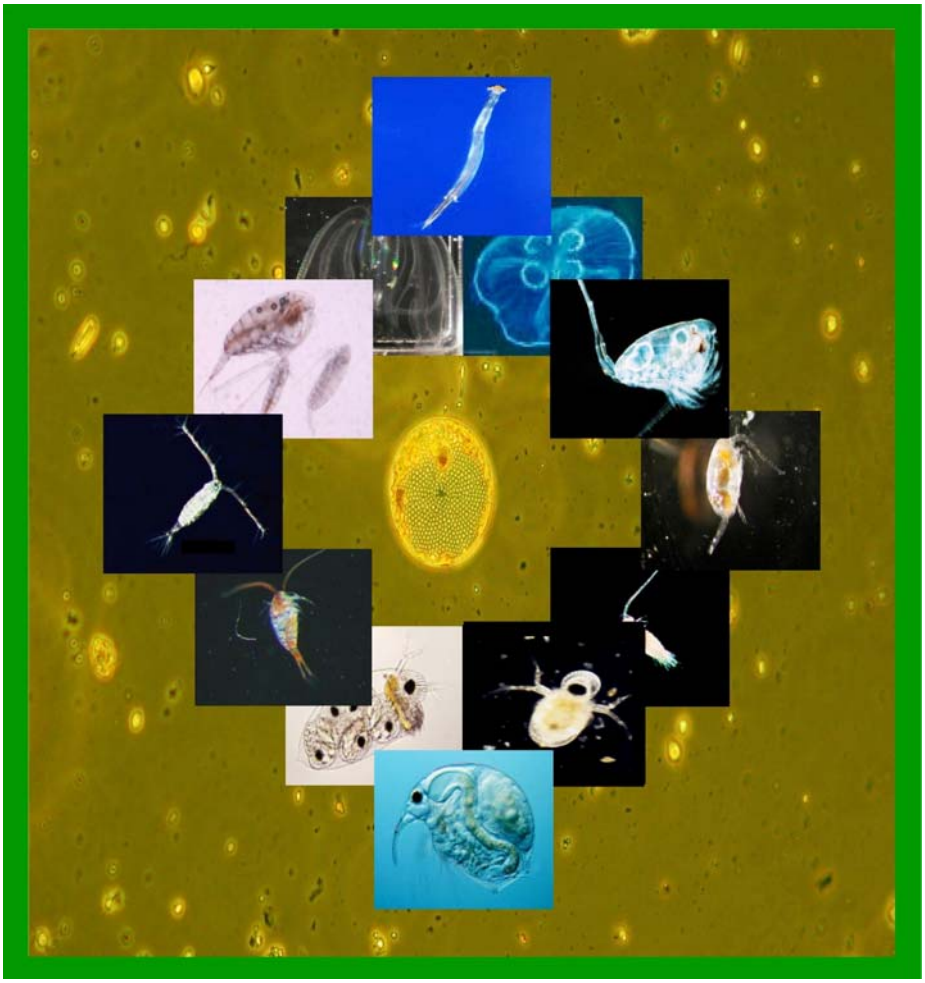
Assessing mesozooplankton trophic levels in the Baltic Sea and North Sea: A stable isotope study



by

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Kiel
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Assessing mesozooplankton trophic levels in the Baltic Sea and North Sea: A stable isotope study

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Contents

Zusammenfassung

Seit Jahrzehnten untersuchen Ökologen die trophischen Interaktionen in aquatischen Systemen und haben traditionell die Struktur des Nahrungsnetzes an Hand von Mageninhaltsanalysen dominanter Gruppen beschreiben. Unsere Sichtweise dieser Interaktionen hängt jedoch stark von der Erfassung trophischer Beziehung innerhalb des „microbial loop“ und der Fehlerquellen direkter Nahrungsanalysen (z. B. den Unterschieden zwischen der Verdaulichkeit verschiedener Nahrungstypen) ab. In dieser Arbeit habe ich das planktische Nahrungsnetz mithilfe einer alternativen Methodik, der Messung der Abundanzen und Fraktionierung von stabilen Isotopen, untersucht. Ich habe die natürlichen Abundanzen der stabilen Isotope von Stickstoff (δ^{15}N) und Kohlenstoff (δ^{13}C) gemessen, um die Struktur des Nahrungsnetzes der Mesozooplanktongemeinschaften in der Zentralen Ostsee und der Nordsee zu erfassen. Dabei habe ich mich auf die räumliche und zeitliche Variabilität des trophischen Niveaus ($\Delta\delta^{15}$N and $\Delta\delta^{13}$C) dominanter Mesozooplanktonarten in diesen beiden marinen Systemen konzentriert. Die Messung der Stabilen Stickstoff- und Kohlenstoffisotope stellt ein wirkungsvolles Instrument zur Ermittlung des trophischen Niveaus eines Organismus sowie zur Erfassung des Kohlenstoffflusses zu Konsumenten in Nahrungsnetzen dar. Allerdings ist alleine die isotopische Signatur eines Konsumenten ohne eine angemessene Definition der „baseline“ für die Ermittlung des trophischen Niveaus oft unzureichend.

Obwohl die Verwendung von Seston als “baseline” die methodisch am einfachsten wäre, ist sie eigentlich nur dann angemessen, wenn die Mischung der einzelnen Sestonkomponenten die δ^{15}N- and δ^{13}C-Signaturen der Hauptfutterquellen widerspiegeln und wenn Mesozooplankter nicht selektiv fressen. Wenn aber Mesozooplankter selektiv fressen und sich diese taxonomischen Gruppen isotopisch unterscheiden z.B. Phytoplankter, Ciliaten, Bakterien, würde die Verwendung von Seston für die Berechnung des trophischen Niveaus von Zooplanktern in die Irre führen. Dieser Umstand und die zeitliche Entkopplung zwischen den isotopischen Signaturen des Sestons und der Mesozooplankter erschwert die Berechnung des trophischen Niveaus von Zooplanktern. Daher habe ich die Cladoceren, die vorwiegend herbivor sind, (*Evadne nordmanni* und *Bosmina coregoni*) als „baseline“ bzw. Referenzorganismen verwendet. Die so ermittelten trophischen Niveaus (TL) von Zooplanktern im Bornholmbecken (Kapitel IV) ließen sich in die folgenden 4 Gruppen in Reihenfolge zunehmender Trophie einteilen: *T. longicornis* (0.7 ± 1.1‰, TL=2.2) < *Podon* spp. (1.3 ± 0.7 ‰), *Acartia* spp. (1.5 ± 1.4 ‰) und *C. hamatus* (1.6 ± 1.2 ‰), alle TL~ 2.5 < *P. acuspes* (3.4 ± 1.5, TL=3) < *Sagitta* spp. (7.9 ± 2.2, TL=4.3). In einem Transekt vom Danziger Tief zum Gotland Becken im Juli 2003 (Kapitel V) konnte die

Mesozooplanktongemeinschaft in die folgenden 5 homogenen Gruppen eingeteilt werden: *T. longicornis* (0.9 ±1.0, TL=2.3) < *C. hamatus*, *Acartia* spp. (both, 1.5 ±0.8, TL=2.4) < *Podon* spp. (1.9 ±1.1, TL=2.6) < *P. acuspes* (4.6 ± 0., TL=3.4) < *L. macrurus* (5.9 ± 0.2, TL=3.7). Insgesamt zeigte sich eine ähnliche trophische Struktur des Mesozooplanktons in beiden Untersuchungsgebieten, die einen Gradienten von Herbivorie zu starker Carnivorie nahe legen. Was den Kohlenstoff betrifft, zeigten sich signifikante Unterschiede in der Anreicherung, die eine Nutzung unterschiedlicher Kohlenstoffquellen nahelegen. Die höchste trophische Anreicherung der Copepodenarten *P. acuspes* (Kapitel IV und V) und *L. macrurus* (Kapitel V) unter den Krebstieren zeigt an, dass sich diese Arten nicht nur herbivor ernährt haben könnten, sondern sich auch von kleinen heterotrophen Protisten und Copepoden oder sogar von Artgenossen bzw. koprophag ernährt haben.

Da herbivore Cladoceren während der Untersuchszeit in der Deutschen Bucht, abwesend waren (Kapitel VI), habe ich das trophische Niveau von Mesozooplanktern mit Seston als „baseline“ berechnet. Das allgemeine Muster der Stickstoffanreicherung zeigte 3 homogene Gruppen: *T. longicornis* (3.4±0.9‰, TL=2.5), Ctenophoren (4.4±2.0‰, TL=2.8) und *P. elongatus* (4.6±3.4‰, TL=2.9) < *C. helgolandicus* (5.3±3.2‰, TL=3.1), *C. hamatus* und *Acartia* spp. (both 6.0±3.1‰, TL=3.3) < *Sagitta* spp. (6.6±3.3‰, TL=3.4) und Quallen (9.2±0.4‰, TL=4.2). In den Kohlenstoffisotopenwerten zeigte sich kein signikanter Unterschied zwischen den Arten, die also eine einzige homogene Gruppe darstellten. Überraschenderweise waren die Unterschiede in den δ^{15}N (-0.9 bis 13‰) und δ^{13}C (-2 bis 9.3‰) zwischen Seston und den Copepoden ungewöhnlich groß. Hiefür kommen mehrere mögliche Erklärungen in Betracht: (i) Innerhalb der Sestonfraktion versteckten sich mehrere intermediäre trophische Gruppen des “microbial loop” bzw. Microzooplanktons, (ii) Bacterien, die gelöste organische Materie der Elbe oder Copepoden “fecal pellets” kolonisierten immobilisierten ^{15}N-reichen Stickstoff aus der Wassersäule, sodass die Copepoden insgesamt angereicherter waren, und (iii) Seston aus Süßwasserabflüssen enthält vorwiegend terrestrisches und refraktäres Material mit angereichertem ^{13}C- and ^{15}N-Gehalt, was den quantitativ geringen Anteil des von Copepoden gefressenen Phytoplanktons isotopisch verdeckten.

In Bezug auf die Haupthypothesen meiner Arbeit, ziehe ich folgende Schlussfolgerungen: (1) Die stabilen Kohlenstoff- und Stickstoffisotopsignale von Mesozooplanktern unterscheiden sich signifikant zwischen den Arten und über die Zeit, jedoch starker in der Zentralen Ostsee als in der südlichen Nordsee. (2) Die stabilen Isotopenwerte und trophische Niveaus der gleichen oder ähnlichen Art unterscheiden sich signifikant in den beiden Systemen, was wahrscheinlich eine Folge der Unterschiede in den Phytoplanktonbiomassen (Chlorophyll *a*-Gehalt) bzw. der Primärproduktion ist. (3) Das

trophische Niveau der Mesozooplankter stieg mit der Zunahme des Anteils von Mikrozooplankton an der Gesamtbiomasse. (4) Geringere δ^{15}N-Werte (^{15}N-Verarmung) des Sestons fielen mit einer Zunahme von Sommerblüten Stickstofffixierender Cyanobacterier in der Zentralen Ostsee und mit einem höheren Anteil von Detritus in the südlichenn Nordsee zusammen. (5) Saisonelle Unterschiede (Sommer/Herbst vs. Winter/Frühjahr) und Unterschiede in der vertikalen Verteilung von Seston und Mesozooplankton-δ^{15}N lassen darauf schließen, dass sich interspezifische Variabilität in den δ^{15}N-Signalen in der Ostsee teilweise durch Unterschiede in der artspezifischen vertikalen Positionierung und der damit verbundenen Verfügbarkeit unterschiedlicher Nahrungsquellen widerspiegeln. (6) Zeitliche Variabilität im Lipidgehalt von Mesozooplanktern (Kapitel VII) kann einen signifikanten Einfluss auf die Variabilität der δ^{13}C-Signale einiger Mesozooplankter in Nord- und Ostsee haben. Schließlich, trotz der Schwierigkeiten in der Interpretation isotopischer Daten hat sich die Messung stabiler Isotope als ein brauchbares Instrumentarium in der Erforschung von Nahrungsnetzbeziehung im aquatischen Ökosystem erwiesen.

Zusammenfassung

Summary

For decades, ecologists have studied trophic interaction in aquatic systems, and described the food web structure of dominant ecological groups based on gut content analyses. The conception of these interactions may, however, be biased by the lack of couplings to the microbial food web and direct errors in diet analyses (e.g. differences in digestion rate between food types). In this thesis, I examined the planktonic food web by analyzing the trophic structure (i.e. trophic levels) with an alternative technique, the abundances and fractionation of stable isotope. I used natural abundances of stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to describe the food web structure of mesozooplankton communities in the Central Baltic Sea and North Sea. I focused on assessing spatial and seasonal isotopic variation with respect to trophic levels ($\Delta\delta^{15}N$ and $\Delta\delta^{13}C$) of the dominant mesozooplankton species in both marine systems. The stable isotopes of nitrogen and carbon provide powerful tools for assessing trophic levels of and carbon flow to consumers in the food web. However, the isotopic signature of a consumer alone is not always sufficient to infer trophic level without an appropriate definition of a baseline.

Although using seston as baseline is methodologically simple, it is only suitable if the mixture of seston components reflects the $\delta^{15}N$ and $\delta^{13}C$ of the primary food source, and when mesozooplankton species feed non-selectively on seston. In contrast, if mesozooplankton species feed selectively on seston groups and these taxonomic groups are isotopically distinct (e.g. phytoplankton, ciliates, bacteria), using seston could introduce error into the assessments of mesozooplankton trophic levels. This circumstance and the frequent temporal decoupling between isotopic signatures of seston and mesozooplankton complicate the calculation of mesozooplankton trophic levels. Thus, in order to obtain an accurate trophic level indication, I used predominantly herbivorous cladocerans (e.g. *Evadne nordmanni* and *Bosmina coregoni*) as baseline reference organism. Hence, the averages of mesozooplankton nitrogen trophic enrichments and their assessed trophic level in the Bornholm Basin (Chapter IV) revealed 4 groups increasing in the order: *T. longicornis* (0.7 ± 1.1‰, TL=2.2) < *Podon* spp. (1.3 ± 0.7 ‰), *Acartia* spp. (1.5 ± 1.4 ‰) and *C. hamatus* (1.6 ± 1.2 ‰), all TL~ 2.5 < *P. acuspes* (3.4 ± 1.5, TL=3) < *Sagitta* spp. (7.9 ± 2.2, TL=4.3). In a transect from Gdansk Deep to Gotland Basin during July 2003 (Chapter V), trophic enrichment allowed species to be divided into 5 homogenous groups increasing in a similar order: *T. longicornis* (0.9 ±1.0, TL=2.3) < *C. hamatus*, *Acartia* spp. (both, 1.5 ±0.8, TL=2.4) < *Podon* spp. (1.9 ±1.1, TL=2.6) < *P. acuspes* (4.6 ± 0., TL=3.4) < *L. macrurus* (5.9 ± 0.2, TL=3.7). Overall, these results showed that the trophic structure of mesozooplankton was similar in both study areas, suggesting a gradient from herbivory to strong carnivory. In terms

of carbon, however, the data were significantly different suggesting that mesozooplankton in both areas used different carbon sources. The highest trophic enrichment of the copepods *P. acuspes* (chapter IV and V) and *L. macrurus* (chapter V) among crustaceans indicates that these species were not exclusively herbivorous, because they might feed also on small heterotrophs, other copepods or con-specifics and/or may possibly be the result of coprophagy or starvation.

Because herbivorous cladocerans were absent during the study period in the German bight, Southern North Sea (chapter VI), I assessed the trophic enrichment using seston as baseline for this ecosystem. The general pattern of nitrogen trophic enrichment allowed species to be divided into 3 homogenous groups increasing in the order: *T. longicornis* (3.4±0.9‰, TL=2.5), ctenophores (4.4±2.0‰, TL=2.8), and *P. elongatus* (4.6±3.4‰, TL=2.9) < *C. helgolandicus* (5.3±3.2‰, TL=3.1), *C. hamatus* and *Acartia* spp. (both 6.0±3.1‰, TL=3.3) < *Sagitta* spp. (6.6±3.3‰, TL=3.4) and medusae (9.2±0.4‰, TL=4.2). In terms of carbon, no differences were detected between mesozooplankton species (i.e. 1 large homogenous group). Surprisingly, there were unusually large differences in δ^{15}N (-0.9 to 13‰) and δ^{13}C (-2 to 9.3‰) between seston and copepods. Several explanations are plausible: (i) There are several intermediate trophic linkages within the microbial loop/microzooplankton food web leading from riverine detrital material to copepods, (ii) bacteria that colonize detrital riverine material or copepods feces immobilize ^{15}N-enriched N from the water column, such that copepods consuming these bacteria have higher δ^{15}N, and (iii) riverine seston is largely terrestrial and refractory to food web use, and the large quantity of this material masks a relatively minor ^{13}C- and ^{15}N-enriched riverine/estuarine/marine phytoplankton component selectively used by copepods.

According to the main hypotheses of this thesis, I conclude that (1) stable carbon and nitrogen isotope signatures of mesozooplankton differ significantly between species over time, particularly in the Central Baltic Sea, being less evident in the Southern North Sea, (2) stable isotope signatures and trophic levels of the same or congener species in both systems were not similar, likely as response to the phytoplankton standing stock (Chl *a*) and therefore to the primary production regime, (3) the mesozooplankton trophic levels increased at higher microzooplankton biomass, (4) the switch to lower δ^{15}N (^{15}N-depletion) of seston coincided with an increasing abundance of summer blooms of nitrogen fixing cyanobacteria in the Central Baltic Sea and with larger amounts of detritus in the Southern North Sea, (5) seasonal (summer-autumn versus winter- spring) and a vertical differences in seston and mesozooplankton δ^{15}N suggest, that the interspecific δ^{15}N variability in the Baltic Sea may be, in part, explained by differences in species-specific vertical position and spatial differences in the availability of different food types (6) temporal variation in

mesozooplankton lipid content (chapter VII) can significantly influence the temporal variation of $\delta^{13}C$ signatures of some mesozooplankton species from the Baltic Sea and North Sea.

Finally, although there are some difficulties in the data interpretation, stable isotope analysis has been proven to be a useful tool in elucidating the food web structure in aquatic systems.

Summary

I. General introduction

Feeding by herbivorous mesozooplankton at the base of pelagic food webs links primary production and the microbial loop to higher trophic levels. Yet, since all “herbivorous” mesozooplankton species are known to be opportunistic feeders, i. e. omnivorous to some degree (Kleppel 1993, Wiackowski et al. 1994, Sommer & Sommer 2006), depending on their feeding strategy (Greene 1988), prey size and motility (Tiselius & Jonsson 1990), turbulence (Saiz & Kioerboe 1995) and food web structure (Ohman & Runge 1994), it is often difficult to disentangle trophic relationships in pelagic food webs.

Fundamental to an understanding of the trophic structure of mesozooplankton is the knowledge of feeding relationships among species and their respective trophic levels through time and across space at the whole-community level. The trophic level concept, however, is limited by the strict use of discrete trophic levels and its limited ability to capture the complex interactions and trophic omnivory that are prevalent in many ecosystems (Paine 1988, Persson 1999, Vander Zanden & Rasmussen 1999). Trophic omnivory is defined as consumption of prey by an organism on different trophic levels (Kling et al. 1992). A primary consumer preys upon a basal resource as well as on a so-called ‘ intermediate consumer’ (Diehl & Feissel 2000). The main reason for the ubiquity of omnivory in planktonic food webs arises from scale overlap (in size) within and between functional groups (e.g. osmotrophs, phagotrophic protists and metazoan zooplankton)

Traditionally, organisms are grouped into discrete trophic levels. However the fact that most pelagic herbivores feed on several trophic levels at a time make it necessary to define the “effective” trophic level of a given species as a fractional number. One way to do so is to estimate it as the mean number of trophic links that relate a given species to basal species or baseline, i.e. species that have no prey (Yodzis 1989, Begon et al. 1996). Another approach that additionally takes into account the relative importance of these links is to define the trophic level as the mean length, weighted in proportion of energy flows, of all chains from primary producers to consumers (Yodzis 1989). Nevertheless, both ways require an enormous amount of work, and all this information will finally be aggregated into a single number representing the fractional trophic level of this species (Ponsard & Arditi 2000). Other approaches include the use quantitative gut content data and weighted average formulas to assign organisms a continuous measure of trophic level, which represents the energy-weighted mean path length leading to a consumer (Vander Zanden & Rasmussen 1999).

