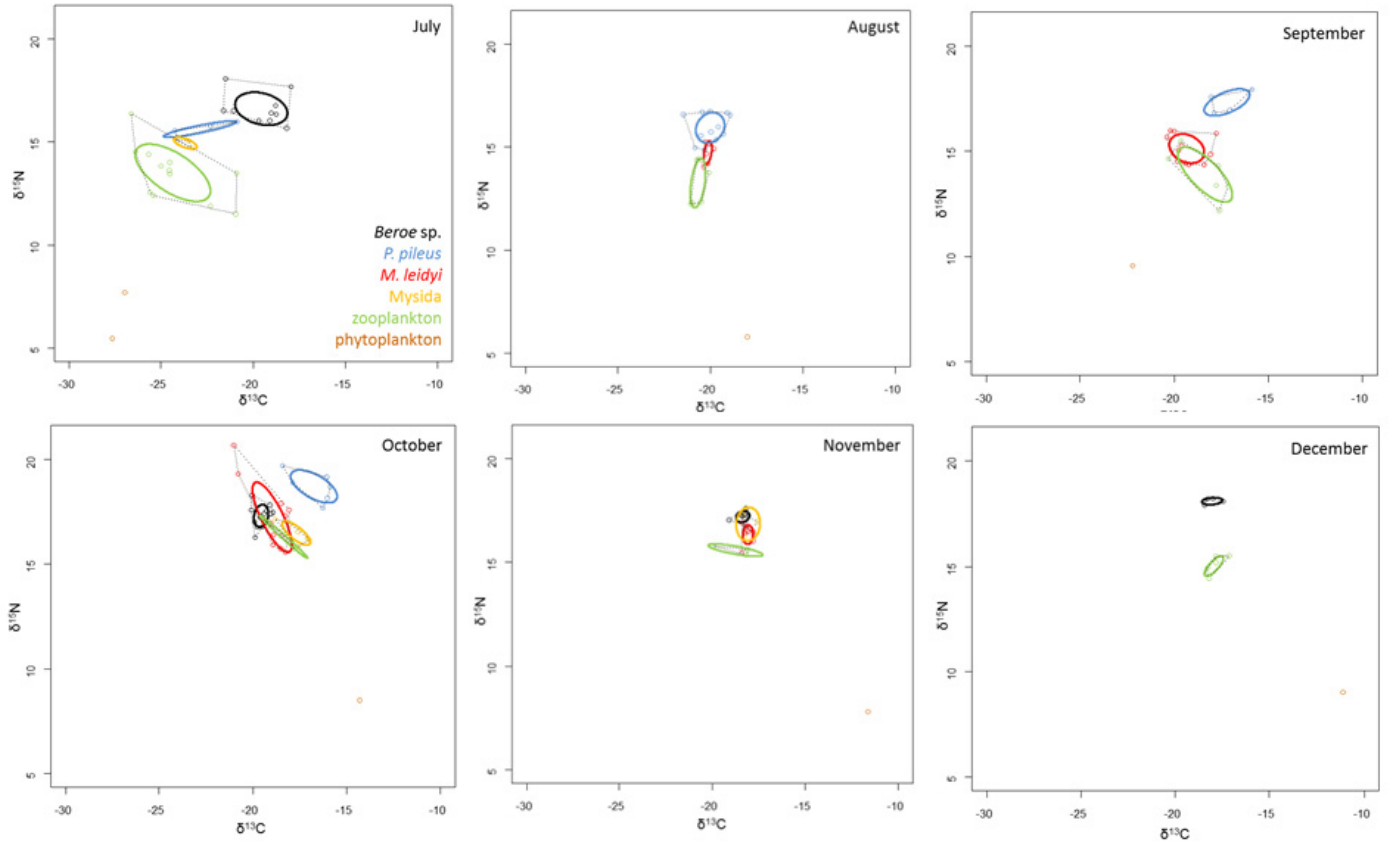

Figure 5.5 Variation in the monthly isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of *M. leidyi*, two native ctenophores and potential food sources in the BPNS. Bivariate ellipses (approximately 40% credibility interval) and convex hulls, demonstrating common isotopic niche areas or resource differentiation.

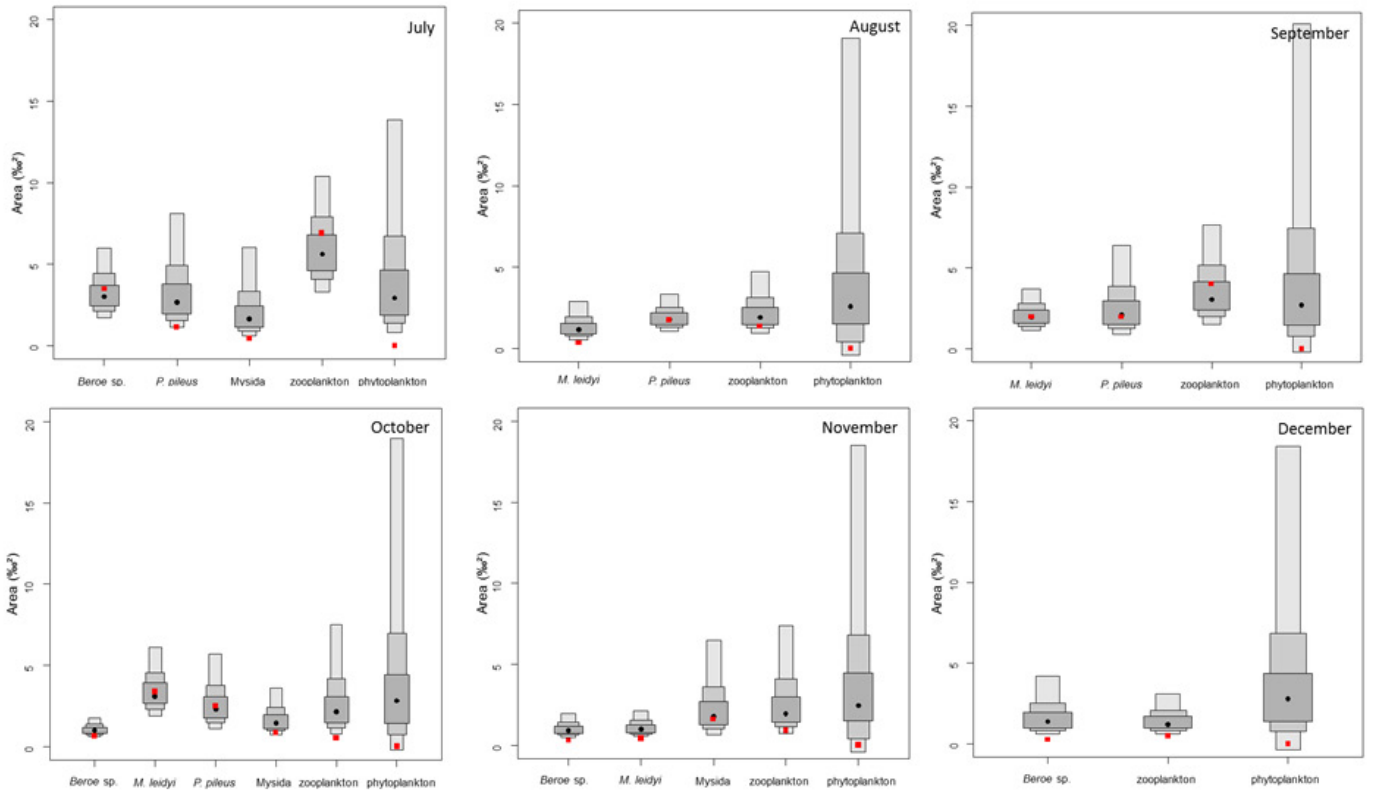

Figure 5.6 Surface ellipse area (SEA) measurements per species calculated using Bayesian inference based on 100000 posterior draws. Measures of uncertainty and central tendency showing 95, 75 and 50% credibility intervals from light to dark grey respectively (black dots = mode based on SEA; red squares = mode based on SEAc (corrected for small sample size); note that the modes do not always overlap as a result of small sample sizes, which also results in much uncertainty e.g. for phytoplankton)

The most abundant fatty acids in the three ctenophores were DHA, 16:0, EPA and 18:0 (Table 5.3). *Beroe* sp. had significantly higher concentrations compared to *M. leidyi* (Table 5.3; PERMANOVA, pseudo-F = 10.97 p = 0.0001) for all but one FA (ALA) (MWU tests with Bonferroni correction, p <0.02). Similarly, FA profiles of *P. pileus* differed significantly from *M. leidyi* (PERMANOVA, pseudo-F = 6.01 p = 0.005), more specifically in concentrations of 16:1ω7 (MWU, p = 0.003), 18:1ω9 (p = 0.02), 18:1ω7 (p = 0.03), 18:2ω6 (p = 0.001), ARA (p = 0.006) and EPA (p = 0.0009). *Mnemiopsis leidyi* had the lowest total average FA concentration (2150 ± 2050 µg.g DW^{-1}), being four times lower than *Beroe* sp. (9442 ± 6254 µg.g DW^{1}) and three times lower than *P. pileus* (7001 ± 6343 µg.g DW^{-1}). The species-specific analysis on the selected trophic and dietary FA markers showed significantly higher values for *Beroe* sp. compared to *M. leidyi* for 15:0+17:0, PUFA/SFA and 18:1ω9/18:1ω7 (Figure 5.7). The other ctenophore *P. pileus* differed significantly from *M. leidyi* in 18:2ω6, DHA/EPA, PUFA/SFA and D/F. *Beroe* sp. differed significantly from *P. pileus* for DHA/EPA, 18:1ω9/18:1ω7 and D/F.

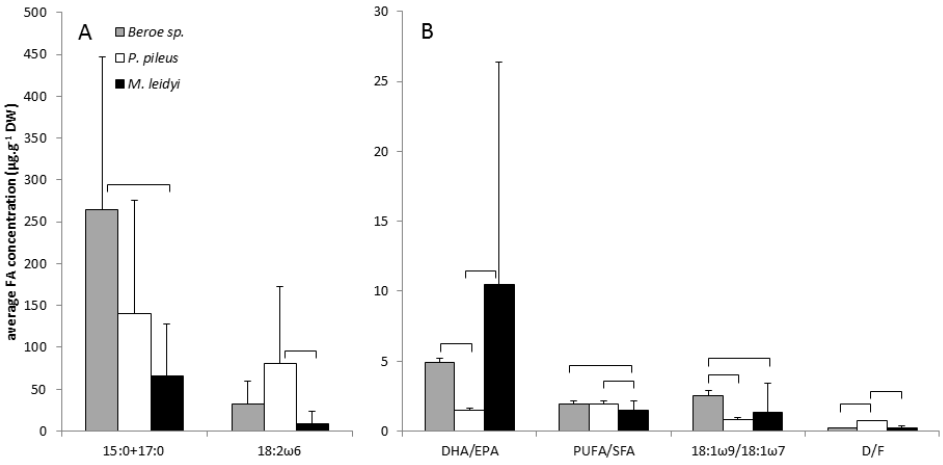

Figure 5.7 Trophic and dietary fatty acid markers per ctenophore species: (A) average FA concentrations (± standard deviation); (B) FA biomarker ratios. Significant differences between species indicated by brackets. (see Material and Methods for explanation of the FA names)

Table 5.3 Absolute (µg.g DW^{-1}) and relative (%) concentrations of FAs in three ctenophore species in the southern North Sea

Fatty acid		***Beroe* sp.**			***P. pileus***			***M. leidyi***		
systematic name	ω reference	mean	± SD	%	Mean	± SD	%	mean	± SD	%
		n = 7			n = 9			n = 52		
cis-4,7,10,13,16,19-docosahexaenoic acid	22:6ω3 (DHA)	4333.7	2908.0	45.9	2406.5	2163.0	34.4	974.6	951.7	45.3
hexadecanoic acid	16:0	1818.7	1225.8	19.3	1352.1	1142.1	19.3	498.2	461.2	23.2
cis-5,8,11,14,17-eicosapentaenoic acid	20:5ω3 (EPA)	879.6	583.9	9.3	1573.5	1466.4	22.5	196.4	230.6	9.1
octadecanoic acid	18:0	479.4	316.7	5.1	486.0	446.1	6.9	158.4	144.9	7.4
tetradecanoic acid	14:0	259.4	167.2	2.7	216.2	191.9	3.1	77.2	67.2	3.6
cis7-octadecenoic acid	18:1ω7	134.8	99.0	1.4	141.6	123.0	2.0	43.0	48.1	2.0
heptadecanoic acid	17:0	149.5	99.9	1.6	89.7	88.8	1.3	42.6	39.0	2.0
cis-5,8,11,14-eicosatetraenoic acid	20:4ω6 (ARA)	470.3	297.0	5.0	250.1	285.6	3.6	38.7	47.9	1.8
cis9-octadecenoic acid	18:1ω9	317.0	205.9	3.4	130.6	130.6	1.9	34.2	31.7	1.6
cis9-hexadecenoic acid	16:1ω7	218.1	136.9	2.3	178.6	146.6	2.6	30.0	32.2	1.4
pentadecanoic acid	15:0	115.0	83.6	1.2	50.5	47.5	0.7	23.7	23.6	1.1
cis-11-eicosenoic acid	20:1ω9	116.5	76.8	1.2	24.7	22.9	0.4	9.6	14.2	0.4
cis/trans-9,12-octadecadienoic acid	18:2ω6	32.6	27.5	0.3	80.9	92.3	1.2	8.7	14.9	0.4
cis-9,12,15-octadecatrienoic acid	18:3ω3 (ALA)	25.0	27.7	0.3	14.2	19.5	0.2	8.7	15.3	0.4
eicosanoic acid	20:0	92.8	73.9	1.0	5.9	7.8	0.1	6.1	8.8	0.3
	Total	**9442.4**		**100.0**	**7001.0**		**100.0**	**2150.2**		**100.0**

5.4 Discussion

5.4.1 Spatial, temporal and ontogenetic variation in trophic ecology of *M. leidyi*

Two types of trophic biomarkers, SI and FA, revealed spatial, temporal and ontogenetic variation in the trophic ecology of *M. leidyi* in the southern North Sea. The spatial variation in isotopic composition did not so much reflect geographical differences, but rather alterations at the base of the food web in the different systems. The $\delta^{13}C$ values of marine coastal organic matter are typically situated between -18 and -22 ‰ (Thornton and McManus, 1994 and references therein). A similar range was noted in the samples of *M. leidyi* originating from the coastal areas (BPNS and DPNS), the Belgian ports (Zeebrugge and Oostende) and the Grevelingen (GR) and Oosterschelde (OS) (highly saline) estuaries. In contrast, *M. leidyi* samples from the Westerschelde estuary (WS) and port of Dunkerque (DK) were more depleted in $\delta^{13}C$. Dunkerque receives riverine water through a sluice from the Canal de Bergues. This not only affects salinity but also the organic matter input in this system. Organic matter originating from terrestrial, sewage estuarine or riverine sources is generally more depleted in $\delta^{13}C$ values, with values between -26 and -27 ‰ for terrestrial material (Thornton and McManus, 1994), between -28 and -23 ‰ for sewage (Andrews *et al.,* 1998) and between -30 and -40 ‰ for riverine sources (Hamilton *et al.,* 1992). The Westerschelde is also influenced by a river (Schelde), but *M. leidyi* samples were collected in the polyhaline part of the estuary. These samples were probably more influenced by estuarine sources with $\delta^{13}C$ values between -21 and -24 ‰ (Middelburg and Nieuwenhuize, 1998). Through incorporation by microorganisms, the depleted carbon is further transferred to higher trophic levels (Thornton and McManus, 1994; Middelburg and Herman, 2007). As such, the influence of the depleted resources was also reflected in *M. leidyi* $\delta^{13}C$ values of WS and DK.

Still, the diet of *M. leidyi* in the Westerschelde probably does not depend on the bacterial and terrestrial detritus-based food web alone. A higher proportion of odd-chained fatty acids 15:0+17:0 and 18:2ω6 would then be expected when compared to the marine (coastal) samples (Kaneda, 1991; Fukuda and Naganuma, 2001; Dalsgaard *et al.,* 2003). However, this was not the case, as the FA profiles of *M. leidyi* specimens from the marine samples were not significantly different from the estuarine samples. Perhaps, the smaller amount of FA samples (n = 45) compared to SI analysis (n = 267) might have obscured some of the spatial variation. However, most likely, *M. leidyi* also feeds on the phytoplankton based food web in the Westerschelde (Heip *et al.,* 1995).

The $\delta^{15}N$ values are generally used to determine trophic position in the food web (Minagawa and Wada, 1984; McCutchan *et al.,* 2003). However, the higher $\delta^{15}N$ values in the WS samples do not necessarily reflect a longer food chain. Again, an alteration in baseline values, this time for $\delta^{15}N$, seems more likely. At the riverine part of the Schelde, high concentrations of ammonium and a preferential uptake of ^{14}N by microorganisms have been

observed. This results in more ^{15}N in the Westerschelde, which is even further enhanced by the long residence time of water (1-3 months) throughout the system (Mariotti *et al.,* 1984; Middelburg and Herman, 2007). The incorporation of this ^{15}N is reflected in the enriched SI values of higher trophic levels, including *M. leidyi*. When accounting for the effect of fractionation (3.4 ‰; Vander Zanden and Rasmussen, 2001) per trophic level, the coastal (BPNS and DPNS) and Belgian port (Zeebrugge and Oostende) samples again reflected the marine origin at the basis of the food web in these systems, with a typical nitrogen isotopic range for marine organic matter between 8 and 10 ‰ (Sweeney and Kaplan, 1980; Mariotti *et al.,* 1984). The more depleted $\delta^{15}N$ values in the OS and GR estuaries and surely in the DK samples could point to a considerable detritus and sewage ($\delta^{15}N$ = 1.5-2.5 ‰) influence in those systems (Sweeney and Kaplan, 1980; Mariotti *et al.,* 1984).

Temporal variation in SI composition of *M. leidyi* has been observed in several areas (e.g. Hamer *et al.,* 2011; van Looijengoed, 2011; Nagata *et al.,* 2015). Notwithstanding the short seasonal occurrence of *M. leidyi* in Belgian waters (Chapter 4), we found temporal differences in SI composition. Both $\delta^{13}C$ and $\delta^{15}N$ values of *M. leidyi* tended to increase from August towards November, which can be explained by several simultaneously working processes. After the spring phytoplankton bloom, high amounts of suspended matter (dead phytoplankton cells) are present in the water column, enhancing microbial processes (Mariotti *et al.,* 1984; Thornton and McManus, 1994; O'Brien *et al.,* 2011). This enriches the isotopic baseline, and consequently the higher trophic levels including *M. leidyi*. Changes in phytoplankton and zooplankton species composition over time (O'Brien *et al.,* 2011; Van Ginderdeuren *et al.,* 2014) result in temporal variation in prey availability. Consequently, the diet composition of *M. leidyi* and lower trophic levels may change over time. Moreover, calanoid copepods from temperate regions (e.g. *Acartia tonsa*) may change from a herbivorous to a carnivorous diet to survive winter (Lonsdale *et al.,* 1979). Such dietary shifts may also lead to enrichment in ^{15}N, reflected in the isotopic composition of higher trophic levels.

Some ontogenetic variation was observed both in SI and FA, but a clear enrichment as a result of ontogenetic shifts from larvae to adults was not fully confirmed by our results. Larvae of *M. leidyi* (<10 mm) feed on microplankton (including autotrophic and heterotrophic prey), normally resulting in more depleted $\delta^{13}C$ and $\delta^{15}N$ values (Sullivan and Gifford, 2007; van Looijengoed, 2011). This was not the case in our study and may partly due to the limited amount of samples. Rapoza *et al.* (2005) stated that after metamorphosis from the tentacular cydippid larvae to the lobate adult, the diet and consequently the $\delta^{15}N$ values in *M. leidyi* remained the same for all adults >30 mm. Both SI and FA profiles in our samples largely corroborated these findings for the adult length classes. The lower concentrations of FAs 15:0, 17:0 and 18:2ω6 might indicate that specimens between 21 and 35 mm were less dependent on a detritus or bacteria-based food web in comparison to the larger adults (Kaneda, 1991; Fukuda and Naganuma, 2001; Dalsgaard *et al.,* 2003). However, length class

does not always reflect adult age and smaller adults might have been poorly fed (shrinking; Anninsky *et al.,* 2005).

5.4.2 Trophic interactions of *M. leidyi* with co-occuring ctenophores and potential food sources in the planktonic food web of Belgian coastal waters

Based on similar isotopic compositions and overlapping isotopic niches, the ctenophores *M. leidyi* and *P. pileus* seemed to share resources in the BPNS. Isotopic niche width was larger in *P. pileus*, indicating that this native ctenophore is more of a generalist than the non-indigenous *M. leidyi*. However, when considering temporal variation, niche overlap between these two species seemed to be avoided, and common isotopic niche areas were rare.

This resource differentiation could be explained by competition between the two ctenophores, which is supported by the fact that the isotopic niche of *P. pileus* seemed to enrich more in ^{13}C over time compared to *M. leidyi*, especially when the two species co-occur. However, this could also be the result of different diets. The food source of *P. pileus* would then be more enriched in ^{13}C, for example as a result of changes at the base of the food web. The FA data supported this and showed that for the indigenous ctenophore *P. pileus*, the diatom (low DHA/EPA and high D/F ratios) and detritus (high 18:2ω6) based food web seemed to be more important (Budge and Parrish, 1998, Dalsgaard *et al.,* 2003). In contrast, the FA profile for *M. leidyi* revealed an omnivorous diet (low 18:1ω9/18:1ω7 ratio) with a strong dependency on the dinoflagellate-driven food web, as can be derived from the high DHA/EPA and low D/F ratios (Budge and Parrish, 1998; Stevens *et al.,* 2004; Dinasquet *et al.,* 2012). This is further supported by the fact that both ctenophores exhibit different hunting mechanisms. *Pleurobrachia pileus* is an ambush predator and stretches its tentacles into a wide 'net' entangling highly mobile prey (Gibbons and Painting, 1992; Costello and Coverdale, 1998). The lobate *M. leidyi* on the other hand generates a feeding current through beating of the cilia on the four auricles, which directs prey towards its colloblasts (Waggett and Costello, 1999; Colin *et al.,* 2010). Additionally, *M. leidyi* captures prey when they collide with the inner surface of its oral lobes. The combination of both techniques support a broader diet for *M. leidyi*, as it can catch less mobile microzooplankton as well as highly mobile mesozooplankton (Costello and Coverdale, 1998; Waggett and Costello, 1999).

In the eastern North Sea, Hamer *et al.* (2011) observed competition between these ctenophore species based on overlapping prey spectra (metazoan prey between 150 and 1000 µm), but also seasonal niche differentiation as *P. pileus* feeds on fish eggs at certain times of the year. Frost *et al.* (2012) identified mesozooplankton >300 µm as prey for *P. pileus* in the central North Sea, whereas *M. leidyi* was shown to have a variable diet ranging from microzooplankton and slowly swimming zooplankton to calanoid copepods (Javidpour *et al.,* 2009b; Granhag *et al.,* 2011). This would imply that *M. leidyi* is more of a generalist (having a larger niche width) than *P. pileus*, which was not confirmed by our data (isotopic niche areas were not significantly different between *M. leidyi* and *P. pileus*).

The ctenophore *Beroe* sp. has been described as a predator of both *P. pileus* and *M. leidyi* (Greve and Reiners, 1988; Hosia *et al.,* 2010; Frost *et al.,* 2012). However, this was not fully supported by our data and probably some temporal variation in the isotopic niches occurred. The isotopic composition between *Beroe* sp. and *M. leidyi* differed significantly over all samples of the BPNS (regardless the temporal variation) and *Beroe* sp. probably fed on *P. pileus* in July. However, in October $\delta^{15}N$ of *Beroe* sp. was lower than that of *P. pileus* and overlapped with *M. leidyi*. Hosia *et al.* (2010) showed that *M. leidyi* of 20 mm (oral-aboral length) or larger could only be partially consumed by *Beroe* sp. (handling error). Consequently, *Beroe* sp. probably fed on the smallest ctenophores or on small hydromedusae in the BPNS in October. Its carnivorous diet was corroborated by high proportions of the specific FA ratios DHA/EPA, PUFA/SFA and C18:1ω9/C18:1ω7 (Budge and Parrish, 1998; Stevens *et al.,* 2004).

The overall FA concentration in *M. leidyi* was considerably lower compared to the other ctenophores, which labels it as a lipid-poor species (Lee *et al.,* 2006). *Mnemiopsis leidyi* has a low reserve capacity and is characterised by high turnover rates of reserve compounds and fast shrinkage (Lee *et al.,* 2006; Augustine *et al.,* 2014). Although *P. pileus* had three times higher FA concentrations than *M. leidyi* in our study, Lee (1974) labelled it also as a lipid-poor species. A more detailed analysis through fractionation of the lipids may elucidate what part of the FA is used for storage to clarify the interspecific differences.

We also aimed to investigate potential food sources of *M. leidyi*. In July, August and September, the sampled zooplankton could be identified as a food source for both *M. leidyi* and *P. pileus*. However, it is unclear what these ctenophores have been feeding on from October until December. Probably, the limited amount of zooplankton samples available and the fact that they represented a mixture of different taxa with a herbivorous, carnivorous or detritivorous diet influenced this outcome. Mysids showed niche overlap with *M. leidyi* and probably also feed on zooplankton (Mauchline, 1980 as referred to in Verslycke *et al.,* 2004). The large isotopic variation ($\delta^{13}C$) noted in the phytoplankton samples (primary producers) was probably the result of particulate organic matter also being retained on the glass fibre filters (Montoya *et al.,* 1990; Thornton and McManus, 1994). Consequently, the $\delta^{13}C$ and $\delta^{15}N$ values for the primary producers may be too low.

5.4.3 Using biomarkers to study trophic ecology of ctenophores

Biochemical markers such as SI composition and FA profiles have proven to be useful tools to elucidate planktonic food web ecology (e.g. Petursdottir *et al.,* 2010; Ying *et al.,* 2012; Nagata *et al.,* 2015). Still, a number of studies showed that the SI outcome may differ, depending on several factors, such as the preparation and preservation of the samples (Pitt *et al.,* 2009; Fleming *et al.,* 2011), the species studied, its feeding strategy (herbivorous or carnivorous) or the body part that is retained for the analyses (Vander Zanden and Rasmussen, 2001; Fleming *et al.,* 2011; D'Ambra *et al.,* 2014). In our study we treated all

samples in the same way to reduce this processing bias as much as possible. Also, the amount of salt (NaCl) might influence the identification of SI composition and FA concentrations (µg.g DW^{-1}), as salt partly accounts for the dry weight (DW) of each sample. The amount of organic material for SI analysis was sometimes close to the border of detection. However, thanks to sufficient replicates, we could determine whether the obtained values from these small samples were comparable and reliable, which was mostly the case. For the FA samples, De Clippele (2012) conducted a small test to remove the salt in 11 *M. leidyi* samples (washing with deionised water and centrifugation to separate the salt form the samples) and concluded that the amount of salt was more or less the same in all 11 samples (av. 0.03 ± 0.01 g), meaning that this error did not influence the main results for the FA concentrations.

The advantages of simultaneously performing SI and FA analyses are clear. In summary, variation in *M. leidyi's* isotopic niche and FA profiles seemed to be influenced by the variation at the base of the food web, temporal shifts in the zooplankton community composition and/or different resource use. Trophic interactions between *M. leidyi* and the co-occurring native ctenophores showed considerable resource differentiation, while a mixture of zooplankton seemed to function as a food source for *M. leidyi*.

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Table Appendix A Overview of the amount of replicates for stable isotope and fatty acid analyses per taxonomic 'group'; M = *Mnemiopsis leidyi*, B = *Beroe* sp., P = *Pleurobrachia pileus*, z = zooplankton, c = copepods, m = mysids, p = phytoplankton

		Stable isotopes																													Fatty acids		
		July					August					September					October							November						December					September		
	Station code	B	P	z	m	p	M	B	P	p	z	M	B	P	p	z	M	B	P	z	m	c	p	M	B	z	m	c	p	M	B	z	c	p	M	B	P
Dunkerque port (FR)	P4																9																				
Oostende port (BE)	P2											37			1	3	5					3	1	5				3	1			3		1	9		
Zeebrugge port (BE)	P1	1					7	8	3	1	3	12	10		1			3				3	1					3					3	1	7	7	
Belgian part of the North Sea	BE1																5	5			3		2														
	BE2	10	4	12	3	1	6		12	1	6	11		4	1	6	6	8	4	3	3		1	8	8	3	3		1		4	6		1	9		
Westerschelde (NL/BE)	WS2											21					6													3							6
	WS3											14					3																		8		3
	WS4											15																							12		
Oosterschelde (NL)	OS1						17										7																				
	OS2						10										5																				
	OS3						18										6																				
	OS4																7																				
Grevelingen (NL)	GR1																5																				
	GR2																9																				
	GR3																5																				
Dutch part of the North Sea	NL1																5	2	3		3	3															

Table Appendix B Pair-wise tests per fatty acid between different length-classes (3: 21-35 mm, 4: 36-55 mm and 5: >55 mm); significant differences (p <0.05) indicated in bold

Fatty acid	Length class		
ω reference	3 vs. 4	3 vs. 5	4 vs. 5
14:0	0.109	**0.001**	0.318
15:0	**0.018**	**0.020**	1.000
16:0	0.052	**0.001**	0.318
16:1ω7	**0.004**	**0.006**	1.000
17:0	**0.009**	**0.021**	1.000
18:0	0.057	**0.001**	0.707
18:1ω9	**0.001**	**0.000**	1.000
18:1ω7	**0.001**	**0.008**	1.000
18:2ω6	**0.013**	**0.000**	0.092
20:0	0.107	**0.000**	**0.044**
18:3ω3 (ALA)	**0.017**	**0.002**	0.903
20:1ω9	**0.025**	**0.001**	0.283
20:4ω6 (ARA)	**0.001**	**0.006**	1.000
20:5ω3 (EPA)	**0.031**	**0.010**	1.000
22:6ω3 (DHA)	**0.001**	**0.001**	1.000

6

EFFECTS OF PREY TYPE AND QUALITY ON *MNEMIOPSIS LEIDYI* FEEDING AND CARBON ASSIMILATION: AN EXPERIMENTAL APPROACH

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ABSTRACT

One of the factors contributing to the invasive success of the ctenophore *Mnemiopsis leidyi* is its broad and flexible planktivorous diet consisting of micro-, meso- and ichthyoplankton. Traditionally, gut content analyses are used to study the diet, but this only provides a snapshot of the diet. Grazing experiments and trophic biomarkers largely contribute to increase the understanding of *M. leidyi's* feeding ecology. In this study, grazing experiments were executed to determine the feeding rates of *M. leidyi* on two mesozooplankton species (*Artemia salina* and *Acartia tonsa*) and different life stages (eggs and larvae) of European sea bass *Dicentrarchus labrax.* No significant differences in clearance rates between prey types or sizes were observed (av. 0.2 ± 0.1 $L.mL_{M.leidyi}^{-1}.h^{-1}$). The assimilation of carbon by *M. leidyi* for these different prey types and the pelagic diatom *Phaeodactylum tricornutum* was determined using ^{13}C tracer experiments. Highest carbon assimilation was observed for *Acartia* and sea bass larvae (most efficiently assimilated), and lowest for the pelagic diatom *Phaeodactylum tricornutum*. To further elucidate the prey-dependent variation in carbon uptake, we investigated the effect of each prey type in terms of fatty acids as a proxy for food quality. The consumption of sea bass larvae, characterised by higher levels of DHA (an essential fatty acid), resulted in significantly higher FA concentrations in *M. leidyi*. As *M. leidyi* does not convert excess food into storage lipids, survival, growth and reproduction are likely enhanced by the higher food quality, which might contribute to its invasive success. As global warming may result in an earlier appearance of *M. leidyi* and thus temporal overlap with high quality prey such as fish larvae, a substantial impact on the ichthyoplankton community in the southern North Sea might be expected.

6.1 Introduction

The ctenophore *Mnemiopsis leidyi* A. Agassiz 1865, indigenous to the Atlantic coasts of North and South America (Purcell *et al.,* 2001), has been observed in northern Europe since 2005 (Oliveira, 2007; Antajan *et al.,* 2014). Its introduction in the Black Sea, in addition to

overfishing and eutrophication, led to a collapse of the major fisheries in the area in the late 1980s, causing vast ecological and socio-economic losses (GESAMP, 1997; Knowler, 2005; Daskalov *et al.,* 2007). Consequently, the spread of this non-indigenous species in northern European waters, whose coastal areas have been subject to intense anthropogenic activities for over a century (including eutrophication and overfishing; Serchuk *et al.,* 1996; Jackson *et al.,* 2001; Vasas *et al.,* 2007) gave rise to the necessary concern (Faasse and Bayha, 2006; Javidpour *et al.,* 2006; Tendal *et al.,* 2007; Van Ginderdeuren *et al.,* 2012b; van Walraven *et al.,* 2013; Hosia and Falkenhaug, 2015).

The invasive success of *M. leidyi* can be attributed to its high tolerance to several environmental parameters (Purcell *et al.,* 2001; Decker *et al.,* 2004), high feeding rates and rapid population growth related to high egg production rates and short generation times (Baker and Reeve, 1974; Granhag *et al.,* 2011; Riisgård *et al.,* 2012). Moreover, several studies confirmed the broad planktivorous diet of *M. leidyi*, consisting of micro-, meso- and ichthyoplankton (reviewed in Costello *et al.,* 2012). Stomach analyses identified copepod adults and nauplii, mollusc larvae, early life stages of fish, cladocerans and appendicularians in varying abundances, depending on the period of the year and the ontogenetic stage of *M. leidyi* (Larson, 1987; Purcell *et al.,* 1994; Mutlu, 1999; Rapoza *et al.,* 2005; Javidpour *et al.,* 2009b; Kellnreitner *et al.,* 2013). Granhag *et al.* (2011) showed that the gut content of *M. leidyi* formed a good representation of the mesozooplankton composition in the ambient water. This all confirms the dietary flexibility of *M. leidyi*, allowing it to survive in a variety of habitats (Purcell *et al.,* 2001). Apart from gut content analysis, which only provides a snapshot of the diet, laboratory grazing experiments can help to understand more of *M. leidyi's* feeding ecology. Several laboratory studies showed that ingestion, digestion and growth rates vary according to prey type and concentration, predator size and varying abiotic variables (Baker and Reeve, 1974; Kremer, 1979; Decker *et al.,* 2004; Granhag *et al.,* 2011;).