Recently, natural biomarkers such as fatty acids and stable isotopes have been used as an alternative tool to gut content analysis and feeding experiments. Stable isotope analysis has become an important technique for elucidating energy flow pathways through

food webs, examining trophic interactions and elucidating the trophic structure in an ecosystem (Peterson & Fry 1987, Lajtha & Michener 1994). This technique can provide a continuous measure of trophic level that integrates the assimilation of energy or mass flow through all the different trophic pathways leading to an organisms. Moreover, stable isotopes have the potential to simultaneously capture complex interactions, including trophic omnivory (Peterson & Fry 1987, Kling et al. 1992, Cabana & Rasmussen 1996).

Since isotopic abundance (δ^{15}N and δ^{13}C) in animals generally reflects that of their diet - plus some off-set due to trophic fractionation - (DeNiro & Epstein 1978, 1981), the isotopic signature of an organism can be used as an indicator of its integrated consumption at different trophic levels over time (Kling et al. 1992, Cabana & Rasmussen 1994, Ponsard & Arditi 2000). Thus, the isotopic signature of an organism does not tell which species it consumes, but directly indicates the mean number of trophic transfers that occurred between the baseline species and this organism, weighted in proportion to the flow of matter, i.e., the mean trophic level on which it feeds (Ponsard & Arditi 2000). Typically, the small isotopic difference in carbon isotopic ratios (Fig.I.1) between a consumer and its diet (0.1 – 1‰, Rau et al. 1983, Fry & Sherr 1984, Peterson & Fry 1987, Hobson & Welch 1992, France & Peters 1997) provides information on the primary energy source of the consumer, while the larger difference in nitrogen stable isotope ratios (3 – 4 ‰, Minagawa & Wada 1984, Michener & Schell 1994, Post 2002) allows for discrimination among trophic levels (Fig.I.1, Peterson & Fry 1987). Additionally, as gut content analysis is inapplicable for organisms smaller than a millimeter and since isotope signatures record material that is actually assimilated and not just ingested (Michener & Schell 1994), stable isotope analysis has become a successful tool in elucidating trophic relationships.

Although stable isotope analysis has become increasingly popular in the past decades, there are some drawbacks. Some ecosystems have multiple organic inputs and consumers (e.g. copepods) often have more than two food sources (e.g. diatoms, ciliates and detritus), which cannot always be discerned by using one or two isotope tracers (Schmidt et al. 2003). Even for a single primary carbon source such as phytoplankton, the isotope ratio can change with species composition, metabolic pathway of photosynthesis, growth rate, season, geographical region (Michener & Schell 1994) and dietary carbon content for consumer (DeNiro & Epstein 1978). Additionally, nitrogen isotope fractionation is not constant, but can vary according to dietary nitrogen content (Adams & Sterner 2000). nutritional stress (Hobson 1993), food source (Gorokhova & Hansson 1999), and species (DeNiro & Epstein 1981). Nevertheless, stable isotope analysis can be a powerful approach and has been applied successfully in the field as well as natural tracer and trophic status

survey of mesozooplankton species (e.g. Fry 1988, 1991, Kling et al. 1992, Hansson et al. 1997, Rolff 2000, Rolff & Elmgren 2000, Sommer et al. 2005).

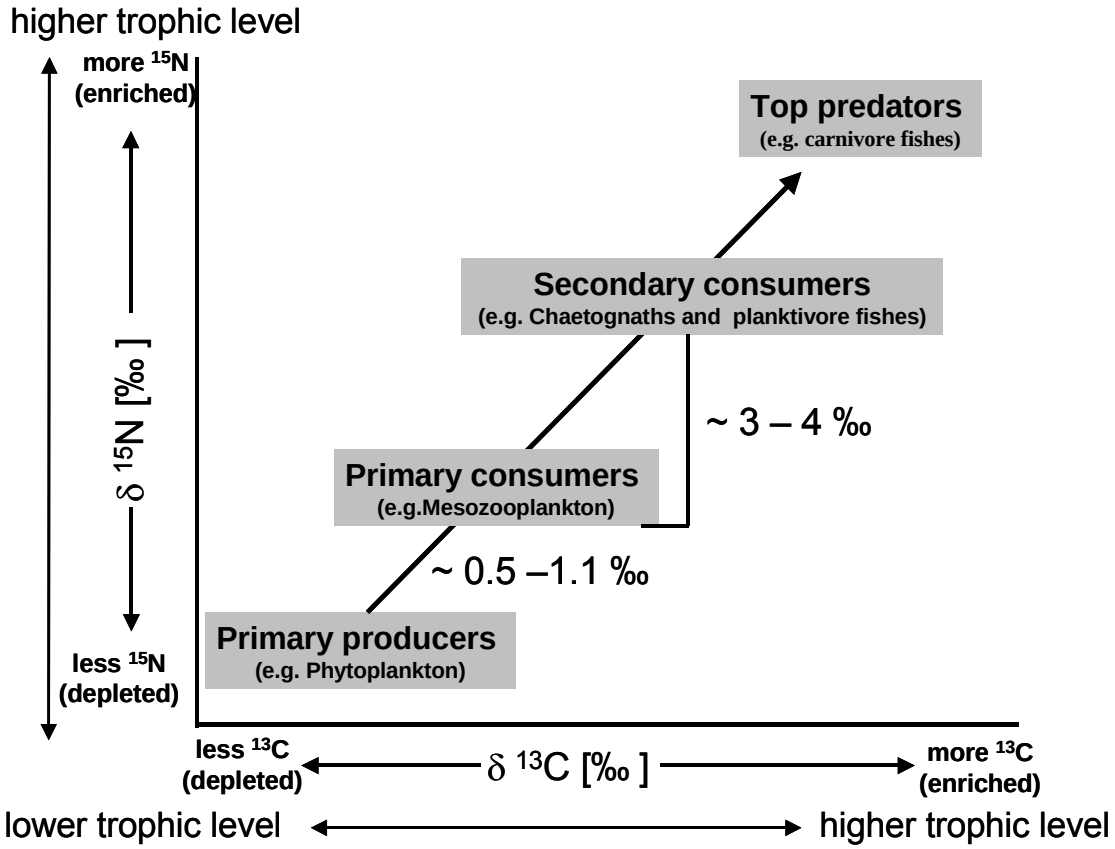

Figure I.1. Conceptual model of trophic enrichment per trophic level in the marine foodweb using stables isotope of carbon and nitrogen.

Mesozooplankton stable isotope signatures (δ^{15}N and δ^{13}C) have been assessed previously in the Baltic Sea (e.g. Hansson et al. 1997, Rolff 2000, Rolff & Elmgren 2000, Sommer & Sommer 2004, Gorokhova et al. 2005, Sommer et al. 2006) and North Sea (e.g. Knotz 2006), yet usually species are lumped into larger groups defined by size, allowing for only a poor resolution of intra-guild differences among mesozooplankton species. However, large intra-guild variation may be expected because different foraging strategies of mesozooplankton species may lead to dietary preferences for immobile (autotrophic) versus motile (often heterotrophic) prey (Tiselius & Jonsson 1990) and because these species have different vertical distribution patterns (Schmidt 2006 and references herein). Therefore, this suggests the need for a better understanding of the trophic structure at the lower trophic level of the pelagic food web in both marine systems.

Purpose and scope

The purpose of this study was to apply the stable isotopic technique to the dominant mesozooplankton species and seston in the Central Baltic Sea and North Sea, attempting to assess the trophic levels on a broad temporal and spatial scale. Six main hypotheses were tested: (1) stable carbon and nitrogen isotope signatures of different mesozooplankton species differ, (2) stable isotope signatures and trophic levels of the same or closely species in both systems are similar, (3) the mesozooplankton trophic levels increase at higher microzooplankton (e.g. ciliates) densities, (4) seston isotopic ratios are depleted at higher detritus amounts and during blooms of diazotrophic cyanobacteria, (5) seasonal and vertical patterns of mesozooplankton $\delta^{15}N$ reflect the intake of food-particles fuelled by different nitrogen sources, i.e. recycled nitrogen during thermal stratification and in the upper water layers (low $\delta^{15}N$) versus new nitrogen (excluding N_2-fixation by cyanobacteria) during spring conditions and in deeper layers (high $\delta^{15}N$) and (6) temporal changes in body composition (e.g. C:N ratio as lipid content indicator) can significantly affect the $\delta^{13}C$ of mesozooplankton.

Specifically, my goals were (i) to examine spatial and seasonal variations in mesozooplankton $\delta^{15}N$ and $\delta^{13}C$ signatures; (ii) to assess the extent and temporal consistency of trophic level differences among mesozooplankton species, (iii) to compare species-specific trophic enrichment with the species' respective foraging strategies; and (iv) to relate the observed mesozooplankton isotopic signatures to their vertical distribution patterns in the water column, (v) to compare the mesozooplankton trophic enrichment in nitrogen and carbon per trophic level relative to differences baselines (vi) to see how many trophic levels can be distinguished in the mesozooplankton community and (vii) to assess the effect of lipid content (C:N) on $\delta^{13}C$ and discuss the mesozooplankton lipid correction of $\delta^{13}C$.

Thesis outline

This thesis presents the results of isotopic analyses of seston and mesozooplankton species from the Central Baltic Sea and the Southern North Sea and discusses the use of stable carbon and nitrogen isotopes to assess mesozooplankton trophic levels. I have arranged my thesis in chapters according to general aspects (chapters I,I,III,VII and VIII) and to the study areas (chapters IV,V,VI). Chapter I gives a general introduction and Chapter II introduces and reviews terms and concepts of stable isotopes in ecology. Chapter III describes the study areas and presents the material and methods used in this study. The chapters IV,V and VI show the results of isotopic analyses from the Bornholm Basin, Gdansk Deep/Gotland Basin in the Central Baltic Sea and from the German Bight in the Southern North Sea, respectively, which include the expected outcome of the measurements and

discussion. Chapter VII presents and discusses the lipid correction of mesozooplankton $\delta^{13}C$. The findings of these chapters are summarized in the abstract at the beginning of each chapter. Chapter VIII concludes the main part of this thesis.

II. General aspects of stable isotope ecology

Overview.

Stable isotopes can be employed in two ways. First, as tracers in which reactions involving chemical elements (e.g. C and N) are followed after addition of quantities of stable isotopes (e.g. ^{13}C and ^{15}N) in various chemical forms. The second, is the investigation of the concentration variations in natural materials.

Stable isotope analysis (SIA) at natural abundance levels is an increasingly useful tool in ecological studies and can provide information in a wide variety of applications such as ecological management of terrestrial, marine and freshwater systems, the hydrologic cycle, the carbon cycle and primary productivity, the nitrogen and sulfur cycle, microbial ecology, nutrient cycling, pollution studies, precipitation analyses across broad spatial scales, fish and bird migrations, palaeoecology and terrestrial and aquatic trophic level and diet studies. This chapter gives an introduction to general aspects of stable isotope ecology.

Isotopes and their elements.

Isotopes are forms of the same element that differ in the number of neutrons in the nucleus (Fig.II.1). Extra neutrons in the nucleus of an element generally impart only subtle chemical differences. Nevertheless, an extra neutron makes the nucleus more massive or "heavier" (Fig.II.2), but does not affect most chemistry that is related to reactions in the electron shell. The real differences among elements lie in the numbers of protons and electrons. The negatively charged electrons react to form the bonds between atoms. The electrons also balance the number of positively charged protons in the nucleus. Thus the neutrons are the peacekeepers of the nucleus, keeping the highly charged, mutually repulsive protons from getting too close together (Fry 2006).

The word "isotope" comes from consideration of the periodic table of the elements, and means that isotopes of an element all occupy the same (*iso*) place (*topo*) in this table. Stable isotopes are safe isotopes that do not decay and unlike the radioactive isotopes, are not at all hazardous to human health. In fact, stable isotopes are quite abundant and natural parts of each one of us (Fig.II.3, Wada et al. 1995).

Stable isotopes often have skewed distributions on Earth, mostly reflecting details of their synthesis long ago in stars. For example, the lightest stable isotope accounts for more than 95% of all the isotopes for elements such as hydrogen (H), carbon (C), nitrogen (N), and sulfur (S) (Table II.1). But the reverse is true for some elements as boron (B) and lithium (Li) where the heavy stable isotopes are the abundant isotopes, > 80% of the total. Only a

few elements such as bromine (Br), silver (Ag), and europium (Eu) show a roughly equal, 50 – 50, distributions between light and heavy isotopes. The element tin (Sn) has the most stable isotope (10 isotopes), and there are elements such as fluorine (F) and phosphorus (P) that are endowed with only a single stable isotope.

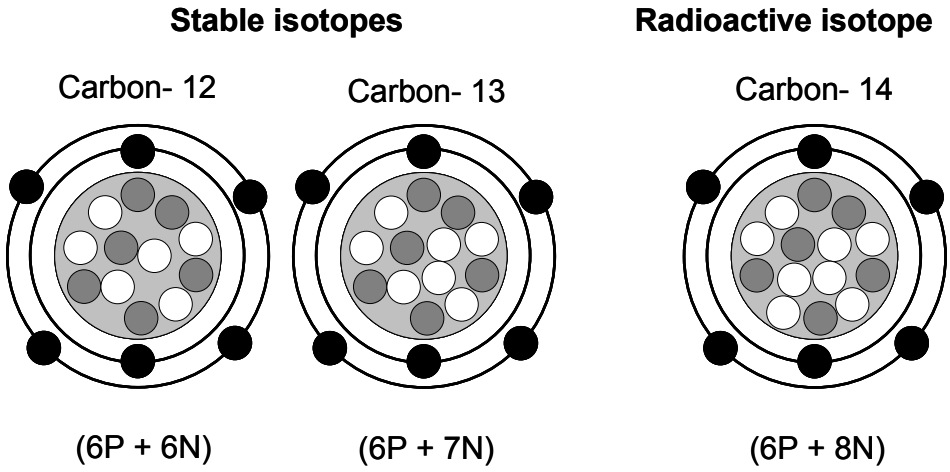

Figure II.1. Isotopes are just atoms of the same element that differ in mass number. Some isotopes are stable (e.g. ^{12}C and ^{13}C), while other are radioactive (e.g. ^{14}C)

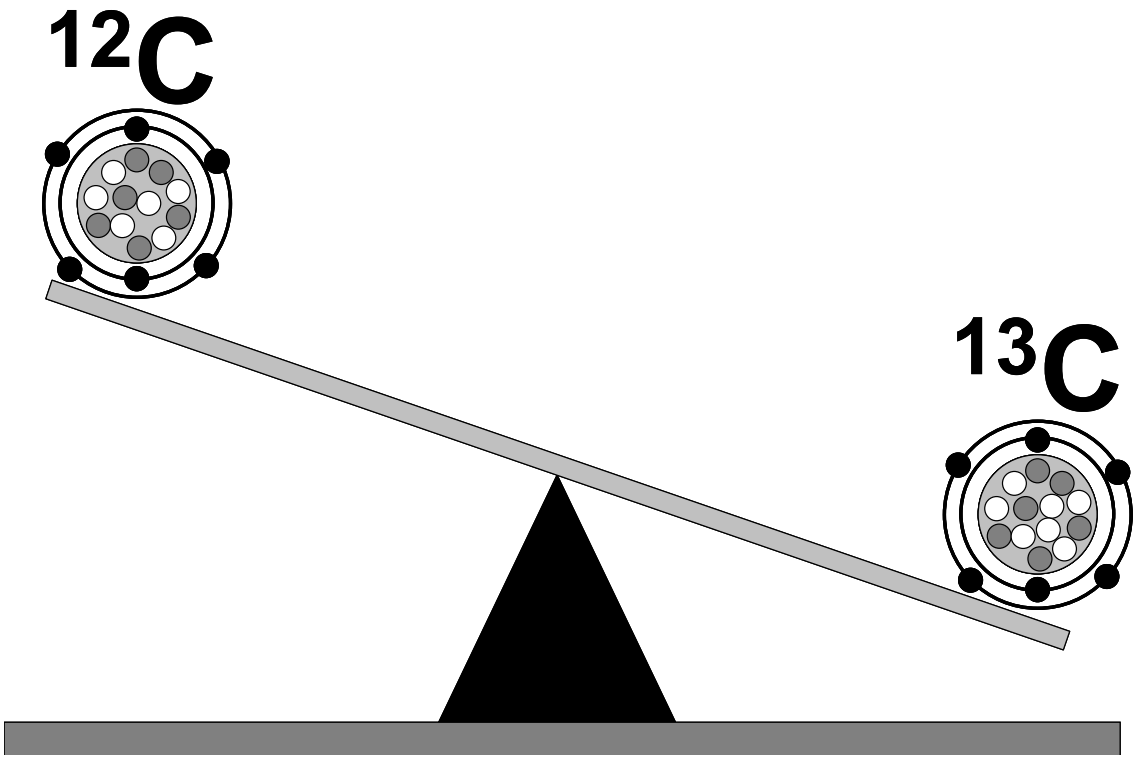

Figure II.2. ^{13}Carbon has one more neutron than ^{12}Carbon in its nucleus, therefore an extra neutron in the ^{13}C isotope makes the nucleus more massive or "heavier" than the ^{12}C isotope, but does not affect most chemistry that is related to reactions in the electron shell.

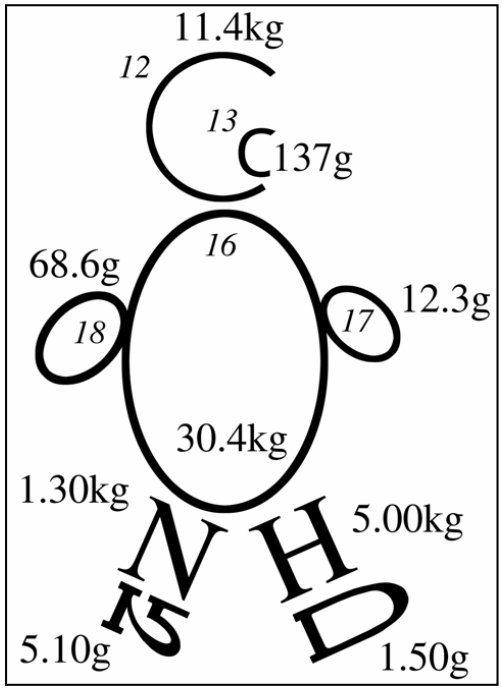

Figure II.3. You are what you eat - stable isotopes in a 50 kg human who is composed of mostly of light isotopes with a small amount of heavy isotopes. People are mostly water, so hydrogen and oxygen isotopes dominate at >35kg. Next come C isotopes at >11 kg, then N isotopes. S isotopes are missing – they should be here at about 220g for the light isotope ^{32}S and 10g for the heavy isotope ^{34}S. (from Wada & Hattori 1990).

Isotopic measurements.

Isotope-ratio analysis involves precise measurement, usually by mass spectrometry (Bowen 1988), of the more abundant light isotope relative to the less abundant heavy isotope (e.g. ^{13}C:^{12}C and ^{15}N:^{14}N) in carbon dioxide (CO_2) or nitrogen gas (N_2), generated from combustion of the sample material. This ratio is reported relative to the isotopic ratio in a reference standard (Table II.2) (Fritz & Fontes 1980, Mariotti 1983, Peterson & Fry 1987, Bowen 1988). The isotopic composition is expressed in terms of the isotopic ratio delta value (δ), defined as:

$$\delta^{H}X\ [‰] = [(R_{sample}/R_{standard}) - 1] \times 1000, \qquad \text{(II.1)}$$

In this definition, the δ notation is specified for a particular element (X = C or N), the superscript H gives the heavy isotope mass of that element (^{13}C or ^{15}N), and R is the ratio of the heavy isotope to the light isotope for the element, $^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the sample or standard.