In light of overexploited fish stocks, the importance of fish eggs and larvae in the diet of *M. leidyi* has gained special attention (e.g. Shiganova and Bulgakova, 2000; Bilio and Niermann, 2004). Lobate ctenophores, such as *M. leidyi*, are considered to be more effective than other ctenophores or pelagic cnidarians to capture fish eggs and larvae, as they simultaneously use two complementary feeding mechanisms (Purcell, 1985; Waggett and Costello, 1999; Colin *et al.,* 2010). Still, there are quite some differences between the clearance and ingestion rates of fish eggs *versus* larvae, between different fish species, and between laboratory *versus* field studies (Burrell and Van Engel, 1976; Monteleone and Duguay, 1988; Cowan and Houde, 1990; Mutlu, 1999; Jaspers *et al.,* 2011; Kellnreitner *et al.,* 2013). The prevailing abiotic conditions could explain some of these differences. Higher sea water temperature for example may affect feeding efficiency and result in higher clearance rates as respiration rates and energetic demands increase (Jaspers *et al.,* 2011; Purcell *et al.,* 2001). Other abiotic factors such as salinity and dissolved oxygen levels seemed to have a less clear effect

(Decker *et al.,* 2004; Hosia *et al.,* 2012), but low dissolved oxygen levels may favour *M. leidyi* as it can result in reduced escape abilities in prey (Decker *et al.,* 2004). Nevertheless, prey selectivity and varying clearance and digestion rates indicate that *M. leidyi* might prefer some prey over others, which is likely related to prey behaviour (including its escape abilities), prey size and prey palatability (Kremer 1979; Javidpour *et al.,* 2009b; Regula *et al.,* 2009; Madsen and Riisgård, 2010; Granhag *et al.,* 2011; Jaspers *et al.,* 2011).

To further elucidate the effect of the prey to *M. leidyi's* feeding ecology, Reeve *et al.* (1989) investigated the assimilation efficiency of *M. leidyi* and showed that it varied with prey concentration. However, it is less clear whether *M. leidyi* assimilates each prey species/type in the same way. A biomarker approach, using stable isotopes (SI) and fatty acid (FA) analyses, can elucidate the energy flow through the food web over a longer period of time, based on the 'you are what you eat' principle (De Niro and Epstein, 1976; Pitt *et al.,* 2009).

In this study, we first executed baseline grazing experiments to determine the feeding rates of *M. leidyi* on a variety of prey species. Then tracer experiments using ^{13}C pre-labelled prey as food were conducted to reveal the assimilation of carbon from different prey by *M. leidyi*. Finally, fatty acid concentrations were determined to investigate the effect of food (prey) quality on *M. leidyi*. The combined experimental results contribute to our understanding of the invasive success of *M. leidyi* and more specifically of the potential impact of this non-indigenous ctenophore species on the ichthyoplankton – and by extension to commercial fisheries – in the Belgian part of the North Sea.

6.2 Material and methods

6.2.1 Test organisms

Mnemiopsis leidyi ctenophores were qualitatively collected from the sluice dock in the port of Oostende (Belgium; 51.23°N 2.95°E) during the blooming season (July until December; salinity 30 ± 3; temperature: 17 ± 5 °C) with a hand-held dip net and bucket. We refer to Chapter 4 for more information on location and sampling methodology. In the lab, the ctenophores were kept alive in aquaria filled with filtered seawater (2 µm bacterial filter, salinity 33) and were acclimatised to 17 °C for at least 12 h. Only undamaged animals in good shape with an oral-aboral length ranging between 25 and 45 mm were further used for the different experiments.

Diatoms, brine shrimp nauplii, adult copepods and fish eggs and larvae were offered as food sources to *M. leidyi* to study the effect of prey type. The pelagic diatom *Phaeodactylum tricornutum* Bohlin 1897 (further referred to as *Phaeodactylum*; ± 20 µm in length; ± 7.10^{-6} µg C.cell^{-1} (Laws *et al.,* 1997)) was obtained from the CCAP culture collection (1055/14; bccm.belspo.be) and further cultured in f2 medium (seawater with additional nutrients; Guillard, 1975) in a 12:12h light:dark regime at 15 °C. Nauplii of brine shrimp *Artemia salina* Linnaeus 1758 (further referred to as *Artemia*; ± 400 µm in length; ± 0.30 µg C.ind^{-1}) were

grown from cysts in aerated, filtered seawater (26 °C, salinity 33) and supplied with excess light (Ocean Nutrition™, ONE-AC_ST-1108-2, Great Salt Lake). Adult *Acartia tonsa* Dana 1849 copepods (further referred to as *Acartia*; ± 1 mm in length; ± 0.40 µg C.ind^{-1}) were obtained from the Dutch Fry-Marine (www.frymarine.nl), where they had been reared with *Rhodomonas baltica* Karsten 1898 algae. Eggs and larvae (1 and 11-day-old) of European sea bass (*Dicentrarchus labrax* Linnaeus 1758) were obtained from the French Ecloserie Marine de Gravelines hatchery (SYSAAF, 2013). Fish eggs and 1 and 11-day-old larvae had a total length of ± 1, 3 and 4 mm respectively. Swimming behavior was observed for the 11-day-old larvae (12.71 µg C.ind^{-1}).

This study was carried out in accordance with the guidelines of the Belgian Council for Laboratory Animal Science (BCLAS), and the experimental protocol was approved by the ethical committee of the institute (ILVO) under permit number 2013/204.

6.2.2 Experimental set-up

Baseline grazing experiments

To investigate feeding rates, grazing experiments were conducted for each prey type except for the diatom *Phaeodactylum*, by adding prey and predators together in 9 L-cylinders filled with filtered seawater (17 °C, salinity 33). Different concentrations of each reared prey type were incubated as different treatments (2-4 replicates), together with one 24h-starved *M. leidyi* per cylinder as predator (Table 6.1). Cylinders were further filled to the brim and firmly closed with household film and a lid. The cylinders (8 at a time, randomly chosen) were incubated on a rolling table (1 rotation min^{-1}) in a dark environment to avoid light-driven behaviour. After approximately 4 h, *M. leidyi* was removed and measured (oral-aboral length, mm); the remaining prey were concentrated by reverse filtration (mesh size 30 µm) and counted. Control treatments, comprising only prey, were incubated randomly with the other treatments.

Feeding rates were measured by means of clearance (F) and ingestion rates (I) and calculated as in Møller *et al.* (2010), using the following formulas:

$$F = \frac{V}{(t \times n)} \times \ln\left(\frac{C0}{Ct}\right) \text{ and } I = \frac{(Ct - C0)}{(t \times n)}$$

with V = container volume (L), t = incubation time (h), n = number of predators or predator biovolume (mL), and C_0 and C_t = prey concentration in individuals.L^{-1} at time 0 and t respectively. Biovolume (mL) was calculated based on the wet weight (WW, g) as $biovolume = \frac{(WW + 0.122)}{1.017}$, and wet weight was determined using the oral-aboral length (L, mm) regression: $WW = 0.009L^{1.872}$ (Kremer and Nixon, 1976). Ingestion and clearance rates were standardised to the biovolume of the *M. leidyi* individuals, as both rates increase with ctenophore size (Kremer, 1979; Granhag *et al.,* 2011). Ingestion rate is thus expressed as prey.mL $_{M.leidyi}^{-1}$.h^{-1}, *i.e.* the number of prey ingested per biovolume of *M. leidyi* per hour;

clearance rate as L.mL $_{M.leidyi}^{-1}$.h^{-1} or the water volume cleared per biovolume of *M. leidyi* per hour. The remaining prey concentration was corrected for the loss of prey in the control treatments: 9.4% for the *Artemia*, 0% for the sea bass eggs, 7.4% for the 1-day-old sea bass larvae and 3.7% for the 11-day-old sea bass larvae. For the controls of *Acartia*, 1.7% more prey were retained and this was also corrected for by dividing the remaining prey in the different treatments by the percentage (average of the replicates) that was left in the controls for each treatment.

Tracer experiments

To evaluate the assimilation of carbon by *M. leidyi* from different prey types, we conducted tracer experiments through artificial enrichment of three trophic levels. First, diatoms (*Phaeodactylum*) were cultured and enriched with ^{13}C by adding $NaH^{13}CO_3$ to the f2 growth medium. After two weeks the labelled growth medium was replaced by filtered seawater (salinity 33). Subsequently, the enriched diatoms were fed to *Acartia* and *Artemia* for 4 days, ensuring a clear and stable signal (De Troch *et al.,* 2005). Then, the enriched *Artemia* were washed and fed to 8-day-old sea bass larvae over 5 days. The different enriched food sources (*i.e. Phaeodactylum, Artemia, Acartia* and sea bass larvae) were offered to 24h-starved *M. leidyi* in beakers with little aeration during parallel treatments of 3 and 6 h (Table 6.2).

To measure the specific and total uptake of ^{13}C by the different prey and by *M. leidyi,* stable isotope analyses were performed as described in Chapter 5. Specific uptake was calculated as the difference of $\delta^{13}C$ values between the labelled and control specimens ($\triangle\delta^{13}C = \delta^{13}C_{enriched} - \delta^{13}C_{control}$; Middelburg *et al.,* 2000) to verify whether enrichment was successful. Isotopic composition of unlabelled controls were obtained from the reared stocks for *Phaeodactylum* and *Acartia*, from Spero *et al.* (1993) for *Artemia*, from Beata *et al.* (2010) for sea bass larvae and from Chapter 5 for *M. leidyi* (47 specimens; Table 6.2).

Total uptake by *M. leidyi* (T, µg $^{13}C.ind^{-1}$) was calculated as T = E x B, *i.e.* the product of the excess ^{13}C (E; above background) and the biomass (B; organic carbon per individual ctenophore), where $E = Fr_{sample} - Fr_{control}$, with Fr (the fraction ^{13}C) calculated as $Fr = {}^{13}C/({}^{13}C+{}^{12}C) = R/(R+1)$, and R (the carbon isotope ratio) derived from the measured $\delta^{13}C$ as $R = ((\delta^{13}C/1000)+1) \times R_{standard}$, with $R_{standard}$ = 0.0113272 according to Vienna Pee Dee Belemnite (Middelburg *et al.,* 2000; De Troch *et al.,* 2005). The total uptake of *M. leidyi* was corrected for the different atomic percentages (at. %) in each prey (*i.e.* proportion of ^{13}C atoms relative to the total number of C atoms in each prey in percentage) and expressed as µg C.ind^{-1}. Furthermore, we corrected for the unequal amount of carbon in each ctenophore and standardized to unit carbon of *M. leidyi* (total uptake in µg C.unit C^{-1}).

Table 6.1 Baseline grazing experiments executed in 9 L-cylinders with *M. leidyi* feeding on different prey at varying prey concentrations. Control treatments only include prey (no predator); results are averages ± standard deviations per treatment; biomass estimates (µg C.ind^{-1}) were provided for *Acartia*, *Artemia* and 11-day-old sea bass larvae

Treatment				Predator (*M. leidyi*)		Clearance rate		Ingestion rate	
Prey type	Prey concentration (ind.L^{-1})	Replicates (n)	Duration (h)	Length (mm)	Calculated biovolume (mL)	total F (L h^{-1} ind^{-1})	specific F (L.mL $_{M.leidyi}^{-1}$.h^{-1})	total I (prey h^{-1} ind^{-1})	specific I (prey.mL $_{M.leidyi}^{-1}$.h^{-1})
Acartia (± 0.4 µg C.ind^{-1})	2	4	4.1 ± 0.1	29.0 ± 3.2	5.0 ± 1.0	0.9 ± 0.4	0.2 ± 0.1	1.4 ± 0.6	0.3 ± 0.1
	5	4	4.2 ± 0.1	31.8 ± 2.6	5.9 ± 0.9	1.2 ± 1.0	0.2 ± 0.1	4.1 ± 2.6	0.7 ± 0.4
	8	4	4.2 ± 0.1	31.0 ± 3.4	5.6 ± 1.2	1.0 ± 0.4	0.2 ± 0.1	6.3 ± 2.3	1.2 ± 0.6
	16	4	4.1 ± 0.2	31.8 ± 3.8	5.9 ± 1.3	1.1 ± 0.7	0.2 ± 0.1	12.8 ± 6.4	2.1 ± 0.7
control	5	4	4.1 ± 0.2	-	-	-	-	-	-
Artemia (± 0.3 µg C.ind^{-1})	2	4	4.3 ± 0.3	38.3 ± 6.1	8.4 ± 2.5	1.7 ± 1.0	0.2 ± 0.2	2.2 ± 0.8	0.3 ± 0.2
	5	4	4.4 ± 0.4	37.5 ± 2.9	8.0 ± 1.1	1.0 ± 0.4	0.1 ± 0.0	4.0 ± 1.2	0.5 ± 0.1
	8	4	4.4 ± 0.4	33.0 ± 3.7	6.3 ± 1.3	1.2 ± 0.6	0.2 ± 0.1	7.0 ± 2.8	1.2 ± 0.5
	16	4	4.2 ± 0.2	36.3 ± 5.2	7.6 ± 2.1	1.4 ± 0.2	0.2 ± 0.0	16.6 ± 1.3	2.3 ± 0.5
control	5	4	4.1 ± 0.1	-	-	-	-	-	-
sea bass eggs	1	3	4.3 ± 0.1	36.7 ± 5.5	7.7 ± 2.2	2.4 ± 1.4	0.3 ± 0.2	1.3 ± 0.6	0.2 ± 0.1
	5	2	4.2 ± 0.2	38.0 ± 1.7	8.4 ± 0.9	3.1 ± 1.1	0.4 ± 0.1	7.9 ± 1.4	1.0 ± 0.1
control	1	3	4.5 ± 0.2	-	-	-	-	-	-
sea bass 1-day-old larvae	1	3	4.3 ± 0.2	35.7 ± 7.8	7.4 ± 2.8	2.0 ± 0.8	0.3 ± 0.1	1.3 ± 0.3	0.2 ± 0.1
	5	3	4.3 ± 0.3	38.7 ± 6.0	8.5 ± 2.5	1.9 ± 0.6	0.2 ± 0.1	6.0 ± 1.0	0.8 ± 0.3
control	1	3	4.4 ± 0.4	-	-	-	-	-	-
sea bass 11-day-old larvae	1	3	4.0 ± 0.1	37.0 ± 5.0	7.8 ± 1.9	1.2 ± 0.6	0.2 ± 0.1	0.9 ± 0.4	0.1 ± 0.1
(± 12.7 µg C.ind^{-1})	5	3	4.0 ± 0.1	37.0 ± 2.0	7.8 ± 0.8	2.3 ± 0.9	0.3 ± 0.2	7.0 ± 1.5	0.9 ± 0.3
control	1	3	4.0 ± 0.1	-	-	-	-	-	-

Table 6.2 Tracer experiments with *M. leidyi* feeding on different enriched prey types; 'control' represents unenriched *M. leidyi* from the field obtained from Chapter 5

	Treatment					Predator (*M. leidyi*)	
Enriched prey type	Duration (h)	Prey concentration (ind L^{-1})	Biomass (µg C)	Replicates (n)	Volume (L)	Number (n)	Length ± SD (mm)
Phaeodactylum	3	42.10^6	± 294	4	2.5	1	34.8 ± 6.3
	6	42.10^6	± 294	4	2.5	1	38.3 ± 5.1
Artemia	3	125	± 37.5	3	4	1	38.3 ± 5.7
	6	125	± 37.5	3	4	1	37.7 ± 5.5
Acartia	3	200	± 80	4	2.5	1	35.3 ± 8.4
	6	200	± 80	4	2.5	1	36.3 ± 9.0
sea bass larvae	3	31	± 394	3	4	1	31.3 ± 5.1
	6	31	± 394	3	4	1	28.7 ± 3.8
control						47	26.8 ± 15.8

Table 6.3 Fatty acid food quality experiments with *M. leidyi* feeding on different prey types for a period of 13 days; control samples represent *M. leidyi* from the field sampled at the start of the experiments (23 July 2014). Two other field samples were taken from the sluice dock at different moments (30 July 2014 and September 2012, respectively) to evaluate temporal variation in the field.

	Treatment				Predator (*M. leidyi*)		
Prey type	Duration (d)	Replicates (n)	Prey concentration (prey d^{-1})	Volume (L)	Number (n)	Length start (mm)	Length end (mm)
Artemia	13	3	2x 200	8	3	36.6 ± 2.1	22.9 ± 4.1
Acartia	13	3	2x 200	8	3	36.4 ± 2.7	29.6 ± 4.6
sea bass larvae	13	3	2x 200	8	3	37.3 ± 2.4	35.3 ± 5.1
control		3			1	37.3 ± 2.5	
field 1		3			1	36.7 ± 2.9	
field 2		12			1	34.0 ± 20.5	

Food quality experiments

A third series of grazing experiments was executed to determine the effect of food quality, in terms of fatty acids (FA) of the different prey types, on the FA composition and concentration of *M. leidyi*. *Artemia, Acartia* and 6 to 11-day-old sea bass larvae were offered to *M. leidyi* for 13 days in separate treatments in 8 L-beakers filled with filtered seawater and provided with little aeration (Table 6.3). Each beaker contained three predators to ensure the survival of at least one individual. At the start of the experiments, predators were measured and control samples for *M. leidyi* were collected in the sluice dock (23 July 2013). Two other field samples (field 1 and field 2) were also taken from the sluice dock at different moments (30 July 2014 and September 2012, respectively) to evaluate temporal variation in the field. Control samples of the prey types were also collected in 2 to 3 replicates (n=100) for *Artemia, Acartia,* and sea bass eggs and larvae, and in 4 replicates for *Phaeodactylum* (average 15 ± 0.5 10^6 cells per replicate). All control samples were frozen at -80 °C. Thirteen days after the start of the experiments, all *M. leidyi* predators were measured again and prior to FA analyses frozen at -80 °C.

FA extractions and analyses were performed as described in Chapter 5. However, some samples were injected in split 10 and split 50 mode in the gaschromatograph mass spectrometer, which corresponded to 0.1 and 0.02 µL, because FA concentrations in splitless mode were too high. Shorthand FA notations of the form A:BωX are used, where A represents the number of carbon atoms, B the number of double bonds and X the position of the double bond closest to the terminal methyl group (Guckert *et al.,* 1985). Fatty acid concentrations are expressed as µg.g DW^{-1}.

6.2.3 Statistical analyses

Significant differences in ingestion and clearance rates of *M. leidyi* were calculated by means of a two-way Anova in R v 3.1.3 (R Core Team, 2015) with factors 'prey type' and 'prey size', as the parametric assumptions were met. Significant differences corresponded to *p*-values less than 0.05. To evaluate tracer and food quality experiments, the parametric assumptions were not met, and the non-parametric alternative PERMANOVA (Permutational ANOVA Primer v6 with PERMANOVA add-on software) was used to test for significant differences (Anderson *et al.,* 2008). For total uptake (µg C.unit C^{-1}), we used a Euclidean distance similarity matrix and tested for the factors 'diet' (*Phaeodactylum*, *Artemia*, *Acartia* and sea bass larvae), 'duration' (3h or 6h treatment) and the interaction factor ('diet x duration'). To compare FA profiles of *M. leidyi* fed with different food sources, the Bray-Curtis similarity matrix based on FA concentrations was tested for the factor 'diet' (*Artemia*, *Acartia* and sea bass larvae). Significant effects were further analysed through pair-wise tests, applying Monte Carlo corrections when the number of permutations was too low (<100) (Anderson *et al.,* 2008). To evaluate shrinkage of *M. leidyi* in relation to prey quality in terms of FA, both

one-way Anova and Kruskal-Wallis tests were executed depending on the normality of the data distribution (R Core Team, 2015).

6.3 Results

6.3.1 Baseline grazing experiments

All prey types offered during the grazing experiments were ingested by *M. leidyi*. The proportion of prey that was removed during the experiment was 35 ± 15% for *Acartia*, 45 ± 13% for *Artemia*, 68 ± 20% for sea bass eggs, 59 ± 12% for 1-day-old larvae and 50 ± 19% for 11-day-old larvae. Specific ingestion rates (prey.mL$_{M.leidyi}^{-1}$.h^{-1}) increased with prey concentration (Figure 6.1A; Table 6.1; no saturation), but no significant differences were found between prey types and sizes when comparing the concentrations of 5 prey.L^{-1} (two-way Anova: F = 1.65; p = 0.23). Furthermore, even though 11-day-old sea bass larvae had a much larger biomass than *Acartia* and *Artemia*, still higher specific ingestion rates were found when offered the same prey concentration (5 prey.L^{-1}; Figure 6.1B; no data available on biomass for sea bass eggs and 1-day-old larvae). Clearance rates decreased with increasing mobility of the prey (Figure 6.1C). Non-mobile eggs were cleared from the water at an average specific rate of 0.33 ± 0.14 L.mL$_{M.\ leidyi}^{-1}$.h^{-1}, while highly mobile *Acartia* copepods were cleared at 0.18 ± 0.09 L.mL$_{M.\ leidyi}^{-1}$.h^{-1}. However, specific clearance rates were not significantly different between the different prey types and sizes (two-way Anova, F = 2.08; p = 0.15). Although sea bass eggs were easily ingested and cleared from the water, regurgitation of entire eggs coated in mucus was observed.

6.3.2 Tracer experiments

All different prey types showed considerable ^{13}C uptake (Figure 6.2A), which was largely reflected in the specific uptake of *M. leidyi* feeding on these prey, except for *Phaeodactylum* (low specific ^{13}C uptake by *M. leidyi*) (Figure 6.2B). In spite of the lower ^{13}C uptake by sea bass larvae themselves, the specific ^{13}C uptake by *M. leidyi* when fed with sea bass larvae was relatively high.

After standardizing to unit carbon (considering the individual biomass of each *M. leidyi* and correcting for different enrichment levels of the prey), the highest total uptake was measured for *M. leidyi* with an *Acartia* diet, followed by the sea bass larvae diet (Figure 6.2C). The factors 'diet', 'duration' and the interaction factor showed significant differences (PERMANOVA, pseudo-F = 12.95; 4.00; 3.68 and p = 0.0002; 0.045; 0.02 respectively). Pair-wise tests only identified significant differences within the sea bass diet for the factor duration (p = 0.01). Significant differences within the factor duration for diet are shown in Table 6.4A.

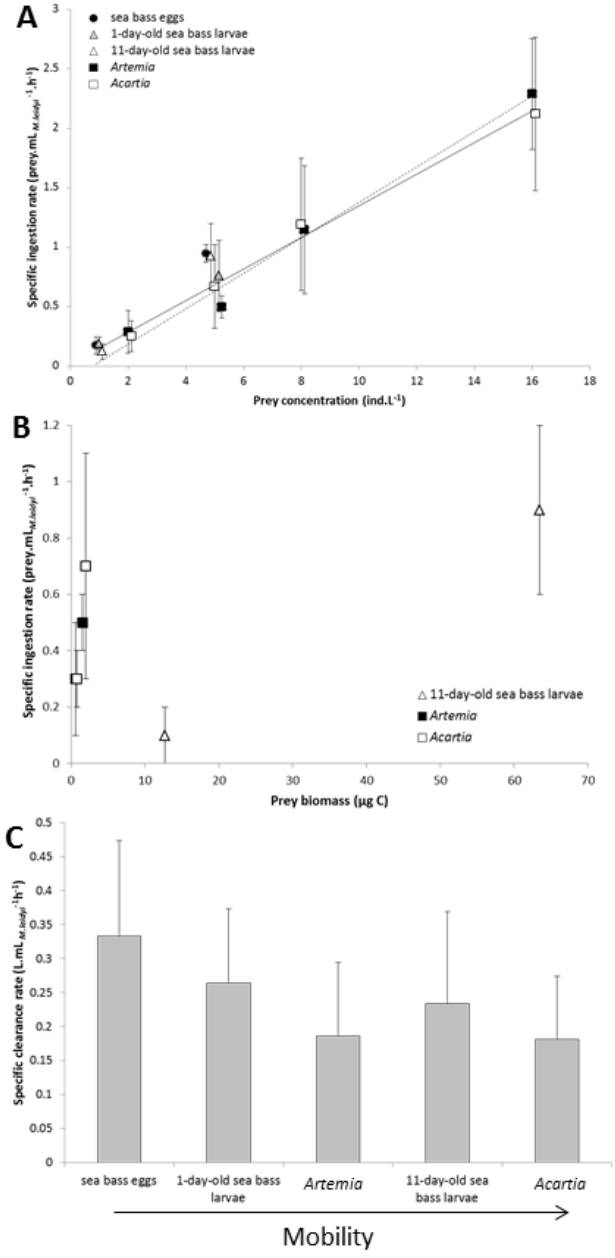

Figure 6.1 Specific ingestion rates (prey.mL $_{M.leidyi}^{-1}$.h^{-1}, ± SD) relative to prey concentration (A; prey.L^{-1}) and prey biomass (B; µg C) and C) specific clearance rates (L.mL $_{M.leidyi}^{-1}$.h^{-1}) of *M. leidyi,* based on baseline grazing experiments with different concentrations of *Artemia, Acartia,* sea bass eggs and 1 and 11-day-old sea bass larvae; regression lines in (A) are shown for *Artemia* (dashed line; y = 0.139x; R^2 = 0.98) and *Acartia* (solid line; y = 0.135x; R^2 = 0.99) and data points are slightly moved for clarity; prey biomass in (B) is shown for 2 and 5 prey.L^{-1} for *Acartia* and *Artemia* and for 1 and 5 prey.L^{-1} for 11-day-old sea bass larva

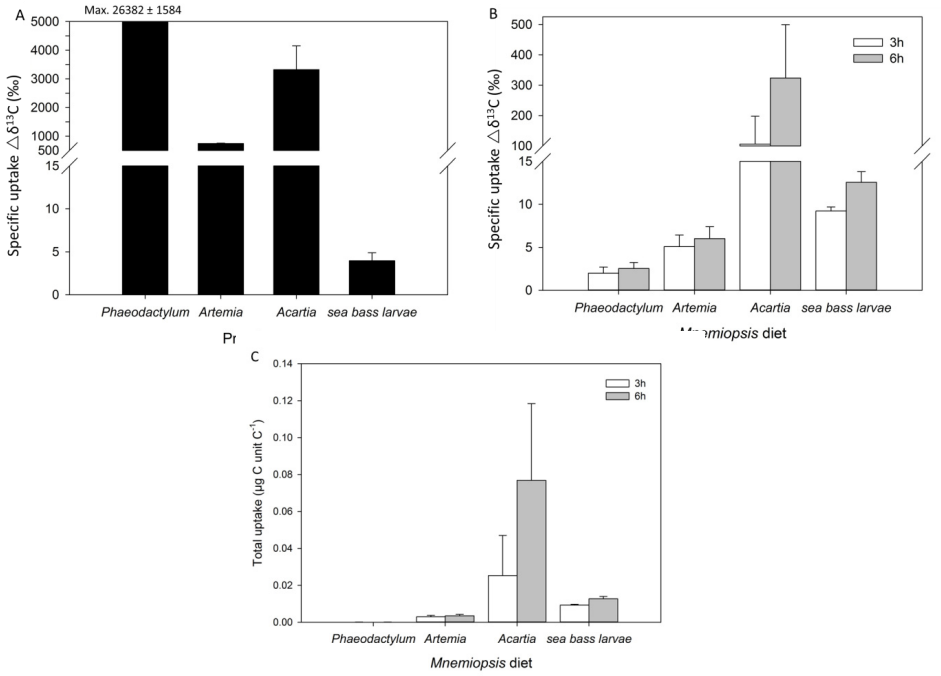

Figure 6.2 Specific ^{13}C uptake ($\triangle\delta^{13}C$, ‰) (A) by selected prey types, and (B) by *M. leidyi* after 3 and 6h feeding on these enriched food sources. (C) Total C uptake per unit carbon of *M. leidyi* (µg C.unit C^{-1}). Error bars = SD. Note the different y-axes scales.

6.3.3 Food quality experiments

The different prey types showed significant variation in their FA composition (Figure 6.3A; PERMANOVA, pseudo-F = 13.20 p = 0.0001). The FA profile of *Phaeodactylum* diatoms as primary producers were characterised by high proportions of 16:1ω7 and EPA (Table 6.5A) and differed significantly from all other prey types (pair-wise tests: Table 6.4B). The FA profiles of the primary consumers (*i.e. Artemia* and *Acartia*) differed in particular in the lower proportion of DHA and the higher proportion of ALA compared to those of sea bass larvae (secondary consumers) and eggs (Table 6.5B).

After 13 days, the total FA concentration of *M. leidyi* fed with sea bass larvae was significantly higher than the control (PERMANOVA pair-wise tests p = 0.006), and also higher than for *M. leidyi* fed with an *Artemia* (p = 0.0004) or *Acartia* diet (p = 0.0005) (Figure 6.3B). Also the FA composition in *M. leidyi* with a sea bass larvae diet differed from the control (p = 0.006), field 1 (p = 0.03), the *Artemia* (p = 0.0003) and *Acartia* diet (p = 0.001) (Table 6.4C). For *M. leidyi* feeding on *Artemia* or *Acartia* no significant differences in FA composition were found when compared to the control (p = 0.48 and p = 0.92 respectively). However, both profiles differed significantly from the field 2 sample (September 2012; p = 0.0005 and p =

0.02, respectively) in contrast to the samples with a diet of sea bass larvae (p = 0.10). The control and other field *M. leidyi* samples were characterised by temporal variation in total FA concentration, but this difference was not significant (Table 6.4C).

When comparing prey and predator FA profiles, the higher food quality of sea bass larvae, as shown by its high levels of DHA, is clearly reflected in the FA profile of *M. leidyi* feeding on this diet (Table 6.5B). The higher concentrations of ALA in *Artemia* and *Acartia* was reflected to a smaller extent in the respective *M. leidyi* FA profiles. The observed shrinkage (decrease in oral-aboral length) was highest for *M. leidyi* individuals feeding on *Artemia* (16.67 ± 3.87 mm), intermediate for *Acartia* (6.89 ± 5.11 mm) and lowest for a fish larvae diet (1.5 ± 3.89 mm). Moreover, significant differences between the start and end measurements of *M. leidyi* with *Acartia* and *Artemia* diet were observed (one-way ANOVA F = 14.96 p = 0.001; F = 79.52; p <0.001, respectively). This was not the case for *M. leidyi* with a fish larvae diet (KW df = 1; p = 0.58).

Table 6.4 *p*-values of PERMANOVA pair-wise tests for (A) total C uptake per unit carbon of *M. leidyi* for different prey types within the factor 'duration' for 'diet'; (B) for FA profiles of different prey types; and (C) FA profiles of *M. leidyi* fed with different prey types, including a control and 2 field profiles sampled at different moments (see Fout! Verwijzingsbron niet gevonden.**); numbers in bold represent significant differences**

A	3 h				6 h			
	Phaeodactylum	*Artemia*	*Acartia*	sea bass larvae	*Phaeodactylum*	*Artemia*	*Acartia*	sea bass larvae
Phaeodactylum								
Artemia	**0.0005**				**0.0005**			
Acartia	0.06	0.14			**0.01**	**0.03**		
sea bass larvae	**0.0001**	**0.0002**	0.28		**0.0001**	**0.0004**	0.05	

B	*Phaeodactylum*	*Artemia*	*Acartia*	sea bass egg	sea bass larva
Phaeodactylum					
Artemia	**0.0003**				
Acartia	**0.007**	0.24			
sea bass egg	**0.0001**	**0.002**	**0.02**		
sea bass larva	**0.0002**	**0.006**	**0.03**	**0.006**	

C	*Artemia*	*Acartia*	sea bass larvae	control	Field 1	Field 2
Artemia						
Acartia	0.23					
sea bass larvae	**0.0003**	**0.001**				
control	0.48	0.92	**0.006**			
Field 1	**0.003**	0.10	**0.03**	0.05		
Field 2	**0.0005**	**0.02**	0.10	0.05	0.14	

Table 6.5 Overview of average fatty acid concentrations (± SD) of (A) the five different prey types (ng.ind^{-1} or µg.ind^{-1}) and of (B) *M. leidyi* after 13 days feeding on *Artemia*, *Acartia* and sea bass larvae (µg.g DW^{-1}); see Table 6.3 for explanation on the control and field samples.

(A) Fatty acid		Prey				
systematic name	ω reference	*Phaeodactylum*	*Artemia*	*Acartia*	sea bass egg	sea bass larva
		ng.ind^{-1}	µg.ind^{-1}	µg.ind^{-1}	µg.ind^{-1}	µg.ind^{-1}
cis-4,7,10,13,16,19-docosahexaenoic acid	22:6ω3 (DHA)	0.08 ± 0.01	-	0.03 ± 0.03	33.91 ± 1.32	13.76 ± 4.83
hexadecanoic acid	16:0	0.66 ± 0.19	0.02 ± 0.02	0.04 ± 0.02	6.28 ± 4.62	3.72 ± 1.45
octadecanoic acid	18:0	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	3.52 ± 0.12	1.32 ± 0.48
cis-5,8,11,14,17-eicosapentaenoic acid	20:5ω3 (EPA)	0.77 ± 0.08	<0.01 ± <0.01	0.01 ± 0.01	7.58 ± 0.25	2.34 ± 0.79
cis9-octadecenoic acid	18:1ω9	0.06 ± 0.03	0.03 ± 0.02	0.03 ± 0.04	18.90 ± 0.69	6.89 ± 2.43
heptadecanoic acid	17:0	-	<0.01 ± <0.01	<0.01 ± <0.01	0.23 ± 0.01	0.05 ± 0.05
tetradecanoic acid	14:0	0.30 ± 0.05	<0.01 ± <0.01	<0.01 ± <0.01	1.31 ± 0.08	0.34 ± 0.13
cis7-octadecenoic acid	18:1ω7	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	2.66 ± 0.09	0.90 ± 0.30
cis-5,8,11,14-eicosatetraenoic acid	20:4ω6 (ARA)	-	<0.01 ± <0.01	<0.01 ± <0.01	2.12 ± 0.07	0.84 ± 0.32
cis-9,12,15-octadecatrienoic acid	18:3ω3 (ALA)	-	0.07 ± 0.04	0.06 ± 0.10	1.08 ± 0.05	0.27 ± 0.11
cis9-hexadecenoic acid	16:1ω7	1.38 ± 0.45	<0.01 ± <0.01	0.01 ± <0.01	4.15 ± 2.77	1.26 ± 0.67
pentadecanoic acid	15:0	0.02 ± 0.00	<0.01 ± <0.01	<0.01 ± <0.01	0.17 ± 0.01	0.06 ± 0.06
cis/trans-9,12-octadecadienoic acid	18:2ω6	0.03 ± 0.01	0.01 ± 0.01	0.01 ± 0.02	9.13 ± 0.29	2.95 ± 1.00
cis-11-eicosenoic acid	20:1ω9	-	<0.01 ± <0.01	<0.01 ± <0.01	0.87 ± 0.04	0.28 ± 0.25
eicosanoic acid	20:0	-	<0.01 ± <0.01	<0.01 ± <0.01	-	-
cis-11,14-eicosadienoic acid	20:2ω6	-	<0.01 ± <0.01	<0.01 ± <0.01	0.46 ± 0.01	0.19 ± 0.07

Table 6.5

(B) Fatty acid		Predator (*M. leidyi*)					
systematic name	ω reference	*Artemia* diet	*Acartia* diet	sea bass larva diet	Control	Field 1	Field 2
		µg.g DW^{-1}	µg.g DW^{-1}	µg.g DW^{-1}	µg.g DW^{-1}	µg.g DW^{-1}	µg.g DW^{-1}
cis-4,7,10,13,16,19-docosahexaenoic acid	22:6ω3 (DHA)	254.68 ± 133.32	494.40 ± 399.44	952.78 ± 417.00	371.15 ± 282.30	541.14 ± 78.65	1286.67 ± 1027.83
hexadecanoic acid	16:0	318.96 ± 150.59	348.32 ± 196.98	617.20 ± 172.95	334.12 ± 207.25	489.40 ± 67.90	532.54 ± 421.39
octadecanoic acid	18:0	94.59 ± 40.70	100.44 ± 57.33	160.95 ± 47.29	95.02 ± 61.81	136.34 ± 13.99	152.00 ± 124.03
cis-5,8,11,14,17-eicosapentaenoic acid	20:5ω3 (EPA)	48.34 ± 21.17	88.92 ± 56.00	124.65 ± 43.92	88.62 ± 66.35	157.42 ± 21.38	221.00 ± 236.64
cis9-octadecenoic acid	18:1ω9	44.90 ± 19.61	42.88 ± 62.17	185.27 ± 107.82	28.02 ± 18.96	53.09 ± 8.91	46.66 ± 34.67
heptadecanoic acid	17:0	18.81 ± 7.92	26.71 ± 13.16	24.70 ± 6.50	25.47 ± 8.69	34.02 ± 8.57	53.48 ± 39.02
tetradecanoic acid	14:0	20.70 ± 10.04	26.59 ± 17.05	43.24 ± 14.95	23.14 ± 14.64	32.97 ± 5.73	104.82 ± 81.62
cis7-octadecenoic acid	18:1ω7	12.87 ± 7.48	12.81 ± 10.38	29.86 ± 11.57	14.82 ± 11.22	26.51 ± 4.19	43.65 ± 37.02
cis-5,8,11,14-eicosatetraenoic acid	20:4ω6 (ARA)	12.29 ± 5.92	20.40 ± 25.34	62.62 ± 23.03	13.56 ± 10.11	37.58 ± 3.97	43.56 ± 36.49
cis-9,12,15-octadecatrienoic acid	18:3ω3 (ALA)	33.62 ± 16.00	11.61 ± 7.63	6.80 ± 3.81	11.73 ± 9.70	18.75 ± 9.27	23.00 ± 24.25
cis9-hexadecenoic acid	16:1ω7	1.66 ± 0.86	5.75 ± 12.17	40.89 ± 35.48	9.90 ± 7.14	17.97 ± 12.98	28.33 ± 27.03
pentadecanoic acid	15:0	6.38 ± 2.92	7.87 ± 5.28	14.81 ± 4.31	9.40 ± 5.62	18.24 ± 3.62	34.60 ± 30.85
cis/trans-9,12-octadecadienoic acid	18:2ω6	10.37 ± 4.54	13.35 ± 20.67	75.83 ± 49.19	8.35 ± 6.46	14.13 ± 2.34	12.78 ± 16.44
cis-11-eicosenoic acid	20:1ω9	9.55 ± 4.46	11.94 ± 10.98	38.61 ± 21.40	7.91 ± 5.52	12.86 ± 1.48	11.41 ± 11.14
eicosanoic acid	20:0	7.90 ± 3.84	8.46 ± 4.34	17.15 ± 8.23	6.86 ± 4.55	10.33 ± 1.54	7.86 ± 9.77
cis-11,14-eicosadienoic acid	20:2ω6	3.12 ± 1.76	5.50 ± 5.08	13.18 ± 5.04	4.60 ± 3.49	8.97 ± 1.45	-

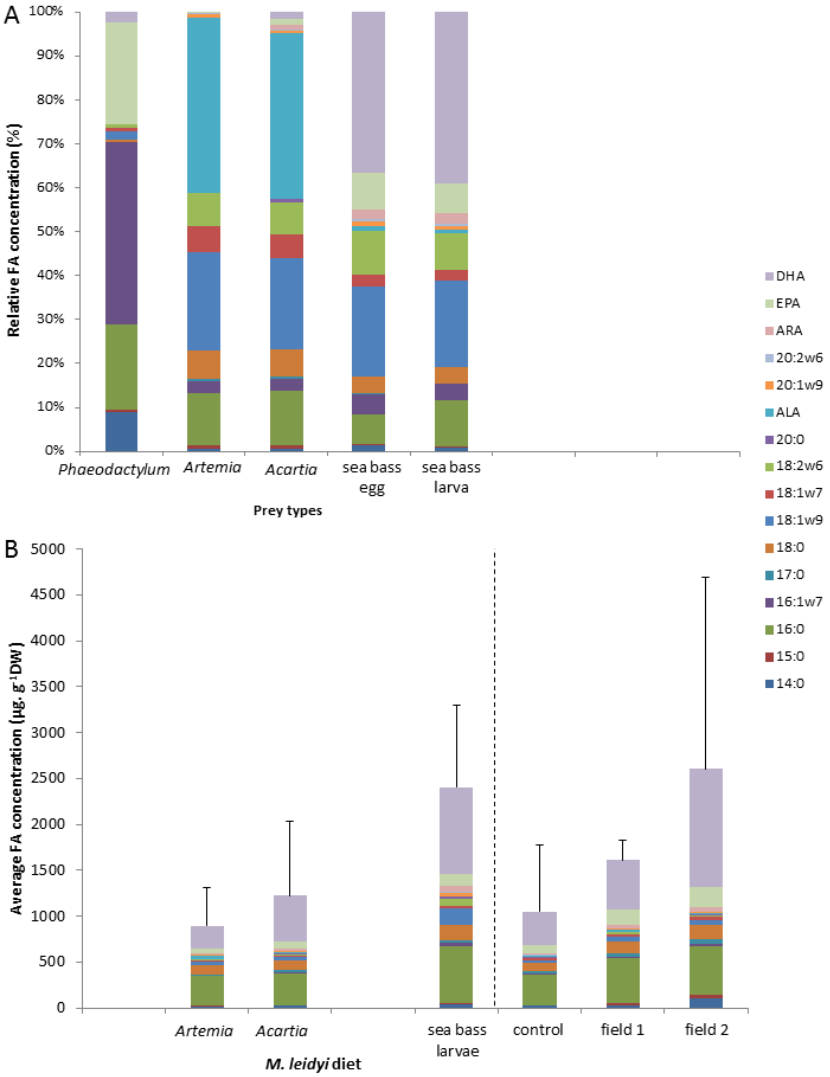

Figure 6.3 (A) Proportional representation of the measured fatty acid concentrations in all prey types (%; calculated per ind.) and (B) total FA (±SD) for *M. leidyi* after 13 days feeding on *Artemia, Acartia* and sea bass larvae (µg.g DW^{-1}) with the contribution of the measured FAs indicated with the colours; see Table 6.3 for explanation on the control and field samples.

6.4 Discussion

6.4.1 Feeding efficiency

The grazing experiments in this study provide evidence for predation of *M. leidyi* on ichthyoplankton (sea bass eggs and larvae) and mesozooplankton (*Artemia* and *Acartia*).

However, both clearance and ingestion rates were not significantly influenced by these prey types or their sizes. Ingestion rates of *M. leidyi* linearly increased with prey concentration, which means that higher encounter rates, result in more consumption. In our experiments, *M. leidyi* did never reach a point of saturation and Purcell *et al.* (2001) noted that even up to much higher prey densities (ca. 3600 copepods.L^{-1}) the ingestion was directly proportional to prey concentration. *Mnemiopsis leidyi* generates a feeding current (beating of cilia on the four auricles), which directs less mobile prey towards the colloblasts on the tentillae. Also more mobile prey, such as *Acartia* and 11-day-old sea bass larvae, can be entrained by the feeding current, after which they are captured when they collide with the inner surface of the oral lobes of the ctenophore during an escape attempt (Costello and Coverdale, 1998; Waggett and Costello, 1999; Colin *et al.,* 2010; Madsen and Riisgård, 2010). Both feeding techniques support the broad diet of this non-indigenous species, including mobile and less mobile prey and seem to work equally efficient as shown by the grazing experiments. Still, the decreasing clearance rates (although not significant) for mobile prey could be related to the better escape abilities of prey with a higher mobility (Titelman and Hansson, 2006). However, a higher mobility of the prey could result in higher encounter rates and thus predation. Additionally, the larger biomass of the fish larvae did not seem to hinder ingestion. On the contrary, clearance and ingestion rates appeared higher than for *Acartia* and *Artemia*. More prey concentrations should be tested to verify the consistency and significance of this trend.

One-day-old sea bass larvae were more efficiently cleared than the actively swimming 11-day-old sea bass larvae, which corroborates the results of Jaspers *et al.* (2011). The clearance rates on sea bass were comparable to other studies with *M. leidyi* feeding on anchovy (Monteleone and Duguay, 1988; Cowan and Houde, 1990), but were ten times higher than when fed with Baltic cod larvae (Jaspers *et al.,* 2011). Furthermore, we showed that *M. leidyi* easily ingested sea bass eggs, while Jaspers *et al.* (2011) found that the capture response of *M. leidyi* was barely triggered by Baltic cod eggs. However, shortly after ingestion, we observed regurgitation of the eggs, which indicates that *M. leidyi* also had difficulties to digest sea bass eggs. Probably, the larger size of Baltic cod eggs (1.5 mm) and larvae (5 mm) contributed to this difference (Jaspers *et al.,* 2011). Moreover, the experiments performed by Jaspers *et al.* (2011) were executed at 7 °C, which has an impact on the feeding efficiency of this temperate ctenophore species (Purcell *et al.,* 2001).

Clearance rates for *Artemia* reported in this study were lower compared to Madsen and Riisgård (2010) (0.19 ± 0.11 *versus* 0.32 ± 0.10 L.mL $_{M.leidyi}^{-1}$.h^{-1}). In contrast, *Acartia* clearance rates (0.18 ± 0.09 L.mL $_{M.leidyi}^{-1}$.h^{-1}) were twice as high compared to other studies (Miller, 1970 as reported in Jaspers *et al.,* 2011; Decker *et al.,* 2004). Granhag *et al.* (2011) illustrated that clearance rates under laboratory conditions could be underestimated compared to the field, due to confinement effects of small container volumes. *Mnemiopsis leidyi* individuals of 30-40 mm (*i.e.* conform the size range in our study) had clearance rates between 6 and 8

$L.ind^{-1}.h^{-1}$ in the field (Granhag *et al.,* 2011), which is up to 8 times higher than in our study. Purcell (2009) suggested to use a container to ctenophore ratio of 2500:1 L. This ratio was not obtained in our incubation experiments (ranging from 880:1L to 2070:1 L), which might have led to some underestimation of the clearance rates. This could also partially explain the differences with other studies.

6.4.2 Carbon assimilation

The ^{13}C tracer experiments showed that high feeding rates are followed by fast carbon assimilation. After 3h of feeding on ^{13}C enriched prey, the uptake of this heavy isotope was already measured in the tissue of the ctenophore. Similarly, Granhag *et al.* (2011) illustrated that *M. leidyi* processes its food fast and showed that the digestion times for *Acartia* ranged between 0.9 ± 0.2 h and 4.8 ± 0.6 h, depending on the initial prey concentration in the gut and the size of the ctenophore. The assimilation of ^{13}C was significantly dependent on the prey type and duration. Total carbon uptake in *M. leidyi* was highest when fed on *Acartia* (especially after 6 h) and lowest for *Phaeodactylum* (small amount of ^{13}C in *M. leidyi* was probably acquired through leakage by *Phaeodactylum*). Herbivory in adult *M. leidyi* is limited, as hitherto only one large diatom species *Ditylum brightwellii* (T. West) Grunow, 1885 has been found in their guts (Deason and Smayda, 1982). A diet only consisting of diatoms was found to be inadequate to support growth and maintenance and resulted in a considerable shrinkage of *M. leidyi* (Baker and Reeve, 1974). On the other hand, carbon assimilation from sea bass larvae was highly efficient and resulted in a higher specific uptake in *M. leidyi* than the ^{13}C concentrations measured in the enriched larvae themselves. This implied that sea bass larvae may be a good food source e.g. in terms of palatability.

6.4.3 Food quality

The reason for a prey-dependent variation in carbon uptake was further elucidated by investigating each prey type in terms of fatty acids as a proxy for food quality. It should be noted that the sixteen FAs reported in this study do not represent the complete FA profile of an organism, and the outcome and standardisation of the FA analyses may also be influenced by the employed extraction protocol and equipment (Chapter 5). Still, our results showed that several essential fatty acids (such as ALA and DHA) were present in *Artemia*, *Acartia*, sea bass eggs and larvae. The limited concentrations of these essential FAs or the shape or size of *Phaeodactylum* could explain why a diatom diet is insufficient to support *M. leidyi's* metabolism (Baker and Reeve, 1974).

The FA profiles of the zooplankton (*Artemia* and *Acartia*) and ichthyoplankton (sea bass larvae) food sources were clearly reflected in the FA profile of *M. leidyi*. The sea bass larvae diet in particular resulted in significantly higher FA concentrations, including the essential fatty acid DHA. As a high quality food source, sea bass larvae may prevent shrinkage. Shrinkage occurs when not enough food or when food of poor quality is provided. Then, *M.*

leidyi starts to use its body proteins to meet its metabolic demands, leading to organic dilution of the tissues and body shrinkage (Reeve and Walter, 1978; Reeve *et al.,* 1989; Anninsky *et al.,* 2005). This was the case for *M. leidyi* with a diet of only *Acartia* or *Artemia*.

Mnemiopsis leidyi was described as a lipid-poor species that does not invest in lipid reserves, even during periods with excess food (Lee*,* 1974; Lee *et al.,* 2006; Augustine *et al.,* 2014; Chapter 5). Nevertheless, the total FA concentration in *M. leidyi* was higher compared to the control samples when feeding on sea bass larvae, and more or less comparable when feeding on *Acartia*. This might imply that the prey concentration in the field is quite similar to the concentrations offered in the experiments, and that *M. leidyi* in the field gets most of its energy from mesozooplankton prey such as *Acartia*. This was also confirmed by the fact that carbon uptake by *M. leidyi* was highest through a diet on *Acartia* (and probably other copepod species as well).

Finally, the FA profiles for *M. leidyi* from the control and field samples showed a clear temporal variation, not only in total FA concentrations, but also in FA composition. This is most likely driven by changing prey distributions and dynamics throughout the summer period (Van Ginderdeuren *et al.,* 2014; Chapter 3). As such, *M. leidyi* collected in September (field 2, highly abundant) were probably able to feed on higher quality prey than those collected in July (control and field 1). As temporal overlap between *M. leidyi* and copepods was high, the latter probably serve as the most important food source for *M. leidyi* in Belgian waters (Van Ginderdeuren *et al.,* 2014).

6.4.4 The impact of *M. leidyi* on the food web in Belgian waters

The broad diet and opportunistic feeding strategy allow *M. leidyi* to quickly obtain and assimilate energy when encountering prey (Costello *et al.,* 2012; Kremer and Reeve, 1989). This feeding strategy is very effective in both its native and invaded distribution range, and may give *M. leidyi* populations the opportunity to rapidly expand under favourable conditions (Purcell *et al.,* 2001). In this study, we show that especially sea bass larvae are rapidly ingested and efficiently assimilated. Their high biomass and food quality in terms of FA may directly contribute to a better survival, growth and reproduction of *M. leidyi*, as excess food is not converted into large lipid reserves (Augustine *et al.,* 2014; Lee *et al.,* 2006).

The impact of *M. leidyi* on the associated zooplankton communities (including ichthyoplankton) can be quite substantial (Purcell, 1985; Shiganova and Bulgakova 2000; Purcell *et al.,* 2001). Although sea bass eggs were regurgitated by *M. leidyi*, an impact on this prey type is still suspected as their survival could be hampered when being caught in mucus strands released by *M. leidyi*. In Belgian waters, *M. leidyi* normally occurs from August until December (Chapter 4). However, due to higher winter temperatures in 2013-2014, high densities of *M. leidyi* were found from the end of June onwards in 2014 (Chapter 4). Many

temperate fish species spawn in early spring (Munk and Nielsen, 2005), resulting in high egg densities in May and high larval densities in July (Greve *et al.,* 2005; Vansteenbrugge *et al.,* unpublished data). Climate change might even further intensify the temporal overlap between *M. leidyi* and the early life stages of fish (van der Molen *et al.,* 2015), most likely enhancing the invasive success of *M. leidyi* and the potential impact on certain fish species in the southern North Sea and beyond.

6.5 Acknowledgements

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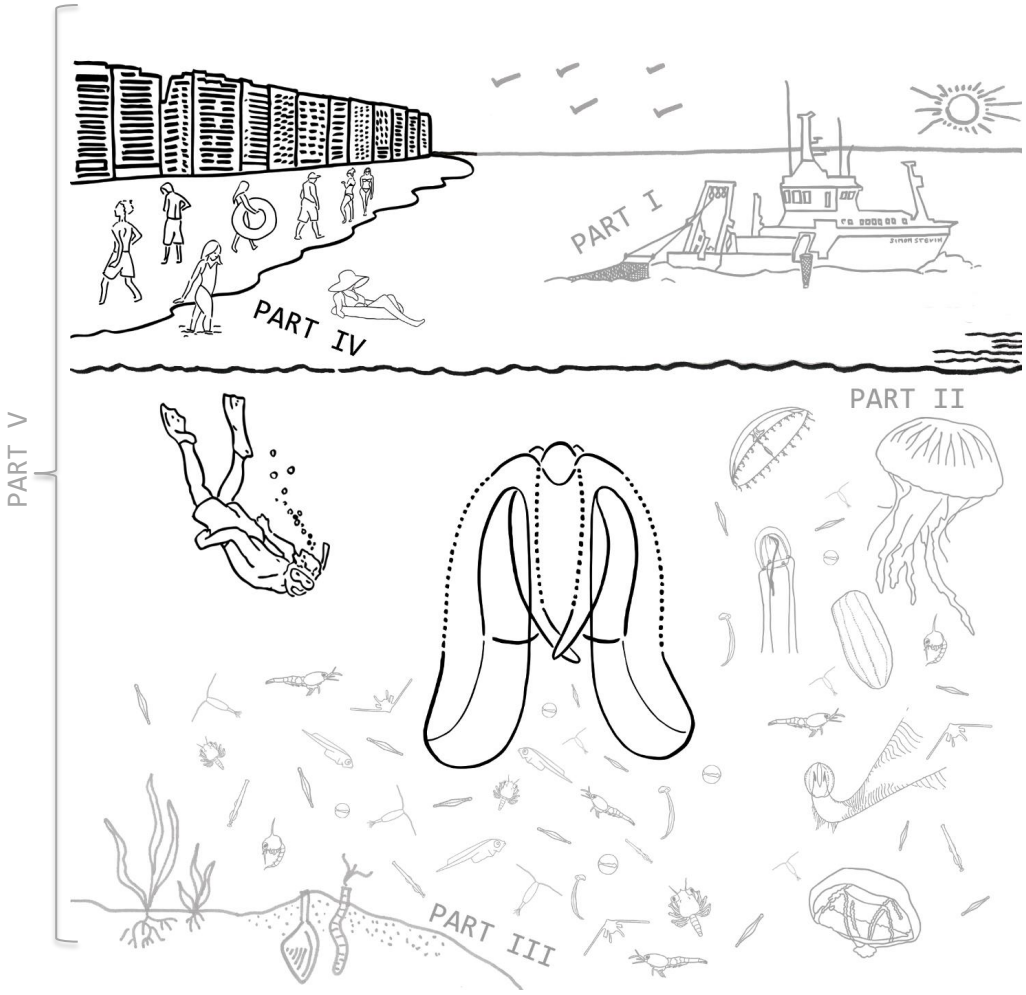
PART IV



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7

JELLYFISH, JELLYPRESS AND JELLYPERCEPTION

Modified from:

Vandendriessche, S., Vansteenbrugge, L.[3], Derweduwen, J., Maelfait, H., Hostens, K., in revision. Jellyfish, Jellypress and Jellyperception. Journal of Coastal Conservation.

ABSTRACT

Since the early 2000's, invasions and blooms of jellyfish have been increasingly reported in scientific literature as well as in the general media. Despite this increased coverage, the global jellification issue remains unsolved due to the scarcity of extended time series. The aim of this study was to determine to what extent the main messages about jellyfish (increase, causes, threats, solutions, etc.) in the Flemish (Belgian Dutch-language) media correspond with the knowledge and perception in the tourism sector at the Belgian coast. The number of articles in the Flemish media (140) increased from <5 in 2000 to 27 in 2010, half of them reporting on jellyfish from the Belgian part of the North Sea. Almost 75% of these articles reported on the causes (overfishing being mentioned as the main cause) and economic consequences of jellyfish blooms. Articles about the dramatic consequences of stinging, poisonous and non-indigenous species were also common. A questionnaire-based survey carried out at the Belgian coast in summer 2012 indicated that jelly perception is only partly driven by the general media, while personal experience seemed at least equally important as driver. Information on causes, threats, consequences and solutions for problems caused by jellyfish corresponded to a large extent with the answers of the tourist respondents. There was also agreement that all underlying causes of a potential jellification problem should be addressed and tackled at an international level. With key words like "pain", "smell" and "slime" used to describe jellyfish, they receive little sympathy from most actors, and most recreational users of beaches and coastal waters are extremely careful with any type of jellyfish, especially when children are involved. Species-specific knowledge (names, ecology, stinging vs. harmless species) provided by the media is not assimilated by most tourists or local officials, except for divers, who have a very different perception of jellyfish than most recreational users. This lack of knowledge appeared to be a key issue in perception among tourists. As public perception is a key driver in policy decisions, integrated

[3] Lies Vansteenbrugge co-authored this paper by contributing to the development of the concept and design of the study, participating in follow-up meetings during preparation and analysis, and actively assisting in the literature study and the writing of the manuscript.

coastal zone management and measures should provide good and easily understandable information, for example by distributing leaflets, putting up informative signs or demonstration aquaria with jellyfish on the beach. This will result in a better understanding and acceptance of jellyfish, as well as high-quality data from citizen science programs. Better and more information on jellyfish will thus benefit all the actors and sectors potentially affected by jellification.

7.1 Introduction

During the past decades, the number of reports on jellyfish blooms has increased in the general media (Condon *et al.*, 2012). Although jellyfish blooms are a common life cycle characteristic, mainly driven by natural fluctuations in the climate (Lynam *et al*., 2004; van Walraven *et al*., 2014), local blooms can have a substantial impact on human activities, including clogging of fishing nets and cooling water intakes in power plants, an increased number of tourists stung by jellyfish, economic losses for the tourism industry, or damage to aquaculture systems (Purcell *et al.*, 2007; Purcell, 2012; Boero, 2013). Given the sometimes dramatic consequences, the number of reports in the media increased, and 'jellification' of the oceans was suspected. Human impacts such as eutrophication, climate change, overfishing, addition of hard substrates, aquaculture and transport of non-indigenous species can be related to larger and more jellyfish blooms (Richardson *et al.*, 2009; Purcell *et al.*, 2007; Baxter *et al*, 2011; Purcell, 2012). However, historic data on jellyfish blooms are scarce, and although local increases in jellyfish have been observed (Brodeur *et al.*, 1999; Licandro *et al.*, 2010; Brotz *et al.*, 2012), a global rise of gelatinous zooplankton has been questioned by Condon *et al.* (2012). Public perception is also potentially driven by the media, and considering the importance of the public in management discissions, their perception on jellyfish should be investigated.

Studies on public perception about jellyfish have recently been carried out in Germany (Baumann, 2009; Baumann & Schernewski, 2012), France (Bonnet, 2013) and California (Kaneshiro-Pinheiro, 2013). These were based on jellyfish abundance data and questionnaire surveys. Condon *et al.* (2012) compared the number of Google News articles on gelatinous zooplankton with the number of scientific papers in Web of Science in the period 1941 – 2010. A similar analysis from four newspapers over the last 30 years was performed in Germany (Baumann, 2009). Both studies found an increased number of media reports on jellyfish. However, the influence of the general media on public perception concerning jellyfish was not addressed in any of these studies. Nonetheless, public perception is a key driver in policy decisions, including coastal zone governance and research funding. Consequently, it is useful to investigate the variability within public perception and the relationship between media and public perception in the light of policy.

In this study we focused on the public perception of jellyfish blooms and the consequences for commercial activities such as fisheries and tourism along the Belgian coast. The study

was triggered by the occurrence and the potential threat of the non-indigenous ctenophore *Mnemiopsis leidyi* in Belgian waters (Van Ginderdeuren *et al.*, 2012b). A thorough overview of the gelatinous zooplankton (jellyfish *sensu lato*) community of the Belgian part of the North Sea and the adjacent Westerschelde estuary is presented in Chapter 3. The socio-economic consequences of *M. leidyi* presence and abundance were investigated as part of the INTERREG IVa-2 Seas MEMO project.

The current study was designed to answer the following research questions: 1) What are the main messages spread by the general media (Flemish newspapers) about jellyfish? 2) What is the perception of tourists, recreational users, and the tourism industry on jellyfish and on the socio-economic threats associated with jellyfish blooms? and 3) Does the perception about jellyfish of the tourism sector correspond to the messages about jellyfish in the media? The answers to these questions were then discussed in the context of integrated coastal zone management.

7.2 Materials and Methods

7.2.1 Study area

The study was carried out on the Belgian coast. The Belgian part of the North Sea (BPNS) is situated in the southern bight of the North Sea and is characterized by an intense exploitation of its natural resources (e.g. fisheries, sand extraction, renewable energy) and a high level of disturbance (e.g. dredging, beam trawl activity, shipping, tourism) (Maes *et al.*, 2005). As such, the BPNS can be categorized as a region with high human impact, and according to Purcell (2012) as a region where jellyfish could proliferate and cause problems. Jellyfish blooms of *Chrysaora hysoscella, Aurelia aurita* or *Cyanea lamarckii* have been reported repeatedly in the general media, but the recent scientific interest in jellyfish was triggered by the observation of the non-indigenous and potentially invasive ctenophore *Mnemiopsis leidyi* along several North-European coasts (Van Ginderdeuren *et al*., 2012b; Chapter 4).

7.2.2 Media search

Using the digital press archive Mediargus (www.mediargus.be), all Flemish newspapers issued between January 2000 and September 2012 were searched for articles featuring jellyfish (Dutch key words used were "*kwal*" or "*kwallen*" in title or text). All results were entered in a database listing title, date, source, species and region (if specified), category (health, science, consequences of blooms, dramatic effects), and key words (defined by the first author and reflecting the main topics and tone of the article). Every article was scanned for mentions of causes of, threats of, and solutions to jellyfish blooms. For the analysis, only articles in which jellyfish were the key news item were retained. Mentions of jellyfish in travel reports, in satiric columns (where 'jellyfish' is mostly used as a reproach), advertising and sports items were not used.

7.2.3 Questionnaire survey

The survey targeted the tourism industry, i.e., tourists, recreational users, professionals and local officials of seaside communities. For this study, we adapted a questionnaire developed as part of the GELAMED project (Bonnet, 2013; the survey used (translated from Dutch) is provided in addendum III). In a trial survey, only few people were aware of the different taxonomic names, so the survey was generalized to the term "jellyfish", comprising the medusa phase of the phylum Cnidaria (classes Hydrozoa and Scyphozoa) and the phylum Ctenophora. The questionnaire is based on multiple-choice and closed questions, and can be divided in (1) personal information (gender, age, relation to the coast), (2) personal perception on jellyfish (experiences, emotions, observations), and (3) personal opinion on the importance of increasing numbers of jellyfish; causes; consequences and policy measures.

The questionnaire is split into a general part for all participants as well as specific parts for tourists and recreational users, professionals from the tourism industry, and local officials. These parts were analysed separately and considered as specifications about either tourist or professional activities (timing, type), impact and perceived threats of jellyfish abundance on these activities, or possible policy measures. Stories, personal experiences and remarks from respondents were listed and used while interpreting the results. The survey was performed during the summer of 2012. Questionnaires were distributed both physically (field survey at the beach and on the dike of Ostend) and digitally (e-mail survey). At the time of the field survey, jellyfish (*Chrysaora hysoscella*) were present in the water and on the beach. Their abundance was moderate, i.e., tourists and recreational users never saw more than five individuals in the water or on the beach on the day of the survey.

7.3 Results

7.3.1 Jelly press

The search for jellyfish related key words in the Mediargus database delivered 140 articles that could be used in our analyses. Since 2000, the total number of articles increased steadily up to a peak in 2010 (Figure 7.1). About 25% of the articles addressed local jellyfish news from the Belgian Part of the North Sea (BPNS). These local articles showed two peaks in 2005 and 2012, but the numbers had actually been increasing since 2007.

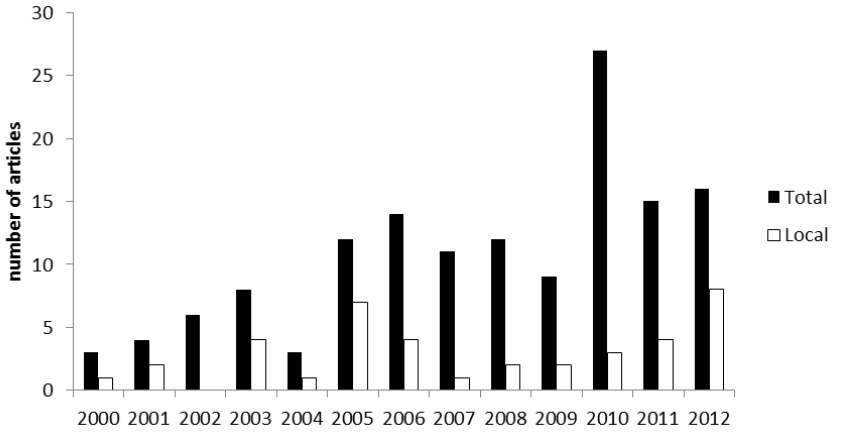


Figure 7.1 Evolution of the number of articles featuring jellyfish in the Flemish general media since 2000 (total = news from all over the world; local = news only related to the Belgian Part of the North Sea)

In about half of the articles, the jellyfish species was specified. The representation of species differed substantially over the years. The North Sea jellyfish species were mentioned in many articles, including *Aurelia aurita, Chrysaora hysoscella, Cyanea lamarckii, Cyanea capillata* and *Rhizostoma octopus*. One article on *M. leidyi* first appeared in 2000, reporting on its deleterious effects in the Caspian Sea. The presence of this species in the North Sea was first reported by the press in 2010 and received further attention in the following years. Reports on other species mostly originated from the Atlantic (UK and USA), the Mediterranean (especially Spain, a popular holiday destination for Belgian tourists), and from the Pacific (especially Australia and Japan).

Most articles reported on the occurrence and consequences of jellyfish blooms (71% of all articles, with 87% of the articles concerning the BPNS). Scientific findings (e.g., "Scientists make jellyfish from a rat") accounted for 14%, and health related topics (e.g., cures for stings) for 4% of all articles (corresponding with 10 and 3% of the BPNS articles, respectively). Reports on dramatic encounters with jellyfish accounted for 12% of all articles, but not a single dramatic article was published for the BPNS. The headlines of these drama articles were usually quite spectacular and alarmist (e.g., "*Jellyfish kills woman in Sardinia*"), and predominantly reported on encounters with *Carukia barnesi* and *Chironex fleckeri* in Australia or with *Physalia physalis* in southern Europe.

The top 30 key words reflect the general messages of the press releases (Table 7.1). They mostly refer to (1) causes and economic consequences of jellyfish blooms and (2) personal risks involved with (poisonous) jellyfish encounters. When only considering articles featuring *Mnemiopsis leidyi*, the key words reflect the ecological and economic threats posed by this non-native species.

Table 7.1 Top 30 key words, all referring to either jellyfish blooms or personal risks. Words in italics represent the most recorded key words; words in bold typeface represent the following top four key words in both categories.

jellyfish blooms	personal risks
overfishing	*washed ashore*
infestation	**tourist**
warmth	**swim**
global warming	**beach**
wind	**poison**
fisheries	children
food	dead
pollution	innocent
temperature	lethal
catch	sea water
climate change	summer
nets	small
plankton	sting
study	pain
	tentacles
	allergic

Specific causes of jellyfish blooms were mentioned in 47% of the articles. Natural causes such as weather, currents or population dynamics were most important, followed by 'lack of natural predators due to overfishing' and 'global warming' (Figure 7.2a). Specific threats and impacts were mentioned in 41% of the articles, with the most important being impacts on fisheries and aquaculture and the consequences for tourism (Figure 7.2b). Solutions were mentioned in only 14% of the articles: removing jellyfish (in some cases for consumption), installing fences in swimming zones and (re)introducing predators such as turtles were the most common (Figure 7.2c). The relative importance of causes, threats and solutions were not mentioned in any of the articles.