The adoption of a comparative method for describing isotope abundance requires that a reference material (standard) be used, against which all subsequent measurements are compared. The internationally recognized primary reference material for carbon and nitrogen are Pee Dee Belemnite (PDB: $CaCO_3$) and atmospheric dinitrogen (N_2), respectively, which

Table II.1. Isotopes for Light Elements HCNOS (Hydrogen, Carbon, Nitrogen, Oxygen and Sulfur).[a]

Element	Isotope Abundance Low Mass		Isotope Abundance High Mass		Mass Difference[b] (Relative)	Range in δ[c] (‰)
Hydrogen[d]	^{1}H	99.98	^{2}H	0.016	2.00	700
Carbon	^{12}C	98.89	^{13}C	1.11	1.08	110
Nitrogen	^{14}N	99.64	^{15}N	0.36	1.07	90
Oxygen	^{16}O	99.76	^{18}O	0.20	1.13	100
Sulfur	^{32}S	95.02	^{34}S	4.21	1.06	150

[a] For each of these elements, the low-mass or "light" isotope is by far the most abundant of the isotopes, >95%. These fundamental isotope abundances prevailing on our planet Earth were determined long ago during element synthesis at the start of our universe, in interstellar space and in stars (Penzias 1979, 1980, Clayton 2003).
[b] Mass difference = high mass/low mass, e.g., 2/1 = 2 for the hydrogen isotopes.
[c] The listed range in δ values is representative for most natural samples that have not been artificially enriched with heavy isotopes (adapted from Fry 2006). δ values are the common way to express isotope abundances.
[d] Hydrogen isotopes especially are in a different class in the isotope world, with large fractionations associated with the large 2× mass difference between protium (^{1}H) and deuterium (^{2}H, or also "D").

are given an arbitrary delta value of zero. The ^{13}C and ^{15}N content of PDB and atmospheric N_2 have been found to be constant within the limits of current techniques, thus its use as a primary reference ensures that all results obtained are comparable. Most investigators also use a secondary reference material or working standard, such as NBS19 (TS-limestone), NBS22 (oil), USGS24 (graphite), IAEA-NO-3 (KNO_3), NSVEC (air), USGS32 (KNO_3), etc. The secondary reference is generally used as an internal check on consistency of sample preparation and mass-spectrometer performance.

Isotope notation.

There are actually four notations used in isotope work. These notations are: δ, R, F and AP or atom %. The absolute abundance of an isotope is described as the percentage of atoms (AP or atom %) which occur as the various isotopes. For example, approximately 0.3663% of nitrogen atoms occur as ^{15}N, the remainder as ^{14}N. Natural variations in the absolute abundance of ^{14}N and ^{15}N occur at 10^{-3} atom % level.

The definition of δ already involves 3 of these notations: δ, R, and F (see equation 1), where

$$R = {}^{H}F/{}^{L}F \qquad \textbf{(II.2)}$$

and F = fractional abundance of heavy (^{H}F) or light (^{L}F) isotope.

Table II.2. Isotope compositions of international reference standards for C and N.

Standards	Ratio, *H*/*L* [a]	Value, *H*/*L* [a]	% *H*	% *L*
PeeDee Belemnite (PDB)	$^{13}C/^{12}C$	0.011180	1.1056	98.8944
Air (AIR)	$^{15}N/^{14}N$	0.0036765	0.36630	99.63370

[a] *H* and *L* indicate heavy and light isotope components, respectively.
Source:Hayes (2002).

The δ definition involves a final multiplication by 1000 (equation 1), and this multiplication amplifies very small differences measured between samples and standards. Small differences of 1 percent become 10 permil δ units, because of the final multiplication by 1000. Thus, the δ definition makes the small neutron-related isotope differences seem large. The units of δ are "‰" or "permil" (also per mill), from Latin roots (*per mille*) for parts per thousand, just as "percent" or "%" is derived from Latin roots for parts per hundred. A sample that measures 10‰ (ten permil) is only 1% (one percent) different than the standard, and even a seemingly large 100‰ difference is still only a 10% difference.

Most δ values range between~ -100 and +50‰ for natural samples, the so-called "natural abundance" range, with the exception that δ measurements for hydrogen span a broader range. Many δ values are negative values, and these negative δ values indicate relatively less heavy isotope than is present in the standard. Standards have a δ value of 0‰, which makes sense from the δ definition because when a standard is measured versus itself, the difference will be zero. Standards contain appreciable, nonzero amounts of heavy and light isotopes (Table II.2), so that 0‰ means no difference from the standard, not "0% isotope," not "no isotope," and not "no heavy isotope."

Samples with higher δ values are relatively enriched in the heavy isotope and are "heavier." Samples with lower δ values are relatively enriched in the light isotope (least enriched or depleted in the heavy isotope) and are "lighter". This leads to the convenient

mnemonic for δ values, "higher heavier, lower lighter" (Fry 2006).

The δ definition actually contains two separate ratios (R_{Sample} and $R_{Standard}$) and a ratio-of-ratios, $R_{Sample}/R_{Standard}$. This leads many scientists to write about isotope variations in terms of ratios. Although use of the ratios has its advantages, practical use of the δ values does not involve a focus on "ratios." Instead, δ values are straightforward indicators of "% heavy isotope" because there is a simple, essentially linear relationship between δ values and isotope content. Thus, the terms "heavier" and "enriched" refer to samples that have a higher % heavy isotope and higher δ values, whereas "lighter" and "depleted" refer to samples that have lower % heavy isotope and lower δ values.

Isotope effect.

An isotope effect is associated with the majority of all biological, chemical and physical reactions involving isotopes. It is important to note the distinction between fractionation and isotope effect since although an isotope effect usually occurs, its physical manifestation, fractionation, may not be observable. For example, the principal cause of the nitrogen isotope effect is the higher vibrational frequency of bonding that occurs between the lighter isotope (^{14}N) than the heavier isotope (^{15}N). This leads to an increase in the probability of bond fission between the lighter isotopes and hence the relative reactivity of ^{14}N over ^{15}N. Most reactions involving nitrogen conform to this general model and hence the products of a reaction are invariably depleted in ^{15}N relative to the substrate.

Isotope mass balance.

Isotopic compositions expressed as δ are additive, such that the isotopic composition of the reactant or substrate must equal that of the products when summed in stoichiometric proportions. If δ is the isotopic composition and Q the mass, then the mass and isotopic balances are, respectively,

$$\mathbf{Q_r = Q_a + Q_b} \qquad \textbf{(II.3)}$$

and

$$\mathbf{\delta_r \, x \, Q_r = (\delta_a \, x \; Q_a) + (\delta_b \, x \; Q_b)} \qquad \textbf{(II.4)}$$

Equations II.3 and II.4 apply to stoichiometric chemical reactions, for example, where Q_r are the moles of N or C in a reactant or substrate, and Q_a and Q_b are the moles of N or C in the products. The equations also apply to simple mixing of two N- or C-containing materials (Qa and Qb, where Q = V x C, and V is volume and C is concentration) having different isotopic compositions (δ_a and δ_b) to produce the final mixture (Q_r) (Mariotti et al. 1981, Mariotti et al. 1988); the mixture will have an intermediate isotopic composition (δ_r)

depending on the relative contributions of added materials. Equations 3 and 4 can be combined as

$$Q_b = Q_r \times [(\delta_r - \delta_a) / (\delta_b - \delta_a)] \qquad (II.5)$$

or

$$\delta_b = [(\delta_r \times Q_r) - (\delta_a - Q_a)] / (Q_r - Q_a) \qquad (II.6)$$

Equation II.5 can be used to estimate the mass in a product (Q_b) contributing to the measured total N or C in substrate (Q_r), if isotopic compositions of N- or C-containing materials (δ_a and δ_b), and intermediate isotopic composition (δ_r) samples are known. Equation 6 can be used to estimate the isotopic composition of a product (δ_b), if the masses (Q_a, Q_r) and isotopic compositions (δ_a , δ_r) of a product and substrate, respectively, are known.

DeNiro & Epstein (1978) used a similar isotope mass balance between animals and their diets. Thus the isotopic composition of carbon which an animal eats (input) must equal the integrated isotopic composition of the carbon which is incorporated into the body and that which is lost by respiration and excretion (output). Therefore, the ^{13}C enrichment of the whole animal relative to its diet must be balanced by a ^{13}C depletion, relative to the diet, of the respired CO_2, the excreted carbon, or both.

Isotope fractionation.

The extra neutron in an atom does make a very slight difference in some reactions; having an extra neutron usually results in slower reactions. This reaction difference is fractionation. Many chemical and physical processes have a significant isotopic fractionation, which generally refers to an enrichment or depletion of the heavy isotope. The fractionation can occur during time-dependent or kinetic processes, as well as during equilibrium processes.

Fractionation during equilibrium (reversible) or disequilibrium (unidirectional kinetic reaction) processes results because atomic masses and bond strengths differ for different isotopes. Isotopic equilibrium exchange reactions involve redistribution of isotopes of an element among phases or chemical species (Coplen 1993). At isotopic equilibrium, the forward and backward reaction rates of the lighter isotopic species or molecules are equal and those of the heavier isotopic species or molecules are also equal (e.g. volatilization or dissolution of gases such as CO_2 and ammonia (NH_3). Equilibrium processes generally take place in closed or semiclosed systems.

Kinetic fractionation can result in nonequilibrium systems in which reaction rates are mass dependent. As a general rule, the lighter isotope reacts faster than the heavier isotope (e.g. evaporation or sublimation of gases, Coplen 1993). Most biologically mediated

reactions are unidirectional, resulting in isotopically heavier reactants and isotopically lighter products during the course of a reaction (Letolle 1980, Peterson & Fry 1987, Coplen 1993). Fractionation during unidirectional kinetic reaction is expressed as a ratio of reaction rates.

$$\alpha = {}^{L}K / {}^{H}K \quad \textbf{(II.7)}$$

where by convention ^{L}K and ^{H}K are reaction rates of the light and the heavy isotopes, respectively. However, several researchers place the heavy isotope in the numerator and the light isotope in the denominator (e.g. $^{H}K/^{L}K = {}^{13}C/{}^{12}C$ or $^{15}N/^{14}N$).

During a single-step, unidirectional reaction, the isotopic composition of the reactant or substrate and instantaneously formed product is a simple function of the progress of the reaction in accordance with the following Rayleigh equation (Mariotti et al. 1981, Mariotti 1983, Peterson & Fry 1987, Mariotti et al. 1988):

$$\delta_r = \delta_{r0} - \Delta_{r/p} \times \ln [C_r / C_{r0}] \quad \textbf{(II.8)}$$

where C_{r0} and C_r are the reactant or substrate concentration at time t = 0 and time t, respectively, and δ_{r0} and δ_r are the isotopic composition of the reactant at time t = 0 and time t, respectively. Δ is the isotopic discrimination of the reaction, which is related to the isotopic kinetic fractionation factor, α ($^{13}C/^{12}C$ or $^{15}N/^{14}N$ in the residual reactant divided by that in the product).

In kinetic reactions the fractionation factor is an inherent or instantaneous measure of isotopic fractionation. Nevertheless, the isotopic composition of the product can vary depending on the extent of the reaction. Thus researchers have variously defined $\Delta\delta$, often referred as Δ, isotopic discrimination or fractionation:

$$\Delta_{r/p} = (\delta_r - \delta_p) / [1 + (\delta_p / 1000)] \quad \textbf{(II.9)}$$

The equation II.9 has unit of per mil (‰), as does δ, yet refers to a difference between product and reactant rather than a value for a single compound. Also in most cases the denominator of this definition is very close to 1.0, and thus many authors have simplified the above definition of $\Delta_{r/p}$ to:

$$\Delta \cong \delta_r - \delta_p \quad \textbf{(II.10)}$$

Finally, isotopic segregation can also be expressed as the isotope enrichment factor, expressed as per mil:

$$\varepsilon = (\alpha - 1) \times 1000 \quad \textbf{(II.11)}$$

Values of ε are positive in sign when the lighter isotope reacts faster than the heavier isotope and can be closely approximated as the per mil difference between an instantaneous product and reactant (see equation II.10, Peterson & Fry 1987, Coplen 1993). Therefore, when substrate or reactant is not limiting and the expression of the isotope factor is at a maximum,

$$\Delta \approx \varepsilon \quad \textbf{(II.12)}$$

Such approximate values of ε or Δ have been determined by previous investigators to derive apparent kinetic fractionation factors α for many of the C or N transformation reactions in the environment (Letolle 1980, Hubner 1986). Thus, if the extent of the transformation reaction and the corresponding fractionation factor are known, isotopic effects from fractionation may be computed by use of a combined form of equations II.8 and II.11.

$$\delta_r = \delta_{r0} - [1000 \times (\alpha - 1)] \times \ln [C_r / C_{r0}] \quad \textbf{(II.13)}$$

The figure II.4 was constructed on the basis of equations II.8 or II.13 to show the effect of processes having fractionation factors (α) greater than 1.0, which is appropriate, for example, for most N-cycle processes (Fig. II.5B).

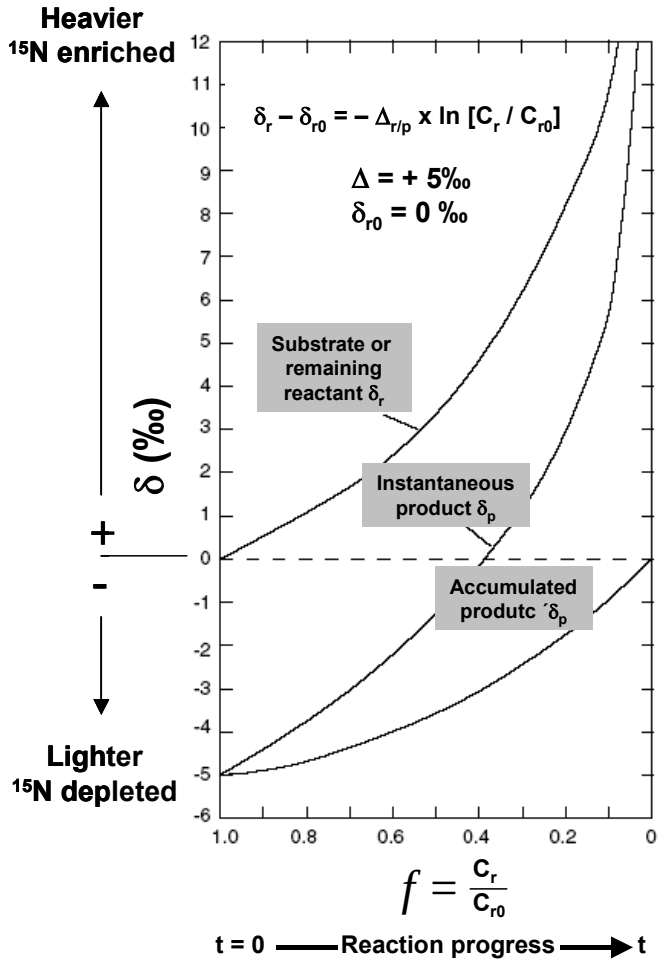

Figure II.4. Effect of kinetic fractionation on isotopic compositions of reactant and product. Curves are based on Rayleigh distillation and show theoretical evolution of isotopic compositions of components during a single-step, first-order process where the lighter isotope reacts faster than the heavier isotope ($\alpha > 1$) and f (C_r / C_{r0}) is the proportion of reactant remaining. The upper curve indicates the composition of the remaining reactant ($´\delta_r$). The middle curve indicates the composition of the instantaneous product formed (δ_p). The lower curve indicates the composition of the accumulated product ($´\delta_p$). (adapted from Fritz & Fontes 1980)

When a small amount of reactant or substrate has been converted to the product, both the accumulated and instantaneous products are depleted in the heavier isotope and have similar delta values. As the reaction proceeds, (i) the remaining reactant, instantaneous product, and accumulated product become progressively more enriched in the heavier isotope, and (ii) the per mil difference becomes larger between the remaining reactant and the accumulated product and smaller between the remaining reactant and the instantaneous product. When all the reactant is consumed, the accumulated product has the isotopic composition of the initial reactant ($\delta_p = \delta_{r0}$).

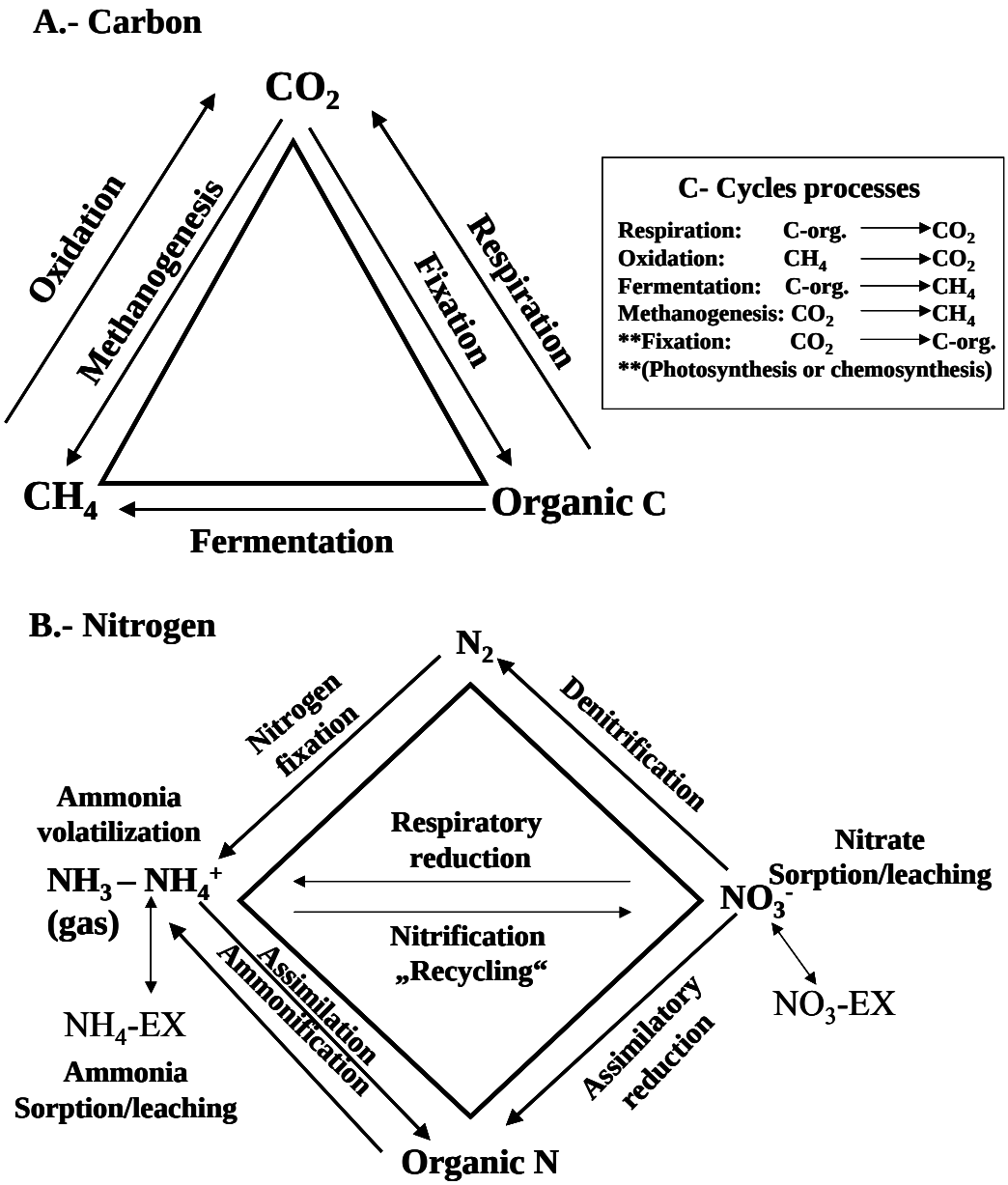

Figure II.5. Biochemical cycles of carbon (A) and nitrogen (B)

Stable isotope and biochemical cycles of C and N.