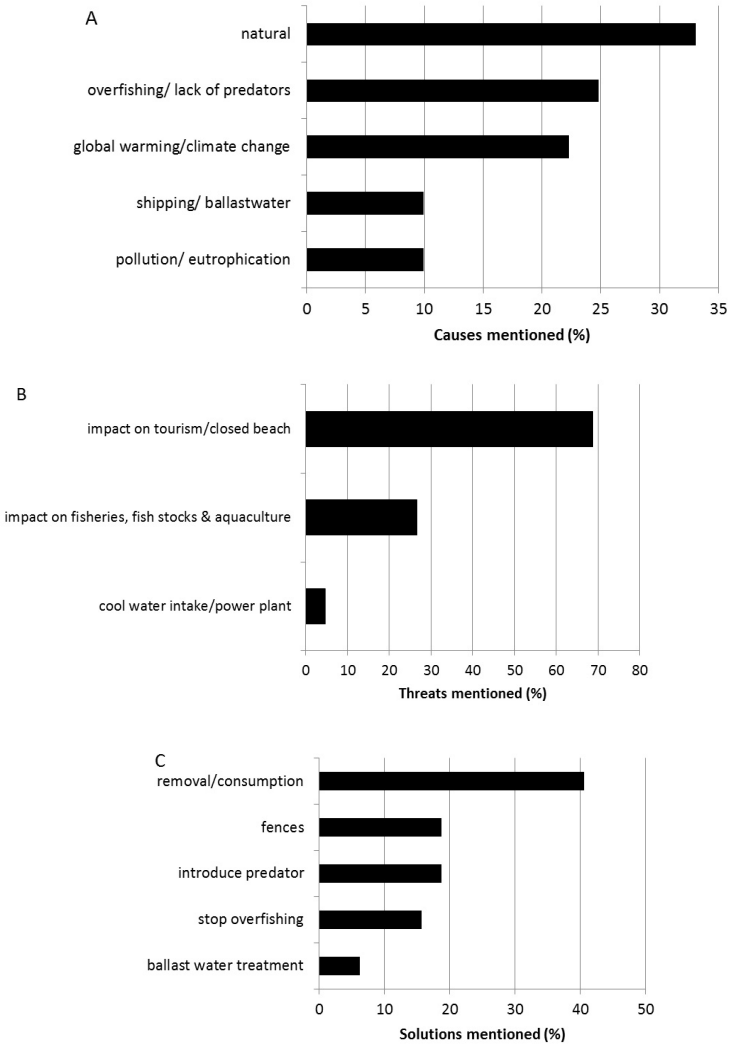


Figure 7.2 The relative importance of causes (A), impacts (B) and solutions (C) for jellyfish blooms, as derived from 140 articles in the Dutch-language Belgian public media between 2000-2010.

7.3.2 Jelly perception

A total of 69 questionnaires was completed for the tourism industry survey performed in the summer of 2012. The gender and age distribution among participants was balanced in terms of age and gender. The respondents were 53% men and 47% women; 13% were 18 - 29 years old, 24% were 30-39, 25% were 40-49, 19% were 50-59, and 19% were over 60 years old. Most respondents (65%) visited the Belgian coast year-round for recreational purposes, while 20% visited the coast only during the summer months. Commercial activities, such as running a surfing club or a bar, continued year-round. Main recreational activities included

running and walking (64% of respondents), swimming (32%), diving (41%), sunbathing (18%), followed by sailing, surfing, shopping, eating, fishing, sleeping, etc. Since the responses by divers were abundant (32) compared to all other responses from the tourism industry (37), both groups were treated separately in the analyses.

When asking respondents which five words they associate with jellyfish, the results are quite different for the divers versus the other recreational users. Divers focus on anatomic characteristics (including beauty), distinguish among species, and mention the need for caution (Figure 7.3). Other recreational users do not distinguish between species and are almost completely focused on negative aspects such as stings, the smell, the feeling when stepping on a jellyfish, etc. Key words related to economic and ecological consequences of blooms were virtually absent from the list.

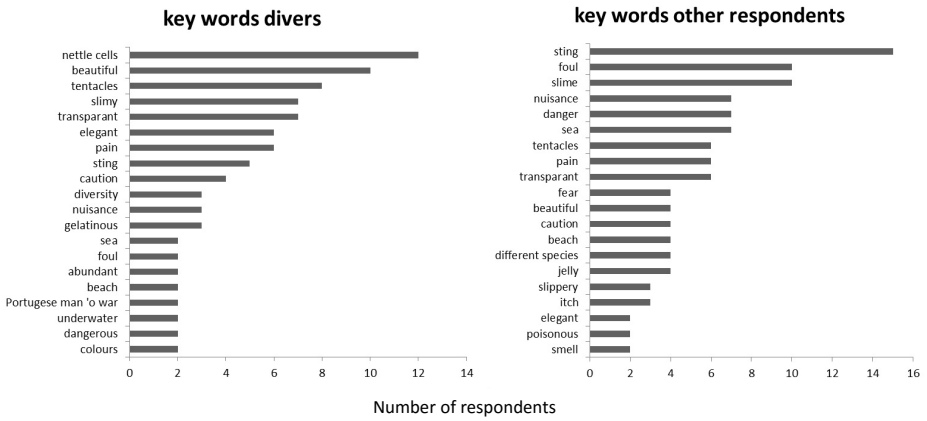


Figure 7.3 Key words associated with jellyfish, as derived from the questionnaire survey completed by divers (left) and other tourism respondents (right) during summer 2012.

All of the divers and 86% of the other respondents had seen jellyfish during the last five years, although the latter admitted they pay little attention to the presence of jellyfish. Seventeen percent of the respondent divers had the impression that the number of jellyfish had increased, while this was only 5% for the other respondents. One beach tourist remarked that he might have the wrong impression because the beaches are regularly cleaned. When persons who perceived an increase were asked whether the number had increased with factor 2, 5, 10 or 100, most admitted to having no idea or nuanced the question based on interspecific or seasonal differences. Two divers specifically mentioned an observed increase of *M. leidyi*.

Using a number of questions, respondents were asked to establish their frame of mind during a (hypothetical) encounter with a number of jellyfish. Most people stay calm and confident, some get nervous and tense or even downright scared, but caution is the dominant emotion (Figure 7.4). Several respondents stated that no risks are taken when

children are involved, especially as most of the tourists cannot tell the difference between stinging and harmless species. In contrast, divers seemed more at ease during a jellyfish encounter, as they usually have a good species knowledge.

Global change and overfishing were indicated most often as causes of the ocean jellification process (35% and 29% of the respondents, respectively). Ballast water transport and life cycle characteristics of jellyfish were both mentioned by 15% of the respondents (Figure 7.5). As for personal involvement, most people were concerned about local and global jellyfish increases, but they also felt they could personally do little about it. Potential jellyfish increases were considered to be a major issue. One respondent made the comparison with toxic algal blooms. Most respondents expressed the understanding that all processes are linked and that multiple interacting factors are at the base of local and global jellyfish increases. Sixty-five percent of all respondents felt that they did not know enough about recent changes in jellyfish abundance, and indicated they would like to receive more information.

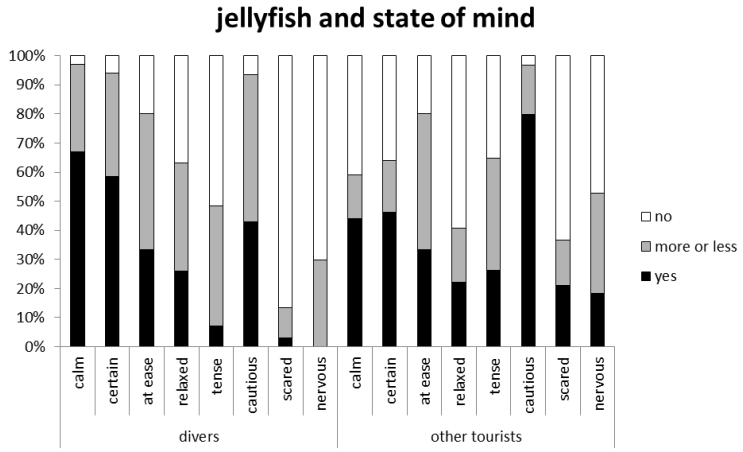


Figure 7.4 Variations in the state of mind of divers and other respondents from the tourism industry during a (hypothetical) jellyfish encounter

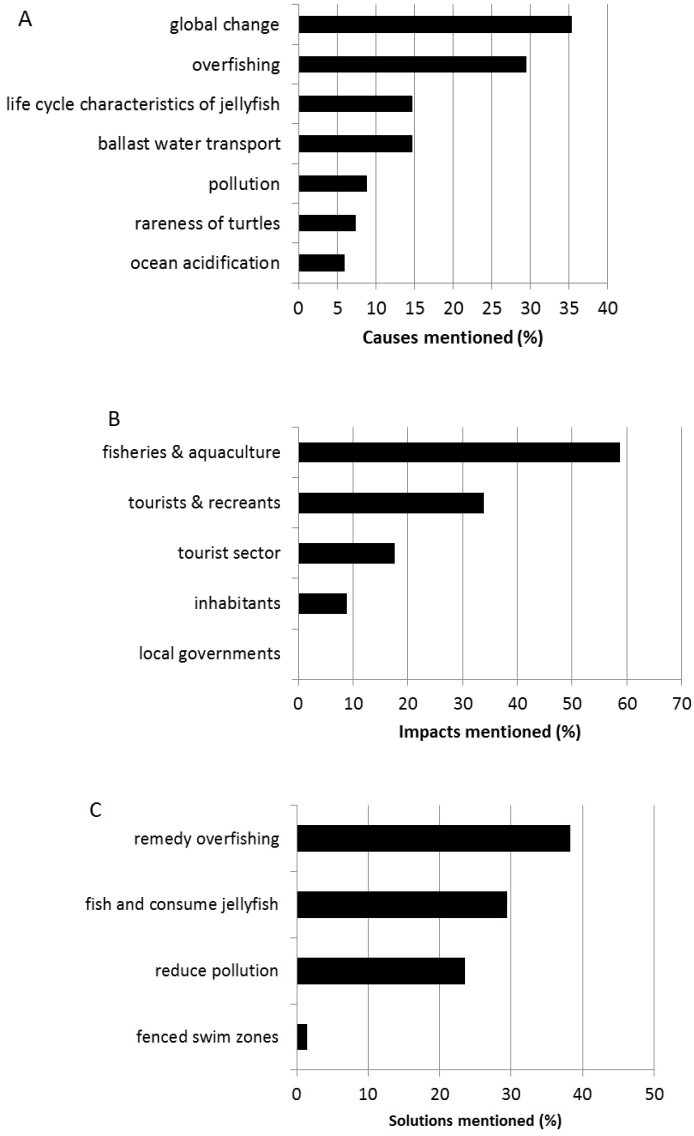


Figure 7.5 Causes (A) of, sectors affected by (B) and possible solutions and measures for (C) jellification, as perceived by 69 tourist respondents during a questionnaire survey in summer 2012

Tourists and recreational users were asked a specific question to find out which industry they thought would be most affected by increased numbers of jellyfish. Most respondents (58%) chose fishermen and aquaculture farmers, 34% chose tourists and recreational users, 18% chose the tourism industry (restaurants, campground owners, etc.), and 9% chose local inhabitants (Figure 7.5). None of the respondents checked the local governments.

Two respondents remarked that tourists will experience little effect because they will simply go elsewhere. However, this opinion was not confirmed by the other survey results: only 27% of the tourists and recreational users (including divers) would choose another destination if jellyfish were abundant (>10 visible on the beach or in the water), although 17% stated they would change their activity (e.g., give up swimming). If the jellyfish are harmless and these people would be informed of that, then half of this 17% would change their mind. For others, only seeing jellyfish would be enough to change their activity. The risk of jellyfish stings was the most important factor for tourists, recreational users and divers when making this decision. Most respondents thought that increased jellyfish densities will result in a substantial increase of stings. Tourists estimated the risk of a beach closure to be low, although local officials indicated beach closures as a potential consequence of jellyfish blooms. Respondents performing commercial activities at the coast were most concerned about an increase in jellyfish stings and decreased catches of commercial fish, factors that might negatively impact the returns from tourism and fisheries and the reputation of the coastal region. Local officials gave similar answers and confirmed these concerns, but also added the concern of increased prevention costs. According to 89% of the respondents, all costs resulting from jellyfish blooms (jellyfish fences, clean-up, damage, etc.) should be paid for by society through taxes. A minority thought the tourism industry (6%) or the fisheries industry (13%) should carry the costs.

Most respondents (52%) felt that any potential remedy for jellyfish increases should aim for long-term results and should deal with the underlying causes, such as overfishing and pollution (Figure 7.5. Still, 29% of the respondents thought it would be a good idea to start fishing for jellyfish or processing them into food, medicine and cosmetics. Only 1% saw fences around swimming zones as a solution. Local officials indicated that initiatives for preventive and mitigating measures should be taken on both national and global levels.

7.4 Discussion

7.4.1 Jelly perception in the tourism industry

The questionnaire survey on public perception should be seen as a small-scale local research for the Belgian coast. The number of respondents was therefore relatively small compared to the surveys carried out in Germany (Baumann & Schernewski, 2012), California (Kaneshiro–Pinheiro, 2013) or France (Bonnet, 2013). However, the main results are quite similar. In agreement with the GELAMED survey (France), jellyfish mainly raised a negative image. Beach tourists in particular harbor little warmth for these 'jelly' creatures. In contrast, our results showed that the perception of recreational divers is quite different from other recreational users and is more positive in general. This indicates a substantial variation in perception amongst recreational users. Although yacht sailors, recreational anglers, surfers and divers are vastly outnumbered by beach tourists *sensu stricto*, it is worthwhile to include all recreant groups in surveys like the one presented here to get a more representative view

of public perception. Because we performed our survey only in one city (Ostend), we were not able to investigate regional differences (coastal cities or regions). It might be worthwhile to include such results in future research. The same holds for information on professional status or social differences between recreational users (Bonnet, 2013).

Another result that agrees with the surveys from other countries is the request for information about jellyfish species, their ecology and the problems they can cause. Most tourists (except for divers) could not tell the difference between jellyfish species, nor between stinging and harmless jellyfish types. So most tourists remain cautious with all jellyfish *sensu lato*, although a number of tourists would not relocate in case of high jellyfish abundances if they were informed that the jellyfish were harmless. Additionally, the majority of respondents felt as they knew too little about recent changes in jellyfish presence to answer questions on causes, threats or solutions, and wanted to receive more information on this matter. Baumann and Schernewski (2012) showed that information provision is an effective way to increase the beach users' acceptance of jellyfish. Therefore, beach management measures coping with high jellyfish abundances should include different communication tools for the broad public and for beach users in particular. Such tools may include warning flags, leaflets, informative signs and forecasts, but also demonstration aquaria with jellyfish, as they are more beautiful in the water than on the beach.

7.4.2 Jelly press versus jelly perception

The second aim of this study was to identify the influence of the press on public jellyfish perception. Our results indicate that the perception in the tourist sector is only partly driven by the press regarding jellyfish. Personal experience was at least an equally important driver. This study confirmed the results of Gershwin (2013), who found that jellyfish problems are related to stings for most people, and that tourists experience the public health aspects of jellyfish blooms as stressful. Especially our analysis concerning the state of mind during a jellyfish encounter strengthens this statement: key words given by tourists almost completely focused on negative aspects such as stings and smell, while key words related to economic and ecological consequences of jellyfish blooms were nearly absent from the list. In contrast, consequences of blooms were the main topic in 71% of the public press articles. Articles describing dramatic encounters with jellyfish (none of which happened on the Belgian coast) made up 12% of all jellyfish related publications in the general media. Condon *et al.* (2012) stated that the general media probably raises the general apprehension towards jellyfish by publishing such dramatic stories. The German survey results of Baumann & Schernewski (2012) showed that the public was well-aware of the lack of life-threatening jellyfish on their coast. This is probably also the case for tourists on the Belgian coast.

Another difference between press and public is the perception that the number of jellyfish has increased (Table 7.2). Most articles in the Flemish (Dutch-speaking), but also in the French general media (Bonnet, 2013) indicated a regional or global jellyfish increase and

presented the “rise of slime” as a real fact. According to our survey results, only 10% of the respondents perceived an increase in jellyfish, thereby relying on their personal observations, or they indicated that they didn’t pay much attention to changing number of jellyfish. Similarly, Bonnet (2013) found that the majority of respondents did not perceive an increase in the number of jellyfish.

The question of jellyfish species recognition clearly emerged in the press vs. tourist perception comparison. While 50% of the general media articles specified one or more jellyfish species, differences between species were unknown to most respondents, and especially to beach tourists (Table 7.2). This means that species-specific information provided by the media is not assimilated in the general knowledge about jellyfish. On the other hand, information on causes, threats, consequences and solutions for jellyfish problems given in the Flemish media agrees with the answers extracted from the questionnaire survey. In this aspect, the public knowledge seems to be influenced by the media, which seems logical, as such information can hardly be derived from personal experience, while the general media is used as the main source of information by the majority of the public.

Table 7.2 Main results of the comparison between public press information and public perception concerning jellyfish (≈: similar; ↔: different)

	Jelly press	versus	**Jelly perception**
species	species specific in about 50% of articles	↔	general, except for results of divers
top 10 key words	washed ashore, overfishing, poison, beach, wind, global warming, warmth, swim, infestation, tourist	↔	sting, icky, slime, nuisance, danger, sea, tentacles, pain, transparent, fear
perception of jellification	increase	↔	only 10% of respondents perceive increase
most important causes	Natural causes + global change & overfishing	≈	global change & overfishing
most important threats	fisheries & tourism	≈	fisheries & tourism
best solutions	jellyfish removal and consumption	≈	stop overfishing and pollution, jellyfish fishing

7.4.3 Relevance to integrated coastal zone management and research

The cumulative impact of multiple human activities causing jellyfish blooms is likely to require a multifaceted integrated management response (Purcell, 2012; Richardson *et al.*, 2009). This should be based on quantitative data on the public perception of jellyfish and the influence of jellyfish on our society (Kaneshiro-Pinheiro, 2013). In Europe, such management is framed within the process of Integrated Coastal Zone Management (ICZM), which is based on eight principles (2002/413/EC). Especially the principle about "the involvement of all parties concerned" is important in the context of jellyfish presence and jelly perception. We noted a large variation in perception among surveyed groups concerning jellyfish presence, and in the reactions and emotions evoked by the term jellyfish. Therefore, it is important to extend future perception surveys to all groups directly and indirectly affected by jellyfish. In doing so, opportunities for cooperation between scientists, policymakers and public parties may emerge. So-called "citizen science" has been described as an alternative method to evaluate the presence and abundance of gelatinous zooplankton (Boero, 2013). Good results of citizen science are given for the Mediterranean in www.jellywatch.org and in "Spot the jellyfish" at www.ioikids.net. Similarly, the jellyrisk.eu program resulted in the discovery of a new jellyfish species by Italian fishermen (Piraino *et al*., 2014).

Our study indicates that the public relies on personal observations and experiences with jellyfish. Such observations may be used as a monitoring tool or alert system for jellyfish along the Belgian coast, e.g., through a smartphone app for people who swim (see Gershwin, 2013). However, for retrospective analyses the public memory has proven not to be very useful, since people's memories are influenced by their current perception. Baumann and Schernewski (2012) found that the answer to the question 'have jellyfish increased during the last five years?' was highly influenced by the number of jellyfish in the water at the time of the interview. Of course, science based on citizen perception and knowledge can only produce useful data when participants have at least a basic knowledge on jellyfish ecology and differences between species. This is yet another reason why the provision of species-specific information should be one of the first management actions concerning increasing numbers of jellyfish. This can be achieved by distributing leaflets and putting up informative signs or demonstration aquaria with jellyfish on the beach. This would likely result in a higher acceptance of jellyfish (Baumann & Schernewski, 2012), better communication between scientists and the public (Bonnet, 2013) and better quality of data resulting from citizen science programs (Boero, 2013).

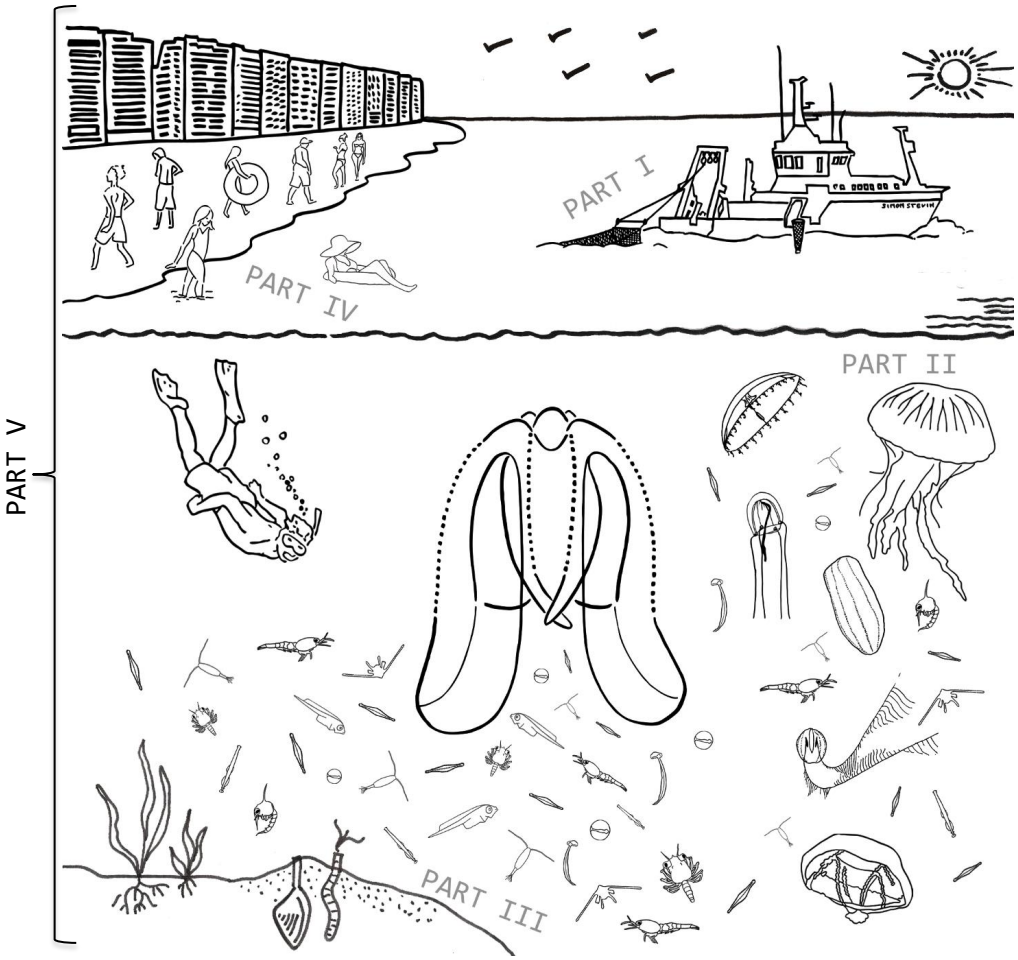
Communication about jellyfish is a coping strategy that can be organized on a local or regional level. However, such communication measures do not resolve the jellification issue itself. The Belgian part of the North Sea is increasingly and intensively used for many human activities. Since jellyfish seem to benefit from human activities and environmental perturbations (Brotz, 2011; Purcell, 2012), jellyfish blooms of local and non-indigenous

species are likely to occur more frequently in the future. Therefore, ICZM should address all underlying causes and the whole process leading to these blooms, as indicated by the survey results. Mitigating and prevention strategies, such as the removal of jellyfish in coastal waters (short-term) or the reduction of eutrophicated waters (long-term), should be included in any jellyfish management plan. Because the jellification problem crosses national boundaries, it should be addressed on an international level.

7.5 Acknowledgements

This study was executed within the framework of the Interreg IVa 2 Seas project MEMO (*Mnemiopsis* ecology and modeling: Observation of an invasive comb jelly in the North Sea). The authors sincerely thank all survey respondents for their contribution to this study, and Miriam Levenson, Karl Van Ginderdeuren and reviewers for their comments and suggestions.

PART V



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8

GENERAL DISCUSSION

8.1 Changing oceans

Since the Holocene, human proliferations have impacted ecosystems – including the oceans – around the world (Purcell, 2012). Especially coastal ecosystems moan under anthropogenic pressure as more than one third of the world's human population live in coastal areas that make up just over 4% of Earth's total land area (UNEP, 2006). Although scientific evidence is abundant (e.g. overfishing: Pauly *et al.,* 1998; Jackson *et al.,* 2001; introduction of non-indigenous species: Knowler, 2005; Figure 8.1), not all of the impacts are immediately perceptible by the broader public. Eutrophication and climate change for example, are most certainly altering the ecosystems in their function, structure and services, but for now only indirectly affect human populations (Arai, 2001; Daskalov *et al.,* 2007; Diaz and Rosenberg, 2008; Hoegh-Guldberg and Bruno, 2010). Jellyfish abundances may increase in altered ecosystems, because these organisms can directly or indirectly benefit from the conditions created by anthropogenic stressors (Uye, 2008; Richardson *et al.,* 2009). However, as blooms form a normal part of the jellyfish life cycle, it is difficult to determine whether an increase in jellyfish abundance is the result of natural variation related to environmental fluctuations or whether it is a true response to changing ecosystems, especially since long-term jellyfish datasets are scarce in many regions (Mills, 2001; Purcell, 2012). Nevertheless, several studies reported an increasing number of problems with jellyfish (Purcell *et al.,* 2007; Purcell, 2012). Once a certain threshold of disturbance is reached, a regime shift towards jellyfish-dominated ecosystems may occur, a process also known as 'jellification' (Uye and Ueta, 2004; Daskalov *et al.,* 2007; Richardson *et al.,* 2009; Brotz, 2011).

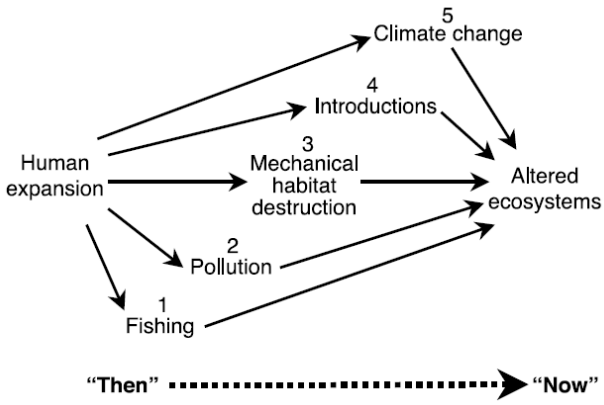


Figure 8.1 Historical sequence of human disturbances affecting coastal ecosystems; steps 2-5 may vary in order (from Jackson *et al.,* 2001)

The observation of such regime shifts resulted in worldwide concern (e.g. Shiganova *et al.,* 2001; Uye, 2008). Also in northern Europe, and more specifically in the southern North Sea, the ecosystem is subject to many anthropogenic pressures: overfishing (O'Brien *et al.,* 2000), pollution (De Witte *et al.,* 2014), physical disturbance (De Backer *et al.,* 2014), introductions of non-indigenous species (Kerckhof *et al.,* 2007) and climate change (Reid *et al.,* 2003; Richardson, 2008) (Figure 8.1). In light of the jellification paradigm and the observation of the non-indigenous ctenophore *Mnemiopsis leidyi* in Belgian waters since 2007 (Dumoulin, 2007; Van Ginderdeuren *et al.,* 2012b), this PhD study aimed to assess the structural and functional role of the non-indigneous ctenphore *Mnemiopsis leidyi* in the southern North Sea. More specifically, we investigated the current distirubtion of *M. leidyi* in Belgian marine waters, the adjacent ports and the Westerschelde estuary, related to other gelatinous zooplankton. Problems concerning sampling and preservation were encountered and recommendations for best practice were made. Furthermore, the trophic ecology and interactions of *M. leidyi* in the planktonic food web were examined through field samples and grazing experiments by means of biochemical markers, and the socio-economic effects of jellyfish at the Belgian coast were determined.

8.2 Risk assessment of *M. leidyi*

Considering the notorious reputation of *M. leidyi* in the Black Sea ecosystem (Kideys, 2002; Knowler, 2005), the question remains whether this non-indigenous ctenophore has an impact on the environment and the human activities in the southern North Sea ecosystem. Therefore, an objective risk assessment was executed using the Harmonia+ protocol (D'hondt *et al.,* 2015; Text box 4) based on all information gathered in this PhD thesis. For this risk assessment, we focused on the Belgian part of the North Sea (BPNS) including the coastal and offshore areas, the coastal ports along the Belgian coast, and the Westerschelde

estuary. However, this evaluation also takes into account some results from other partners within the MEMO project, and is as such representative for the broader southern North Sea.

Text box 4: The Harmonia+ protocol

Damage and control of invasive species entails considerable costs, for example 1.3 billion euro per year in The Netherlands (van der Weijden et al., *2007 as referred to in D'hondt* et al., *2015). Therefore, it is helpful to have tools available that allow to condense information on a certain species into its perceived risks according to a common framework. Bearing this in mind, the Harmonia+ protocol was constructed to allow a rapid screening of the risks of a potentially invasive alien species and to prioritise these risks accordingly.*

The primary focus of Harmonia+ is risk screening of potentially invasive plants and animals on land. Consequently, some adjustments to the interpretation of certain questions had to be made in order to meet the requirements for evaluation of marine non-indigenous species, such as M. leidyi*. For example, 'the impacts on cultivated plants and domesticated animals' were interpreted as 'the impact on aquaculture of algae and (shell)fish', respectively. The Harmonia+ protocol is freely available, easy to use, and can be accessed via ias.biodiversity.be/harmoniaplus.*

In the first part of this chapter, we answer the questions of the Harmonia+ protocol (overview of the questions is provided in Addendum IV). Eventually, the protocol converts these answers into scores: exposure and impact scores. This allows an objective assessment of the risk that *M. leidyi* poses to the ecosystem. In the second part of this chapter, recommendations towards policy makers and management options are discussed on how to deal with the established *M. leidyi* population in our waters. Finally, remaining challenges for future research are proposed.

8.3 The Harmonia+ protocol applied to *M. leidyi*

8.3.1 Exposure risk of *M. leidyi* in the southern North Sea

In order to determine the exposure risk, we need to evaluate the introduction, establishment and spread of *M. leidyi*. To assess the introduction and spread, both natural pathways and (un)intentional human actions are discussed. For the establishment of *M. leidyi*, climate and habitat suitability of the southern North Sea were evaluated (Addendum IV).

Introduction and spread of M. leidyi

While *intentional* human actions are unlikely to have enhanced the introduction of *M. leidyi* (low probability; no commercial value, not common for aquaria purposes), several studies suggest that *unintentional* human actions formed the basis of its introduction and spread (Vinogradov *et al.,* 1989; Shiganova *et al.,* 2001; Costello *et al.,* 2012). As a planktonic non-indigenous species, *M. leidyi* (in the form of eggs, larvae or adults) was most likely

transported by means of ballast water from its native distribution area across the Atlantic to Eurasia (Chapter 1). Genetic evidence showed that at least two separate introduction events have occurred (Reusch *et al.,* 2010; Ghabooli *et al.,* 2011). Considering the large amounts of ballast water transported across the world's oceans on a daily basis (Globallast, 2015), this human action can result in regular re-introductions from the native distribution area as well as from already invaded areas (secondary introduction and spread). The proximity of large international ports, the fact that *M. leidyi* was first observed in the port of Zeebrugge in Belgium (Van Ginderdeuren *et al.,* 2012b) and the intense shipping traffic in the Westerschelde estuary (Faasse and Bayha, 2006) all support the hypothesis that *M. leidyi* has been introduced into the southern North Sea through ballast water.

Introduction of *M. leidyi* from its native area to Eurasia through *natural means* (e.g. currents) is rarely discussed in the available literature and is regarded as highly improbable. An exception is the study by Oliveira (2007), who explored this pathway with proof from divers' observations at several oceanic monitoring stations 500 km off the US coastline (Harbison *et al.,* 1978). Theoretically, *M. leidyi* can be transported with the superficial waters of the North Atlantic Current to the UK coast and the North Sea (Hughes and Holliday, 2006 as referred in Oliveira, 2007). However, as a predominantly coastal species, *M. leidyi* needs a high prey availability (reviewed in Costello *et al.,* 2012). When starved for a longer period of time, shrinkage occurs due to the lack of substantial energy reserves and the predominance of proteins in their organic composition (Anninsky *et al.,* 2005; Chapters 5 and 6). Consequently, oligotrophic oceanic waters (<3 µg $C.L^{-1}$) such as the Atlantic ocean form a natural geographic barrier for *M. leidyi* (Kremer, 1994).

As a planktonic organism, *M. leidyi* may also be introduced or spread by *local* currents from already invaded 'source' areas (Costello *et al.,* 2012). More specifically in the southern North Sea, the coastal area, which is regarded as a 'sink' area, can be (re-)colonised when population outbreaks occur in the ports of Oostende (sluice dock), Zeebrugge and the Westerschelde estuary (Chapter4). Furthermore, using models, van der Molen *et al.* (2015) showed that *M. leidyi* can survive throughout the North Sea and can be transported by dominant currents over considerable distances. This facilitates the connectivity between metapopulations (e.g. within the southern Dutch estuaries) and the spread to new areas. For example, in Chapter 4 we showed that *M. leidyi* can be found in the vicinity of the Thorntonbank, up to 30 km offshore from the Belgian coast.

Genetic analyses of the western Mediterranean *M. leidyi* population indicated that both ballast water and current-driven transport, resulting in introduction and spread, work simultaneously, as alleles from both the Black Sea and the Gulf of Mexico were identified (Ghabooli *et al.,* 2013). A similar analysis still needs to be performed for the southern North Sea to expose the pathways responsible for the presence of *M. leidyi* in these waters.

Establishment of M. leidyi

Once introduced into an area, the climate and habitat conditions need to be suitable in order to establish a viable and reproductive population. The *climate* is defined as the 'prevailing weather conditions of the area'. For the assessment, the effect of temperature, air pressure (North Atlantic Oscillation, NAO) and wind conditions were considered. Similar to other invasive species, *M. leidyi* is known for its tolerance to a broad temperature range (0-32 °C; Purcell *et al.,* 2001; Costello *et al.,* 2006; Haraldsson *et al.,* 2013). Its native distribution area also covers a large latitudinal gradient, from temperate to sub-tropical areas (Costello *et al.,* 2012). Nevertheless, temperature plays an important role in winter survival and reproduction of *M. leidyi* (Chapter 1). Several studies have suggested that colder winters can cause *M. leidyi* populations to disappear or to develop smaller blooms (e.g. Sea of Azov (Purcell *et al.,* 2001) and Black Sea (Purcell, 2005)). Similarly, warmer water temperatures (related to positive NAO indices) in Narragansett Bay (north-eastern US) resulted in a longer period of occurrence and increased *M. leidyi* abundances (Purcell, 2005). In Chapter 4, we showed that *M. leidyi* occurred between August and December in Belgian waters (including several ports) and the Westerschelde estuary. However, in 2014, high *M. leidyi* densities were already observed in June in the sluice dock in the port of Oostende (personal observation). Undoubtedly, the exceptionally warm winter of 2013-2014 in Belgium (the 2nd warmest since the measurements; KMI, 2015) contributed to this earlier appearance. Furthermore, as reproduction of *M. leidyi* is favoured when temperatures are higher than 12 °C, a warming climate may lead to larger outbreaks (Purcell *et al.,* 2001; Purcell, 2005; van der Molen *et al.,* 2015).