For ecologists, stable isotopes provide a natural way to directly follow and trace details of element cycling. The isotopes function as natural dyes or tracers and their use can resolve many environmental and ecological questions. The environmentally significant stable isotopes of C and N, common chemical forms, and abundance of C and N in the biosphere (e.g. atmosphere, freshwater, soils, and plants) are summarized in table II. 3. Important biochemical transformations of C and N are shown in figure II. 5.

The use of C and N isotopes to identify C and N sources is based on the concept that these elements are interrelated in the biochemical C and N cycle (Bolin & Cook 1983, Peterson & Fry 1987), and that measurable differences in the isotopic composition of C- and N-source materials will persist as C- and N-containing compounds are transported from the source. The isotopic compositions and forms of C and N in terrestrial and aquatic systems may resemble those of a nearby C and N sources. However, this isotopic composition not only reflects the composition of the original source, or of mixed sources having different compositions, but can be influenced by isotopic fractionation during the transport and chemical transformation of C and N compounds.

The most important C forms in the biosphere are gaseous CO_2 and CH_4, dissolved CO_2 (carbonate species), solid carbonate minerals, and organic compounds (Table II.3). Major biochemical C-cycle processes (Fig.II.5A) include photosynthesis and chemosynthesis, whereby CO_2 is converted into organic matter; respiration, whereby organic compounds are oxidized to CO_2; and methanogenesis or fermentation, which may be considered reduction of CO_2 to CH_4. The most important factor affecting C-isotopic compositions of natural compounds in the biosphere is the effect of absorption and photosynthesis. Additional biological mechanisms for fractionation of C isotopes include microbial decay processes, such as the formation of CH_4 during anaerobic decomposition and of CO_2 during aerobic respiration. These processes enrich the product gases in ^{12}C and can leave the organic-C reactant enriched in ^{13}C.

The most important N forms in the biosphere are N_2; dissolved nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), mineral-fixed NH_4^+ and organic-N compounds. Organic N consists primarily of amino acids and amide (proteinaceous). Major biochemical N-cycle processes (Fig. II.5B) include N_2-fixation, ammonification, nitrification, uptake or assimilation, respiratory nitrate reduction, and denitrification. Some N-cycle processes (e.g. N_2-fixation) tend to cause depletion of the heavier isotope in the products relative to the reactants or substrate (Letolle 1980, Hubner 1986). Some cumulative and large fractionations do occur in the nitrogen cycle. An isotope cumulative faster loss of ^{14}N than ^{15}N during particulate N decomposition

results in ^{15}N increases of 5 to 10‰ with increasing depth both in soils and in the oceans. Nitrification and denitrification in the sea both proceed with substantial isotope effects (Δ =10 to 40‰), and where nitrate is abundant, assimilation by phytoplankton proceeds with a smaller effect (Δ = 4 to 8‰)

Table II.3. Geochemical characteristics of carbon and nitrogen[1]

	Carbon (C)	Nitrogen (N)
Atomic number (Atomic weight, amu):	6 (12.011)	7 (14.0067)
Stable isotopes (Abundance, percent)	^{12}C (98.89) ^{13}C (1.11)	^{14}N (99.634) ^{15}N (0.366)
Common chemical forms		
Gaseous compounds	CO_2, CO, CH_4	NO_2, N_2O, N_2, NH_3
Aqueous species	$H_2CO_3^0$, HCO_3^-, CO_3^{2-}, CH_4	N_2, NO_3^-, NO_2^-, NH_4^+
Mineral compounds	$CaCO_3$, $CaMg(CO_3)_2$	KNO_3, NH_4^-EX
Organic compounds	$C_6H_{12}O_6$ = carbohydrates	$CO(NH_2)_2$ = urea
Typical abundance (ppm):		
Atmosphere	CO_2 322 to 332	N_2 780,900
Freshwater	HCO_3^-C 2 to 30	NO_3^-N 0.1 to 5
Soils	4,000 to 120,000	440 to 5,440
Plants	450,000 to 500,000	2,000 to 55,000
Isotopic composition (δ, ‰):		
Atmosphere	CO_2 −6 to −8	N_2 0
Freshwater	HCO_3^-C −15, POM −35	NO3−N +4 to +7
Soils	−18 to −31	−4 to +14
Plants	−12 to −30	−8 to +2
Ocean	POM -22, DOM -23	N_2 +1, POM -2 to +11 Deep NO_3^- +4 to +6
Isotopic standard reference:	Pee Dee Formation belemnite ($CaCO_3$)	Atmospheric gas nitrogen (N_2)
Standard abundance ratio:	$^{12}C/^{13}C$ = 88.99	$^{14}N/^{15}N$ = 272.0

[1]Sources: (Faure 1977, Fritz & Fontes 1980, Hem 1985, Peterson & Fry 1987, Coplen et al. 1992, Coplen 1993).

Stable isotopes and diet.

Stable isotope analysis has more recently been used as an alternative to standard approaches in ecology (e.g. gut contents analysis, direct observation both in the field and laboratory, and radiotracer techniques), being in some cases a more efficient tool for food web analysis.

Diet is the primary determinant of animal C- and N-isotopic compositions. Carbon isotopic compositions of animals reflect those of the diet within about 1 ‰ (Haines 1976, DeNiro & Epstein 1978, Fry et al. 1978, Fry & Parker 1979, Haines & Montague 1979, Teeri & Schoeller 1979, Rau 1980, Rau & Anderson 1981, Fry & Arnold 1982, Tieszen et al. 1983, Checkley & Entzeroth 1985, Peterson & Fry 1987, Hobson & Welch 1992, France & Peters 1997, Post 2002). Overall, there appears to be a slight (0,5 – 1 ‰) enrichment in the animal relative to its diet. There are several possible processes which might contribute to this enrichment: (i) preferential loss of $^{12}CO_2$ during respiration, (ii) preferential uptake of ^{13}C compounds during digestion or (iii) metabolic fractionation during synthesis of different tissue types (DeNiro & Epstein 1978, Rau et al. 1983, Tieszen et al. 1983, Fry & Sherr 1984). There is evidence for each case, but no general consensus.

The conservative transfer of carbon isotopic compositions (<1 ‰) to the animal from the diet can be useful in tracing food webs in system where there are food sources with large differences in $\delta^{13}C$ values, such as C_3 versus C_4 plants or marine versus terrestial systems (Fry et al. 1977, DeNiro & Epstein 1978, Fry et al. 1978, Rau & Anderson 1981, Schoeninger & DeNiro 1984). However, the researcher must also be aware of isotopic variations in different tissues within an organism, as well as the different rates of tissue turnover when an organism is selectively feeding. This can have implications for systems where there is more than one food source. Despite the seeming difficulties, there are several excellent studies of food web systems using $\delta^{13}C$ measurements (see references above).

In contrast to carbon, an animal's nitrogen stable isotope becomes enriched by ~ 3 to 4 ‰ relative to its diet (Rau 1981, DeNiro & Epstein 1981, Macko et al. 1982, Minagawa & Wada 1984, Checkley & Entzeroth 1985, Owens 1987, Peterson & Fry 1987, Checkley & Miller 1989, Michener & Schell 1994, Post 2002). As with carbon, DeNiro & Epstein (1981) found that the $\delta^{15}N$ in the organism reflects the $\delta^{15}N$ of the diet, but in most cases the whole animal is enriched in ^{15}N relative to the diet, because "heavy" ^{15}N is preferentially retained in the consumer, while light ^{14}N is excreted. Thus when enrichment occurs, there has been found to be a preferential excretion of ^{15}N-depleted nitrogen, usually in the form of urea and ammonia (Minagawa & Wada, 1984). Differences in ^{15}N retention varies according to species, diet and nutritional stress (Hobson 1991). On the other hand, starvation may equally

result in increases of an animal's $\delta^{15}N$ (Hobson et al. 1993, Adams & Sterner 2000), as animals are then physiologically "living on their own flesh".

Previous studies found that animal urine was depleted in ^{15}N relative to diet as well as to blood, faeces and milk (Steele & Daniel 1978), and that various tissues measured were enriched relative to the diet, with $\delta^{15}N$ increasing from kidney to hair to liver to brain (DeNiro & Epstein 1981). Part of these differences in tissue $\delta^{15}N$ may be due to isotopic fractionation during amino acids trans and deamination (Gaebler et al. 1966, Macko et al. 1986, Macko et al. 1987), e.g. during synthesis of dietary proteins (Bada et al. 1989). As with carbon, analysis of several tissue types or whole organism $\delta^{15}N$ should be performed when comparing animal and diet $\delta^{15}N$.

Many studies show a range from 3 to 4‰ enrichment in animal $\delta^{15}N$ versus diet, which is reflected as a trophic level effect in food web studies (Fig.I.1). Minagawa & Wada (1984) found a ^{15}N enrichment of 3.4 ± 1.1‰ as function of the presumed trophic level, independent of habitat. A survey of bone collagen by Schoeninger & DeNiro (1984) for 66 species of vertebrates resulted in an average 3 ‰ enrichment per trophic level. Recently Post (2002) has confirmed that the mean trophic enrichment of $\delta^{15}N$ is 3.4 ± 1‰ per trophic level, and that even though variable, is widely applicable.

III. Materials and methods

The study areas.

The study was conducted in the Bornholm Basin (BB), Gdansk Deep/Gotland Basin (GDGB) in the Central Baltic Sea (CBS) and in the German Bight (GB) in the Southern North Sea (SNS). Both systems (CSB and SNS) are marginal seas in the temperate northern hemisphere and are influenced by the Northern Atlantic climate system (Janssen et al. 1999, Alheit et al. 2005).

The Central Baltic Sea characterized as a semi-enclosed brackish-water region is a transition area between the western Baltic (Arkona Sea), which contains higher saline water (salinity of 15-25, inflow from North Sea through Kattegat Sea), and the northern Baltic with lower saline water (salinity of 1-2, influenced by freshwater run-off) (Matthäus 1995). As a consequence, the water column is vertically structured, composed normally of 2 to 3 layers (Kullenberg 1981) depending on the season, i.e. a surface layer with low salinity, the Baltic Intermediate Water (BIW) layer and the bottom layer with high saline water, but deficient of oxygen. Thus during summer stratification, the Baltic Intermediate Water (BIW) may be formed, which is bordered to the top and the bottom by the seasonal thermocline and the permanent halo/oxycline at ~60-70m depth, respectively (Kullenberg 1981, Thomas et al. 2003).

Nitrogen limitation occurs in the central regions of the Baltic Sea whereas phosphor can become limiting in the coastal areas (Thomas et al. 2003). These forms of nutrient limitation occur regularly in the upper layer of the water column. After the spring bloom of phytoplankton (mainly diatoms), a bloom of nitrogen fixing cyanobacteria occurs regularly in summer (July and August) in the central nitrogen depleted areas (Wasmund et al. 2001, Nausch et al. 2004) and heterotrophic protozoa, mostly ciliates, can reach high abundances (Johansson et al. 2004, Höglander 2005). A second diatom bloom, accompanied by high abundances of dinoflagellates (Wasmund & Uhlig 2003, Wasmund et al. 2004) in late summer or autumn that supports the subsequent growth of mesozooplankton can occur in the coastal zones in the central Baltic Sea (Rolff 2000). The Bornholm Basin, Gdansk Deep and the Gotland Basin are also the major spawning grounds for cod (*Gadus morhua callarias* L.) and sprat (*Sprattus sprattus balticus* S.) in the Central Baltic Sea. (Möllmann & Köster 2002, Koster et al. 2003, Möllmann et al. 2003).

The German Bight is a shallow part of the North Sea with an average depth of about 20 meters and where tidal currents lead to a mixing of different water masses. The German Bight in the southern North Sea is strongly influenced by the coastal areas with large rivers

such as the Rhine and the Elbe (Allen 1981, van der Zee & Chou 2005) and it is connected to the Northern Atlantic at the English channel and between Scotland, Shetland and Norway (Reid et al. 2003). Temperature differences between summer and winter are extreme in the continental coastal waters as compared to areas that are influenced by North Atlantic water masses. The temperature varies spatially also across the German Bight , particularly in February from west (~ 10°C) to east (~ 4°C) (see Barz & Hirche 2007). Frontal zones can develop in areas where water masses of different density meet. According to Krause et al. (1986)and Otto et al. (1990), three types of frontal zones can occur in the German Bight: (i) Tidal mixing fronts forced by tidal friction mixing, wind stress and temperature as along the Frisian coast; (ii) river plume fronts along the Danish-German coastline of Jutland between freshwater and oceanic water and (iii) upwelling fronts west of Helgoland where bottom water advected from the central North Sea is forced upward by easterly wind stress.

The main nutrient input into the German Bight is from the freshwater systems of the Elbe, Weser and Ems rivers (Brockmann & Eberlein 1986). Therefore, there are nutrient gradients from high concentrations at the coast towards lower concentrations at the central North Sea (Raabe et al. 1997). The nutrient distribution and cycling in the North Sea is hydrographically influenced (Brockmann et al. 1990). In the frontal zones, the waters contain the highest nutrient values and turnover rates, triggering the growth of phytoplankton, compared with the adjacent stratified and mixed waters (Maguer et al. 2000).

Several studies have demonstrated considerable changes in phytoplankton and zooplankton abundance and composition during the last 50 years in the North Atlantic and the North Sea (e.g. Gillbricht 1988, Hickel et al. 1997, Beaugrand 2004a, b) and tried to correlate changes of phytoplankton to ambient nutrients levels, river discharge and climate (e.g. Hickel et al. 1989, Gieskes & Schaub 1990, Cadee & Hageman 1993, Beaugrand & Ibanez 2004). The temperature has significantly increased over the last years and caused shifts in the phytoplankton species succession (Franke et al. 2004, Wiltshire & Manly 2004). The spring bloom in the German Bight is generally dominated by diatoms, followed regularly by blooms of *Phaeocystis globosa* (Riegman et al. 1992, Hansen et al. 1993, Mills et al. 1994, Riegman & van Boekel 1996, Stelfox-Widdicombe et al. 2004), followed by a second increase in diatoms. Flagellates form major blooms during summer. Large dinoflagellates like *Ceratium* species occur regularly from mid June to mid September. Zooplankton distribution in the North Sea differs between three main water bodies: Atlantic water masses, central North Sea water mass, and coastal water masses (Krause et al. 1995).

Mesozooplankton sampling.

Mesozooplankton samples for nitrogen and carbon stable isotope analysis from the Central Baltic Sea were collected in May, July, August, November/December (Nov/Dec.) 2003 and in March 2004 at 7 stations in the Bornholm Basin (Fig.III.1) and in July 2003 at 5 stations in a transect from Gdansk Deep to Gotland Basin (Fig.III.2).

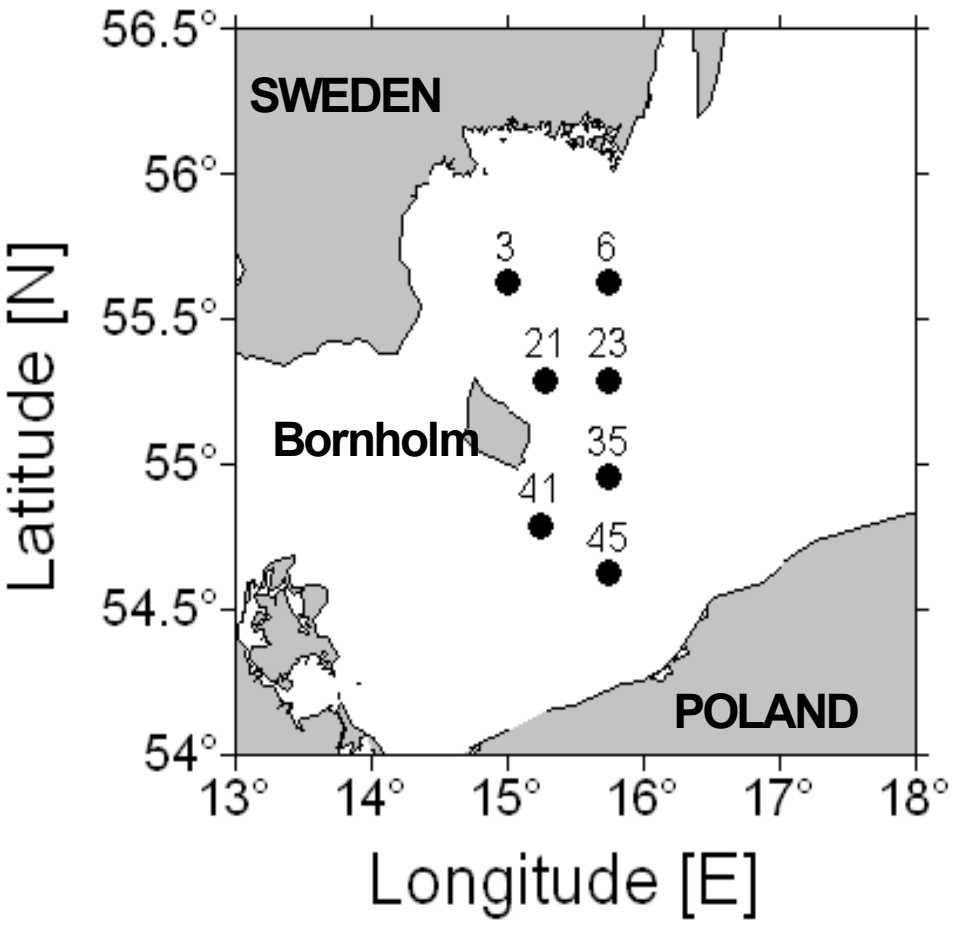

Figure III.1. Location of the Bornholm Basin in the Central Baltic Sea: Closed circles represent stations sampled.

Depth-integrated samples in the CBS were obtained from 3 replicate hauls at each station with a 200µm WP2 net from near bottom to the surface. In order to account for stratification, additional depth-specific samples were collected with a Multinet (300 µm mesh size; Hydrobios Kiel, Germany) at 5 to 10 discrete depth intervals at a central, deep station (station no. 23) in August 2003 (stratified summer conditions) and March 2004 (mixed early-spring conditions). Sampling depths in August 2003 and March 2004 were 5, 15, 25, 65 and 75 m, and 10, 20, 45, 65 and 80 m, respectively. Surface samples were collected with a Bongo net trawled at the immediate sea surface.

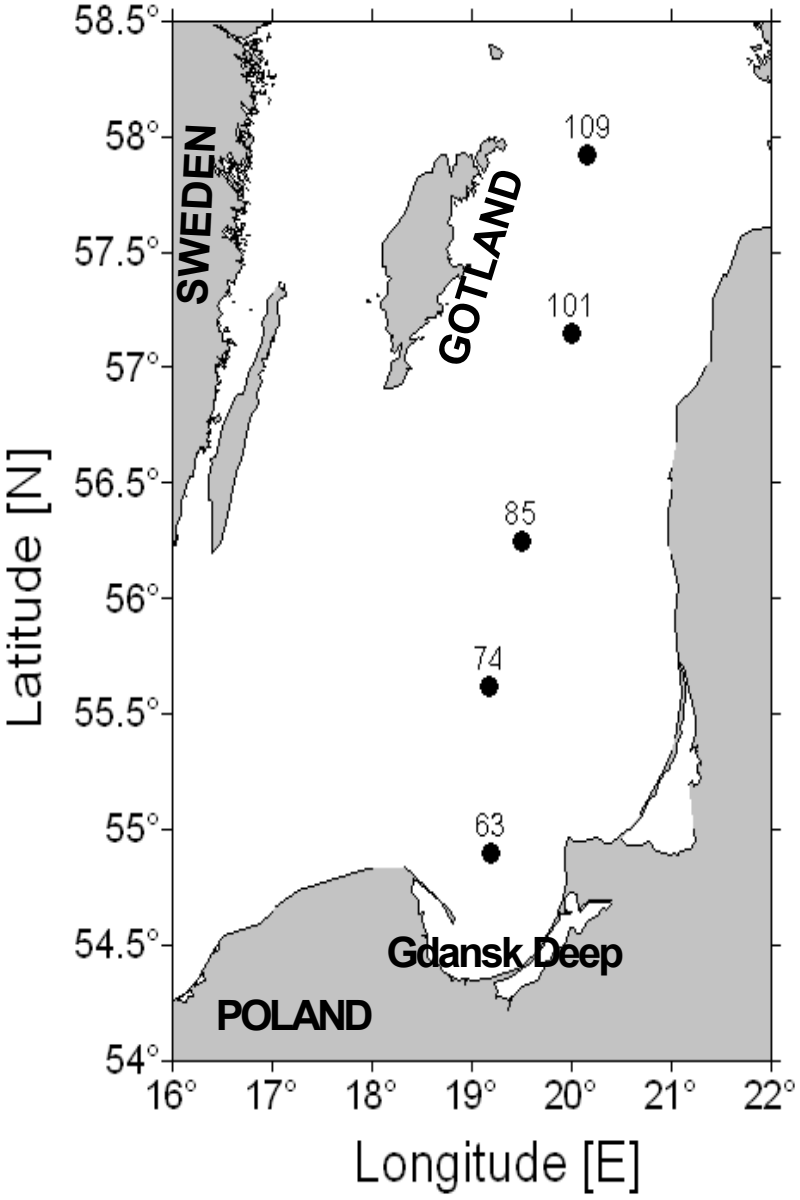

Figure III.2. Location of the Gdansk Deep and Gotland Basin in the Central Baltic Sea: Closed circles represent stations sampled.