Other factors, such as low wave height, often as a result of calmer wind conditions, may also contribute to the population dynamics and to higher *M. leidyi* densities (Chapter 4). This corroborated the findings of Mianzan *et al.* (2010), who suggested that turbulent conditions interfere with the feeding current created by *M. leidyi*, resulting in a downward migration to the sea bottom. The latter makes it less likely that *M. leidyi* will be caught in traditional plankton nets (Chapter 2). Overall, climate conditions in the southern North Sea are similar to those of the temperate native area of *M. leidyi*, and therefore can be considered as suitable for the establishment of *M. leidyi* populations.

The *habitat* is defined as 'the place where a species occurs and where abiotic and biotic factors meet the requirements for survival, growth and reproduction'. Several studies have shown that *M. leidyi* occurs in estuaries and coastal waters where salinity ranges between 0 and 40 (Mutlu, 1999; Purcell *et al.,* 2001; Shiganova *et al.,* 2001; Fuentes *et al.,* 2010), where oxygen levels are larger than 1 mg.L^{-1} (Decker *et al.,* 2004), and where sufficient food allows rapid population growth (> 24 µg C.L^{-1}; Kremer and Reeve, 1989). All these factors contribute to the invasive success of *M. leidyi* in areas with a favourable climate (Purcell *et al.,* 2001). Costello *et al.* (2012) demonstrated that within a 'favourable' habitat, source-sink dynamics

are present, suggesting that some parts of the habitat are sub-optimal, for example for winter survival. Also in the southern North Sea, where environmental conditions are optimal for the establishment of *M. leidyi* populations (Daro *et al.,* 2006; Van Ginderdeuren *et al.,* 2014), these source-sink dynamics were observed (Chapters 3 and 4). Source areas (coastal embayments and estuaries) are characterised by low advection and low water exchange and form an optimal habitat for survival throughout the year compared to sink areas (coastal waters), which have shorter retention times causing the ctenophores to flush out of the habitat (Costello *et al.,* 2006). Since the study of Van Ginderdeuren *et al.* (2012b), winter survival of *M. leidyi* was not observed in the southern North Sea (Chapter 4, no observations before August). However, recent observations may prove that *M. leidyi* is present in the area year-round, as ILVO-divers detected large *M. leidyi* individuals (± 90 mm oral-aboral length) in the port of Oostende (sluice dock) in April 2015. These few winter survivors may found the next summer-autumn population outbreaks, and could imply that the *M. leidyi* population is 'established'. Appropriate management actions are therefore recommended.

8.3.2 Impact risk of *M. leidyi* in the southern North Sea

Besides the exposure risk, the impact on and the consequences of *M. leidyi* for the environment (including native organisms, habitats and ecosystems), and for human activities (such as tourism, fisheries, aquaculture and coastal industries) in the southern North Sea are evaluated using the Harmonia+ protocol (impact risk; Addendum IV).

Impact on environment

Considering its broad diet, high feeding rates and fast carbon assimilation (Chapters 5 and 6), the effect of *M. leidyi* on native species through predation and competition is thought to be substantial (Mutlu, 1999; Granhag *et al.,* 2011; Costello *et al.,* 2012). As a non-indigenous species, *M. leidyi* arrived in a diverse gelatinous zooplankton community in the southern North Sea ecosystem (33 gelatinous zooplankton taxa (Chapter 3), compared for example by the 11 species recorded in the Black Sea (Kovalev and Piontkovski, 1998)). All gelatinous taxa display variation in their abundances in both space and time, but highest densities were observed in the coastal locations in autumn. Some of the co-occurring species feed on the same prey (and can be considered as competitors), while others have been identified as direct predator of *M. leidyi* (Hamer *et al.,* 2011; Hosia *et al.,* 2010). In Chapter 5, isotopic niches were investigated for *M. leidyi* and the co-occurring ctenophores *Beroe* sp. and *P. pileus* and resource differentiation was observed. The indigenous *Beroe* sp. is known to feed on gelatinous zooplankton and especially on the ctenophore *Pleurobrachia pileus* (Greve, 1975), which was confirmed in Chapter 3 through density observations and in Chapter 5 by stable isotopes for the month July (BPNS). Predation by *Beroe* sp. on *M. leidyi* was described by Hosia *et al.* (2010), but was not not fully supported by our data (Chapter 5). *Beroe* sp. probably only fed on the smallest ctenophores (and hydromedusae) due to handling limitation. As a predator, a time lag in the isotopic composition of *Beroe* sp. may occur

(Woodland *et al.,* 2011), but even then, the $\delta^{15}N$ values (as indicator for trophic position) did not support predation on *M. leidyi* (Vander Zanden and Rasmussen, 2001). Additionally, Purcell *et al.* (2001) argued that *M. leidyi* might use its high tolerance to low salinities as a mechanism to escape less-euryhaline predators. The rare observations of *Beroe* sp. in the Westerschelde estuary agree with this hypothesis (Chapter 3).

Resource differentiation between *P. pileus* and *M. leidyi* could be the result of competiton or both ctenophores could have different diets (Chapter 5). The latter is further supported by the fact that both ctenophores exhibit different hunting mechanisms. While *P. pileus* uses its tentacles to target large mesozooplankton prey (>300 µm), *M. leidyi* uses a feeding current and its lobes resulting in a broader diet (Granhag *et al.,* 2011; Hamer *et al.,* 2011; Frost *et al.,* 2012). Both less mobile microzooplankton as well as highly mobile mesozooplankton may form a part of this diet (Costello and Coverdale, 1998; Waggett and Costello, 1999). Nevertheless, 8 years after its introduction in the area, also competition between the two ctenophores might have influenced these different diets.

Dietary flexibility allows *M. leidyi* to exploit a variety of food sources including ichthyoplankton (Purcell et al., 1994; Purcell and Arai, 2001; Jaspers et al., 2011). The impact of *M. leidyi* on early life stages of fish through competition and predation has been studied intensively, especially in light of overexploited fish stocks (e.g. Shiganova and Bulgakova, 2000; Bilio and Niermann 2004). Baltic cod larvae (Jaspers *et al.,* 2011), as well as anchovy eggs and larvae (Monteleone and Duguay, 1988; Cowan and Houde, 1990) were ingested by *M. leidyi*, while for Baltic cod eggs only low clearance rates have been observed (Jaspers *et al.,* 2011). Some authors have reported that eggs and larval fish make only a minor contribution to its diet in the field (Burrell and Van Engel, 1976; Mutlu, 1999; Kellnreitner *et al.,* 2013). Nevertheless, in Chapter 6, we showed that mobile sea bass larvae were most efficiently assimilated and served as high quality food in terms of fatty acids for *M. leidyi*. A brief analysis of ichthyoplankton densities in ring trawl samples from the Belgian part of the North Sea indicated that fish eggs were most abundant in May and fish larvae in July (1.4 and 2.9 $ind.m^{-3}$ respectively; unpublished data). Nevertheless, fish larvae were present in the water column the whole year. At least for some fish species, there is temporal overlap with the occurrence of *M. leidyi*. For sole *Solea solea* for example both eggs and larvae occur in the water column when *M. leidyi* reaches its highest densities (Chapter 4; Munk and Nielsen, 2005; Van Ginderdeuren *et al.,* 2012b). Van der Molen *et al.* (2015) calculated that in light of rising sea temperatures, *M. leidyi* may occur earlier and longer in the water column and in higher densities. As such, the impact on the early life stages of fish might increase, as fish probably respond slower to environmental changes compared to jellyfish, the latter having shorter generation times (Purcell, 2005).

Pathogens or parasites in *M. leidyi* have not yet been observed in the southern North Sea. However, Selander *et al.* (2010) found a parasitic sea anemone larvae *Edwardsiella* sp.

infecting the *M. leidyi* population in Sweden. As these parasitic larvae are common in the native habitat of *M. leidyi*, they probably also survived transatlantic transport (Reitzel *et al.,* 2007; Selander *et al.,* 2010). Furthermore, hyperiid amphipods such as *Hyperia galba* are known to infest gelatinous zooplankton, such as scyphozoan jellyfish (Fleming *et al.,* 2014) and have occasionally been reported in *M. leidyi* specimens (personal observations in The Netherlands by Lodewijk van Walraven, NIOZ). Overall, the consequences of hosting pathogens or parasites on native species are considered to be very low.

High *M. leidyi* densities can have a substantial impact on the mesozooplankton population with cascading effects throughout the ecosystem, as has been observed in the Black Sea (Daskalov *et al.,* 2007). Although *M. leidyi* can reach high densities in the ports along the Belgian coast and the Westerschelde estuary, these areas are actually characterised by low ecological values. Additionally, only low densities have been observed in the richer coastal area (Chapter 4; nursery grounds for fish Ellis *et al.,* 2012; rich benthic communities Derous *et al.,* 2007; Vanden Eede *et al.,* 2014). Therefore, the current effect of *M. leidyi* on the environment of the southern North Sea seems rather low. Furthermore, the competition and predation interactions within the gelatinous zooplankton community might counter the predatory potential of *M. leidyi* (Chapters 3 and 5).

Impact on human activities

Jellyfish are especially 'unpopular' when they directly interfere with human activities, such as tourism (stinging swimmers), fisheries (clogging nets, competition and predation on fish eggs and larvae), aquaculture (fish mortality, inhibited inoculation in shellfish farms), and coastal industries (clogging cooling-water intake screens of power plants) (Purcell *et al.,* 2007).

Tourists encounter jellyfish in the water when swimming or after stranding on the beach. Strandings of cnidarians are regularly observed along the Belgian coastline (e.g. Rappé, 1989). In Chapter 7, we showed that the knowledge of beach tourists in Belgium on the different jellyfish species is rather limited and that only few people know which species are actually stinging. Ctenophores catch their prey by adhesive cells, so-called colloblasts (Brusca and Brusca, 2003). In contrast to the nematocysts of some cnidarians, colloblasts do not harm humans. Consequently, beach tourists will barely be hindered by *M. leidyi* outbreaks in the water. Moreover, the fragile body of *M. leidyi* is often damaged (Chapter 2) by the wave action in the surf zone, rendering *M. leidyi* strandings a rare phenomenon.

Belgian fishermen have not reported clogging problems with *M. leidyi* yet (unpublished data). In contrast to other jellyfish, these ctenophores are too small and escape through the large meshes of fishing nets. However, during a sampling survey of ILVO in September 2014 several catches with a shrimp beam trawl were dominated by *M. leidyi* ctenophores (Figure 8.2; personal communication Kris Hostens, ILVO). Probably, the smaller mesh size in the cod end of the net (22 mm compared to >70 mm in commercial fishing nets) in combination with

a large outbreak of *M. leidyi* after the warm winter resulted in many gelatinous catches (relatively high numbers of *M. leidyi* and other jellyfish) up to 30 km off shore.

Figure 8.2 Catches from the ILVO campaign in September 2014 with small-meshed beam trawl (22 mm), resulted in some catches full of *M. leidyi* at the Thorntonbank (30 km offshore), left photo no jellyfish for reference

Aquaculture in Belgian marine waters is restricted to the sluice dock in Oostende, where an oyster farmer grows both *Ostrea edulis* and *Crassostrea gigas* (Lescrauwaet *et al.,* 2013). Notwithstanding the high densities of *M. leidyi* in that area, its impact is expected to be low, as the oysters are grown from juveniles. In contrast to oyster larvae, juvenile oysters are unlikely to be consumed by *M. leidyi*.

The effect of jellyfish blooms on power plants by clogging of the cooling water intake screens has been reported from all over the world (reviewed in Purcell *et al.,* 2007). These blooms may cause power reduction or shutdown of plants resulting in economic losses. Blooms of *M. leidyi* have been reported to cause problems with power plants in Israel (Galil *et al.,* 2009). This may also occur during outbreaks, for example at the power plants in Borsele and Doel situated along the Westerschelde estuary.

8.3.3 Risk score for *M. leidyi* in the southern North Sea

The completed Harmonia+ protocol (Addendum IV) resulted in a risk score for *M. leidyi* of 0.286. Intuitively, we categorised this score as low and concluded that the risk posed by *M. leidyi* in the southern North Sea is rather limited. Currently, relatively high densities (up to 18 ind.m^{-3}) were only observed in the ports, which are characterised by a low ecological value, while in the coastal zone and Westerschelde estuary the densities remained below 1 ind.m^{-3}. Moreover, *M. leidyi* showed a clear seasonal outbreak from late summer until late autumn (Chapter 4). The combination of periods with unfavourable environmental conditions, the diverse gelatinous zooplankton community (potential competitors) and the presence of predators such as *Beroe* sp. and the scyphomedusa *Chrysaora hysoscella* (Purcell *et al.,* 2001; Hosia *et al.,* 2011), limits the success of *M. leidyi* (also reviewed in Costello *et al.,* 2012).

Still, this PhD study (including the more recent observations) showed that the population of *M. leidyi* is fully established in the southern North Sea. Moreover, the risk or threat could become considerably higher when the population is able to reach higher densities in the coastal zone, where the ecological value is much higher (e.g. nursery grounds for fish (Ellis *et al.,* 2012)).

Finally, we mention some evaluating remarks about the Harmonia+ protocol. Although one of its goals is to provide managers with an objective tool to assess the risk of potentially invasive species, the interpretation of some questions can be quite subjective (is the answer 'low' or 'medium'?). Furthermore, D'hondt *et al.* (2015) deliberately did not define any risk categories, so the scores only allow to rank species relative to one another. However, in an unpublished memo they presented their view on such a demarcation, which may act as a guidance to Harmonia+ users to interpret the obtained scores in an absolute manner (D'hondt *et al.,* unpublished). In their 5-level categorisation, the risk score of 0.286 for *M. leidyi* is indeed interpreted as low. To conclude, Harmonia+ is a user-friendly tool, and can also be used for marine non-indigenous (potentially invasive) species, but in order to efficiently use this protocol for management purposes, more than one expert assessor should complete the evaluation for a particular species to reduce subjectivity in the scores.

8.4 Management options and recommendations

Although the risk of *M. leidyi* in the southern North Sea was scored low, management actions should not be postponed. On the contrary, some actions should have been taken before the population was fully established. Based on Lodge *et al.* (2006), we made a scheme listing several management options and recommendations for each step in the invasion process of *M. leidyi* (Figure 8.3).

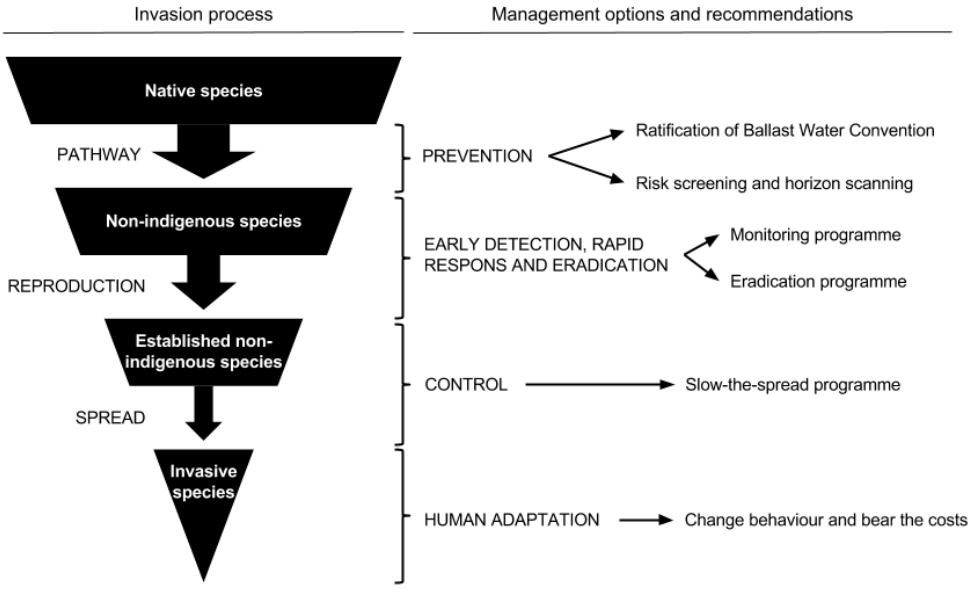


Figure 8.3 Management options and recommendations for each step of the invasion process (adapted from Lodge *et al.*, 2006)

8.4.1 Prevention

To prevent *M. leidyi* from entering Belgian waters, a first step would have been to ratify the Ballast Water Convention (IMO, 2015). Hitherto (dd. 22 September 2015 last update on www.imo.org), 44 countries ratified the convention representing approximately 33% of the world shipping tonnage. In October 2014, six countries including Belgium have indicated to be close to ratification, bringing the percentage of the world's merchant-shipping tonnage above the required 35% threshold (TradeWinds, 24 October 2014). Discussions on exemptions (certain ships may be exempted to comply with the convention's requirements in exchange for monitoring) and economic implications of the convention slow down the ratification process (David and Gollasch, 2008). From the moment the convention is ratified, vessels need to comply with the requirements, forming an additional cost for the shipping industry (David and Gollasch, 2008; IMO, 2015). However, in the end, costs for dealing with the adverse effects of successful invasive species may be avoided (Knowler, 2005). Even when an established population is already present in the area, which is the case for *M. leidyi* in the southern North Sea, this management action will prevent re-introductions and potential further spread.

Furthermore, risk screening and horizon scanning may also contribute to prevent the introduction of non-indigenous species (Lodge *et al.,* 2006). Risk assessment protocols, such as Harmonia+, should be used, but interpreted with care (D'hondt *et al.,* unpublished). Moreover, regular risk assessments will allow to intercept non-indigenous species before they get introduced and established. However, as species do not respect political boundaries, close collaboration with neighbouring countries is required. Since 1 January 2015, the EU regulation 1143/2014 on invasive alien species has entered into force. Although no additional financial means are provided, this initiative may promote awareness and cooperation on a EU scale.

8.4.2 Early detection and eradication

Considering the vast connectivity of the marine ecosystem through dominant and local currents, transport of planktonic organisms is difficult to stop. Regular monitoring may allow early detection of non-indigenous species (Lodge *et al.,* 2006). Monitoring programmes established in function of the Marine Strategy Framework Directive (2008/56/EC) form excellent platforms for an early detection at EU level (*cfr.* second descriptor MSFD). The main problem is that not all ecosystem components are equally monitored. Regardless of the fact that zooplankton hosts the early life stages of many benthic organisms and demersal fish, and although zooplankton forms an essential part of the marine food web (Van Ginderdeuren *et al.,* 2014), there is no long-term tradition to monitor zooplankton in Belgian waters.

Also, jellyfish are largely ignored in monitoring programmes, mainly due to sampling and preservation difficulties (Chapter 2) and the lack of direct economic interest (Laakmann and Holst, 2014). Yet, early detection followed by a rapid response in the form of eradication may prevent worst case scenario's and thus reduce the costs of additional management measures to protect the invaded ecosystem. Richardson *et al.* (2009) suggested short-term measures for direct removal of holoplanktonic jellyfish, through biocontrol, massive harvesting, jellyfish destruction or restocking of predators such as fish. Such measures require sufficient research in order not to aggravate the situation (*cfr.* the debate on introducing another non-native species *Beroe ovata* in the Caspian Sea as a predator of *M. leidyi* (Stone, 2005)). Furthermore, destruction of *M. leidyi* is less recommended, considering its regeneration capabilities (Coonfield, 1936). A more long-term solution is to reduce the impact of multiple anthropogenic stressors, such as eutrophication and overfishing, which can favour jellyfish blooms (Richardson *et al.,* 2009; Purcell, 2012).

8.4.3 Control

Once a non-indigenous species is established, which is the case for *M. leidyi* in our study area, management need to focus on 'control' (Lodge *et al.,* 2006). Dedicated sampling campaigns showed that the source areas of *M. leidyi* are situated in the ports, and especially in the sluice dock of Oostende (Chapter 4). The water level of the sluice dock is regulated by sluices and complete drainage of the sluice dock is possible. Therefore, drainage in winter – in consultation with other sluice dock stakeholders – can be suggested to eradicate all winter surviving specimens. However, since the sluice dock is not the only source area, and since local currents may transport *M. leidyi* from other source areas, complete drainage of the sluice dock in the port of Oostende will only slow down the size and spread of the population.

8.4.4 Adaptation

Lodge *et al.* (2006) stated that hitherto, the default approach of policy makers is adaptation, *i.e.* passively adjusting to the damage caused by an introduced species, even when eradication or control would be more cost-effective in the long-term. The above mentioned management actions may significantly reduce the impact of *M. leidyi* (and other invasive species) and should be adopted in current local (coastal communities), regional (Flanders) and national (Federal Belgium) management. Internationally, Olenin *et al.* (2010) identified a series of global and European policies and conventions related to non-indigenous species (e.g. United Nations Convention on the Law of the Sea (UNCLOS, 1982); Convention on Biological Diversity (CBD, 1992)). However, implementation of the Ballast Water Management Convention is considered as one of the most important steps towards reducing the unintentional spread of non-indigenous species regionally and worldwide, because this convention acts on the initial step in the invasion process, *i.e.* the pathway. Next to the Marine Strategy Framework Directive (MSFD, 2008/56/EC), also the EU regulation

1143/2014 on invasive alien species (into force since 1 January 2015) forms another step forward in the prevention, early detection, eradication and management of non-indigenous (terrestrial and aquatic) species. As the latter is a binding agreement, it obliges all EU Member States to tackle non-indigenous species issues through a variety of measures, such as permits, surveillance and control systems and priority lists.

8.5 Main conclusions

The general aim of this PhD thesis was to assess the structural and functional role of the non-indigenous ctenophore *Mnemiopsis leidyi* in the southern North Sea. More specifically, we focused on (1) the current distribution of *M. leidyi* in Belgian marine waters, the adjacent ports and the Westerschelde estuary, related to other gelatinous zooplankton, (2) the trophic ecology and interactions of *M. leidyi* in the planktonic food web, (3) the potential ecological and socio-economic effects of the presence of *M. leidyi* and other gelatinous zooplankton in these waters, and (4) the overall threat of *M. leidyi* and the implications for non-indigenous species's management. The following main conclusions can be drawn by answering the questions stipulated in Chapter 1.

What are the effects of using different net types for quantitative sampling of M. leidyi *and how do different preservation techniques influence morphological and genetic identification of this fragile species?*

Two different types of plankton nets: a WP2 net (mesh size 200 µm, vertical haul) and ring trawl net (mesh size 1000 µm, undulating trawl) were compared in terms of *M. leidyi* density and size distribution. WP2 nets did not provide a good estimate of *M. leidyi* presence compared to ring trawl nets, when densities were low. Moreover, when *M. leidyi* was present in both nets, much larger density estimates were found by the WP2 net (45.2 ± 114.0 ind.m^{-3} for WP2 net versus 12.8 ± 28.5 ind.m^{-3} for ring trawl net). The ring trawl net gave a good overview of adult population structure, but may underestimate some of the small ctenophores. Consequently, both the filtered volume and the mesh size largely determine the catch.

Different preservation solutions and methods were tested with respect to morphological and genetic identification of *M. leidyi* and in function of stable isotope analyses. Unpreserved samples are preferred for any type of analysis. However, short-term preservation in Lugol's solution or RCL2® may provide a good alternative, but shrinkage was observed in both preservatives. For stable isotope analyses, different preservation methods resulted in significant differences in both $\delta^{13}C$ and $\delta^{15}N$, which should be considered when comparing different isotopic compositions.

What is the current spatial and temporal distribution of M. leidyi *in relation to the associated zooplankton community in the Belgian part of the North Sea (BPNS) and the adjacent Westerschelde estuary?*

The lack of historical quantitative gelatinous zooplankton data hampered the objective assessment of 'jellification' in the southern North Sea ecosystem. Our study identified 33 gelatinous zooplankton species in the Belgian part of the North Sea and the adjacent Westerschelde estuary, setting the baseline for future research. Overall, hydromedusae were the most important group both in terms of diversity and density. The highest densities of gelatinous zooplankton were observed close to the coast in autumn, while the lowest densities were found in the Westerschelde and in spring. Three non-indigenous species were identified: *Nemopsis bachei*, *Lovenella assimilis* and *M. leidyi*. The seasonal occurrence of *M. leidyi* was situated between August and December, although the most recent (unpublished) data revealed that the species may be present year-round in our waters. Highest population densities were measured in the ports (up to 18 ind.m^{-3}), which were identified as 'source' areas. Densities in the coastal area remained quite low (<0.5 ind.m^{-3}) and were identified as potential 'sink' areas. The presence of larvae and the occasional observation of specimens in winter indicate that the *M. leidyi* population is fully established in the southern North Sea. Overall, distribution patterns of gelatinous zooplankton were mainly driven by temperature and salinity, while for *M. leidyi* also wave height (turbulence) played an important role. Competition with and predation by the indigenous ctenophores *Pleurobrachia pileus* and *Beroe* sp. might limit the success of *M. leidyi* in the coastal area.

How does M. leidyi *behave in the food web of the southern North Sea (including the BPNS and Westerschelde estuary)?*

The trophic ecology of *M. leidyi* was investigated in three different systems (coastal waters, ports and estuaries) in the southern North Sea using biochemical markers. This allowed for an integrated analysis of the trophic variation and interactions in the food web of the southern North Sea, based on the 'you are what you eat' principle. Based on the isotopic composition, we found that spatial differences were largely driven by variation at the base of the food web rather than diet changes of *M. leidyi* in the different ecosystems. Temporal variation in *M. leidyi* SI composition was also influenced by shifting baseline values and driven by seasonal changes in the associated plankton communities. Fatty acid (FA) profiles highlighted the omnivorous diet of *M. leidyi* and confirmed the low lipid reserves. Furthermore, trophic interactions between *M. leidyi* and the two co-occuring ctenophores (*P. pileus* and *Beroe* sp.) showed considerable resource differentiation, which could be the result of competition or both ctenophores could have different diets. A mixture of zooplankton was identified as potential food source for *M. leidyi*. FA markers supported the carnivorous diet of *Beroe* sp., but its SI composition did not confirm it as a predator of *M. leidyi.*

Grazing experiments using several prey types allowed to further investigate the trophic ecology of *M. leidyi.* No significant differences in clearance rates between prey types or sizes were observed (av. 0.2 ± 0.1 $L.mL_{M.leidyi}^{-1}.h^{-1}$). Highest carbon assimilation was observed for *Acartia* and sea bass larvae (most efficiently assimilated), and lowest for the pelagic diatom *Phaeodactylum tricornutum*. To further elucidate the prey-dependent variation in carbon uptake, we investigated the effect of each prey type in terms of fatty acids as a proxy for food quality. The consumption of sea bass larvae, characterised by higher levels of DHA (an essential fatty acid), resulted in significantly higher FA concentrations in *M. leidyi*. As *M. leidyi* does not convert excess food into storage lipids, survival, growth and reproduction are likely enhanced by the higher food quality, which might contribute to its invasive success.

What is the perception of the tourism sector at the Belgian coast on jellyfish (blooms), compared to newspaper articles on this matter?

Although there is no direct proof for increased jellyfish blooms in the Belgian part of the North Sea, the number of articles in Flemish media increased from less than 5 to 27 per year over a 10 year period. Especially the causes of jellyfish blooms related to anthropogenic activities were described. A questionnaire survey focussing on the tourist sector of the Belgian coast illustrated that the perception on jellyfish (jellyperception) by the broader public is only partly driven by the media. Personal experience with jellyfish seemed an equally-important factor. However, most beach tourists lacked a good knowledge on the different jellyfish species. Consequently, most people cannot distinguish stinging from non-stinging species. Divers on the other hand had an excellent species knowledge and described the beauty of jellyfish rather than stressing their negative characteristics (e.g. stinging). As public perception is a key driver in policy decisions, providing simple and good information about jellyfish (e.g. billboards on the beach) should be considered in light of integrated coastal zone management.

What is the overall threat of M. leidyi *to the southern North Sea ecosystem and what are the implications for non-indigenous species' management?*

The overall risk score obtained from the risk assessment using the Harmonia+ protocol was categorised as low, indicating that the risk of *M. leidyi* in the area is currently rather limited. The low densities in the coastal zone could be explained by periods of unfavourable conditions, the presence of predators (*Beroe* sp. and *Chrysaora hysoscella*) and competition with a rich gelatinous zooplankton community. However, in light of climate change, the established *M. leidyi* population should remain under close observation. If higher densities are reached in the coastal zone, which has a considerably higher ecological value than the ports (e.g. presence of nursery grounds for fish), the ecological impact will be higher.

Apart from risk screening, another prevention measure for policy makers should be the ratification of the Ballast Water Convention (IMO, 2015), which aims to inhibit further introductions of new species. Monitoring programmes as established within MSFD

(2008/56/EC) may enhance early detection on a EU level and may allow eradication for example through massive harvesting. Once a non-indigenous species is established in the area, control of the populations is advised (e.g. for *M. leidyi*, yearly drainage of the sluice dock in Oostende – as a source area – in winter). The costly 'adaptation' approach of policy makers, *i.e.* to cope with the damages caused by invasive species, should be replaced by a more pro-active approach. However, a broad range of conventions and especially the most recent EU regulation on invasive alien species (1143/2014) provide a framework and a step forward towards a more effective management of non-indigenous species.

8.6 Remaining challenges and opportunities

This PhD thesis sets in many ways the baseline for future research on gelatinous zooplankton in the southern North Sea. However, in order to fully understand this often 'forgotten' group of zooplankton, including its function, dynamics (blooms) and relation to environmental and human perturbations, some challenges for the future remain.

- A first challenge that needs to be urgently addressed is the establishment of dedicated zooplankton monitoring surveys to obtain long-term (gelatinous) zooplankton datasets. As illustrated in this study, long-term data on zooplankton (including ichthyoplankton), and in particular gelatinous zooplankton are scarce. The fact that gelatinous organisms entail restrictions for sampling and preservation should be taken into consideration to avoid underestimation. When resources (in terms of time, and on the long-term also money/budget) for zooplankton monitoring are limited, the following three opportunities should be further developed, favouring fast data acquisition.
    1. Automated tools: Automated zooplankton tools for monitoring such as a Zooscan (Grosjean *et al.,* 2004) and a video plankton recorder (Davis *et al.,* 1996) should be optimised and used to obtain fast, basic zooplankton data (compared to time-consuming microscope analyses). Incorporation of these tools on existing (benthic or fisheries) monitoring surveys can further reduce costs of monitoring, once the tools are optimised.
    2. Metabarcoding: This genetic method allows to determine the species composition of a complete mixed plankton sample, including for example microplankton (Corell and Rodríguez-Ezpeleta, 2014; Vargas *et al.,* 2015).
    3. Citizen science: This method involves the cooperation of citizens or amateur biologists to conduct (part of) the scientific research, in this case the collection of gelatinous zooplankton data. Citizen science initiatives have been proven to be quite successful for example in the Mediterranean Sea (e.g. Jellyrisk) and globally (e.g. Jellywatch) and have resulted in much data on presence, but also on absence of jellyfish on the beach or in the water. In Belgium an online database (waarnemingen.be) exists where amateur biologists can enter their observations

from terrestrial or aquatic nature. Specifically for divers, two platforms exist in The Netherlands ('Stichting Anemoon' and NELOS) to report on their underwater sightings. In 2013, after giving a lecture on *M. leidyi* to a group of interested divers from NELOS, several divers shared their experiences on *M. leidyi* from an underwater perspective.

Citizen science should be considered more often in low-budget scientific research and cooperation between research institutes and existing and new initiatives should be promoted. The only disadvantage on the obtained data is that they are often qualitative rather than quantitative and recorded at irregular times. However, these data at least provide indications on abundances such as 'many', 'few', 'one', and as we all know: 'some data is better than no data at all'.

- A second challenge is to gain more insights in the life cycle dynamics of Scyphozoa and Hydrozoa. As polyps are often disregarded from jellyfish research, it is still unclear where and how widely these sessile stages are distributed and if they profit from the introduction of more hard substrate in the southern North Sea (e.g. wind farms). Also, the (environmental) triggers for strobilation or budding and their response to climate change should be studied. Dulière *et al.* (2014) used a model to for- and hindcast blooms and polyp colonies respectively, based on strandings. Also, insight in the high functional diversity by means of trait analyses is still missing for gelatinous zooplankton (e.g. Beaugrand, 2004).
- Due to short generation times, gelatinous zooplankton quickly responds to changing environmental conditions. Consequently, when more data are available, the calculation of indicators based on gelatinous zooplankton diversity and density may be useful with respect to climate change and may serve as early warning systems.
- For the food web studies in this study, we focused on the potential prey of *M. leidyi* (Chapters 5 and 6). However, a remaining challenge is to investigate the food web with respect to potential predators of *M. leidyi*. For example: are there fish species consuming this ctenophore? Esser *et al.* (2004) showed that *P. pileus* is sometimes consumed by epibenthic organisms, when it occurs close to the sea bed. The same might be true for *M. leidyi*, for example in unfavourable conditions.
- We identified source-sink dynamics in the established *M. leidyi* population based on its distribution. However, studies on population genetics can expose a more detailed image on a small scale, which is useful for example in terms of eradication measures.

Addendum I: Modelling survival and connectivity of *Mnemiopsis leidyi* in the south-western North Sea and Scheldt estuaries

Adapted from:

van der Molen, J., van Beek, J., Augustine, S., Vansteenbrugge, L., van Walraven, L., Langenberg, V., van der Veer, H.W., Hostens, K., Pitois, S., Robbens, J., 2015. Modelling survival and connectivity of *Mnemiopsis leidyi* in the south-western North Sea and Scheldt estuaries. Ocean Science 11: 405-424.

Abstract

Three different models were applied to study the reproduction, survival and dispersal of *Mnemiopsis leidyi* in the Scheldt estuaries and the southern North Sea: a high-resolution particle tracking model with passive particles, a low resolution particle tracking model with a reproduction model coupled to a biogeochemical model, and a dynamic energy budget model. The results of the models, each with its strengths and weaknesses, suggests the following conceptual situation: (i) the estuaries possess enough retention capability to keep an overwintering population, and enough exchange with coastal waters of the North Sea to seed offshore populations; (ii) *M. leidyi* can survive in the North Sea, and be transported over considerable distances, thus facilitating connectivity between coastal embayments; (iii) under current climatic conditions, *M. leidyi* may not be able to reproduce in large numbers in coastal and offshore waters of the North Sea, but this may change with global warming - however this result is subject to substantial uncertainty. Further quantitative observational work is needed on the effects of temperature, salinity and food availability on reproduction and on mortality at different life stages to improve models such as used here.