The sampling in the North Sea were conducted in February, April, May, June and August 2004 at 5 stations in the German Bight (Fig.III.3). Depth-integrated samples were obtained by hauling a WP2 (200 µm mesh) net. Three vertical hauls were collected at each station. Depth-specific samples at station no 35 (NW offshore) were obtained in all months. These samples were collected with a 0.25 m^2 multi opening/closing net system. The gear was operated vertically with a speed of 0.2 m/s, resolving the water column in 10 meters (in February and April) and 5 m (in May and June) intervals from the bottom up to the surface. In August 2004, stations no 11, 22 and 35 were also vertically sampled. Surface samples (<0.5

to 1 m) were collected with a Bongo net (310 µm mesh size), which was emerged halfway at the sea surface and towed for 5 min at 5 m s^{-1}. Samples from >3 m depth were collected with a Multinet composed of 7 individuals nets (335 µm mesh size), which were each towed – starting deepest – for 5 min at 4 m depth intervals (5 m s^{-1}).

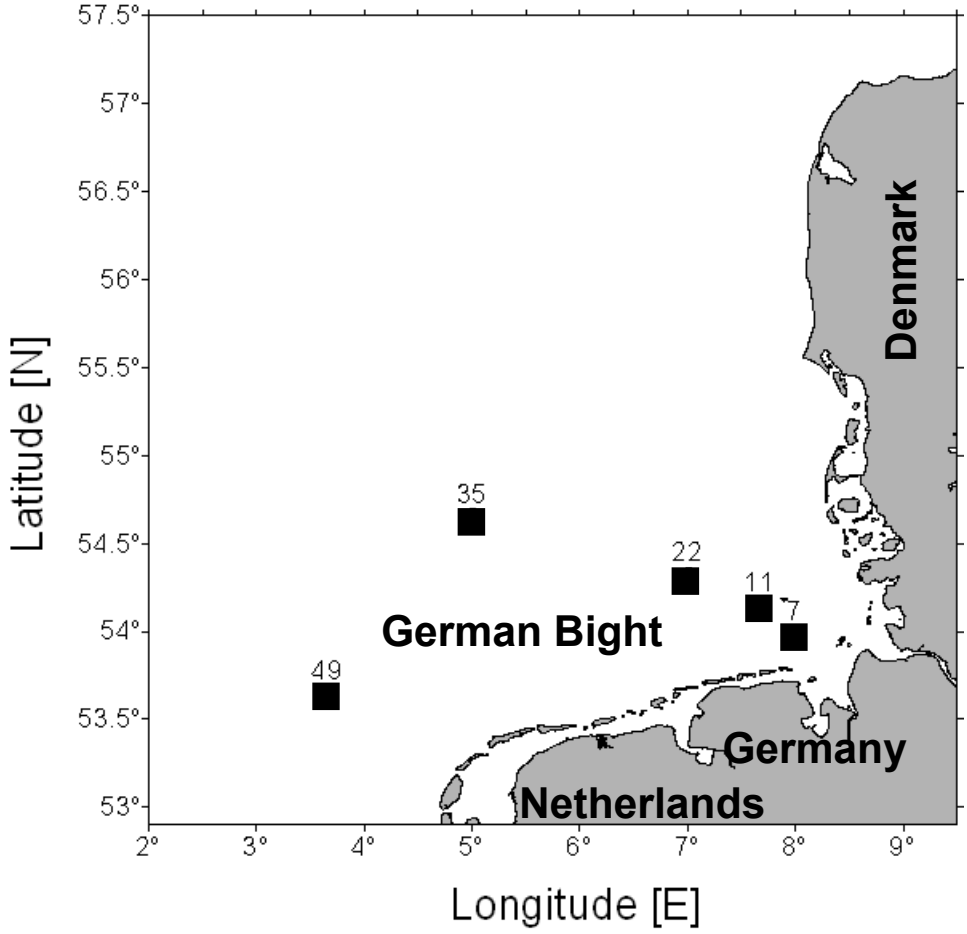

Figure III.3. Location of the German Bight in the Southern North Sea: Closed square represent stations sampled.

After the sampling, mesozooplankton samples were stored at ca. 10 °C in 0.2 µm filtered seawater to allow for gut evacuation (~1 to 2 h). Adult individuals, with the exception *P. acuspes* C5 stages in March 2004, were picked under a dissecting microscope (Wild M3 C) and transferred into tin cups. Between 5-50 (copepods), 50-100 (cladocerans) or 1-5 (chaetognaths, ctenophores, and medusas) individuals of each species were pooled for one joint nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope measurement.

Some cladocerans (only in the CBS) and copepods were identified to species: *Evadne nordmanni, Bosmina coregoni maritima* (also known as *Bosmina longispina maritima)*, *Podon* spp. (*P. intermedius* and *P. leuckarti*), *Temora longicornis* (in the CBS and

SNS), *Centropages hamatus* (in the CBS and SNS), *Pseudocalanus acuspes* (in the CBS), *Pseudocalanus elongatus* (in the SNS), *Calanus helgolandicus* (in the SNS). *Acartia* species were differentiated in *A. bifilosa* and *A. longiremis* only in August 2003 in the CBS. In the SNS *Acartia* species (*A. tonsa* and *A. clausii*) were not separated.

CTD profiles, seston, chlorophyll *a* and nutrients.

Vertical profiles of temperature, salinity and oxygen, and depth-specific seston samples from the CBS (0, 5, 10, 15, 25, 60 and 0, 10, 20, 45, 65, 80 m in August 2003 and March 2004, respectively) were obtained with a CTD-rosette. Seston depth-specific samples from the SNS were obtained at 5 m depth intervals in all months.

Pre-screened (<100 μm) seston samples (500 ml) were filtered onto pre-combusted (550°C, 24 h) Whatman GF/F filters for both the analysis of stable isotope ratios and total carbon and nitrogen content. The latter was determined with a CHN-analyser (Fisons 1500N). Seston subsamples (250 ml) were preserved with acid Lugol's solution for cell counts determined under an inverted microscope (Zeiss Axiovert 200) following Utermöhl (1958). Cell counts were converted to biovolume assuming simple geometrical figures (Hillebrand et al. 1999). The biomass of diatoms, chlorophytes, cryptophytes and dinoflagellates in terms of carbon biomass was calculated from equations provided by Menden_Deuer & Lessard (2000). Cyanobacteria and ciliate biomass were estimated after Verity et al. (1992) and Putt & Stoecker (1989), respectively. Detritus was calculated by subtracting phytoplankton and ciliate biomass from total seston carbon content. Chlorophyll *a* (chl *a*) concentrations were measured *in situ* from fluorescence with a submersible fluoroprobe (bbe Moldaenke; Kiel, Germany). The vertical distribution of inorganic nutrients was obtained from the long time monitoring MUDAB-database maintained by the Deutsches Ozeanographisches Datenzentrum (DOD) and from the Globec-Germany measurements by Alfred Wegener Institute (AWI) at Sylt and Helgoland.

Stable isotope analysis (SIA).

After freeze-drying at -50°C for 24 h onboard, all samples for SIA were stored in a dessicator until combustion in a CHN-analyser (Fison, 1500N) connected to Finnigan Delta Plus mass spectrometer. The ratios of heavy (^{15}N and ^{13}C) to light (^{14}N and ^{12}C) stable isotopes are expressed in the δ notation ($\delta^{15}N$ and $\delta^{13}C$ signatures) which indicates the depletion (negative values) or the enrichment (positive values) of the heavy isotope compared to the lighter isotope relative to a standard according to the formula::

$$\delta X\ [‰] = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad \text{(III.1)}$$

where *X* is ^{15}N or ^{13}C, R is (^{15}N:^{14}N) or (^{13}C:^{12}C), and standards are atmospheric N_2 or PDB carbon, respectively. Pure N_2 and CO_2 gas was calibrated against primary IAEA reference standards (N1, N2, N3, NBS22). A laboratory-internal working standard (acetanilide) was measured after every sixth sample with a precision of ±0.2‰ for both isotopes.

Lipid correction of $\delta^{13}C$.

After correlations between $\delta^{13}C$ and C:N ratios of mesozooplankton, we applied three correction approaches using the C:N ratios as lipid-indicator to the species with negative slopes and significant associations ($p < 0.001$). Therefore, in order to account the effect of lipid content on $\delta^{13}C$, the $\delta^{13}C$ values of *P. acuspes* and *L. macrurus* from the CBS, as well as, *P. elongatus* and *C. helgolandicus* from the SNS were normalized for the species' high lipid content following McConnaughey & McRoy (1979), Fry et al. (2003) and using ANCOVA regressions (see chapter VII).

McConnaughey & McRoy (1979) established a mathematical equation (Eq.VII.1), based on the lipid content (or C:N ratios) and the original $\delta^{13}C$ value, to calculate the lipid-normalized $\delta^{13}C$ of the sample ($\delta^{13}C_{corr}$):

$$\delta^{13}C_{corr} = \delta^{13}C_{original} + D \times [-0.207+3.9] / [1+287/[L]] \quad \text{(III.2)}$$

where *D* (or δ_{lipid}) corresponds to the estimated average depletion of lipids in ^{13}C (6‰) and L corresponds to the assessed lipid content (%):

$$L\ (\%\ lipid) = 93 / [1+ [[0.246 \times C:N] - 0.775]^{-1}] \quad \text{(III.3)}$$

Recently, Fry et al. (2003) proposed a mass balance approach (Eq. III.3) for $\delta^{13}C$ lipid-correction. This approach was deduced from stable isotope in the brown shrimp:

$$\delta^{13}C_{corr} = [[\delta^{13}C_{original} \times C:N_{original}] + [\delta_{lipid} \times (C:N_{original} - C:N_{protein})]] / [C:N_{original}] \quad \text{(III.4)}$$

where $C:N_{protein}$ and δ_{lipid} are assumed to be constant (3.7 and 6‰, respectively).

Trophic level (TL).

Mesozooplankton trophic enrichment was assessed both relative to seston <100μm $\delta^{15}N$ and $\delta^{13}C$ signatures ($\Delta\delta X_{Seston}$) in the CBS, as well as in the SNS and relative to reference mesozooplankton $\delta^{15}N$ and $\delta^{13}C$ ("herbivorous" *E. nordmanni* and *B. coregoni*) closest to the base of the food web ($\Delta\delta X_{EvBo}$). The second approach was used only in the CBS and has the advantages of avoiding the assumption that seston is representative of phytoplankton, and of averaging producer isotopic signatures over time (Vander Zanden et al. 1999, Post 2002).

Trophic enrichment of mesozooplankton species *i* ($\Delta\,\delta Xi$) was calculated as:

$$\Delta\,\delta^{15}N_i = \delta^{15}N_i - \delta^{15}N_X \quad \text{(III.5)}$$

and

$$\Delta\,\delta^{13}C_i = \delta^{13}C_i - \delta^{13}C_X, \quad \text{(III.6)}$$

where $\delta^{15}N_i$ and $\delta^{13}C_i$ are the mesozooplankton $\delta^{15}N$, $\delta^{13}C$ signatures and *X* is either seston <100μm or a reference cladoceran species (*E. nordmanni* in May, July, Nov/Dec 2003; *B. coregoni* in August 2004; due to absence, neither in March 2004).

Mesozooplankton trophic level (TL) was calculated based on the assumption that $\delta^{15}N$ and the $\delta^{13}C$ signatures become enriched by 3.4‰ and 1.0 ‰ per trophic level, respectively, (Minagawa & Wada 1984, Fry & Sherr 1984, Rau et al. 1983; Peterson & Fry 1987, Hobson & Welch 1992, Post 2002), and that the seston and the cladoceran reference baselines have a trophic level of 1.5 (mixture of autotrophs and heterotrophs) and 2 (primary consumers), respectively. The trophic level of mesozooplankton species *i* (TL*i*) was calculated as:

$$TL_i = (\Delta\,\delta^{15}N_i / 3.4) + d \quad \text{(III.7)}$$

and

$$TL_i = (\Delta\,\delta^{13}C_i / 1.0) + d, \quad \text{(III.8)}$$

where *d* is either 1.5 or 2 according to the baseline (seston or reference cladoceran, respectively).

Data analysis.

Differences in the mean $\delta^{15}N$ and $\delta^{13}C$ of seston <100 μm and mesozooplankton species were tested by two-way analysis of variance (ANOVA) with the factors site (sampling station) and month. The data were exponentially transformed by box-cox procedures (Sokal & Rohlf 1995) in order to produce normality and reduce heteroscedasticity. Variance homogeneity and data normal distribution were tested with Levene´s test and normal probability plots, respectively. We further used two-way ANOVA to test for differences in mesozooplankton $\Delta\delta^{15}N$, $\Delta\delta^{13}C$ and TL among species (box-cox transformed) and between months. Post-hoc comparisons were applied using Tukey´s honestly significant difference (HSD) test or the Spjotvoll-Stoline test (a generalization of Tukey's test applicable at unequal samples sizes). The ANOVA and HSD tests were performed using the general linear model (GLM) of SPSS 13. Diverse Spearman correlations, t – tests and Mann-Whitney U test in this thesis were performed using Statistica 6.0.

Model II regressions (including ANCOVA regressions) using the standard major axis (SMART V.1.0) according to Falster et al. (2003) were applied, for example, to test the effect

of diazotrophic cyanobacteria biomass on seston $\delta^{15}N$ in July (in the GDGB) and August (in the BB) 2003 or to define the relationship between carbon and nitrogen trophic enrichments, etc. Some relationships were analyzed by robust linear model regression using an MM estimator (rlm- MASS package, Venables & Ripley 2002) and *F* robust test (sfsmisc package) of R software, the latter aimed at reducing the uncertainty in the estimations due to outliers.

IV. Mesozooplankton trophic levels in the Bornholm Basin (Central Baltic Sea).

Abstract

In order to resolve within-guild trophic level differences, I analyzed the seasonal and spatial variability of carbon and nitrogen stable isotope signatures ($\delta^{13}C$, $\delta^{15}N$) of dominant Baltic mesozooplankton species in the Bornholm Basin. Species-specific mesozooplankton $\delta^{13}C$ and $\delta^{15}N$ proved to be spatially homogeneous within the study area (7 stations, covering ~6000 km^2) and, with the exception of *T. longicornis,* remarkably variable over time. Temporal increases and decreases in both the $\delta^{13}C$ and $\delta^{15}N$ of mesozooplankton were observed during summer and early spring, respectively. However, the relative interspecific differences among mesozooplankton species were preserved over the entire sampling period, such that average mesozooplankton $\delta^{15}N$ increased in the order: *Bosmina coregoni maritima* (2.8 ± 0.2 ‰) < *Evadne nordmanni* (5.3 ± 0.6 ‰) and *Temora longicornis* (5.3 ± 0.4 ‰) < *Acartia* spp. (5.9 ± 1.0 ‰); *Podon* spp. (6.0 ± 0.9 ‰) and *Centropages hamatus.* (6.1 ± 0.6 ‰) < *Pseudocalanus acuspes* (7.6 ± 1.4 ‰) < *Sagitta* spp. (13.7 ± 1.4 ‰), suggesting an increase in carnivory in the same order. The $\delta^{13}C$ signatures, excluding *Acartia* spp, showed similar patterns. A vertical profile of seston and mesozooplankton $\delta^{15}N$ during vertical stratification in summer suggests that the interspecific $\delta^{15}N$ variability may be, in part, explained by differences in species-specific vertical position and hence possibly reflect the intake of food-particles fuelled by different nitrogen sources (new versus recycled nitrogen). The highest trophic enrichment of the copepod *P. acuspes* among crustaceans indicates that this species was not exclusively herbivorous, because *P. acuspes* might feed also on small heterotrophs (bacteria, flagellates, and ciliates), which can be expected to have higher $\delta^{15}N$ values than autotrophic components of seston and/or may possibly be the result of coprophagy. However, the possibility of starvation in adults of *P. acuspes,* predation on early stages of other crustacean zooplankton (*Oithona similis*) or on conspecifics under food limitation below the halocline, may be alternative explanations for the observed pattern. Therefore I conclude that *P. acuspes* displays a omnivorous feeding dominated by carnivory over most of the year.

Results

Spatial and seasonal variability of seston and mesozooplankton $\delta^{15}N$ and $\delta^{13}C$.

Two-way analysis of variance with station and month as independent variables revealed significant differences (Table IV.1, ANOVA, $p < 0.001 – 0.01$) over time and between sites for seston and each mesozooplankton species with the exception of *B. coregoni* $\delta^{15}N$ and *Sagitta* spp. $\delta^{13}C$ (ANOVA, $F = 1.0$ and 1.9, $p = 0.45$ and 0.16, respectively). However, when comparing the stable isotope signatures of a given species within the same month, spatial homogeneity was found among all stations (Fig. IV.1, 2;Tukey HSD, $p > 0.05$). Therefore, the $\delta^{15}N$ and $\delta^{13}C$ of all stations for a given species were pooled by sampling month (Fig. IV.3).

Strong seasonal fluctuations in the $\delta^{15}N$ and $\delta^{13}C$ signatures of seston were observed, with $\delta^{15}N$ values being isotopically less enriched in August 2003 (4.3 ± 1.1 ‰) and March 2004 (3.2 ± 0.8 ‰) (Fig. IV.3A, C). Seston $\delta^{13}C$ increased from May to Nov/Dec. and were most depleted in March 2004 (-28.6 ± 0.3 ‰). Seston $\delta^{15}N$ in August were significantly negatively correlated with the biomass of diazotrophic cyanobacteria (*Nodularia spumigena* and *Aphanizomenon* spp.) (Fig. IV.4, $Y = 5.99 - 0.65X$; $r = 0.86$, $p < 0.0001$) indicating fixation of isotopically light atmospheric nitrogen.

Depth-integrated isotopic signatures were obtained for 4 calanoid copepod species on all sampling occasions and for 3 cladoceran species only during 2003 (Fig. IV.3). *Podon* spp. showed both the highest $\delta^{15}N$ and $\delta^{13}C$ among the cladocerans and exhibited slightly depleted $\delta^{15}N$ and strongly enriched $\delta^{13}C$ in August. The $\delta^{15}N$ and $\delta^{13}C$ of *E nordmanni* were significantly different between months (ANOVA, $F = 230$, $p < 0.001$) and could not be determined in August, when this species was absent. In contrast, *Bosmina coregoni*, which was the most abundant cladoceran species (> 90%) present only in August, had the lowest $\delta^{15}N$ (2.8 ± 0.2 ‰) of all species and also among the lowest $\delta^{13}C$ signature (-23.4 ± 0.9 ‰).

With the exception of *Acartia* sp., copepod $\delta^{15}N$ signatures were relatively stable during thermal stratification in 2003 (Fig. IV.3B). After winter mixing, copepod $\delta^{15}N$ in March 2004 were in all cases strongly depleted. *P. acuspes* was by far the isotopically most enriched copepod species in terms of its $\delta^{15}N$, yet only during the stratified period. Of all copepods, *T. longicornis*, showed the most stable and isotopically least enriched $\delta^{15}N$ during 2003, whereas *Acartia* sp. showed a prominent increase during summer. *C. hamatus* $\delta^{15}N$ appeared also relatively constant and slightly more enriched than *T. longicornis* $\delta^{15}N$. In general, all copepods exhibited higher $\delta^{15}N$ signatures than cladocerans with the exception of *Podon* sp., which was isotopically similar to *Acartia* sp. Also, all copepods showed a

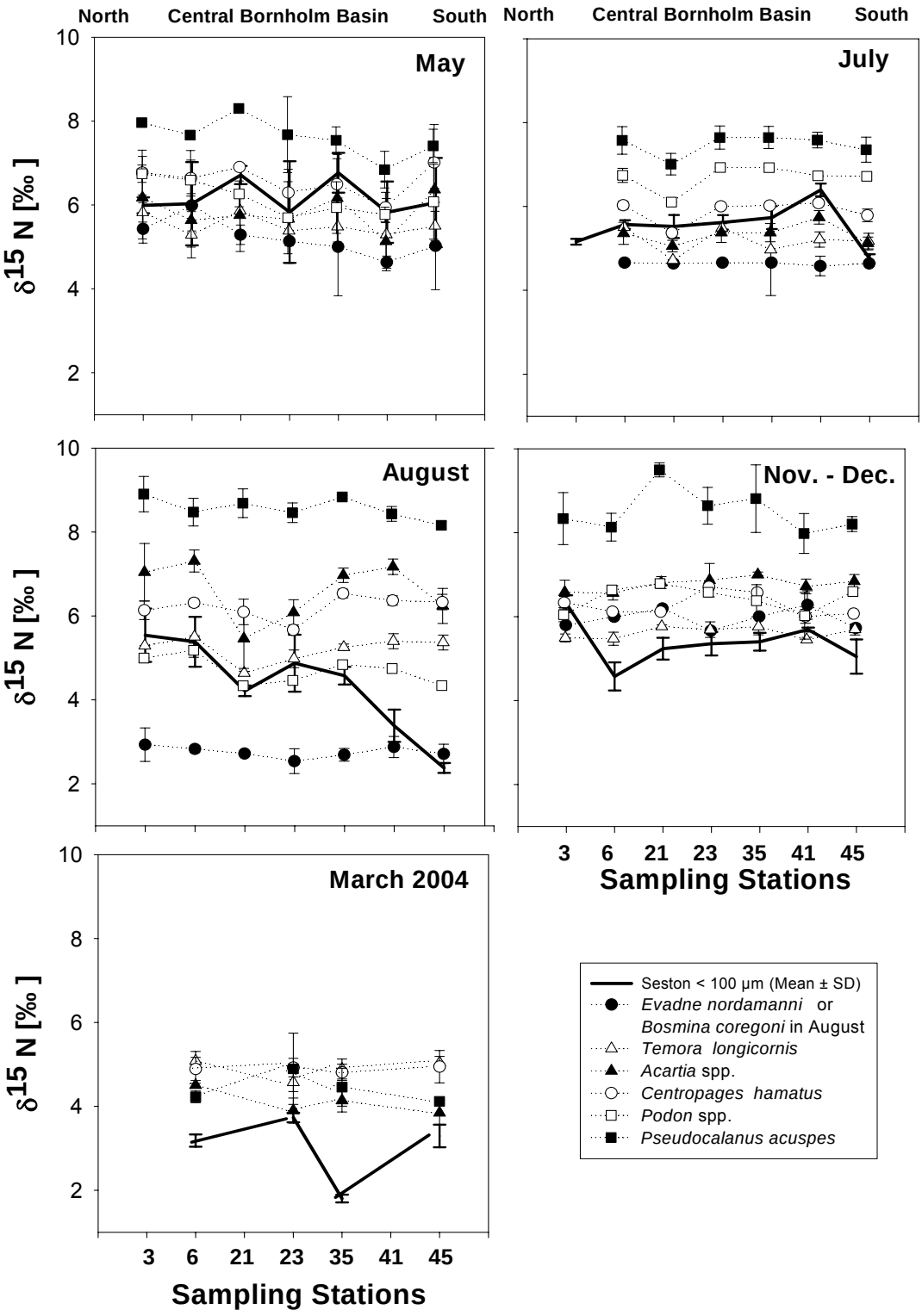

Figure IV. 1. Spatial distribution of Seston and Mesozooplankton $\delta^{15}N$ in the Bornholm Basin during 2003 – 04.

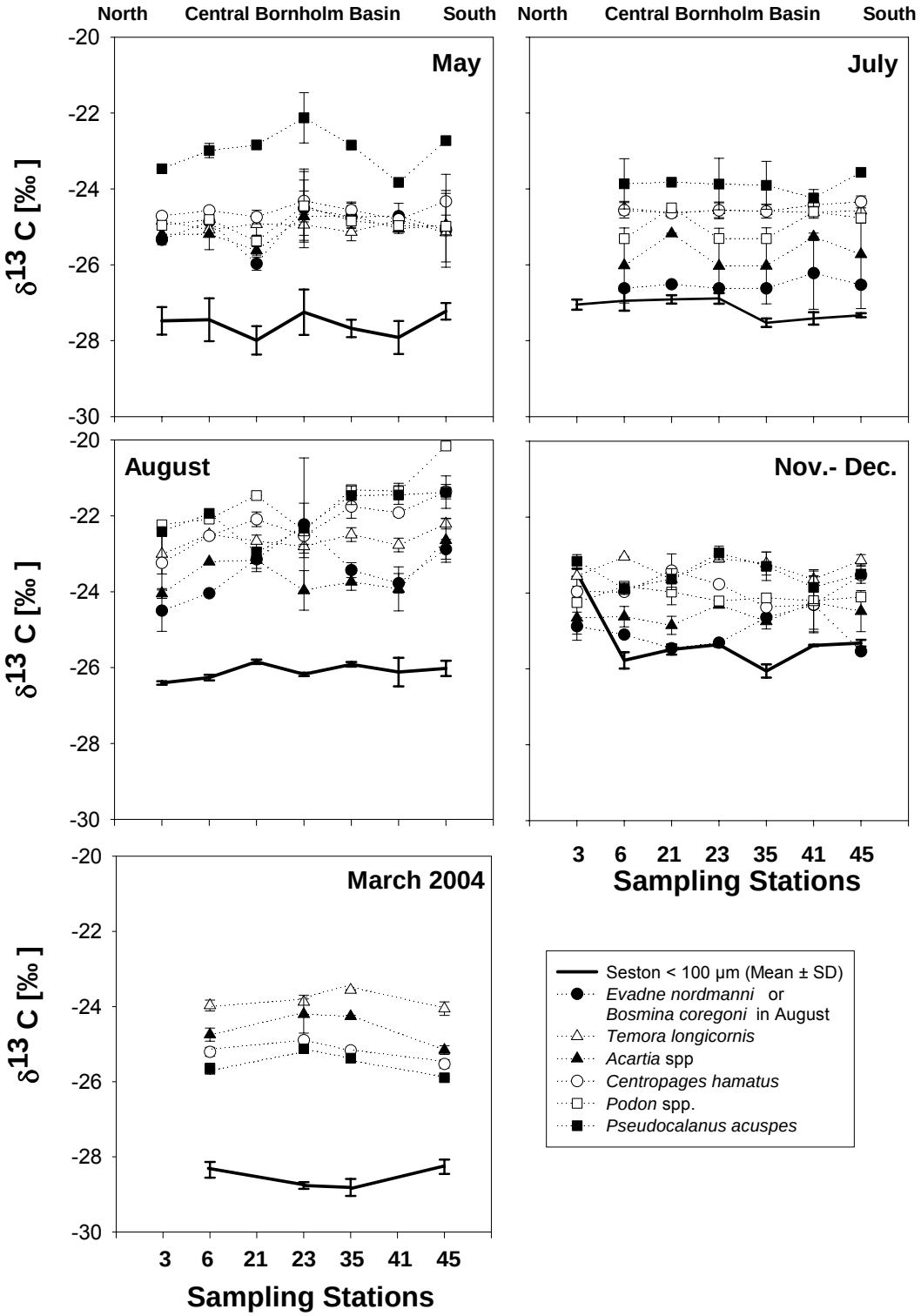

Figure IV. 2. Spatial distribution of Seston and Mesozooplankton $\delta^{13}C$ in the Bornholm Basin during 2003 – 04.

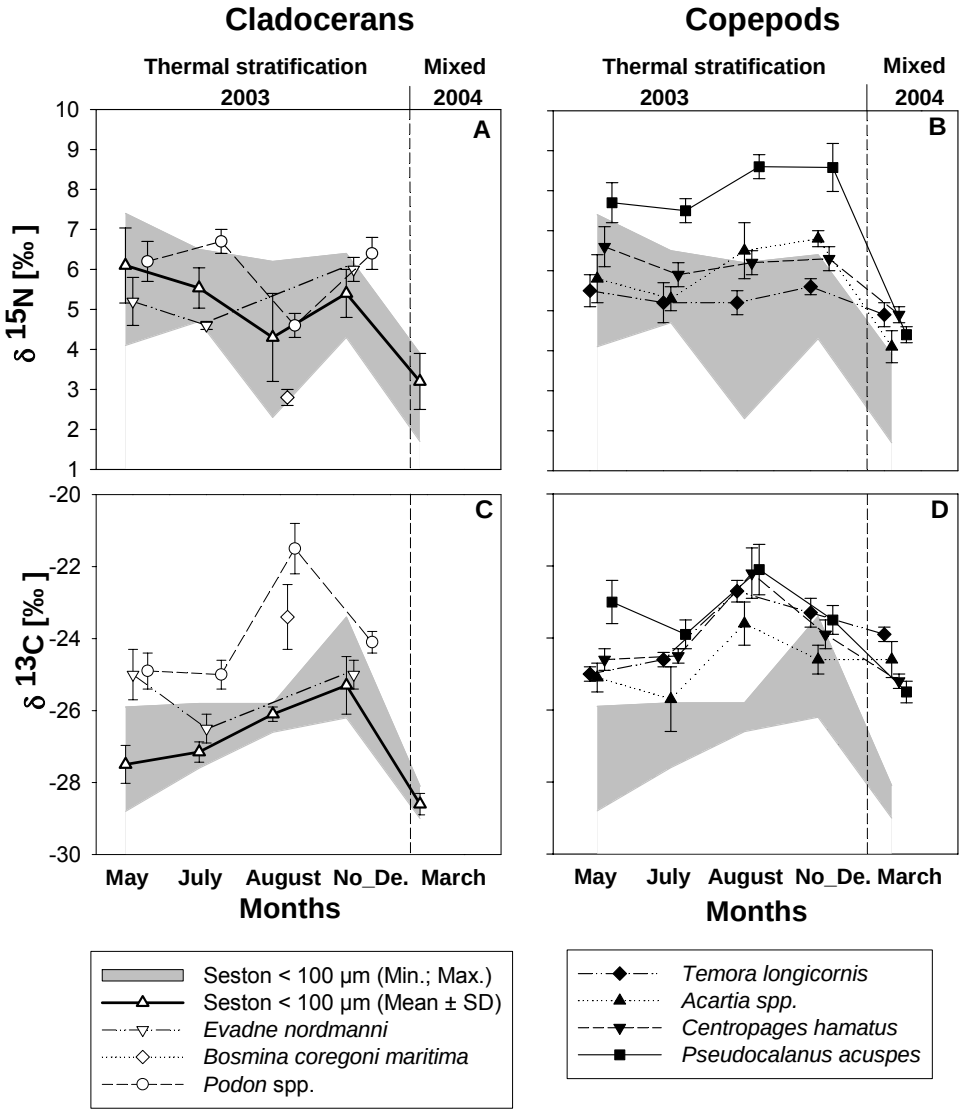

Figure IV. 3. Seasonal depth-integrated δ^{15}N (top) and δ^{13}C (bottom) signatures (mean ± 1SD, all sampling stations pooled by month) of cladocerans (A and C), copepods (B and D) and seston <100µm in the Bornholm Basin. Shaded region represents minima and maxima of seston δ^{15}N and δ^{13}C. Note that species symbols of a given month are shifted relative to each other to improve readability.

similar seasonal trend in their δ^{13}C being most enriched in August (Fig. IV.3D). The chaetognath *Sagitta* spp. showed the highest isotopic signatures in terms of both its δ^{15}N and δ^{13}C (Fig. IV.5). While *Sagitta* δ^{13}C were relatively stable over time, its δ^{15}N signatures showed a strong decrease in Nov/Dec 2003.

Mesozooplankton trophic level (TL).

The calculation of mesozooplankton trophic levels relative to seston $\delta^{15}N$ generally resulted in negative values in May and July 2003 (Fig. IV.6A). Exceptions were *P. acuspes,* which had positive values and *Podon* sp. in July . In the remaining months, calculated trophic levels with seston $\delta^{15}N$ as baseline were positive for all species but *B. coregoni*, which showed the most negative value of all species (-1.6 ± 1‰). Trophic enrichment was highest in August, reaching approximately 2‰ in *Acartia* sp. and *C. hamatus,* and 4‰ (TL≈ 3) in *P. acuspes*. On all occasions, *Podon* spp. showed higher trophic enrichment than *E. nordmanni.* The calculation of mesozooplankton $\delta^{15}N$ relative to the $\delta^{15}N$ of cladocerans (Fig. IV.6B). resulted in positive values for all species, except for *T. longicornis* in Nov/Dec. Again, enrichment was highest in August, with *P. acuspes* $\Delta\delta^{15}N$ being 5.8 ± 0.3 ‰ (TL =3.7 ± 0.1) and the remaining species ranging between 2 and 3.5‰. Enrichment in *Podon* sp. was similar to enrichment in the copepod species.

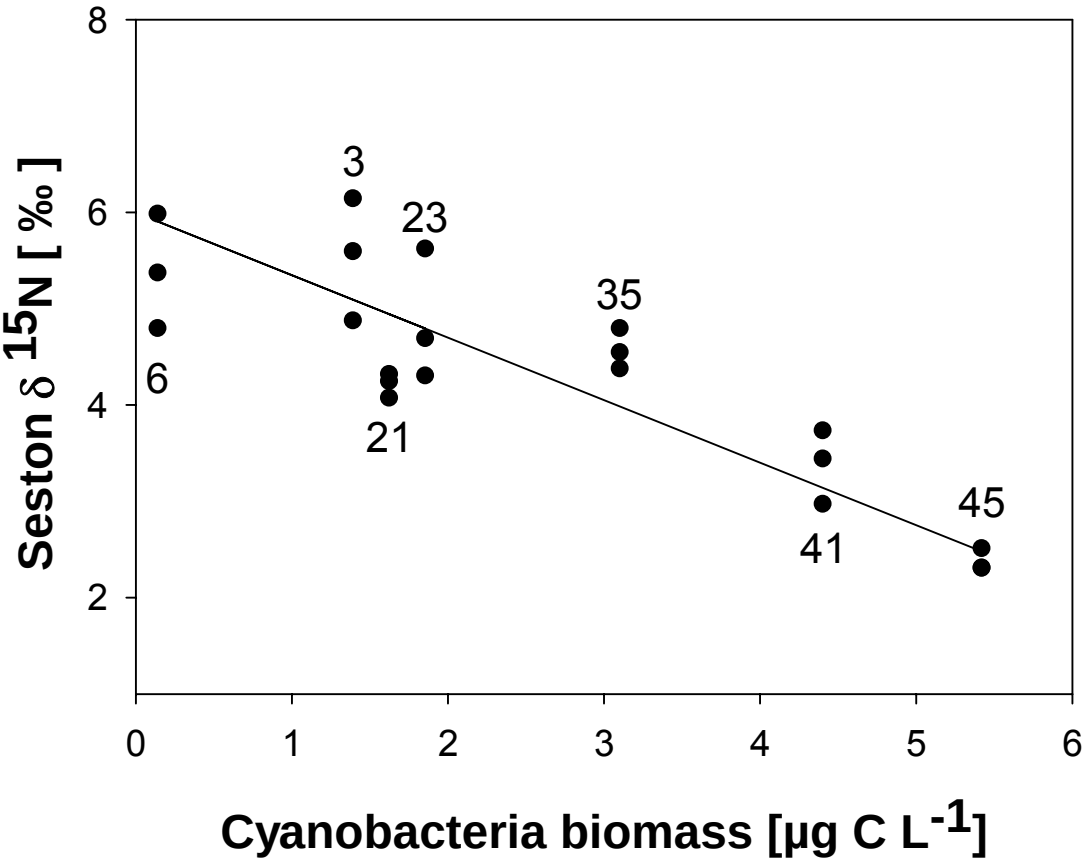

Figure IV. 4. Depth-Integrated seston $\delta^{15}N$ as a function of total cyanobacterial biomass in August 2003. A linear regression model II (SMA: Standardised major axis test) was fitted to the data (Y = 5.99 - 0.65X; n = 21, r = 0.86, r^2 = 0.74, p < 0.001). Numbers above symbols indicate sampling stations.

The average enrichment of mesozooplankton $\delta^{13}C$ relative to seston was positive for all species and generally ranged from 1 to 4.5‰ (Fig. IV.6C). Highest values were found for *P. acuspe*s in May 2003, for *Podon* in July 2003 and for *T. longicornis* in March 2004, (all,4.6‰). In contrast to nitrogen trophic enrichment, *B. coregoni* $\Delta\delta^{13}C$ were clearly enriched in terms of carbon (2.7‰). Using cladocerans as baseline, trophic enrichment in carbon exhibited less seasonal variation and resulted in generally lower values than with seston as baseline (Fig. IV.6D). However, interspecific differences among species were preserved, with *P. acuspes* and *Acartia* spp. being the most enriched and depleted species, respectively.

Trophic enrichment of the chaetognath *Sagitta* spp (Fig. IV.7) relative to both the seston and cladoceran baselines was high in August (10.5±0.6‰ and 12.8±0.6‰, respectively) and March (only seston: 12.0±1‰) and low in Nov/Dec. (7.7±1‰ and 7.2±1‰, respectively). In terms of carbon, trophic enrichment was higher in March (+7.0±0.7‰) than in Nov/Dec (3.8±1‰ and 3.5±0.7‰, respectively) and August (4.1±0.6‰ and 0.2±1.8‰, respectively).

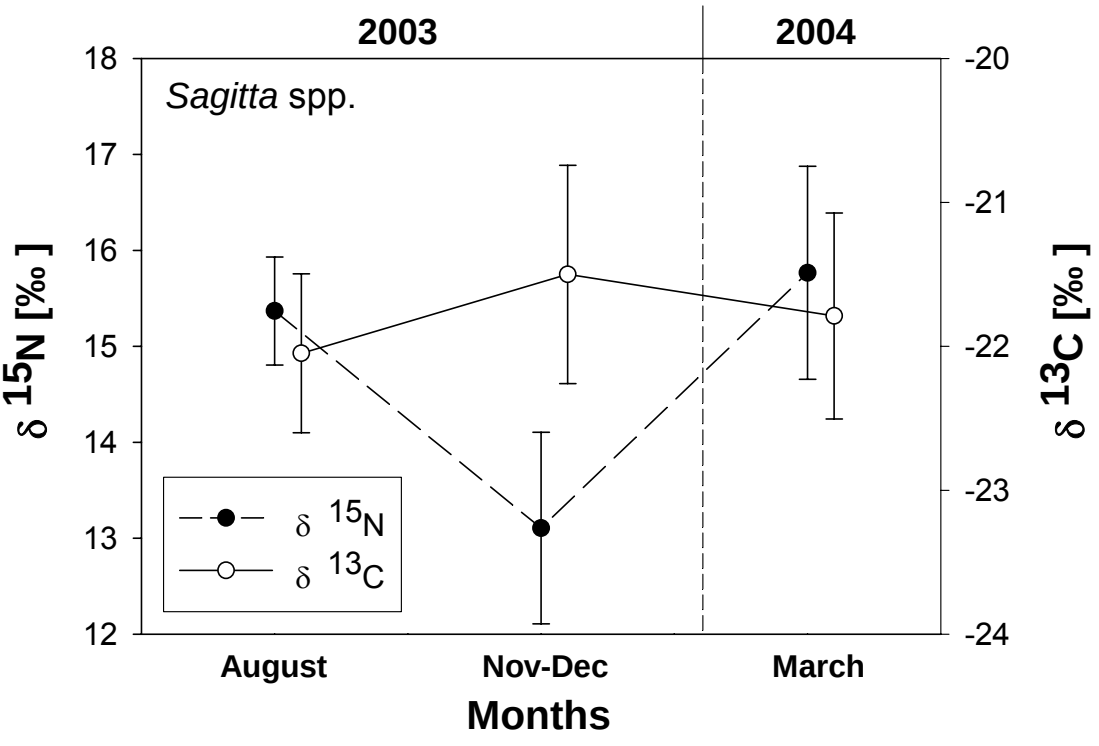

Figure IV. 5. Seasonal depth-Integrated (mean ± 1SD) $\delta^{15}N$ (closed circles) and $\delta^{13}C$ (open circles) signatures of the Chaetognath *Sagitta* spp.