Introduction

Background

The comb jelly *Mnemiopsis leidyi* originates from temperate to sub-tropical waters along the East coast of the American continent (Purcell *et al.* 2001, Costello *et al.* 2012). *M. leidyi* is notorious for its highly adaptive life traits: A fast growth rate combined with high fecundity, early reproduction, the ability of self-fertilization and a euryoecious lifestyle tolerating a wide range of environmental parameters (temperature, salinity, water quality) are characteristics which favour its establishment and fast expansion in invaded areas (Purcell *et al.* 2001, Fuentes *et al.* 2010, Jaspers *et al.* 2011, Salihoglu *et al.* 2011).

M. leidyi was introduced in the Black Sea in the early 80s (See also the comprehensive review by Costello *et al.*, 2012), probably through ballast water (Vinogradov *et al.*, 1989). The presence of *M. leidyi* together with eutrophication and overfishing caused a deterioration of the ecosystem, which finally degraded to a low biodiversified 'dead-end' gelatinous food web (Shiganova, 1998). This led to an economic loss/collapse of the pelagic

fish population, in particular anchovies and sprat fisheries (Kideys, 1994; Kideys, 2002). *M. leidyi* then spread further into the Sea of Azov (Studenikina *et al.* 1991), the Sea of Marmara (Shiganova 1993), the Aegean Sea (Kideys and Niermann 1994), and the Levantine Sea (Kideys and Niermann 1993). In 1999, *M. leidyi* was transported from the Black Sea to the Caspian Sea (Ivanov *et al.*, 2000). *M. leidyi* spread from the eastern Mediterranean to other regions of the Mediterranean: it was recorded in 2005 in the northern Adriatic Sea (Shiganova and Malej, 2009) and in 2009, blooms were reported in waters of Israel (Galil *et al.*, 2009), Italy (Boero *et al.*, 2009), and Spain (Fuentes *et al.*, 2010).

M. leidyi was also transported from the northwestern Atlantic to northern European waters (Reusch *et al.*, 2010); first records date back to 2005 and originates from Le Havre harbour in northern France (Antajan *et al.*, 2014), Danish territorial waters (Tendal *et al.* 2007) and Norwegian fjords (Oliveira 2007). By 2006 *M. leidyi* had been reported in the western Baltic Sea (Javidpour *et al.* 2006), in the Skagerrak (Hansson, 2006), in the Scheldt estuaries and Wadden Sea (Faasse and Bayha 2006) and the German Bight (Boersma *et al.* 2007). In 2007, the species was found in Limfjorden (Riisgård *et al.* 2007) and in Belgian waters in the harbour of Zeebrugge (Dumoulin, 2007; Van Ginderdeuren, 2012). In the following years the species remained present in the western and central Baltic Sea (Javidpour et al 2009, Jaspers *et al.* 2013), Kattegat, Skagerrak and inshore Danish waters (Tendal et al 2007, Riisgard et al 2012) and Wadden Sea (Kellnreitner *et al.* 2013, van Walraven *et al.* 2013). In most of these areas the highest densities are observed in summer, although in the Wadden Sea as well as in the Baltic the species has been observed in all seasons. In the Scheldt area *M. leidyi* is observed in Lake Veere, Lake Grevelingen, the Eastern Scheldt and Western Scheldt. In this area, *M. leidyi* is observed every year, with highest densities in summer as well (Gittenberger 2008).

Since 2009 *M. leidyi* has been observed frequently along the French coast of the North Sea (Antajan *et al.*, 2014). This is particularly worrying because the North Sea is the home of commercially important fish stocks, including spawning and nursery grounds (Ellis *et al.* 2011), and also shares the depleted state of fish stocks that characterized the Black Sea when *M. leidyi* was introduced (Kideys, 1994; Daskalov, 2002; Mutlu, 2009). Furthermore, model predictions from recent work from Collingridge *et al.* (2014) suggest that large parts of the North Sea are suitable for *M. leidyi* reproduction in summer months, with some of the highest risk areas along the southern coastal and estuarine regions of the North Sea, due to a combination of high temperatures and high food concentrations. The presence and potential establishment of *M. leidyi* in the southern North Sea is therefore cause for concern, and there is a need to further expand our understanding on the mechanisms involved in the dynamics of *M. leidyi* populations and its potential spread from source locations where it is established.

In this paper we apply three different models to simulate aspects of transport, survival and reproduction of *M. leidyi* in the Scheldt estuaries and the North Sea. We use the combined results to provide insight into the potential spreading and population dynamics of *M. leidyi* at a range of spatial and temporal scales in the area, which could not have been obtained with each model individually.

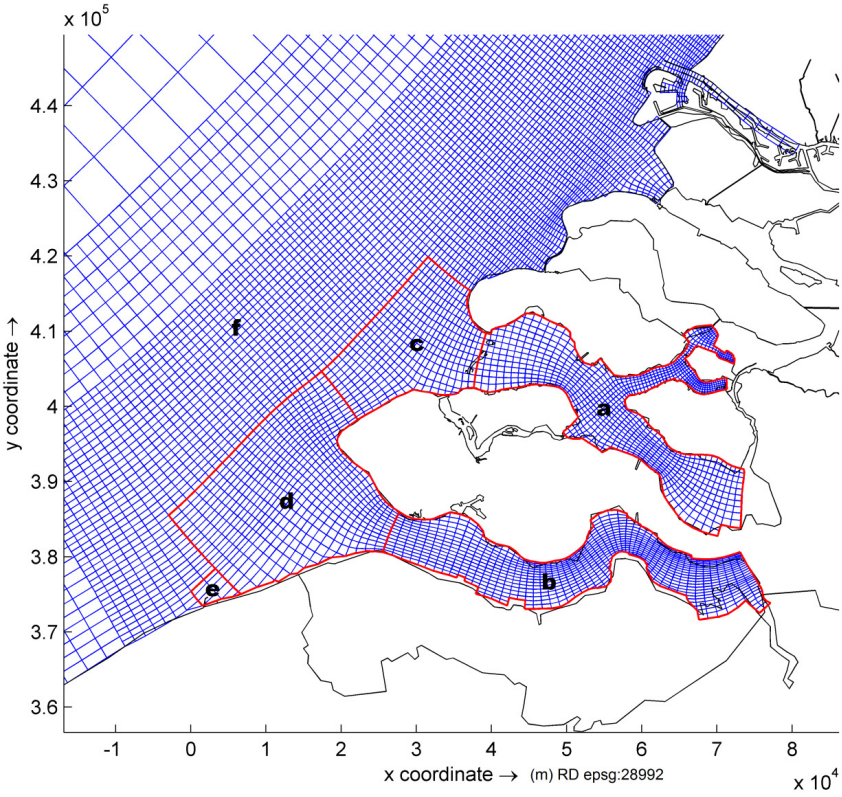


Figure 8.4. Model grid of the Delft model in blue and definition of the areas in red. (a) Eastern Scheldt estuary, (b) Western Scheldt estuary, (c) Eastern Scheldt mouth, (d) Western Scheldt mouth, (e) Zeebrugge harbour area and (f) southern North Sea.

Study Area

Scheldt estuaries

The Western Scheldt is the Dutch part of the estuary of the Scheldt River which flows from France to Belgium and enters the North Sea in the Netherlands, see Figure 8.4. The total surface area of the Western Scheldt is approximately 310 km^2 and it has a length of about 60 km. The average channel depth is 15-20 m (Meire *et al.*, 2005) and the estuary has extensive tidal flats. The Scheldt River has an average fresh-water discharge of 104 m^3s^{-1} and the upstream part in Belgium has the characteristics of a tidal river. The salinity at the Belgian-

Dutch border ranges from 2 to 14 and the maximum tidal range is 5 meters. The Scheldt is considered well mixed, except in periods of peak river discharge (Meire *et al.*, 2005).

The Eastern Scheldt estuary is the former mouth of the Scheldt river and has a connection to the Rhine and Meuse river system, see Figure 8.4. The total surface area of the Eastern Scheldt is approximately 350 km^2 and it has a length of about 40 km. The inner part of the Estuary is forked, with a smaller branch to the north and a wider branch to the south east. Following the 1953 storm surge, waterworks have been constructed which isolate the Eastern Scheldt from most of the fresh water input, transforming the estuary into a well-mixed tidal bay. In the mouth of the estuary a storm surge barrier has been constructed which is usually open, but can be closed under extreme weather conditions. The barrier reduces the exchange of water with the open sea by 28% (Smaal and Nienhuis, 1992).

The two estuaries are only connected by sluiced waterways. Both estuaries have a protected status as nature reserve.

Southern North Sea

The southern North Sea is a relatively shallow shelf sea with depths less than 80 m. The most prominent feature is the Dogger Bank, which rises up to less than 30 m water depth, and is separated from the Norfolk Banks to the southwest by the Silver Pit. The latter has a depth of over 50 m. To the southeast of the Dogger Bank are the Oyster Grounds, with depths of 40-50 m. The Southern Bight is situated further south, and consists of a deep channel (depth up to 50 m) in the west and a shallow area (depths typically less than 30 m) in the east. The channel is connected to the Strait of Dover to the south.

The tides in the southern North Sea are semi-diurnal, with dominant M_2 tidal amplitudes over 2 m along the UK east coast, near Dover Strait, and in the German Bight, and amphidromic points in the central southern North Sea and in the Southern Bight of the North Sea (e.g., Davies *et al.*, 1997). Maximum surface currents at spring tide are about 1.4 m s^{-1} in the western and southern parts of the Southern Bight, reducing to 0.3 m s^{-1} in the central southern North Sea (Hydrographical Survey, 2000).

Wind can induce depth-averaged surge currents of up to 1 m s^{-1} (Flather, 1987). The time and depth-averaged atmospherically-induced residual currents are about 1/3 of the tidal residuals and directed to the north in the Southern Bight, and to the northeast in the southern North Sea (Prandle, 1978). Combined residual current speeds in the Southern Bight are approximately 0.05 m s^{-1} (Prandle, 1978).

Thermal stratification occurs in summer in the northern parts of the southern North Sea, whereas the southern parts remain well-mixed, and are separated by the Frysian Front (Otto *et al.*, 1990). Under stratified conditions, a subsurface jet induced by density differences transports water around the north, east and southeast slopes of the Dogger Bank into the

Oyster Grounds (Brown *et al.*, 1999; Hill *et al.*, 2008). The thermal stratification breaks down in the autumn, and is absent throughout the winter.

On a more local scale, fresh-water outflow of the river Rhine forms a plume along the Dutch coast to the North, resulting in density-driven coastward near-bottom currents of several cm s^{-1} (Visser, 1992). A similar plume is present in the German Bight and associated with the river Elbe (e.g. Schrum, 1997). UK coastal waters converge in the East Anglian plume, which is mostly recognisable by its elevated levels of turbidity. This plume crosses the North Sea in a northeast-ward direction, from the coast of East Anglia to the south of the Dogger Bank (see Dyer and Moffat, 1998 for a detailed description).

Multi-model approach

Three existing models were used: i) Delft 3D (in the results and discussion referred to as Delft model), ii) GETM-ERSEM-BFM model with particle tracking (GITM) (in the results and discussion referred to as GETM model) and iii) the Dynamic Energy Budget (DEB) model (in the results and discussion referred to as DEB model). By deploying the strengths of the individual models, and through combining and intercomparison of the results, this study provides insight into the potential spreading and population dynamics of *M. leidyi* at a range of spatial and temporal scales in the area that could not have been obtained with each model individually, and without the investment required to develop a single model to encompass all. The Delft model implementation at high spatial resolution with its native particle tracking module using passive particles provided insight into the potential role of the Scheldt estuaries as a nursery and source of *M. leidyi*, and in the role of estuarine-marine exchange processes. The GETM model with particle tracking (GITM) was developed to include a simple reproduction model, and was used to study transport, connectivity and population dynamics at the scale of the North Sea. The DEB model was then used for fixed hypothetical locations using prescribed temperatures to simulate in greater detail how temperature and food concentrations dynamically affect the eco-physiology of a growing, developing and/ or reproducing individual. In this model age and size at important life-history can depend on the prior temperature and food experienced by the individual. The DEB model was used to both gain confidence in the simple reproduction model in the GETM model and to expose its limitations.

Material and Methods

Delft3D

Hydrodynamics

Delft3D is an integrated modelling suite used to simulate three-dimensional flow, sediment transport and morphology, waves, water quality and ecology and the interactions between these processes. More specifically, the hydrodynamic module simulates non-steady flows in relatively shallow water, and incorporates the effects of tides, winds, air pressure, density

differences (due to salinity and temperature), waves, turbulence and drying and flooding (Lesser *et al.*, 2004).

The model application of the southern North Sea uses a curvilinear boundary fitted c-grid. The domain decomposition technique creates extra resolution by inserting an intermediate and a fine sized domain near the Dutch coast (Figure 8.4). The horizontal resolution ranges from 0.5 km near the coast to 25 km near the open boundaries, resulting in 22473 active computational elements. The vertical dimension consists of 12 σ transformed layers with the highest resolution near the sea bed and the sea surface. The shallow-water hydrostatic pressure equations are time-integrated by means of an alternating direction implicit (ADI) numerical scheme in horizontal directions and by the Crank-Nicolson method in the vertical direction. The solution is mass-conserving at every grid cell and time step. This code is extended with transport of salt and heat content and with a k-ε turbulence model for vertical exchange of horizontal momentum and matter or heat. Along the open sea boundaries tidal harmonics for water level are imposed consisting of 50 astronomical constituents. The model was forced using meteorological data from the High Resolution Limited Area Model (HIRLAM) run at the Royal Dutch Meteorological Service [KNMI] (Undén *et al.*, 2002): two horizontal wind velocity components, air pressure and temperature, archived every 6 h. The fresh-water discharges from 18 rivers were included in the model. Seven of these discharges varied temporally (historic daily averages) and 11 were constant (based on long-term averages).

The primary focus of the hydrodynamic model is the representation of the water level and tidal flow velocities along the Dutch coast and in the estuaries. The results of the model have been applied and validated against observational data in the modelling of suspended matter (van Kessel *et al.*, 2011), eutrophication (Los *et al.*, 2008) and the transport of fish larvae (Bolle *et al.*, 2009; Dickey-Collas *et al.*, 2009).

Particle tracking in Delft3D

The particle module of Delft3D uses a numerical advection scheme for particles that is fully compatible with the local mass conserving advection properties of the underlying flow field at the discrete level of that field (Postma *et al.*, 2013). Horizontal dispersion is accounted for by a random walk step. The depth varying vertical diffusion as calculated by the hydrodynamic turbulence model is incorporated by a stochastic bouncing-algorithm to move the particles in the vertical. The algorithm closely approximates the analytical solution. For the purpose of this study, passive particles were used.

The particle tracking module is run offline, for this purpose the hydrodynamic results are stored on an hourly basis. The particle model itself runs with a timestep of 5 minutes.

For the simulation of biological vectors a module is available to simulate development and vertical migration behaviour. The development is divided into an unlimited amount of stages

where the duration of the stage is dependent on the age of the particle and the accumulated temperature encountered over that stage (Bolle *et al.*, 2009). For each stage the behaviour can be set with its own parameterisation. Apart from neutral buoyancy the types of behaviour are positive buoyancy, negative buoyancy, diurnal vertical migration, selective tidal transport and settling towards the sea bed. Growth and mortality based on food availability and predation were not incorporated in the model. At the start of this study, we had no information suggesting migration behaviour for *M. leidyi*. Hence, use of passive particles was assumed to be sufficient to study the potential exchange between the estuaries and offshore waters.

Application: estuaries

The Delft3D model was applied to determine the potential connectivity of *M. leidyi* between the Eastern and Western Scheldt estuaries and the North Sea. Applying the hydrodynamic situation from 2008, a run with a uniform initial distribution of particles over the estuary volume (particles m^{-3}) was performed for each estuary and for each month of the year. The boundaries of the estuaries are shown in Figure 8.4. Five-hundred thousand particles were released simultaneously. The horizontal dispersion coefficient was set to 1.0 (m^2 s^{-1}) and no behaviour was included (neutral buoyancy).

The simulations were performed from the first high tide of the month to the first high tide after a period of 30 days, which corresponds with two spring neap cycles. At the end of the simulation the position of the particles within six pre-defined areas was scored and reported as a percentage of the number of particles released, resulting in a connectivity matrix. The areas were the Eastern Scheldt estuary, the Western Scheldt estuary, the Eastern Scheldt ebb-tidal delta, Western Scheldt ebb-tidal delta, the Zeebrugge harbour area and the remainder of the North Sea as far as covered by the outer model domain (Figure 8.4). To test the sensitivity of the results for the release moment, the July runs for both estuaries were also performed from low tide towards low tide over a period of 30 days.

In addition to the simulations described above, model runs were carried out with initial conditions based on observations. These initial conditions were constructed using zero order extrapolation of the measurements in the lateral direction of the estuary and interpolation in the longitudinal direction with a zero value outside the estuary. Model runs were carried out from the date of measurements until the next set of measurements available for comparison.

For the Western Scheldt the model was run from 1 September 2011 to 1 December 2011 . The initial field was based on samples collected on 1 September 2011 and 1 December 2011 in the Western Scheldt onboard RV Zeeleeuw at three different locations using a WP3 net (Ø 1 m, mesh size 1 mm) in oblique hauls. Ctenophores, among which *M. leidyi*, were isolated from the samples and morphologically identified, counted and measured (oral-aboral length) on board (Vansteenbrugge *et al.* 2015).

For the Eastern Scheldt the initial condition was constructed from measurements on 28-09-2012 (Van Walraven *et al.*, 2014) onboard RV Luctor using the same gear and method. The model was compared with data from the MEMO cruise on 20-10-2012 (include reference MEMO cruise lead by France). The model was run with 2011 hydrodynamics for the same period because a hydrodynamics simulation for 2012 was not available. The runs with non-uniform initial condition will be referred to as the realistic runs.

Particle tracking IBM coupled to GETM-ERSEM-BFM

Particle tracking IBM (GITM)

The Individual Behaviour Model (IBM) GITM (General Individuals Transport Model) includes physical particle advection and diffusion, and biological development and behaviour. The advection-diffusion elements of GITM were based on a re-coded version of the lagrangean semi-analytical advection-diffusion method developed by Wolk (2003). This method ensures that particles follow stream lines exactly. Furthermore, a random walk method with advective correction (Visser, 1997) was included to simulate diffusion (Hunter *et al.*, 1993). This method uses a constant diffusion coefficient in the horizontal direction and a variable diffusion coefficient in the vertical direction. The latter is based on the vertical diffusivity obtained from the turbulence closure model in the hydrodynamics model GETM (see also Section 0). The combined hydrodynamics model (GETM) and particle tracking model (GITM) were applied recently to simulate the transport of plaice larvae in the North Sea (Tiessen *et al.*, 2014).

The biological development and behaviour module of GITM allows particles to progress through a user-defined number of egg and larval development stages, using physical and biological information from the GETM-ERSEM-BFM model (e.g. temperature and food fields). However, these mechanisms were not used here. Instead, the model was modified to include a simplified version of the reproduction mechanism suggested by Salihoglu *et al.* (2011), elements of which originate from the model of Kremer (1976). This reproduction mechanism was implemented to affect the number of individuals represented by a super-individual (particle). The main simplifications were: (i) each super-individual was assumed to represent a number of adults of average mass; (ii) egg and juvenile stages were assumed to be infinitely short to allow for (i); (iii) food stocks were assumed not to be impacted upon by *M. leidyi*. Including the latter would require either inclusion of a comb jelly functional type in ERSEM-BFM, or development of full, on-the-fly coupling and feedback between ERSEM-BFM and GITM. These options were considered to be beyond the scope of this study. As a result, the survival and reproductive success of individuals simulated by the present model implementation should be considered an over-estimate. The reproduction mechanism was implemented as follows; all values and constants were taken from Salihoglu *et al.* (2011) unless specified otherwise. Genetic evidence suggests differences between northern and southern populations (Reusch *et al.*, 2010). However we have not found corresponding

evidence in the literature for differences in physiological response to temperature, hence it is assumed that the parameter values suggested by Salihoglu *et al.* (2011) are a reasonable first approximation for populations in the North Sea.

Eggs were only produced if temperature and salinity were above the thresholds of 12 °C and 10, respectively (Lehtiniemi *et al.*, 2012; see, however, Section 0). *M. leidyi* exhibits synchronised spawning (Pang and Martindale, 2008). In the model, this behaviour was not included, and egg production was spread over time. As in the model eggs were not released as separate particles, and predation processes were not explicitly included, the influence of this simplification on the modelled adult population is expected to be small. The number of eggs produced per time step n_e depended on food availability:

$$n_e = \frac{fF_a}{w_e} \quad (1)$$

with F_a the food intake of the adult population represented by the super-individual [mg C timestep^{-1}], w_e=0.1 µg C the average mass of an egg, and f the proportion of food turned into eggs. The adult food intake was calculated as:

$$F_a = n_a \frac{f_a}{1000} c_{cd} w_a G_a A_a \frac{dt}{24*3600} \quad (2)$$

with n_a the number of adults represented by the super-individual, f_a the adult food concentration [mg C m^{-3}] (taken here as mesozooplankton from the GETM-ERSEM-BFM model, see Section 0), w_a=2.8 mg C the average mass of an adult, dt the time step [s], c_{cd}=73 mg mg^{-1} C a factor to convert carbon weight to dry weight for high salinities, A_a=0.72 the adult assimilation efficiency, and G_a the adult clearance rate [l mg^{-1} dry weight day^{-1}]:

$$G_a = a_0 \left[\left(\frac{w_a}{c_{w2c}} \right)^{-b} \right] e^{kT} \quad (3)$$

with a_0=0.09 l mg^{-1} d^{-1} an empirical constant, b=0.5 a power, k=0.05 °C^{-1} a decay coefficient, c_{w2c}=0.574 mg C g^{-1} a conversion factor of wet weight to carbon weight, and T temperature [°C].

In (1), the proportion of food turned into eggs f was calculated as:

$$f = 0.01 T_f e^{c_f (w_a / c_{w2c})} \quad (4)$$

with c_f=0.115 mg^{-1} an empirical constant, and T_f a temperature function given by:

$$T_f = a_T e^{b_T T} \quad (5)$$

with $T_{f,min}$=0.01 a minimum introduced here to prevent negative values, and a_T=0.03 and b_T=0.14 empirical constants. Out of the three functions suggested by Salihoglu *et al.* (2011)

we have chosen this one over the linear function preferred by Salihoglu *et al.* (2011), which has a cut-off at a rather high temperature of approximately 14 °C. For the reference run example of Salihoglu *et al.* (2011), the order of magnitude of the number of eggs (several hundreds) produced using these equations corresponded with the observations for small individuals presented by Kremer (1976) and Reeve *et al.* (1989). Note that a direct comparison is impossible because the conditions of the observations, as far as reported, cannot be fully represented with the current model.

Subsequently, the number of eggs calculated in (1) was subjected to egg and juvenile mortality. The number of surviving eggs n_{es} was calculated using a constant daily mortality rate m_e=0.7 and assuming an egg phase duration of 1 day:

$$n_{es} = (1 - m_e) n_e \qquad (6)$$

Juvenile mortality was calculated as a combination of a daily background mortality m_j =0.27 and food availability. Egg and juvenile daily mortalities were calibrated to reproduce the results of the reference run example of Salihoglu *et al.* (2011). The surviving juveniles n_{js} after application of the background mortality was:

$$n_{js} = (1 - m_j)^{D_j} n_{es} \qquad (7)$$

with D_j a temperature-driven duration of the juvenile stage in days

$$D_j = a_d + b_d T \qquad (8)$$

with a_d=76.0 and b_d=-2.4 constants based on the graphs with model results presented by Salihoglu *et al.* (2011).

Juvenile starvation was implemented by comparing the daily food intake F_j with the average daily weight gain w_g required to reach the mass at the end of the transition stage w_{aj}=1.5 mg C:

$$w_g = \frac{w_{aj} - w_j}{D_j} \qquad (9)$$

with w_j=0.13 mg C the average mass of a juvenile. The daily juvenile food intake was calculated as:

$$F_j = \frac{f_j}{1000} c_{cd} w_j G_j A_j (1 - L_j) \qquad (10)$$

with f_j the juvenile food concentration [mg C m^{-3}] (taken here as microzooplankton from the GETM-ERSEM-BFM model, see Section 0), A_j=0.75 the juvenile assimilation rate, L_j=0.06 a metabolic loss fraction, and G_j the juvenile ingestion rate [l mg^{-1} dry weight day^{-1}]:

$$G_j = 0.4*12.3*\left(\frac{w_j}{c_{w2c}}\right)^{0.574} + 0.1 \quad (11)$$

Then finally, by combining the results of (7), (9) and (10), the number of new adults recruited n_{ar} into the existing population in the time step under consideration (i.e. assuming infinitely short egg and juvenile duration, but including mortality calculated over their normal duration) was calculated as:

$$n_{ar} = \min\left(1, \frac{F_j}{w_g}\right) n_{js} \quad (12)$$

Adults were assumed not to survive temperatures less than 2 °C. For such low temperatures, there is no reproduction in the model. As the maturation in the model is artificially compacted into a single time step, this means that there are then no juveniles, so a similar rule for juvenile mortality is not relevant. For temperatures above that, a background mortality of 2% was imposed for completeness following Salihoglu *et al.* (2011). There is evidence to suggest that *M. leidyi* can survive lower temperatures (Costello *et al.*, 2006), so this element of the model may be refined. As offshore water temperatures in the south western North Sea only very rarely fall to such low levels, however, the results presented here are not expected to change if such a refinement was implemented. Also, a daily starvation mortality rate of 13% for food concentrations less than 3 mg C m^{-3}, based on the observation that *M. leidyi* can survive without food for up to 17 days (Oliveira, 2007), and observations of the lowest concentrations of zooplankton at which *M. leidyi* has been found in the field (Kremer, 1994). The latter results in approximately 10% of the population surviving after 17 days. It is likely that in reality, starvation mortality is temperature-dependent, so subject to the availability of suitable observations, this element of the model may be improved.

GETM-ERSEM-BFM

The coupled physical-biogeochemical model GETM-ERSEM-BFM was used to produce hydrodynamics and food fields for the particle tracking model. GETM (General Estuarine Transport Model) is a public domain, three-dimensional finite difference hydrodynamical model (Burchard and Bolding, 2002; www.getm.eu). It solves the 3D partial differential equations for conservation of mass, momentum, salt and heat. The ERSEM-BFM (European Regional Seas Ecosystem Model - Biogeochemical Flux Model) version used here is a development of the model ERSEM III (see Baretta *et al.*, 1995; Ruardij and Van Raaphorst, 1995; Ruardij *et al.*, 1997; Vichi *et al.*, 2003; Vichi *et al.*, 2004; Ruardij *et al.*, 2005; Vichi *et al.*, 2007; Van der Molen *et al.*, 2013; www.nioz.nl/northsea_model), and describes the dynamics of the biogeochemical fluxes within the pelagic and benthic environment. The ERSEM-BFM model simulates the cycles of carbon, nitrogen, phosphorus, silicate and oxygen and allows for variable internal nutrient ratios inside organisms, based on external

availability and physiological status. The model applies a functional group approach and contains four phytoplankton groups, four zooplankton groups and five benthic groups, the latter comprising four macrofauna and one meiofauna groups. Pelagic and benthic aerobic and anaerobic bacteria are also included. The pelagic module includes a number of processes in addition to those included in the oceanic version presented by Vichi *et al.* (2007) to make it suitable for temperate shelf seas: (i) a parameterisation for diatoms allowing growth in spring, (ii) enhanced transparent exopolymer particles (TEP) excretion by diatoms under nutrient stress, (iii) the associated formation of macro-aggregates consisting of TEP and diatoms, leading to enhanced sinking rates and a sufficient food supply to the benthic system especially in the deeper offshore areas (Engel, 2000), (iv) a *Phaeocystis* functional group for improved simulation of primary production in coastal areas (Peperzak *et al.*, 1998), and (v) a suspended particulate matter (SPM) resuspension module that responds to surface waves for improved simulation of the under-water light climate.

Application: North Sea

The GETM-ERSEM-BFM model was run from 1991 until 2009, and hot-started from a 50-year hindcast carried out with an earlier version (Van Leeuwen *et al.*, 2013). Hourly hydrodynamics and food fields were stored from June 2008 to February 2009. The particle tracking model IBM GITM was run from 1 June 2008 to 31 January 2009, releasing 3 particles per day from the 1st of June until the 30th of October near the surface in each of 6 grid cells just seaward of the Dutch estuaries, corresponding with expected bloom times (eg., Collingridge *et al.*, 2014). The particles were assumed to be passive tracers. Upon release, each particle was assumed to represent 1000 *M. leidyi* individuals. Daily particle positions, particle characteristics and environmental conditions were stored. The results were processed into density contour maps, and into time series of properties aggregated over all the particles. In the following, this run is called the standard run. The standard run did not produce *M. leidyi* bloom conditions, because very few juveniles survived due to a combination of a long juvenile duration and the imposed daily juvenile mortality. Hence, additional runs were carried out to, specifically targeting these factors, to investigate how blooms might occur. To illustrate the effect of temperature on reproduction, and to compare with the response in warmer waters, an additional scenario run was carried out in which the particles experienced 10% higher temperatures. The sensitivity to juvenile mortality was assessed by a model run with two thirds of juvenile mortality at normal temperatures, and a run with four thirds of juvenile mortality at the 10% higher temperatures.

To study interconnectivity between ports and estuaries along the French Channel coast and areas in the southern North Sea, a model run was carried out releasing 20 particles per day at one grid cell in the mouth of the river Seine, and one grid cell in the mouth of the river Somme during the same period as in the previous simulations.

Dynamic Energy Budget (DEB) model

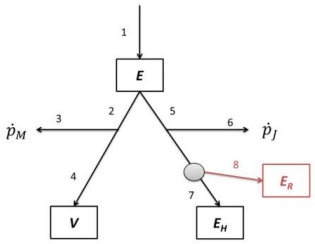
DEB model

Dynamic Energy Budget (DEB) theory (Kooijman 2010) describes the uptake and use of food for all organisms under conditions in which food densities and temperatures vary. The standard DEB model is the simplest of a large family of DEB models. Augustine *et al.* (2014) carried out a literature review on eco-physiological data for *M. leidyi* and estimated DEB model parameters for this species (seeTable 8.1). The formulation of the standard DEB model applied to *M. leidyi* is well documented in Augustine *et al.* (2014). We refer to that study for details.

Table 8.1. DEB model parameters used in the simulations. The parameter values are taken from Augustine et al (2014). * denotes parameters which increase by a factor 8.6 during metabolic acceleration (i.e. $E_H^s < E_H < E_H^j$). The values are given at reference temperature of 20°C. We refer the reader to Figure 8.5 and to the original study (Augustine et al 2014) for the physiological interpretation of the parameters.

E_H^b	1.5 10^{-3} J	κ	0.7	$\dot{k}_J$	0.002 d^{-1}
E_H^s	4.4 10^{-3} J	κ_R	0.95	$\dot{v}$*	0.21 cm d^{-1}
E_H^j	3.2 J	$[\dot{p}_M]$	5.0 J cm^{-3} d^{-1}	$\{\dot{p}_{Am}\}$*	3.0 J cm^{-2} d^{-1}
E_H^p	42.0 J	$[E_G]$	78.0 J cm^{-3}	T_A	1.05 10^4

In short in the DEB theory, the state of the individual is quantified by energy fixed in reserve (E, J), volume of the structural component (V, cm^3) and its maturity level (E_H, J), see Figure 8.5. The model closes the full life-cycle from egg to adult. Stage transitions are assumed to occur at fixed maturity levels, quantified by the cumulated amount of energy invested in maturity. The model encompasses three life-stages: embryos (does not feed, and allocates energy to maturation), juveniles (feeds, and allocates energy to maturation) and adults (feeds, grows, and allocates energy to size-related reproduction). Growth is possible in all of the life stages as long as enough energy is mobilised to cover somatic maintenance costs. Birth is defined as the moment when feeding is switched on (E_H = E_H^b) while puberty (E_H = E_H^p) is defined as the moment juveniles start allocating energy to reproduction (E_R) instead of maturation.



$$\frac{dV}{dt} = \frac{\dot{p}_G}{[E_G]}$$

$$\frac{dE}{dt} = \dot{p}_A - \dot{p}_C$$

$$\frac{dE_H}{dt} = \dot{p}_H$$

$$\frac{dE_R}{dt} = \kappa_R \dot{p}_R$$

1 assimilation: $\dot{p}_A = f\,\{\dot{p}_{Am}\}V^{2/3}$

2 allocation fraction to soma: $\kappa\,\dot{p}_c$ and $\dot{p}_c = E\,(\dot{v}V^{-1/3} - \frac{\kappa\,E\,\dot{v}\,V^{-1/3} - [\dot{p}_M]V}{\kappa\,E + [E_G]V})$

3 somatic maintenance costs: $\dot{p}_M = [\dot{p}_M]V$

4 growth (synthesis of structure): $\dot{p}_G = \kappa\,\dot{p}_c - \dot{p}_M$

5 allocation fraction to maturation/reproduction: $(1-\kappa)\,\dot{p}_c$

6 maturity maintenance costs: $\dot{p}_J = \dot{k}_J E_H$

7 maturation: $\dot{p}_H = (1-\kappa)\,\dot{p}_c - \dot{p}_J$ if $E_H < E_H^p$ else $\dot{p}_H = 0$

8 reproduction: $\dot{p}_R = (1-\kappa)\,\dot{p}_c - \dot{p}_J$ if $E_H = E_H^p$ else $\dot{p}_R = 0$

Figure 8.5. Energy flux scheme of the standard DEB model and model equations (modified from Kooijman, 2010). Boxes: variables. Arrows: energy fluxes in J d^{-1}. The equations for each flux can be found below. Grey circle: metabolic switch associated with puberty: the individual stops allocating towards maturation and starts allocating towards puberty. *E*: reserve (J), *V*: volume of structure (cm^3), E_H: cumulated energy invested in maturation, and E_R: cumulated energy invested in reproduction. The energy fluxes are functions of the model parameters which can be found in table 1.