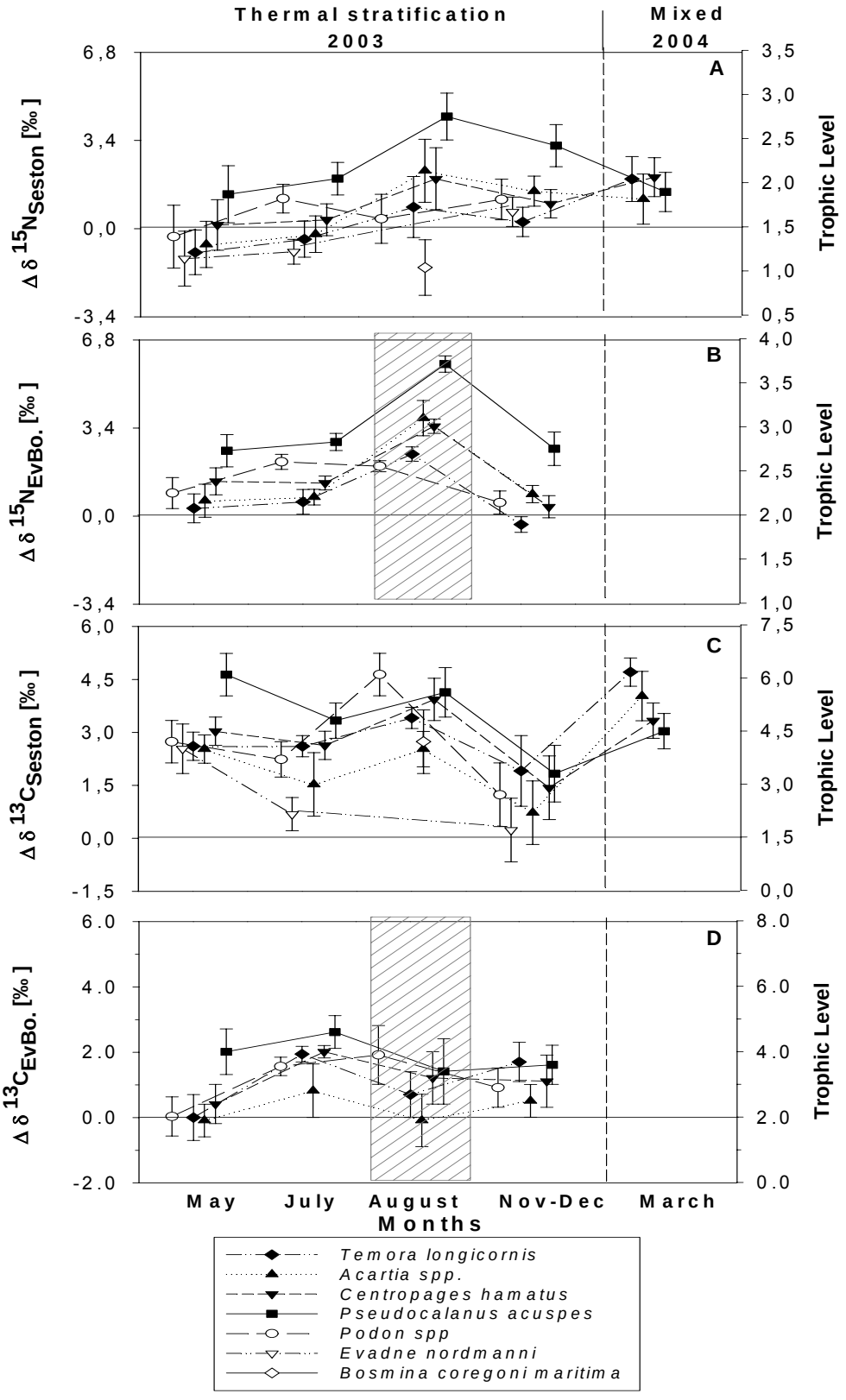

Figure IV. 6. Mesozooplankton trophic enrichment (left axis) in terms of nitrogen ($\Delta\delta^{15}N$) and carbon ($\Delta\delta^{13}C$) relative to seston (A and C) and reference cladocerans (B and D) and trophic level (right axis). Hatched region denotes trophic enrichment relative to *B. coregoni* in August 2003. Solid horizontal lines indicate zero enrichment.

When using seston signatures as baseline, trophic enrichment in nitrogen ($\Delta\delta^{15}N$) and carbon ($\Delta\delta^{13}C$) were significantly and positively correlated only for a few species: *C. hamatus* (Y = 1.05X - 2.0; r^2 = 0.19; p < 0.0001), *T. longicornis* (Y = 1.20X - 3.3; r^2 = 0.35; p < 0.0001) and *Sagitta* spp. (Y = 1.30X + 3.1; r^2 = 0.34; p < 0.002) (Fig. IV.7A). The overall analysis including all data (n = 561) however revealed a positive and highly significant correlation between the $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ with seston as baseline (Y = 1.66X - 3.3; r^2 = 0.14; p < 0.0001). When using *E. nordmanni* as baseline (Fig. IV.7B), significant and positive correlations were again found for few and different species: *P. acuspes* (Y = 0.70X + 1.2; r^2 = 0.29; p < 0.0001), *Acartia* spp. (Y = 0.65X + 0.5; r^2 = 0.10; p < 0.03) and *Podon* spp. (Y = 0.91X + 0.4; r^2 = 0.22; p < 0.0001). The overall analysis including all data again revealed a positive and highly significant correlation between the $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ (Y = 1.6X - 0.5; r^2 = 0.40; p < 0.0001). No significant correlation was found between mesozooplankton $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ (n = 107; p > 0.05) with *B. coregoni* as reference (Fig. IV.7C). The relationship of all mesozooplankton $\Delta\delta^{13}C$ as a function of trophic level (data not shown) revealed significantly positive correlations for both seston (Y = 2.04X - 1.1; n = 561; r^2 = 0.14; p < 0.0001) and *E. nordmanni* (Y = 2.14X – 4.1; n = 321; r^2 = 0.40; p < 0.0001) as reference. The slopes of the regressions indicate a carbon enrichment factor of ~2 ‰ per trophic level.

Interspecific differences.

A two-way ANOVA indicated significant differences (F = 11 to 52; p < 0.0001) between species and months for the $\delta^{15}N$, $\delta^{13}C$ and the trophic enrichments (indicative for trophic levels) of mesozooplankton relative to both seston and cladoceran baselines (Table IV.2). Despite the significant differences detected for temporal variability (ANOVA; F = 72 to 676; p < 0.0001), I decided to pool all values by species in order to determine within-guild trophic enrichment (Table IV.3).

Average mesozooplankton $\delta^{15}N$ signatures (Fig. IV.8) showed significant interspecific differences within 5 groups, with increasing enrichment in the order *B. coregoni* (2.8±0.2‰) < *E. nordmanni.* (5.3±0.6‰), *T. longicornis* (5.3±0.4‰) < *Acartia* spp. (5.9±1.0‰), *Podon* spp. (6.0±0.9‰), *C. hamatus.* (6.1±0.6‰) < *P. acuspes* (7.6±1.4‰) < *Sagitta* spp. (13.7 ±1.4‰). In terms of carbon (Fig. IV.8), average $\delta^{13}C$ increased in a somewhat different order within also 5 groups: *E. nordmanni.* (-25.3±0.9 ‰) < *Acartia* spp. (-24.7±0.9‰) < *C. hamatus* (-24.0±1.1‰), *T. longicornis* (-23.9±0.9‰), *Podon* sp. (-23.8±1.5‰) < *B. coregoni* (-23.4±0.9‰), *P. acuspes* (-23.3±1.2‰) < *Sagitta* spp. (-21.6±0.7‰).

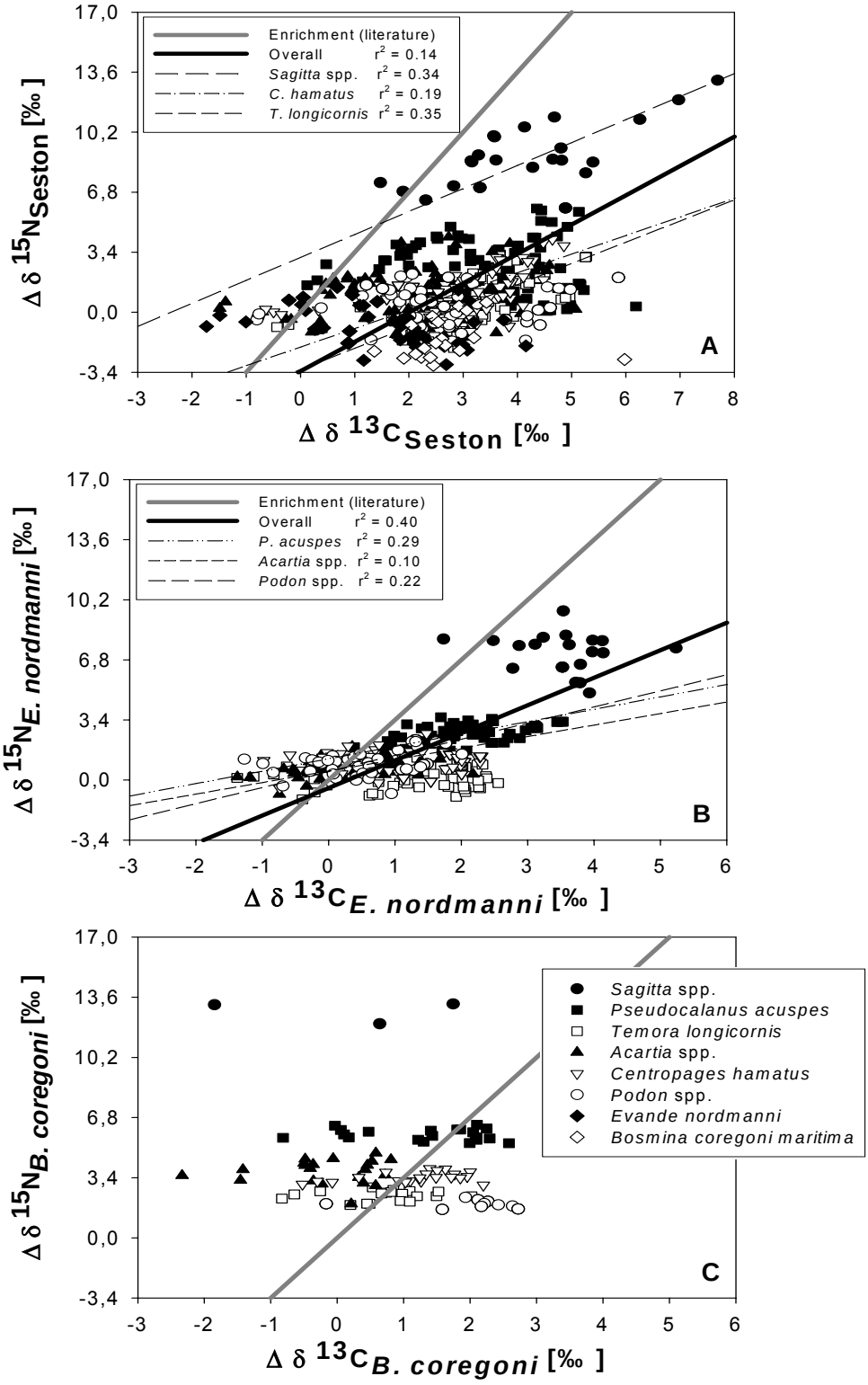

Figure IV. 7. Relationship between mesozooplankton $\Delta\delta^{13}$C and $\Delta\delta^{15}$N using seston (A) *E. nordmanni* (B) and *B. coregoni* (C) as reference baselines. Only significant ($p < 0.002$) correlations are shown. Gray lines represent a trophic enrichment in the nitrogen and carbon signatures of 3.4‰ and 1.0 ‰, respectively, per trophic level.

Mesozooplankton trophic enrichments with seston $\delta^{15}N$ as baseline (i.e. $\Delta\delta^{15}N_{seston}$) allowed species to be divided into 6 groups increasing in the order: *B. coregoni* (-1.6±1.1‰) < *E. nordmanni.* (-0.5±1.1‰) < *T. longicornis* (0.2±1.2‰) < *Podon* spp., *Acartia* spp. (both, ~0.6±1.2‰), *C. hamatus.* (1.0±1.1‰) < *P. acuspes* (2.5±1.4‰) < *Sagitta* spp. (8.5±1.9‰). The increase in carbon trophic enrichment (i.e. $\Delta\delta^{13}C_{seston}$) again resulted in only 5 groups: *E. nordmanni* (1.2±1.3‰) < *Acartia* spp. (2.1±1.2‰) < *B. coregoni*, *Podon* spp. (both, 2.7±1.2‰), *C. hamatus.* (2.8±1.1‰), *T. longicornis* (2.9±1.0‰) < *P. acuspes* (3.4±1.2‰) < *Sagitta* spp. (4.2±1.4‰). Mesozooplankton trophic enrichments with cladoceran $\delta^{15}N$ and $\delta^{13}C$ as baseline (i.e. $\Delta\delta^{15}N_{EvBo}$, $\Delta\delta^{13}C_{EvBo}$) both revealed only 4 groups increasing in the order *T. longicornis* < *Podon* spp., *Acartia* spp. and *C. hamatus* < *P. acuspes* < *Sagitta* spp. However, the $\Delta\delta^{13}C_{EvBo}$ of *Acartia* spp. (0.2±0.8‰) was less enriched than all mesozooplankton species (see Table IV.3).

$\delta^{15}N$ vertical distribution of seston and mesozooplankton.

In August 2003, the water column was strongly stratified and divided into a warm (21°C) surface layer (<10 m), a cold (~4°C) bottom layer (>30 m) and an intermediate layer (10 to 30 m) (Fig. IV.9A). Chlorophyll a (chl *a*) concentrations showed the same trend as temperature with depth, reaching maximum concentrations (1.8 µg l^{-1}) at 8 m. In contrast, during spring conditions in March 2004 (Fig. IV.9B), the water column showed a mixed upper layer extending down to 45 m with uniform temperatures (2.7°C) and low chl *a* concentrations (~0.7 µg l^{-1}). In both seasons, salinity was lower in the upper compared to the lower (>50 m) water column. Also, in both months, concentrations of oxygen and nitrate (Fig. IV.9C, D) decreased and increased similarly with depth, respectively. In August, the biomass of plankton showed a pronounced subsurface peak dominated by diatoms at ~10 m similar to the peak found for chl *a* (Fig. IV.9E). In turn, in March, plankton biomass was lower, dominated by cryptophytes, and distributed more or less homogeneously with depth (Fig. IV.9F).

In August and March, seston $\delta^{15}N$ increased from low surface values (<2‰) to higher values (>4 to 8 ‰) below the mixed layer at ~15 m and ~50 m, respectively (Fig. IV.10A, B). In August, surface seston $\delta^{15}N$ were somewhat more depleted (<1‰), probably due to the occurrence of diazotrophic cyanobacteria (Fig. IV.9E) and to low nitrate (NO_3^-) concentrations (Fig. IV.9C). In August, mesozooplankton $\delta^{15}N$ increased with depth (Fig. IV.10C), since species with higher signatures generally occurred at greater depths. Thus, lowest $\delta^{15}N$ signatures were found for the cladocerans *B. coregoni* (2.5‰) and *Podon* sp. (4.5‰), which were found at depths <10 m. The $\delta^{15}N$ of *T. longicornis* (~ 4.7 ‰), which was

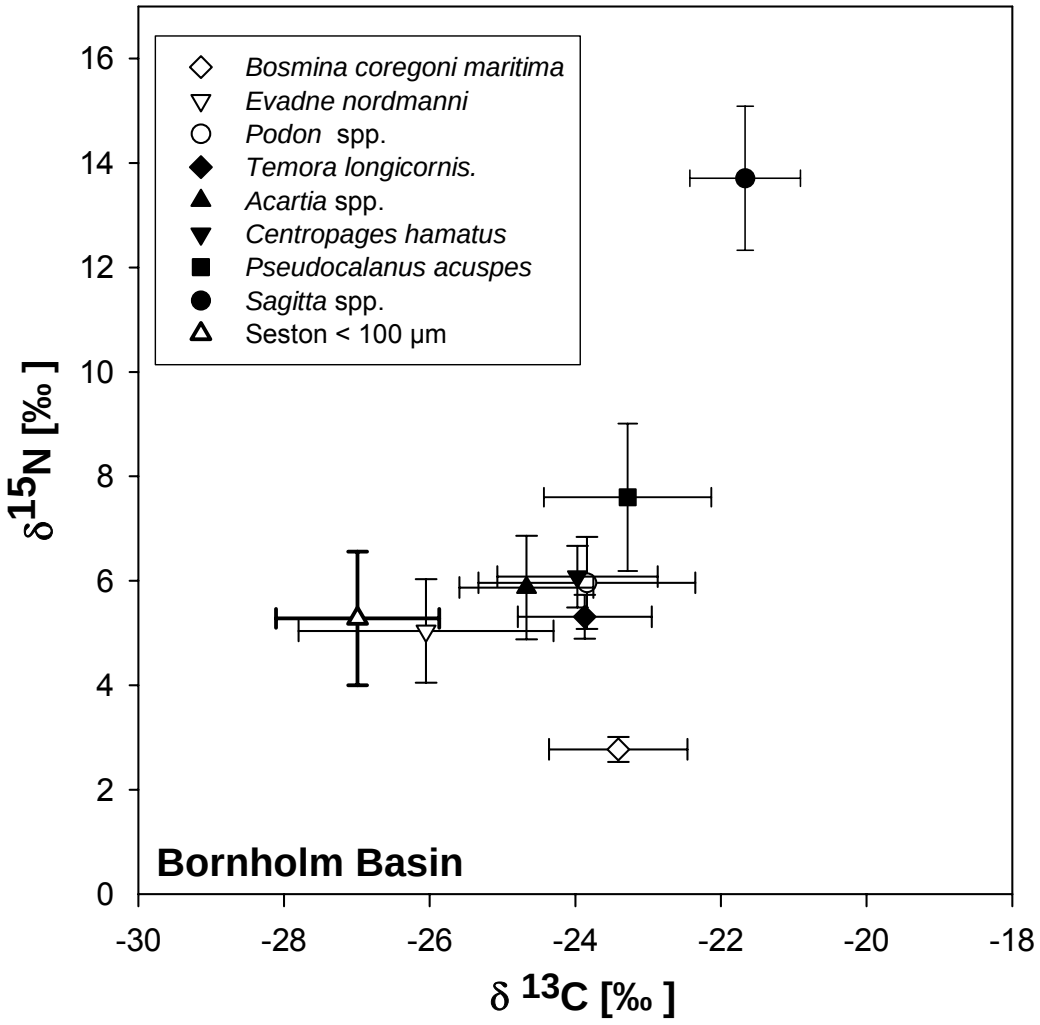

Figure IV.8. General dual isotope diagram of depth-integrated (mean ± 1SD) δ^{15}N and δ^{13}C signatures of mesozooplankton species in the Bornholm Basin. Sample size are: Seston < 100µm (*n* = 132); *Bosmina coregoni maritima*.(August 2003, *n* = 21); *Evadne nordmanni* (only in 2003, *n* = 60); *Podon* spp. (only in 2003, *n*= 81); *Temora longicornis, Acartia* spp., *Centropages hamatus and Pseudocalanus acuspes* (each species *n* = 93); and *Sagitta* spp.(from August to March, *n* = 29).

found between around the thermocline between 10 and 30 m, was lower than that of co-occurring copepod species *C. hamatus* (~5-6‰), *A. longiremis* (~6.8 ‰) and *A. bifilosa* (~ 5.4 ‰). *P. acuspes*, which was the only species found below the thermocline, where the chl *a* concentration was lowest and ciliate biomass was highest (Fig. IV.9) showed the highest δ^{15}N (~8‰, only *P. acuspes* adult stages). In turn, the vertical distribution of copepod δ^{15}N in March showed similarly depleted mesozooplankton δ^{15}N throughout the water column (Fig. IV.10D). Also, in this month, *Acartia* spp. and *C. hamatus* were found deeper in the water

column, probably due to the greater extension of the mixed surface layer, than in August and, particularly, *T. longicornis* was also found below the mixed layer together with *P. acuspes*.