M. leidyi is characterized, along with a variety of other species, by a so-called metabolic acceleration during ontogeny, which means that the embryo and early juvenile stages develop more slowly than later stages (Kooijman, 2014). *M. leidyi* was found to begin to accelerate its metabolism sometime after hatching at maturity level E_H^S $\mathrm{E_H^S}$. The end of the acceleration was found to coincide with the end of the transitional stage defined in the model as: $E_H = E_H^j$ (Augustine *et al.*, 2014). Metabolic acceleration is defined as an increase in energy conductance and surface-area specific assimilation during that phase; this acceleration is implemented in the model by applying a shape coefficient $(V/V_s)^{1/3}$ where V_s is the structure at the onset of acceleration to both of the parameters designated with an asterix in Table 8.1.

Food uptake is taken proportional to organism surface area and is converted into reserves with a constant efficiency. A fixed fraction $\kappa\,\dot{p}_c$ of reserve is mobilised towards growth and somatic maintenance while the remaining fraction $(1-\kappa)\,\dot{p}_c$ is mobilised towards maturity maintenance plus maturation (in embryos and juveniles) or reproduction (in adults). Somatic

maintenance has priority over growth, and hence, growth ceases when $\kappa\, \dot{p}_c$ no longer suffices to cover somatic maintenance.

Setup and Application

The DEB model and parameters presented in Augustine *et al.* 2014 (see Table 8.1) were used to simulate effects of food and temperature on key life history traits of *M. leidyi*. Food and temperature are treated as forcing variables; reproduction, mass and timing of stage transitions are model output.

We performed two original simulation experiments. In the first experiment we simulated juvenile stage duration and reproduction rates as function of temperature for three different levels of constant food availability. In the second experiment we simulated the change in reproduction rates for organisms of three different size classes subject to time varying temperature and food availability. We extracted the temperature and the (juvenile and adult) food densities experienced by a particle in the GETM model. Note that food density from the GETM model was converted from mgC m^{-3} to molC L^{-1} for input into the DEB model.

Food availability for an individual is quantified by the scaled functional response f which relates ingestion to food density in the environment, X:

$$f = \frac{X}{K+X} \qquad (13)$$

$0 < f < 1$ where 0 reflects starvation and 1 optimal food conditions (feeding ad libitum). K (mol C L^{-1}) is the half saturation coefficient where $K = \frac{\{\dot{J}_{XAm}\}}{\{\dot{F}_m\}}$ (L d^{-1} cm^{-2}) is the surface area specific food searching rate. $\{\dot{J}_{XAm}\}$ (mol d^{-1} cm^{-2}) is the maximum surface-area specific ingestion rate. Assuming a digestion efficiency of κ_X = 0.8, and that food has a chemical potential of μ_X= 525 kJ mol^{-1}, we can relate $\{\dot{J}_{XAm}\}$ to the maximum surface-areas specific assimilation rate $\{\dot{p}_{Am}\}$ (a model parameter, see Table 8.1) by the following relationship: $\{\dot{J}_{XAm}\} = \{\dot{p}_{Am}\}/\kappa_X/\ \mu_X$. Thus, K is a very context-specific parameter because it both reflects the capacity of the organism to search for prey, the food quality of the prey and the intrinsic maximum assimilation capacity of the individual. For the purpose of this study we assumed that $\{\dot{F}_m\}$ = 4 L d^{-1} cm^{-2}. Laboratory experiments have shown that Mnemiopsis can exert important behavioural control over feeding rates (Reeve *et al.*, 1978) and feeding rates do not necessarily saturate as function of prey density. To simplify the model we did not extend eqn 13 to consider effects of behaviour on the process of feeding.

All rates and ages were corrected for the effect of temperature using an Arrhenius type relation that describes the rates $\dot{k}(T)$ at ambient temperature, as follows:

$$\dot{k}(T) = \dot{k}(T_1) * e^{\left[\frac{T_A}{T_1} - \frac{T_A}{T}\right]} \qquad (14)$$

where T is the ambient temperature (K), T_A the Arrhenius temperature (K) and T_1=293 is the reference temperature (K). This relationship assumes that the temperature experienced by the organism is within its tolerance range. Below or above that tolerance range physiological performance starts to be negatively impacted (Kooijman 2010), but we do not account for this here.

The weight of the organism is computed as the sum of the weights of E and V. We convert volumes and energy to carbon mass using a carbon density of 0.0015 gC cm^{-3} V, elemental frequencies C:H:O:N taken to be 1:1.8:0.5:0.15 and assuming a chemical potential of E, μ_E = 550 kJ mol^{-1} (Lika et al 2011), see Augustine *et al.* (2014) for a motivation of the choices for these constants and ratios. Age and size at onset of acceleration, end of acceleration, first reproduction are evaluated by integrating over maturity. Reproduction rates R are given by $R = \kappa_R \dot{p}_R / E_0$ where E_0 is the initial energy content of an egg. $\dot{p}_R$ is specified in Figure 8.5 (row 8) and κ_R is the reproduction efficiency (see Table 8.1).

Results

Estuaries (Delft model)

The results of all the monthly Eastern Scheldt model runs with the Delft model using a uniform initial condition are presented in Table 2. The retention within the estuary ranged from 56% to 66% (60% on average), while on average only 10% of the particles remained in the estuary mouth. The connectivity with the Western Scheldt was low, 2% on average. No clear seasonal pattern was found.

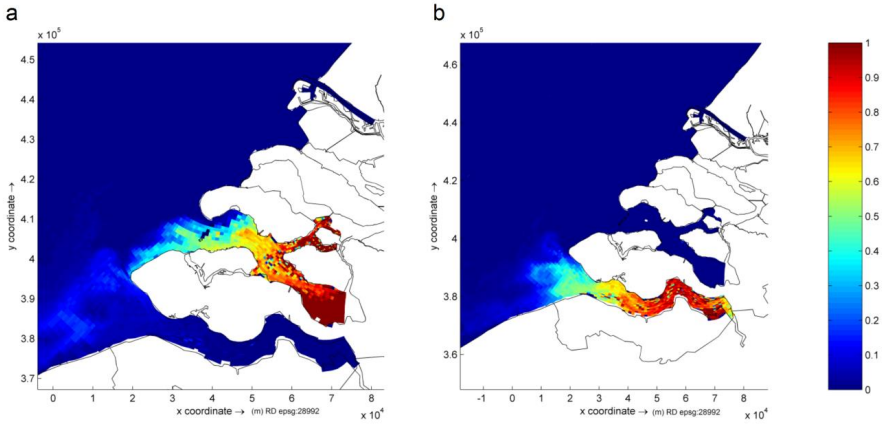


Figure 8.6. a) Final concentration of particles (N. m^{-3}) relative to an assumed initial concentration of 1.0 (N. m^{-3}) for the Eastern Scheldt July simulation, Delft model; b) similar for the Western Scheldt.

The final depth-averaged concentration pattern (m^{-3}) for the Eastern Scheldt July simulation is given in Figure 8.6. The concentration was calculated by counting the particles within a hydrodynamic grid cell, dividing by the volume, averaging over all cells in the vertical, and

scaling relative to an assumed initial concentration of 1.0 m^{-3}. Deep in the estuary the concentrations were still close to 1.0 m^{-3} and no exchange had happened.

Table 2. Percentage of particles from the Eastern Scheldt estuary per area after 30 days.

	Eastern Scheldt	Western Scheldt	Eastern Scheldt mouth	Western Scheldt mouth	Zeebrugge area	Rest North Sea
Jan	62.72	0.32	14.78	3.20	0.00	18.98
Feb	63.72	2.49	13.40	3.76	0.12	16.52
Mar	55.57	2.43	12.91	13.50	0.29	15.30
Apr	62.66	2.90	9.58	10.67	0.32	13.88
May	61.62	3.41	5.54	11.14	0.38	17.92
Jun	57.59	2.32	11.64	12.63	0.17	15.66
Jul	58.64	2.06	8.33	14.96	0.27	15.73
Aug	59.95	1.57	10.92	12.06	0.01	15.49
Sep	62.40	3.55	7.04	15.01	0.46	11.54
Oct	59.81	2.56	8.84	15.16	0.03	13.60
Nov	61.22	2.50	6.94	18.30	0.24	10.80
Dec	61.68	2.66	5.25	14.00	0.27	16.10

The results of all of the monthly Western Scheldt model runs using a uniform initial condition are presented in Table 3. The retention within the estuary ranges from 51% to 69% per month (65% on average), while on average 20% of the particles remained in the estuary mouth. The connectivity with the Eastern Scheldt was low, 2% per month on average. In general, the retention was larger in summer and autumn than in winter, due to lower river discharges. The final concentration pattern for the July run is given in Figure 8.6. Concentrations in the inner part of the estuary were reduced by fresh water inflow from the river Scheldt. The retention was negatively correlated with the river discharge ($r \leq -0.76$, $p<0.001$).

The sensitivity test for the release time showed an increased retention of 5% for the Eastern Scheldt July run and a 3% increase of retention for the Western Scheldt July run. This difference could be explained by the fact that simulations starting at low water begin with inflow, whereas simulations starting at high water begin with outflow.

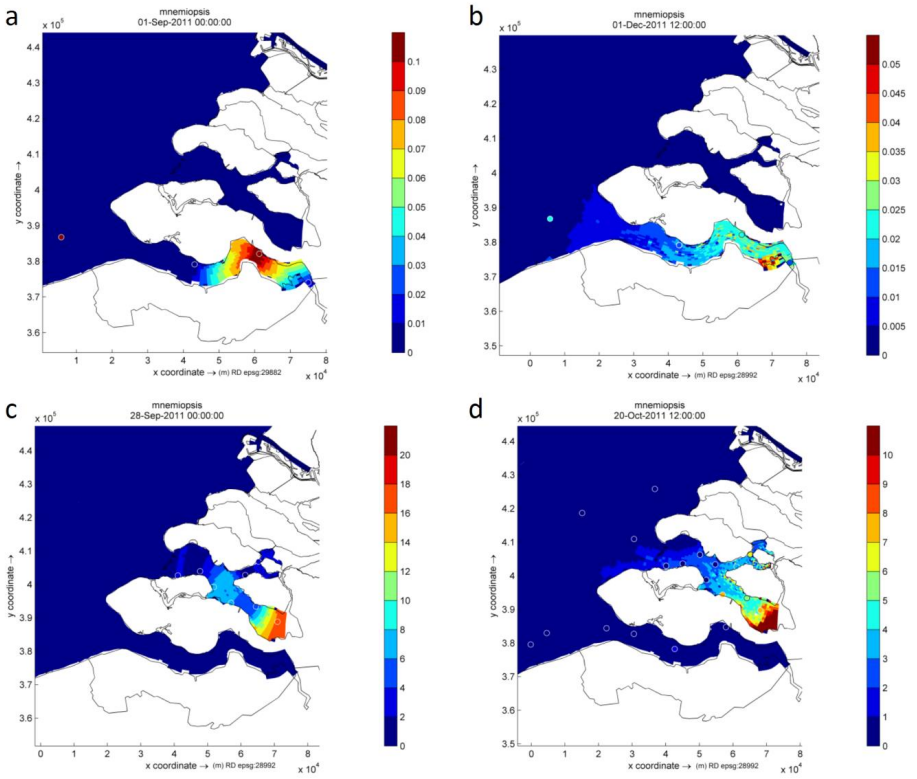


Figure 8.7. Observed density *M. leidyi* (individuals m^{-3}) in realistic runs for 2011 using the Delft model. a) and c): initial density based on field observations (circles) for Western and Eastern Scheldt, respectively. b) and d): final simulated density and field observations (circles) for Western and Eastern Scheldt, respectively.

The initial conditions and results of the realistic runs for the Western and Eastern Scheldt are presented in as contour plots of *M. leidyi* densities (ind. m^{-3}) as calculated by the model together with the observed values as coloured circles using the same scale (Figure 8.7).

For the Western Scheldt there is a good match in the middle of the estuary. At the innermost station there is overestimation of the concentration. The relative high measurement outside the estuary is not met by the model. The correlation coefficient r between the model and observations excluding the station outside the estuary is 0.28.

For the Eastern Scheldt run the model represented the conditions in the inner estuary reasonably well with some underestimation in the northern branch and some

overestimation in the south eastern branch. There is an overestimation of the concentration in the outer part of the estuary. The correlation coefficient r between the model and observations is 0.72.

Table 3. Percentage of particles from the Western Scheldt estuary per area after 30 days.

	Eastern Scheldt	Western Scheldt	Eastern Scheldt mouth	Western Scheldt mouth	Zeebrugge area	rest North Sea
Jan	1.88	57.96	2.62	15.25	0.03	22.26
Feb	0.45	64.59	7.39	12.80	0.24	14.54
Mar	1.04	50.71	5.23	28.31	0.68	14.03
Apr	0.00	66.73	3.09	18.80	0.24	11.13
May	0.00	69.13	0.00	20.60	0.38	9.90
Jun	0.04	65.69	0.61	22.49	0.18	11.00
Jul	0.06	66.78	0.20	22.80	0.33	9.83
Aug	0.77	65.84	1.41	20.08	0.07	11.82
Sep	0.04	68.75	0.08	20.70	0.49	9.93
Oct	0.28	67.14	1.04	20.19	0.16	11.20
Nov	0.55	66.32	0.22	22.61	0.46	9.84
Dec	0.03	64.76	0.16	20.88	0.50	13.65

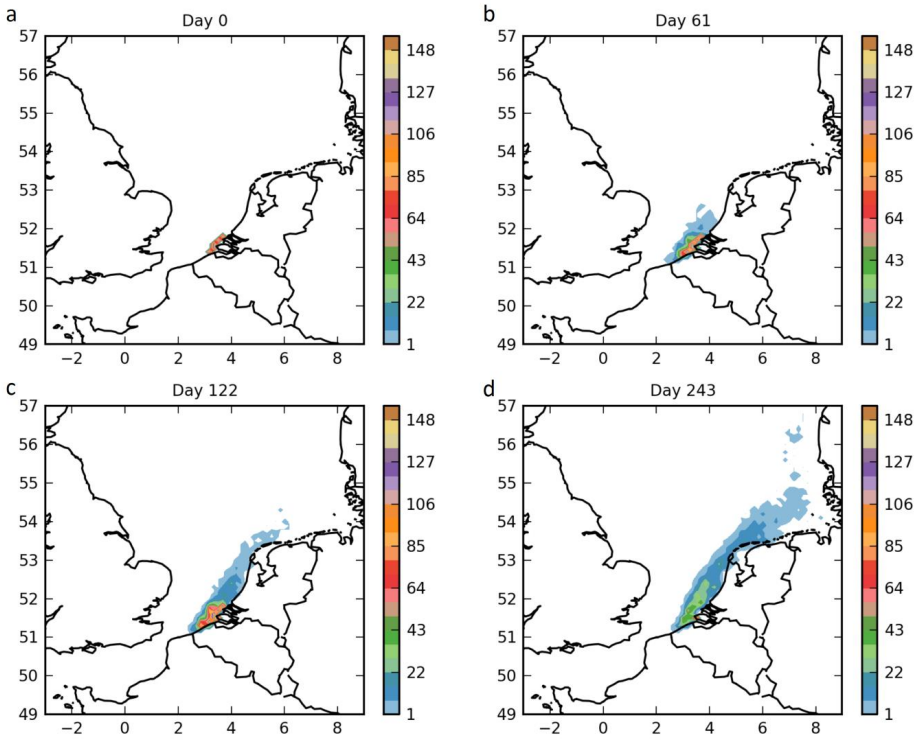


Figure 8.8. Density of particles on the model grid [number of particles per grid cell], GETM model.a) on day 1 of the simulation (1 June 2008), b) on day 61 (31 July 2008); c) on day 121 (29 September 2008); d) on day 240 (25 January 2009).

North Sea (GETM model)

The particles in the GETM model dispersed as a plume along the continental coast to the north, and to a limited extent to the south (Figure 8.8). The plume detached from the coast in the vicinity of the Dutch-German border, and continued to the north at some distance from the Danish coast. The particles that travelled furthest reached approximately the middle of the Danish west coast. The concentration of particles decreased steadily along the plume, in response to both the temporal distribution of the release and dispersion. The associated density of *M. leidyi* individuals showed a similar pattern, but with a strong reduction in densities in winter in response to adult mortality (Figure 8.9).

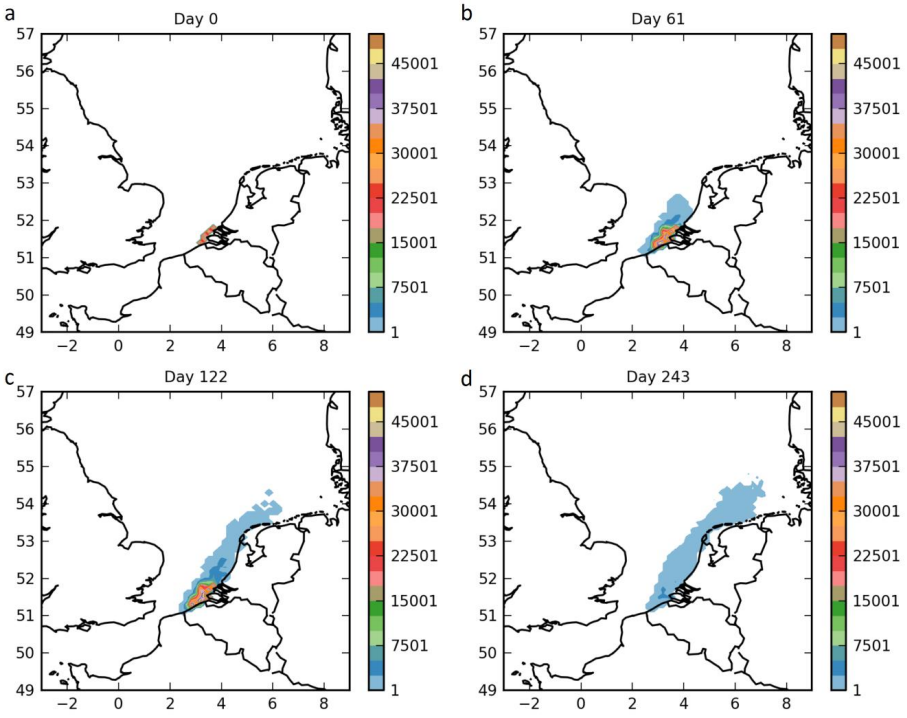


Figure 8.9. Density of simulated *M. leidyi* individuals on the model grid [number of individuals per grid cell], GETM model.a) on day 1 of the simulation (1 June 2008), b) on day 61 (31 July 2008); c) on day 121 (29 September 2008); d) on day 240 (25 January 2009).

The model run releasing particles in the rivers Seine and Somme (Figure 8.10) resulted in moderate transport to the west up to Cap de la Hague, and substantial transport along the continental coast to the north through the Strait of Dover, along the Dutch coast and into the German Bight. Enhanced concentrations were simulated off the Belgian coast, and *M. leidyi* individuals reached the German Bight, similar to the pattern obtained from releasing particles off the Dutch estuaries, but slightly further offshore. Low numbers crossed the North Sea to the UK and were found in the Thames estuary and off the coast of East Anglia. None of the particles crossed the English Channel south of the Strait of Dover.

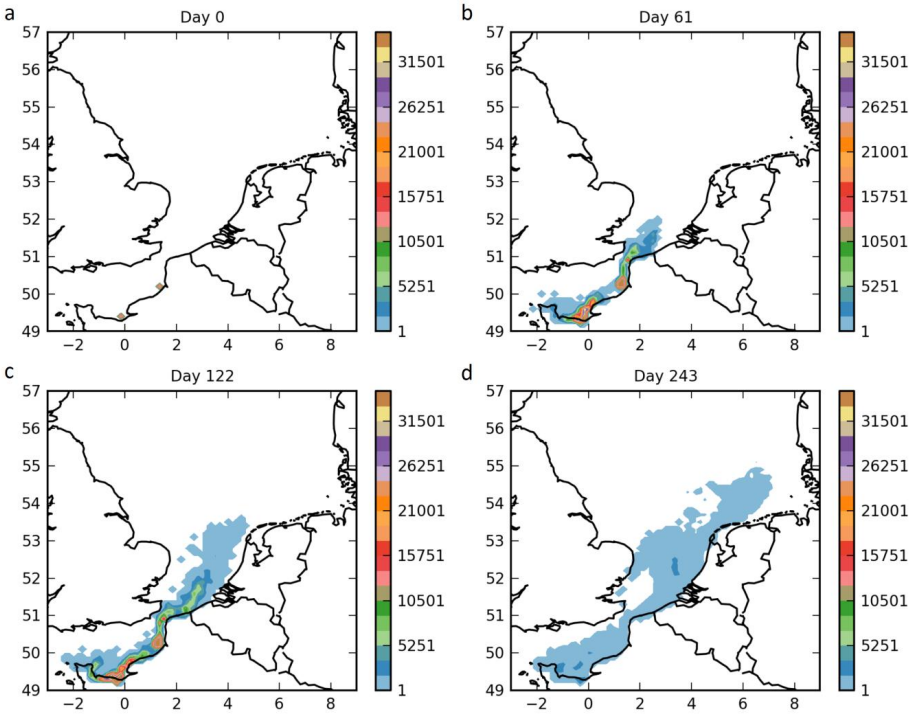


Figure 8.10. Density of simulated *M. leidyi* individuals on the model grid [number of individuals per grid cell] for releases in the rivers Seine and Somme, GETM model. a) on day 1 of the simulation (1 June 2008), b) on day 61 (31 July 2008); c) on day 121 (29 September 2008); d) on day 240 (25 January 2009).

For the standard run, the total number of *M. leidyi* individuals increased steadily as particles were released, levelling out in response to the background adult mortality, and declined when starvation set in December (Figure 8.11a, dark blue line). Food abundance for juveniles and adults was high until the beginning of October, and declined to reach low winter values by December (Figure 8.11b,c). Average temperature experienced by the particles peaked at 20 °C, declining to winter values of 4 °C (Figure 8.11h). Average salinity experienced by the particles increased until the beginning of October, consistent with reduced precipitation in summer and their transport away from the fresh-water source of the river Rhine, and decreased subsequently as river runoff increased in the autumn (Figure 8.11i). Over a million eggs were produced per hour by the population in July, August and September (Figure 8.11d, dark blue line). Roughly a third of the eggs survived to hatching (Figure 8.11e). However, due to primarily juvenile mortality (Figure 8.11f) hardly any new adults were added to the population (Figure 8.11g). An important factor for juvenile mortality as implemented here is the prolonging of juvenile duration for lower temperatures, leading to strongly reduced overall survival. The scenario run with two thirds of juvenile mortality showed some bloom potential, with new individuals contributing to population growth (Figure 8.11, green lines).

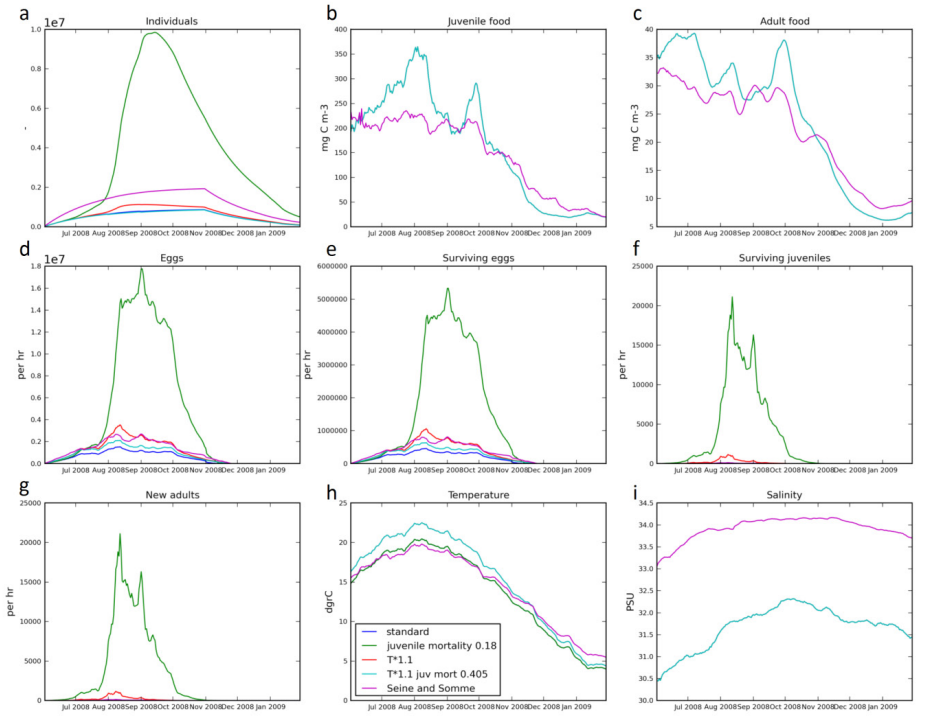


Figure 8.11. Cumulative results (GETM model) over all particles as a function of time for hindcast temperatures (dark blue), 1.1 times hindcast temperatures (red), two-thirds of juvenile mortality (green), combined 1.1 times hindcast temperatures and four-thirds of juvenile mortality (light blue), and release from the Seine and Somme (magenta). a) simulated number of *M. leidyi* individuals; b) average juvenile food concentration available to particles [mg C m^{-3}] (missing lines coincide with light blue line); c) average adult food concentration available to particles [mg C m^{-3}] (missing lines coincide with light blue line); d) total number of eggs released per hour; e) total number of surviving eggs per hour; f) total number of surviving juveniles per hour (missing lines coincide with x-axis); g) total number of adults added to the population through reproduction per hour (missing lines coincide with x-axis); h) average temperature experienced by the particles(missing lines coincide with green line); i) average salinity experienced by the particles (missing lines coincide with light blue line).

The model run in which the particles were made to experience 10% increased temperatures produced significantly different results. The maximum average temperature experienced by the particles was now approximately 22 °C, with winter temperatures nearly the same as in the reference scenario (Figure 8.11h, green line). Over 10 million eggs were produced per hour between the beginning of August and the end of September (Figure 8.11d, green line). This caused a bloom that increased the adult population at a rate far greater than the number of the additional particles that were released (Figure 8.11a). Increasing the juvenile mortality by one third for this experiment, however, prevented the bloom, and the associated model run thus yielded results very much like those of the standard run (Figure 8.11, light blue lines).

For the model run releasing particles in the Seine and the Somme (Figure 8.11, magenta lines), the mean concentration of food encountered was slightly lower. Average salinity was higher, indicating a more seaward trajectory of the particles. Egg production and survival was comparable with the standard run, considering that approximately twice as many particles were released. As for the standard run, hardly any adults were added to the population through reproduction.

DEB model

From the DEB model simulations, the age at the start and the end of metabolic acceleration as well as the age at puberty for f = 1, 0.45 and 0.3 at 22 °C are provided in Figure 8.12A (three bottom rows). These simulations show that the timing of stage transitions is extremely sensitive to the food level experienced by an individual. Indeed, f can be interpreted as the actual ingestion relative to the maximum possible one for an individual of that size. So f is a dimensionless quantifier for food level. The duration of metabolic acceleration ranges from approximately 2 weeks to a little over 1.5 months at 22 °C depending on the food history. Furthermore, the model predicted that an individual would mature even when experiencing food levels only 30 % of the maximum, but that it would take 4 times longer at that low food level than for ad libitum feeding.The adult parameter values depended on the acceleration factor given by the ratios of structure at E_H^j and E_H^s, thus food history has consequent impact on the duration of the acceleration phase, but not so much on the value of the acceleration factor which stays around 8.6 (see Table 8.1).

The predicted carbon mass at the different stage transitions for f = 1 (*ad libitum*) are also shown in Figure 8.12a (grey text). Overall, the mass at the different stage transitions is less sensitive to the prior feeding history than age. The predicted mass at the end of the acceleration phase varies from 0.11 to 0.16 mg C for f = 0.3 and 1 respectively. Carbon mass at puberty goes from 1.8 mg C for f = 1 to 0.8 mg C at f = 0.3.

The DEB model predicts that growth after puberty is extremely sensitive to food level: the predicted maximum carbon mass goes from ca. 80 mg C (f= 1) to 2 mg C at f = 0.3. Finally, the simulations showed that reproductive output was extremely sensitive to size as well as food history (compare values in Figure 8.12c and d). A 1.8 mg C individual might produce around 1500 eggs d^{-1} at 22°C (Figure 8.12c, solid line), while the 0.8 mgC individual would only produce ca. 344 eggs d^{-1} (Figure 8.12c, dotted line).

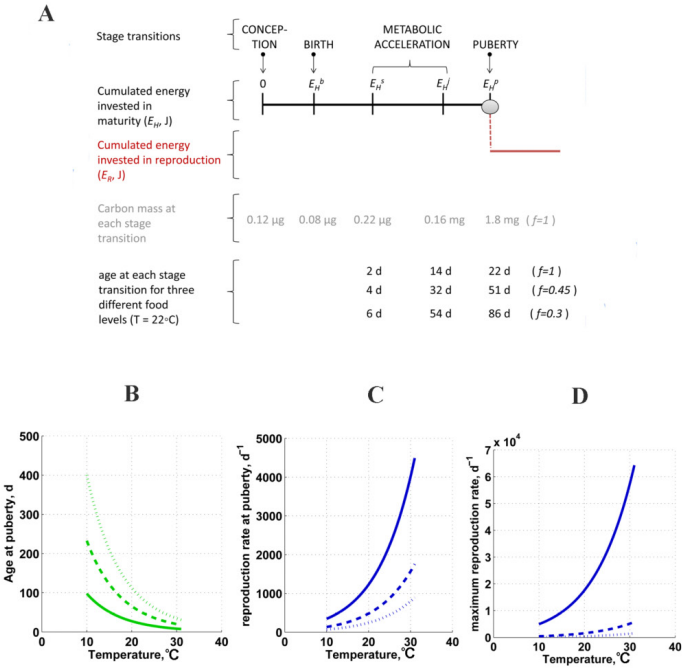


Figure 8.12. Results of the first DEB model simulation – a) Carbon mass and age at each stage transition (modified from Augustine et al 2014). The different stage transitions occur at fixed maturity levels (black horizontal line). At puberty (grey circle) the organism no longer invests in maturity and starts investing in reproduction (red horizontal line). Below, the carbon masses at each stage transition are computed for *f* = 1 (grey text). The three bottom rows show the predicted ages at the start and end of metabolic acceleration as well as the age at puberty for ingestion levels ranging from 1 to 0.3. The ages are all temperature corrected to T = 22 °C using Eqn 14. b) Age at puberty as function of temperature. (c–d) show the predicted reproduction rates at puberty (1.8 mg C) and at maximum size (80 mg C) respectively as function of temperature. (B-D) Values are computed for three different ingestion levels: *f* = 1 (solid line), *f* = 0.45 (dashed line) and *f* = 0.3 (dotted line).

At low food levels in combination with low temperatures, the organism can stay in the juvenile stage for a very long time: at 12°C and *f*= 0.3 it could take up to 300 d to reach puberty Figure 8.12b. Yet the model predicted that at abundant food and temperatures as high as 26°C reproduction would take as little as 14 d to start.

The results of the second simulation experiment are summarized in Figure 8.13(a-c). The values of the food densities and the temperature can be found in Figure 8.13a. By using the relationship (13) we obtain the scaled functional response experience by juveniles and adults (Figure 8.13b).

In Figure 8.13c the reproduction rates for adults of three size classes (2.8, 5 and 10 mg C respectively) were computed. We computed the minimum f needed for each size class to pay its maintenance and found: 0.3, 0.4 and 0.5 for the smallest to the largest individual. Assuming that the organism stops reproducing when *f* decreases below the minimum *f* to

pay its maintenance, it follows that larger individuals are more sensitive to drops in food availability. However, they also reproduce more when food is abundant enough. In summary, the model predicts rapid response to changes in reproduction as function of food level and temperature.

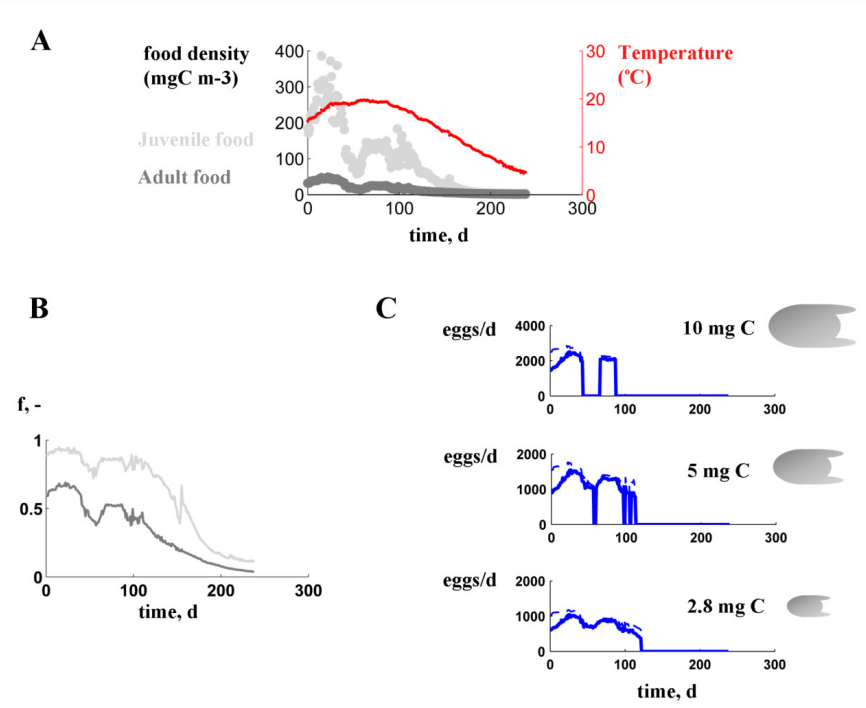


Figure 8.13. a) adult and juvenile food density in combination with temperature experienced by one particle from the GETM model. b) scaled functional response *f* (-) of the DEB model, assuming $\{\dot{F}_m\} = 4$ l d^{-1} cm^{-2} for juveniles (light grey) and adult (dark grey). c) we simulate the combined effects of temperature and ingestion level on the daily reproduction rates of a 10, 5 and 2.8 mg C individual of the DEB model. The dashed lines assume a constant temperature of 20 °C, and coincide mostly with the temperature-varying result (solid lines). For each size class there is a minimum ingestion level for which maintenance can no longer be paid. We assumed that there was no reproduction when f decreased underneath that minimum, see text.

Discussion and conclusions

Interconnectivity

Exchange between estuaries and North Sea

Growth and mortality are not included in the Delft model and might explain some of the mismatch between modelled output and field measurements. E.g., better growth conditions in the inner estuary may have caused an underestimation of the modelled numbers in the northern branch of Eastern Scheldt. On the other hand, the overestimation in the modelled numbers in the outer estuary could be explained by the model not considering mortality.

The initial model conditions were based on a small set of measurements, which do not account for potential local patchiness in density. Also, for the Eastern Scheldt, the hydrodynamics used were from a different year. The schematic runs however show little variability between months within the same year, indicating that there might be little variability between the same period in different years.

The results of the Delft model indicated that about 10-15% of the particles released in the Scheldt estuaries were exported to North Sea on monthly basis. This is enough for a substantial supply of *M. leidyi* to coastal waters of the North Sea on one hand, and on the other hand allows for sufficient retention in the estuaries to facilitate blooms and an overwintering population. The model suggested an increasing level of retention towards the landward end of the estuaries, which contributes to this mechanism. A similar process has been described in other estuaries, such as Narragansett bay, where shallow, shoreward embayments serve as winter refugia for *M. leidyi* (Costello *et al.*, 2006).

The landward (eastern side) of the Western and Eastern Scheldt, where retention of *M. leidyi* was highest in the Delft model, have very different environmental characteristics. The Eastern Scheldt is an enclosed tidal bay with salinities equal to those in the nearby North Sea in the whole area (Smaal & Nienhuis 1993), while the Western Scheldt estuary includes river inflow, resulting in a west-east salinity gradient. The area in the Western Scheldt where M. leidyi retention is highest in the Delft model is a mesohaline area (Meire et al 2005). Salinities in this area are often at or below the values for which *M. leidyi* reproduction appears to be limited (salinities <15, Jaspers et al 2011) and larval mortality is increased (salinities <10, Lehtiniemi *et al.*, 2012). This might explain why observed *M. leidyi* densities are one to two orders of magnitude lower in the western Scheldt than in the eastern Scheldt. At the start of this work, we did not have firm evidence of vertical migration behaviour by *M. leidyi*. Hence, we implemented *M. leidyi* as passive particles in the models. Since then, new evidence has emerged suggesting vertical migration behaviours (Haraldsson *et al.*, 2014). As such behaviour may influence particle dispersal pathways, this should be considered in further work.

Exchange between coastal areas

The GETM model results suggested a general south to north transport along the continental coast, in agreement with the residual flow pattern (e.g., numerical model: Prandle, 1978; radioactive tracers: Kautsky, 1973; various data: North Sea Task Force, 1993). As a result, any estuary or harbour containing an established *M. leidyi* population can, within one year, act as a source area for estuaries and harbours along the coast to the north at distances of tens to many hundreds of kilometres. For colonisation at larger distances, *M. leidyi* will need to establish a year-round population in one of the receiving coastal embayments, which can then in turn act as a source population in the following year. As a result, *M. leidyi* will be able to survive in the connected network of estuaries tens to hundreds of kilometres apart, as

long as there is intermittent winter survival in some of them each year. Although there is occasional transport of *M. leidyi* individuals over limited distances to the southwest, a solidly established, continuous population in the southernmost estuary or harbour is also likely to be required.

To our knowledge, *M. leidyi* has so far not been found in the UK. The model results suggested only minor potential for *M. leidyi* to colonise UK waters through natural transport processes from continental populations. The most likely stretch of UK coast vulnerable to colonisation appeared to be the East Anglian coastline. If such colonisation were to happen, *M. leidyi* is not expected to be able to colonise much further along the UK coast through natural transport processes, because the general residual coastal flow converges from north and south in this area, and then moves offshore across the North Sea towards Scandinavia.

Comparison of DEB model and GETM model *M. leidyi* implementation

There is a need to work with simple characterizations of metabolism when performing ecosystem level modelling. The way the biology of *M. leidyi* was implemented into particle tracking models in this study is a promising way to proceed. At this stage it is difficult to assess what would happen to the output if more complex, albeit more realistic aspects of the individual physiology (e.g. growth) were incorporated. Would such implementations pay off in terms of adding new insight?

Given the predicted plasticity in growth and juvenile stage duration, future studies should consider incorporating these processes into models designed to analyze observations that include the size structure of populations in the field. Simulation studies using ambient temperature and zooplankton biomass could be performed, where one starts with hatched eggs, to study how juvenile stage duration and condition would vary (in the absence of predation). Such results could be compared to data of the type presented by Jaspers *et al.* (2013) who recorded the size structure and abundance of early life stages of *M. leidyi* in the Baltic Sea. Mismatches between data and model might guide research aiming to understand natural mortality and food availability. The results of the GETM model suggest that mortality has a significant effect on the results, and that improved understanding and formulations of mortality are required.

The simulation studies with the DEB model demonstrate the sensitivity of the juvenile stage duration and reproduction rates to differences in food availability and temperature. In light of the predicted plasticity in growth and juvenile stage duration, future studies should consider incorporating these processes.

It is not clear to which extent the timing of the juvenile stage is realistic because there is no clear empirical evidence about how stage duration depends on different food levels, however, the values obtained here for juvenile stage duration are within the range presented in other studies: Baker and Reeve (1974) predict the timing of first reproduction

to be 13-14 days at 26 °C, Jaspers (2012) (Chapter 6, Fig 1A) show that reproduction is starting around 22 – 32 days at 19.5 °C (the DEB model with parameters in Table 1 predicts 30 days).

In previous work, Augustine et al (2014) parameterised and validated the DEB model for *M. leidyi* based on an extensive literature review of eco-physiological data. They showed, among others, that the predictions for reproduction rates and mass as function of length are in accordance with reproduction rates against length and wet mass reported in Baker and Reeve (1974), Jaspers (2012) and Kremer (1976) respectively. The new simulations presented here in Figure 8.12 and Figure 8.13 thus represent the best possible estimate of the metabolism of *M. leidyi* that we can achieve to date.

Separate juvenile and adult food densities were extracted from the biogeochemical module of the GETM model. The GETM model provided the density (in carbon) of two size classes of zooplankton experienced by the particles. Subject to a few additional assumptions to translate this information into carbon ingested per individual per unit time (see Section 2.3.2), the DEB model allowed us to uncouple the problem of effects of varying resources on the metabolism from the problem of how food availability relates to assimilation rates. In turns out that with this set of parameter values for *M. leidyi* juveniles seem to experience higher food levels relative to adults (Figure 8.13b). Moreover, the model results indicated that juveniles can maintain themselves at very low environmental food levels and can wait out the bleak season especially if temperatures are low until conditions are favorable for rapid growth and reproduction. We see from Figure 8.13c that the size structure of the population could strongly impact the dynamics of reproduction.

The value one choses for the food searching rate will also determine how much energy is assimilated by the organism. We found that $\{\dot{F}_m\} = 4$ L d^{-1} cm^{-2} provided theoretical ingestion rates within the range of those recorded by Sullivan & Gifford (2004) [table 4], and have hence assumed this value.

Uncertainties about reproduction rates further hampers finding good estimates for juvenile mortality. Still too little is known about what natural processes affect juvenile mortality in the field. And our study only exacerbates to what extent we need to know more about this.

Comparison between the two models illustrates that although there are similarities, there are also substantial differences. These differences are partly due to the values chosen for key parameters, which, at the current state of knowledge, include substantial uncertainty. They are also partly caused by the more sophisticated processes included in the DEB model. There is clearly room for improvement, for instance in the shape of a particle tracking model with particles that represent 'real' individuals through use of a DEB model for each particle, and that can spawn independent new particles as offspring. Such a model is likely to produce results that differ substantially from the current particle tracking model, and that may be

more realistic. Reducing uncertainty in parameter values through observational and laboratory studies is vital to ensure the required level of confidence in such a model.

Survival and reproduction in the North Sea

The simulations with the GETM model indicated that food levels in coastal waters in the North Sea were sufficient to sustain a *M. leidyi* population in summer and a reduced population until mid-winter. Current offshore water temperatures were too low in summer and autumn for *M. leidyi* to reproduce in large numbers. Further work is required to assess to which extent this result would hold if feedback of *M. leidyi* on food stocks were included. However as the current results suggest negligible offshore reproductive success, we expect numbers to remain low and such feedback to be limited. The presence of *M. leidyi* found near the German Bight corresponds with observations of *M. leidyi* in mid-winter in these waters on the International Bottom Trawl Survey (IBTS; ocean.ices.dk/Project/IBTS) and results from a habitat model on winter survival (David *et al.*, 2015; Antajan *et al.*, 2014). Our results, however, are subject to considerable uncertainty due to the unknown effects of (juvenile) mortality that dominate the reproduction process, and to potential adaptation to lower temperatures. In particular, production of eggs at temperatures too low for juvenile survival does not seem to make evolutionary sense, suggesting that juvenile mortality may be temperature-related, rather than constant as assumed in the GETM model. Further work is required to elucidate these issues.

Two thresholds were included in the model that, on closer inspection, are not in agreement with field observations, and that should not be used in future modelling: the lethal temperature of 2 °C for adults, and the reproduction threshold of 12 °C. The lethal temperature should not be used because *M. Leidyi* is known to overwinter under the ice in its native habitat (Costello *et al.* 2006). The reproduction threshold of 12 °C that can be inferred from Lehtiniemi *et al.* (2012) was based on field data presented by Purcell *et al.* (2001) that did not include temperatures lower than 12 °C, and is thus artificial. Lehtiniemi *et al.* (2012) also refer to Sarpe *et al.* (2007) in connection with reproduction above 12 °C, but this abstract does not contain such a threshold. We do not think that either of these two thresholds has had a significant effect on the model results, however, because i) offshore sea-water temperatures below 2 °C are very rare in the area of interest, and ii) Figure 8.13 shows that the egg production in the model falls to very low levels (in response to reductions in food-availability and temperature-driven reductions in feeding and egg-production efficiency; eq. (1)-(5)) before the average temperature experienced by the particles drops to 12 °C.

The scenario simulation with increased summer temperatures suggested that water temperature is an important limiting condition for blooms in the North Sea. The model results suggest that blooms may occur in some years as a result of interannual variability in temperature, and that such incidences may increase in frequency in the future as a result of

global warming. This result is consistent with the parameterisations in the model, and with observed reproduction behaviour in warmer seas (Shiganova *et al.*, 2001). Moreover, blooms tend to be found in estuaries, which experience higher water temperatures than the surrounding seas (Costello *et al.*, 2006a,b). The simulated blooms for the increased temperature scenario should be considered an upper estimate, as food concentrations are not impacted on by grazing of *M. leidyi* in the present model implementation. Other limiting conditions such as predation may exist as well, but these were not included in the model. Overall, taking account of the limitations of the models used, we conclude that there seems to be very limited potential for an established offshore population and large offshore blooms of M. Leidyi in the southern North Sea under normal conditions. Individuals found offshore most likely originated from estuarine populations, or resulted from minor blooms initiated by exported estuarine individuals under exceptionally favourable conditions of high local water temperatures and abundant food supply (e.g. in eddies) combined with low mortality.

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Addendum II: Larval mantis shrimp *Rissoides desmaresti* (Stomatopoda) in the Belgian part of the North Sea

Adapted from:

Vansteenbrugge, L., Van Ginderdeuren, K., Van Regenmortel, T., Hostens, K., Vincx, M., 2012. Larval mantis shrimp *Rissoides desmaresti* (Risso, 1816) (Stomatopoda) in the Belgian part of the North Sea. Belgian Journal of Zoology 142, 154-158.

The mantis shrimp *Rissoides desmaresti* (Risso, 1816) is a stomatopod crustacean (Stomatopoda: Squillidae), native to the Mediterranean Sea and the North East Atlantic from the southern North Sea to the coasts of Madeira (Portugal) (Manning, 1977; Biscoito, 1985; Lewinsohn and Manning, 1980).

Adult *R. desmaresti* are benthic and burrow in the sediment (Ramsay and Holt, 2001). They occupy sub-littoral habitats to a depth of 75-80m (Manning and Froglia, 1979) and can reach lengths up to 97mm (Herbert, 2011). Adults are fast and efficient ambush predators that use their two toothed, raptorial forelimbs (2nd thoracopods) as a spear to capture small fish and shrimps (Caldwell and Dingle, 1976). They are preyed upon by demersal fish, such as tope *Galeorhinus galeus* (Linnaeus, 1758) and bull-rout *Myoxocephalus scorpius* (Linnaeus, 1758; Griffin *et al.*, 2011; Herbert, 2011).

Figure 1 Picture of the 6th stage megalopa larva of *Rissoides desmaresti* (specimen 1)

The larvae of *R. desmaresti* (Figure 1) are planktonic, have a total body length of 3.6 to 22.5mm, and also possess strong raptorial appendices, which are mainly used to prey upon larvae and eggs of echinoderms and molluscs (Giesbrecht, 1910; Gohar and Al-Kholy, 1957).

Both adult and larval specimens of *R. desmaresti* have been reported infrequently in the southern North Sea and English Channel region (Griffin *et al.*, 2011). In Belgian waters, adults were so far never recorded (VLIZ Belgian Marine Species Consortium, 2010). However, Stomatopoda larvae were collected by G. Gilson during the European ICES (International Council of the Exploration of the Sea) campaigns between 1902 and 1913 (Gilson collection, largely preserved at the Royal Belgian Institute for Natural Sciences (RBINS) in Brussels, Belgium). Several specimens that were identified as *Erichthus* larvae were re-identified in the 1960s as larvae of *Squilla desmaresti* (van der Baan and Holthuis, 1966), nowadays renamed to *Rissoides desmaresti* (Manning and Lewinsohn, 1982). An overview of these findings is shown in Figure 2. Some other specimens could not be re-identified as they were absent from the Gilson collection, but are likely to be larvae of *R. desmaresti*. The latter are presented as '*Erichthus*' observations in Figure 2. Larvae of *R. desmaresti* have been found all over the Belgian part of the North Sea (BPNS) area. However, since the early 1900s, no more recordings of *R. desmaresti* larvae were made or could be uncovered for the BPNS, even not in more recent hyperbenthic and zooplanktonic studies performed in this area (Dewicke *et al.*, 2001; Van Ginderdeuren *et al.*, 2012a).

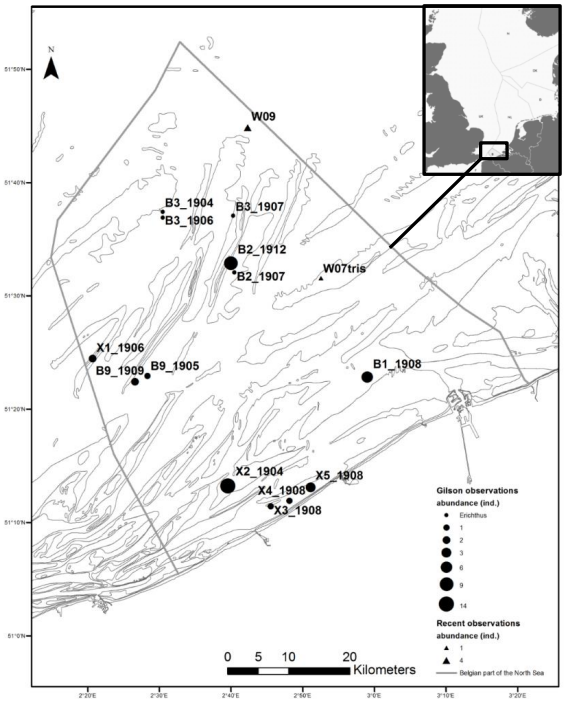

Figure 2 Spatial distribution of larval *Rissoides desmaresti* in the Belgian part of the North Sea. Triangles (▲) indicate recent observations; circles (●) indicate observations by Gilson from the early 1900s. Note: Coordinates of stations labeled 'X' are estimations based on descriptions in van der Baan & Holthuis (1966).

Almost a century later, in August and September 2011, five larval *R. desmaresti* specimens were caught during zooplankton sampling campaigns in the BPNS on board RV Zeeleeuw. Four larvae were found at monitoring station W09 (N 51°45' E 2°42') situated north of 'Hinderbanken' and one at the 'Thorntonbank' monitoring station W07tris (N 51° 31.72' E 2° 52.44') (Figure 2). A CalCOFI net (mesh size 1000µm, Ø 1m) was employed to collect the zooplankton samples. The net was trawled at a speed of approximately three knots, filtering the water column four times from surface to bottom in an undulating haul. Zooplankton samples were preserved in 4% buffered formaldehyde and analysed in the laboratory, using a stereomicroscope.

The larval morphology of *R. desmaresti* can easily be distinguished from another Stomatopoda species *Platysquilla eusebia* (Risso, 1816) that is also found in the North Sea, comparing the shape of the carapax and telson (Giesbrecht, 1910; van der Baan and Holthuis, 1966).

During larval development nine megalopa stages can be morphologically distinguished (Giesbrecht, 1910). The three specimens collected in August could be allocated to the 6th and

7th stage, the two specimens caught in September to the 8th stage. Examined identification characteristics are listed in Table 1.

Table 1 Examined identification characteristics for the five collected specimens (verified with Giesbrecht, 1910), A= antenulla, B= basis, I= ischium, T1= 1st thoracopod or 'cleaning leg', T2= 2nd thoracopod or raptorial leg, T3 – T5= 3rd – 5th thoracopod, na= not applicable, + = present, - = absent.

Characteristics	Spec 1	Spec 2	Spec 3	Spec 4	Spec 5
Location	W07tris	W09	W09	W09	W09
Date of collection	3 Aug 2011	3 Aug	3 Aug	2 Sep	2 Sep
Filtered volume (m^3)	85	242	242	483	483
Density (ind/m^3)	0.012	--------0.008--------		--------0.004--------	
Length (rostrum-telson)	10.7	11.4	10.8	17.0	17.0
# articles dorsal flagellum of	2	2	3	8	8
# articles ventral flagellum of	1	1	2	4	4
Ratio width and length	na	na	4:5	na	na
Ratio B + I of T3 and B of T2	2:7	2:7	1:2	>3:4	>3:4
Gills of T3	na	na	+	na	na
Gills of T4	na	na	+	na	na
Gills of T5	na	na	-	na	na
Gills T1 equal in size as gills	na	na	na	yes	yes
Larval development stage	6	6	7	8	8

The larvae that were re-identified from the Gilson collection were also caught in August and September, but belonged to different development stages, ranging from 2nd megalopa to postlarva stage (Table 2). The duration of larval development in *R. desmaresti* has not thoroughly been investigated yet. However, there are similarities with other Squillidae, in particular *Squilla mantis* (Linnaeus, 1758). In late autumn and winter, female mantis shrimp prepare for reproduction, but spawning only happens in spring (mid-March – mid-April) (Herbert, 2011; Giesbrecht, 1910). After a ten week incubation period (as in *S. mantis*), stage 1 megalopa larvae of *R. desmaresti* should be present in the water in June or July. Stage 8 and 9 larvae should show up in the plankton between August and October (8-12 weeks later, just as in *S. mantis*), which is consistent with our findings for *R. desmaresti* (Table 2). Hereafter, the larva undergoes metamorphosis (four postlarval stages were described by Giesbrecht, 1910), which results in a pubescent adult living in and on the sediment (duration approximately 2-3 months) (Herbert, 2011).

Table 2 Overview of larval stages of *Rissoides desmaresti* found in the Belgian part of the North Sea (M = megalopa, PL = postlarva), recent observations are marked in bold

Station	Date	Larval stages									
		M1	M2	M3	M4	M5	M6	M7	M8	M9	PL
X2_1904	29/08/1904							14			
B9 1905	25/08/1905							1			
X1 1906	23/08/1906		1				1				
B1 1908	25/08/1908						6				
X5 1908	1/09/1908									2	1
X3 1908	16/09/1908								1		
X4 1908	23/09/1908									1	
B9 1909	22/08/1909				1			1			
B2 1912	25/08/1912						9				
W07tris	**6/08/2011**						**1**				
W09_Aug	**7/08/2011**						**1**	**1**			
W09_Sept	**8/09/2011**								**2**		

It is unclear how the larvae of *R. desmaresti* arrived in the BPNS. Adult Stomatopoda have never been observed in the BPNS despite regular benthic monitoring campaigns with Van Veen grabs and an 8m shrimp trawl (mesh size 20mm in the cod end) since the late 1970s (VLIZ Belgian Marine Species Consortium, 2010). Benthic specialists were addressed, but none of them could confirm an observation in the BPNS. Yet, adults were recently observed at the east, south (including English Channel area) and west coast of the UK by divers and in beam trawl and grab samples (Ramsay and Holt, 2001; Griffin *et al.*, 2011; Martin, 2011). There are a few observations in the Dutch part of the North Sea from the early 1900s (van der Baan and Holthuis, 1966) and a few recent unpublished observations. The southern North Sea is known as the northern boundary of the distribution range for *R. desmaresti*. The northernmost sighting of an adult was offshore the Dutch Wadden islands (N 53°42' E 3°52') on 31 January 1963 (van der Baan and Holthuis, 1966).

Since dominant surface currents run in north eastern direction, larvae might be transported to the BPNS and beyond from populations in the English Channel and the south coast of the UK (Verwey, 1966). Increase in sea water temperature due to global warming might favour this larval transport and survival.

The absence of adult Stomatopoda in the BPNS is probably also related to the lack of suitable habitat. Adults require a particular sediment composition (a mixture of mud, sand and gravel) to construct a U-shaped burrow, while they avoid sites with either high mud concentration (> 70%) or sandy sediments with very low mud concentrations (≤ 2%) (Ramsay and Holt, 2001). The BPNS is characterised by mixed sediments, but only the nearshore area (overlapping with the *Abra alba* benthic community (Van Hoey, 2004; Degraer et al., 2008)) contains enough mud to construct cohesive burrows (Van Lancker, 2007). Together with disturbance by ubiquitous demersal fishing activities, the current lack of a proper gravel

concentration in the sediment mixture probably prevents the settlement of stable *R. desmaresti* populations.

In conclusion, this manuscript describes five new recordings of mantis shrimp *Rissoides desmaresti* larvae in the Belgian part of the North Sea, which are the first recordings since the early 1900s. The species *R. desmaresti* and the order Stomatopoda can now be added to the Belgian marine species list (VLIZ Belgian Marine Species Consortium, 2010). The larvae were most probably transported with the currents through the English Channel, possibly favoured by global sea water temperature increase.

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Addendum III: questionnaire to evaluate the perception of the tourism sector concerning jellyfish

Interviewer:

Location of the interview:

Date:

JELLYFISH: a socio-economic study

The Institute for Agricultural and Fisheries Research (ILVO) coordinates the international research project MEMO. Within this project, scientists study the distribution of jellyfish along the coasts of Belgium, France and England. To be able to assess the socio-economic consequences of rising numbers of jellyfish, we would like you to answer a few questions. Your answers will be treated as confidential and will stay anonymous. Thank you for your cooperation.

1/ In order to process your answers , we need some personal information.

Gender

- Male
- Female

Age

- 18 to 29
- 30 to 39
- 40 to 49
- 50 to 59
- 60+

Your connection to the coast:

- government - policy
- commercial
- Recreant/tourist

Your profession (optional):……..

QUESTIONS FOR ALL PARTICIPANTS

2/ What five words do you immediately associate with the word “jellyfish”?

1.
2.
3.
4.
5.

3/ Have you seen any jellyfish on the beach or in the water these last five years?

- Yes
- No
- I’m not sure

4/ Have you experienced an increase in the occurrence of jellyfish over the last decade(s)?

- Yes
- No
- I’m not sure

If you perceived an increase, what do you think is the magnitude of the increase? Please encircle.

- x2 x5 x10 x100 x I’m not sure

5/ You are given eight statements. Respond with "Yes", "A little" or "No":

When I (would) encounter a large number of jellyfish, I (would) feel ….

	Yes	**A little**	**No**
Calm			
Confident			
Tense			
At ease			
Cautious			
Scared			
Nervous			
Relaxed			

6/ Are you aware of the main causes of jellyfish blooms? Indicate

Yes

- Climate change
- Ocean acidification
- Overfishing and removal of natural enemies
- Rareness of turtles
- Pollution
- Invasive species (e.g. through ballast water)
- Natural patterns of jellyfish numbers

No

- Would you like to receive more information? Encircle

  YES NO

7/ Please encircle the number that best reflects your opinion. When you consider the jellyfish issue, do you think that

It does not concern you 1 2 3 4 5 it all depends on you

It only concerns you	1	2	3	4	5	it is a global problem
It is a minor issue	1	2	3	4	5	it is of great importance

8/ Who do you think will be affected most by an increase in jellyfish numbers? Please encircle one possibility.

1. The tourist industry (e.g. restaurant and camp site owners)
2. Inhabitants of the coastal areas
3. Local governments
4. The fisheries and aquaculture industries
5. Tourists and recreants

9/ According to you, what solutions to the jellyfish problem seem the most effective:

- Fishing for jellyfish and processing them into medicine, cosmetics, food…
- Remedying overfishing of jellyfish predators
- Fencing swimming zone with jellyfish nets
- Increasing waste water treatment

10/ Who should finance the implementation of such measures?

- The government
- The fisheries industry
- The tourist industry
- The whole society – through taxes

QUESTIONS FOR TOURISTS AND RECREANTS

11/ In what period do you mainly have holidays and recreation at sea?

- Equally throughout the year
- January to March
- April to June
- July to September
- October tot December

12/ What do you do at sea and on the beach?

- Walking - running
- Swimming
- Sailing - surfing
- Fishing

- Sunbathing
- Other:

13/ Would you continue your activities if you would observe large quantities of jellyfish (more than 10 on the beach or in the water)?

- Yes
- Maybe
- No

If NO: 14/ Would you continue your activities if you would be sure that the jellyfish were harmless?

- Yes
- Maybe
- No

15/ Would the presence of large quantities of jellyfish be a reason for you to change your holiday destination or recreational activities?

- Yes
- Maybe
- No

16/ According to you, what would be the extent of the consequences of an increase in jellyfish numbers? Encircle the number that best reflects your estimation.

Jellyfish stings during bathing and swimming

Negligible	1	2	3	4	5	significant

Closure of beaches and swimming areas

Negligible	1	2	3	4	5	significant

QUESTIONS FOR PROFESSIONALS (COMMERCIAL)

17/ In what period do you mainly carry out professional coastal activities? In welke periode is uw professionele activiteit aan zee voor u het belangrijkst?

- Equally throughout the year
- January to March
- April to June
- July tot September

- October tot December

18/ According to you, what would be the extent of the consequences of an increase in jellyfish numbers? Encircle the number that best reflects your estimation.

Jellyfish stings during bathing and swimming

Negligible 1 2 3 4 5 significant

Clogging of intake screens at hydro-electric and nuclear power plants

Negligible 1 2 3 4 5 significant

Damage to fishing gear

Negligible 1 2 3 4 5 significant

Mortality of shellfish in aquaculture systems (mussels, oysters)

Negligible 1 2 3 4 5 significant

A decrease in the amount of commercially important fish

Negligible 1 2 3 4 5 significant

Closure of beaches and swimming areas

Negligible 1 2 3 4 5 significant

19/ For each of the given criteria, indicate how severe you think the risks of increasing jellyfish numbers will be:

Risk of decrease in revenues for fisheries and shellfish production

low 1 2 3 4 5 high

Risk of decrease in revenues from coastal tourism

low 1 2 3 4 5 high

Risk of damage to the image of coastal areas and products

low 1 2 3 4 5 high

Risk of physical damage, e.g. to fishing nets

low 1 2 3 4 5 high

QUESTIONS FOR GOVERNMENT AND POLICY ACTORS

20/ According to you, what would be the extent of the consequences of an increase in jellyfish numbers? Encircle the number that best reflects your estimation.

Jellyfish stings during bathing and swimming

Negligible 1 2 3 4 5 significant

Clogging of intake screens at hydro-electric and nuclear power plants

Negligible 1 2 3 4 5 significant

Damage to fishing gear

Negligible 1 2 3 4 5 significant

Mortality of shellfish in aquaculture systems (mussels, oysters)

Negligible 1 2 3 4 5 significant

A decrease in the amount of commercially important fish

Negligible 1 2 3 4 5 significant

Closure of beaches and swimming areas

Negligible 1 2 3 4 5 significant

21/ At what level do you think a jellyfish increase will cause the most effect? Encirclel

Personal Local Regional Global

22/ For each of the given criteria, indicate how severe you think the risks of increasing jellyfish numbers will be:

Risk of decrease in revenues for fisheries and shellfish production

low 1 2 3 4 5 high

Risk of decrease in revenues from coastal tourism

low 1 2 3 4 5 high

Risk of damage to the image of coastal areas and products

low 1 2 3 4 5 high

Risk of physical damage, e.g. to fishing nets

low 1 2 3 4 5 high

23/ At what policy level do you think priority actions should be taken to counteract increasing jellyfish numbers?

- Local
- Regional
- National
- Global

Thank you very much for your cooperation!

Addendum IV: Risk assessment of *Mnemiopsis leidyi* using the Harmonia+ protocol

a01. Provide the name(s) of the assessors: Lies Vansteenbrugge

a02. Provide the name of the organism under assessment: *Mnemiopsis leidyi*

a03. Define the area under assessment: southern North Sea with a focus on the Belgian part of the North Sea (BPNS) including the coastal and offshore areas, the coastal ports along the Belgian coast, and the Westerschelde estuary

a04. The Organism is: alien to, and established within The Area's wild

a05. This assessment is considering potential impacts within the following domains: the environmental domain

Question	Weight	Answer	AValue	Confidence	CValue
introduction					
a06. The probability for The Organism to be introduced into The Area's wild by natural means is:	1	high	1	high	1
a07. The probability for The Organism to be introduced into The Area's wild by unintentional human actions is:	1	medium	0.5	medium	0.5
a08. The probability for The Organism to be introduced into The Area's wild by intentional human actions is:	1	low	0	high	1
establishment					
a09. The Area provides ... climate for establishment of The Organism.	1	optimal	1	high	1
a10. The Area provides ... habitat for establishment of The Organism.	1	optimal	1	high	1
spread					
a11. The Organism's capacity to disperse within The Area by natural means is:	1	high	0.75	high	1
a12. The Organism's frequency of dispersal within The Area by human actions is:	1	low	0	medium	0.5
environmental impact					
a13. The Organism has a(n) ... effect on native species, through predation, parasitism or herbivory:	1	high	1	medium	0.5
a14. The Organism has a (...) effect on native species, through competition:	1	medium	0.5	medium	0.5
a15. The Organism has a(n) (...) effect on native species, through interbreeding:	1	no / very low	0	high	1
a16. The Organism has a (...) effect on native species, by hosting pathogens or parasites that are harmful to them.	1	very low	0	medium	0.5
a17. The Organism has a (...) effect on ecosystem integrity, by affecting its abiotic properties.	1	low	0	high	1
a18. The Organism has a (...) effect on ecosystem integrity, by affecting its biotic properties.	1	medium	0.5	medium	0.5
plant impact					
a19. The Organism has a(n) (...) effect on plant targets, through herbivory or parasitism.	n/a	inapplicable	n/a	high	1
a20. The Organism has a(n) (...) effect on plant targets, through competition.	n/a	inapplicable	n/a	high	1
a21. The Organism has a(n) (...) effect on plant targets, by interbreeding with related organisms or with the target itself.	n/a	inapplicable	n/a	high	1
a22. The Organism has a (...) effect on plant targets, by affecting the cultivation system's integrity.	n/a	unanswered	unanswered	unanswered	unanswered
a23. The Organism has a(n) (...) effect on plant targets, by hosting pathogens or parasites that are harmful to them:	n/a	inapplicable	n/a	high	1
animal impact					
a24. The Organism has a(n) (...) effect on individual animal health or animal production, through predation or parasitism.	n/a	inapplicable	n/a	high	1
a25. The Organism has a (...) effect on individual animal health or animal production, by having properties that are hazardous upon contact.	n/a	unanswered	unanswered	unanswered	unanswered
a26. The Organism has a(n) (...) effect on individual animal health or animal production, by hosting pathogens or parasites that are harmful to them.	1	very low	0	medium	0.5
human impact					
a27. The Organism has a(n) (...) effect on human health, through parasitism.	n/a	inapplicable	n/a	high	1
a28. The Organism has a (...) effect on human health, by having properties that are hazardous upon contact.	n/a	unanswered	unanswered	unanswered	unanswered
a29. The Organism has a(n) (...) effect on the health of human targets, by hosting pathogens or parasites that are harmful to them.	n/a	inapplicable	n/a	high	1
other impact					
a30. The Organism has a (...) effect on causing damage to infrastructure.	1	medium	0.5	low	0
services					
a31. The Organism has a (...) effect on provisioning services.		moderately negative	0.25	medium	0.5
a32. The Organism has a (...) effect on regulation and maintenance services.		moderately negative	0.25	medium	0.5
a33. The Organism has a (...) effect on cultural services.		neutral	0.5	medium	0.5
climate					
a34. INTRODUCTION - Due to climate change, the risk for The Organism to overcome geographical barriers and -if applicable- subsequent barriers of captivity or cultivation will (...).		increase moderately	0.75	medium	0.5
a35. ESTABLISHMENT - Due to climate change, the likelihood for The Organism to overcome survival & reproduction barriers will (...).		increase significantly	1	medium	0.5
a36. SPREAD - Due to climate change, the risk of The Organism to overcome dispersal barriers & (new) environmental barriers within The Area will (...).		increase significantly	1	medium	0.5
a37. IMPACTS: ENVIRONMENTAL TARGETS - Due to climate change, the consequences of The Organism on wild animals and plants, habitats and ecosystems will (...).		increase moderately	0.75	medium	0.5
a38. IMPACTS: PLANT TARGETS - Due to climate change, the consequences of The Organism on cultivated plants (e.g. crops, pastures, horticultural stock) will (...).		not change	0.5	high	1
a39. IMPACTS: ANIMAL TARGETS - Due to climate change, the consequences of The Organism on domesticated animals (e.g. production animals, companion animals) will (...).		not change	0.5	medium	0.5
a40. IMPACTS: HUMAN TARGETS - Due to climate change, the consequences of The Organism on humans will (...).		not change	0.5	medium	0.5
a41. IMPACTS: OTHER TARGETS - Due to climate change, the consequences of The Organism on targets not considered in previous modules will (...).		not change	0.5	medium	0.5

Summary

module	score	aggregation method	weight	confidence
introduction score	0.5	arithmetic	1	0.833
establishment score	1.0	arithmetic	1	1.0
spread score	0.375	arithmetic	1	0.75
environmental im. score	0.333	arithmetic	1	0.667
plant im. score	n/a	arithmetic	1	n/a
animal im. score	0.0	arithmetic	1	0.5
human im. score	n/a	arithmetic	1	n/a
other im. score	0.5	arithmetic	1	0.0
invasion score	0.572	geometric		
impact score	0.500	maximum		
overall risk score:	0.286			

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