Average trophic enrichments calculated with seston $\delta^{15}N$ as baseline ($\Delta\delta^{15}N_{seston}$) were in all cases positive in August (Fig. IV.10E), with values greater than one trophic level (i.e., >3.4‰) found both at shallow depths (5 to 10 m) for most species and at depths exceeding 50 m (only *P. acuspes*). Despite the similar vertical pattern in the $\delta^{15}N$ signatures of copepods (only slight differences between species, but consistent across depth intervals, Fig. IV.10D), calculated trophic enrichments in March (Fig. IV.10F) decreased from high (~2 to 4‰) subsurface values to strongly negative values with depth, given the strong increase of seston $\delta^{15}N$ with depth (Fig. IV.10B).

Discussion

I discuss below some aspects that are likely tied to the mesozooplankton isotopic variation: (i) the spatial homogeneity and seasonal variability, (ii) the baseline, (iii) relationship of nitrogen and carbon trophic enrichment, (iv) inter- specific differences, (v) mesozooplankton foraging strategies, and (vi) the $\delta^{15}N$ vertical distribution of seston and mesozooplankton.

Spatial homogeneity and seasonal variability.

I found strong spatial homogeneity (Fig. IV.1, 2) in the isotopic signatures of mesozooplankton species over a large (~6000 km^2) horizontal spatial scale in contrast to many previous studies (Montoya et al. 1990, Schell et al. 1998, Kline 1999, Harvey & Kitchell 2000, Rolff & Elmgren 2000, Davenport & Bax 2002, Montoya et al. 2002, Schmidt et al. 2003, Syväranta et al. 2006). With the only 2 exceptions - *B. coregoni* $\delta^{13}C$ in August and *Sagitta* $\delta^{13}C$ and $\delta^{15}N$ in Nov/Dec. – spatial variance within the study area accounted for only 0.4% of the total variance in mesozooplankton isotopic ratios. This was somewhat higher (4% of the variance explained) for seston isotopic signatures, which was related to horizontal spatial differences in seston signatures during the development of a cyanobacterial bloom in August 2003 (Fig. IV.4).

Temporal differences constituted the main source of isotopic variation (36% to 94%) in this study. These seasonal changes were low for some species (*T. longicornis*) and pronounced for others (*P. acuspes*) and may be related to “autochtonous” factors, such as seasonal changes in diet (phytoplankton versus ciliates or detritus) and nutrient supply at the food base (regenerated production during thermal stratification versus new production in

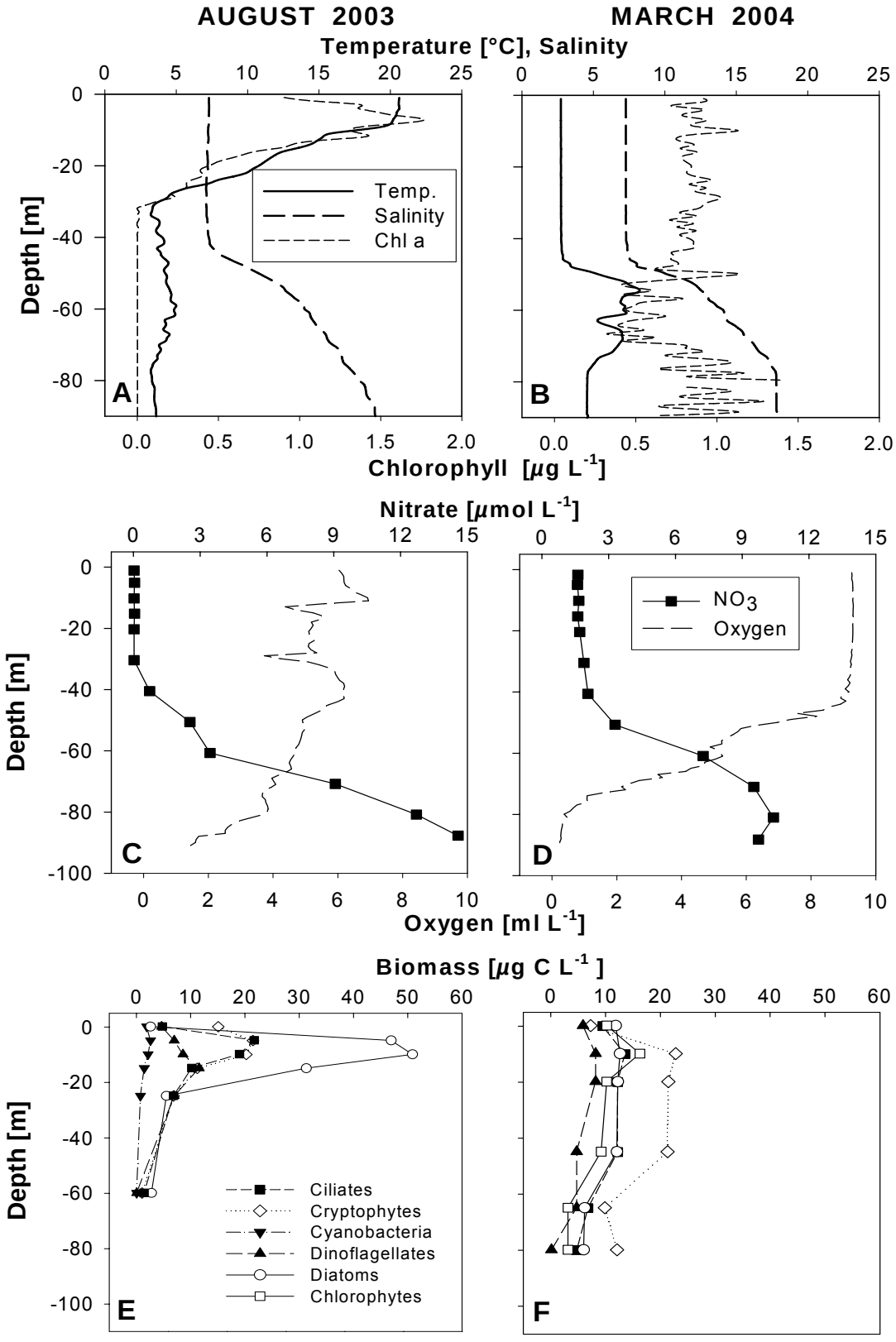

Figure IV. 9. Station 23: Vertical distribution of temperature, salinity, chlorophyll *a* (A and B), oxygen and nitrate concentrations (C and D), and the biomass of major taxonomic groups (E and F) in August 2003 (left column) and March 2004 (right column).

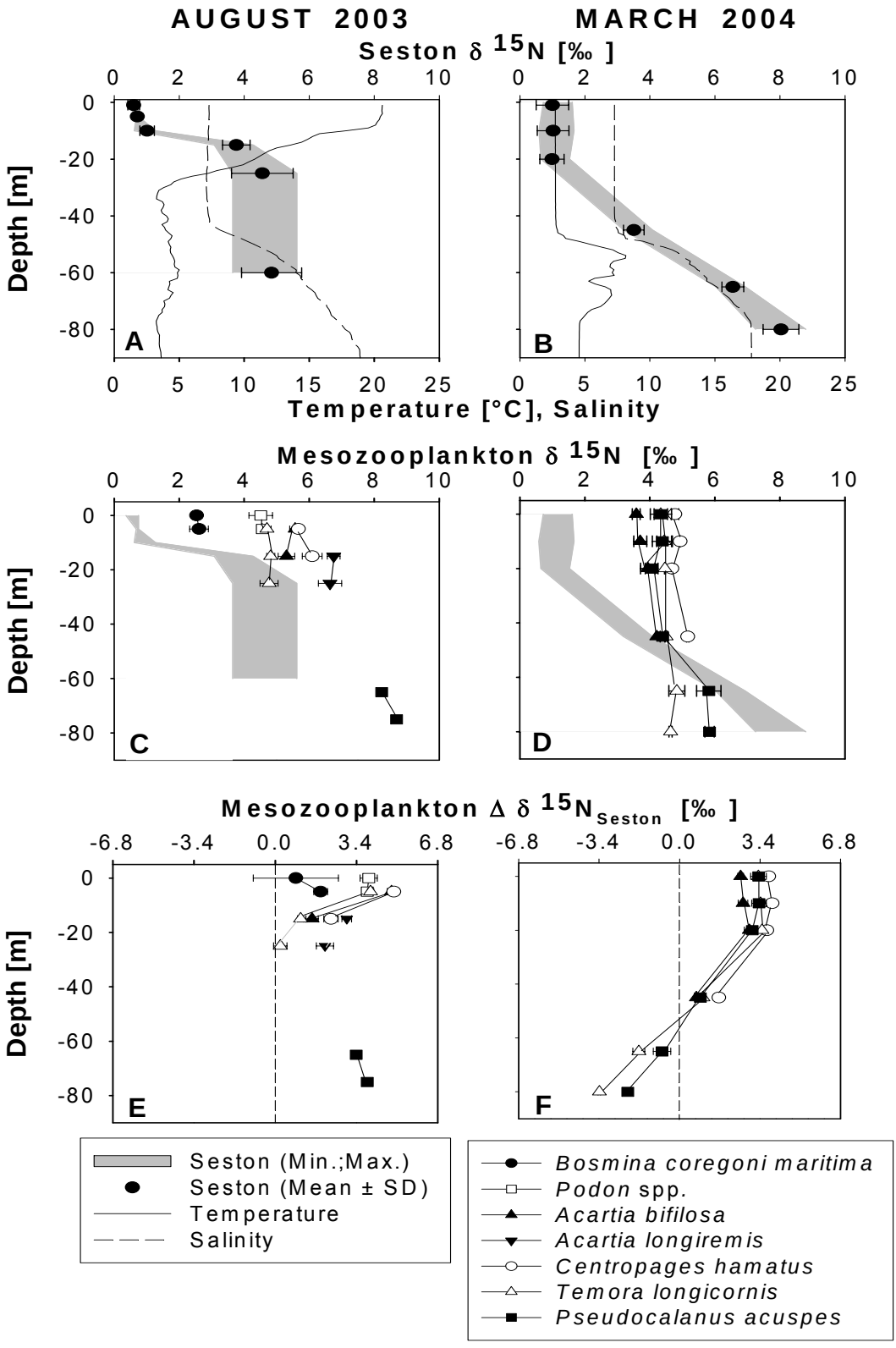

Figure IV. 10. Station 23: Vertical distribution of seston δ^{15}N (A and B), mesozooplankton δ^{15}N (C and D) and mesozooplankton trophic enrichment ($\Delta\delta^{15}$N) relative to seston (E and F) in August 2003 (left column) and in March 2004 (right column). In March 2004, *A. bifilosa* and *A. longiremis* were pooled as *Acartia* spp. Shaded region spans minima and maxima of seston δ^{15}N.

spring). “Allochtonous” factors such as terrestrial input via freshwater run-off, which may cause significant variation in coastal zones (Rolff 2000, Rolff & Elmgren 2000), are however unlikely to affect isotopic signatures of plankton in the Bornholm Basin.

The decrease of seston $\delta^{15}N$ during summer (Fig. IV.3) is consistent with the recurrence of cyanobacterial blooms in the Baltic Sea, which introduces significant amounts of isotopically light nitrogen. Also, the increase of seston $\delta^{13}C$ may be explained by increased productivity due to higher temperatures resulting in decreased discrimination against the heavier ^{13}C isotope during photosynthesis. While the cladocerans closely followed the variations in seston isotopic ratios, we found that copepod species remained relatively unchanged or even slightly increased their isotopic ratios during summer stratification.

Seasonal variability at the base of the food web may complicate comparisons among consumers, because the isotopic signatures of primary producers and consumers may become temporally de-coupled due to the slower growth of the latter. Hence, rapid temporal changes in seston isotopic signatures may partially explain the fact that the calculated trophic enrichments (indicative of TL) were negative in some cases.

In general, the isotopic signatures increased in mesozooplankton during summer and autumn period, but decreased throughout winter and early spring (Fig. IV.3). Such a trend is shown in particular for ^{15}N, while the ^{13}C values were more variable, but also higher in summer. These results are partly consistent with those from ecosystems where the annual primary production is characterized by a second phytoplankton bloom in summer or autumn that supports the subsequent growth of mesozooplankton in the coastal zone in the northern Baltic Proper (Montoya et al. 1990, Rolff 2000).

The baseline.

The goal of a baseline for the food web, is to reflect the isotopic signatures of the primary source of production (Cabana & Rasmussen 1994, 1996, Post 2002, Matthews & Mazumder 2003).The use of stable isotopes as a trophic level indicator in the plankton communities suffers from the problem of usually not being able to isolate pure samples of primary producers (TL= 1). Filterable seston is a mixture of phytoplankton, mixo- and heterotrophic flagellates, ciliates, bacteria and detritus (allochthonous or autochthonous in origin), each occupying a different trophic level and having their own isotopic signatures.

I have tried to overcome this problem by size fractionation of seston (data not shown), but was not successful in obtaining pure phytoplankton fractions. The failure of using seston as a baseline is demonstrated by the fact that the zooplankton trophic level calculated in this way in several cases was clearly <2 (Leggett et al. 2000, O'Reilly et al.

2002).Therefore, I rely more on the alternative approach to assume that the least ^{15}N enriched species in this study (*E. nordmanni.* and *B. coregoni*) represent the closest possible approximation to trophic level 2 (pure herbivory), as has been previously done with the filter feeding cladoceran Daphnia in fresh water (Matthews & Mazumder 2003), with filter feeding mussels (Fry 1999, McKinney et al. 1999, McKinney et al. 2002, Post 2002) and with a size fractionated mixture of zooplankton (Cabana & Rasmussen 1994, Rolff 2000). While the former two approaches are quite similar to my approach, the latter one was only meaningful because the trophic levels above plankton were the focus of study.

I are aware, that the actual trophic levels of my study species might have been higher than calculated according to the extent of carnivory in the two reference species, but I am confident that at least the relative position of the different zooplankton species in the trophic pyramid are well reflected by this method. Additionally, the baseline consumers provide time-integrated isotope values that buffer fluctuations in the quality, assimilation efficiency and preliminary fractionation of phytoplanktonic or detrital food sources (Hart & Lovvorn 2002). In sum, cladocerans assimilate only edible and digestible particles from seston, hence, their tissues reflect the isotopic baseline of the foodweb better than seston collected on a filter does (Hart & Lovvorn 2002).

There are nevertheless, some potential drawbacks of using reference organisms as baseline. First, an ideal scenario is only obtained when the baseline organisms and the omnivore consumers are biologically related and likely have similar temporal and spatial integration of food source isotopic signatures (Kling et al. 1992). Second, a recent experimental study with *Daphnia magna* by Adams & Sterner (2000) has reported that the enrichment of ^{15}N per trophic levels depends on C:N ratio of the ingested food (e.g. algae). Third, *E. nordmanni* could feed not only on phytoplankton, but also on heterotrophic organisms (direct particle capture). This was probably the case in Nov/ Dec. (Fig. IV.3A, B), where the $\delta^{15}N$ of *E. nordmanni* were higher than the $\delta^{15}N$ of *T. longicornis* (stationary suspension-feeding). Thus, variable omnivory may complicate the interpretation of *E. nordmanni* $\delta^{15}N$ as an appropriate baseline. Fourth, the reference organisms were not always presents in the study area. *B. coregoni* was only present in August, while *E. nordmanni* was almost absent in the Basin during the same month and both cladoceran species were completely absent in March. These cladocerans, such as *Daphnia spp.* in limnetic systems, are short-lived organisms, hence they do not provide long temporal isotopic integration like mussels (Fry 1999, Post 2002). However, Matthews & Mazumder (2003) have suggested that cladocerans are better suited for finer scale temporal integration of pelagic stable isotope signatures. In spite of the differences, possible drawbacks and assets between the baselines applied in this study, the assessed trophic enrichment and apparent trophic levels

showed that in both approaches, there were the same inter-specific differences and an increasing enrichment of ^{15}N and ^{13}C (Fig. IV.6, 7).

Relationship of nitrogen and carbon trophic enrichment.

The overall relationship between $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ for planktonic consumers relative to seston (Fig. IV.7A) and to *E. nordmanni* (Fig. IV.7B), although significant ($p < 0.0001$) and positively correlated, yielded a weak predictive capability (r^2 = 0.14 and r^2 = 0.40, respectively), indicating high variation in ^{13}C with respect to the values (-1.5 to +2.7‰) reported by DeNiro & Epstein (1978), as well as, in comparison to the usually assumed trophic shift in ^{15}N (+2.6 to +3.4‰) between diet and consumer (DeNiro & Epstein 1981, Minagawa & Wada 1984, Owens 1987).

I compared the $\Delta\delta^{15}N$ to $\Delta\delta^{13}C$ relative to different baselines (Fig. IV.7). In each case, with the exception of the $\Delta\delta^{15}N$ and $\Delta\delta^{13}C$ relative to *B. coregoni* in August 2003, where no association was detected (Fig. IV.7C), the difference between the $\delta^{15}N$ of the mesozooplankton species and *E. nordmanni* (-1.1 to 9.6‰) was less than between mesozooplankton and seston (-3.0 to 13.1‰), but on average ($\Delta\delta^{15}N_{E.nordmanni}$: 1.5 ± 1.8 ‰; $\Delta\delta^{15}N_{Seston}$: 1.1 ± 2.3‰) both were less than the generally accepted value of 3.4 ‰ per trophic level. The differences in $\delta^{13}C$ between mesozooplankton and seston (-1.7 to 6.2‰) were greater than the differences relative to *E. nordmanni* (-1.4 to 5.2‰), indicating that both $\Delta\delta^{13}C$ approaches should be greater than the generally accepted value of 1‰ per trophic level for open-ocean food webs. Consequently, the significant regressions of all mesozooplankton $\Delta\delta^{13}C$ as a function of trophic level according to ^{15}N, relative to seston (Slope = 2.04; $p < 0.0001$) and relative to *E. nordmanni* (Slope = 2.14; $p < 0.0001$), confirm that the carbon enrichment factor (slopes) per trophic level was higher than the usually proposed average 0.5 ‰ by Post (2002) and Fry (2006), and clearly higher than the ^{13}C trophic enrichments averages among different aquatic ecosystems (e.g. freshwater (0.2‰) , estuarine (0.5‰), coastal (0.8‰) and open-ocean (1.1‰) food webs,) reported by France & Peters (1997).

Although the average 3.4 ‰ and 1‰ of nitrogen and carbon trophic enrichment are consistent with several previous studies, it is important to note that these values are a valid approximation of trophic fractionation only when averaged over multiple trophic pathways (Post, 2002). Trophic level differences of 3 to 4‰ of ^{15}N are common for organisms at high trophic levels, but are not always observed for organisms lower in the food web, such as cladocerans and copepods in this study, feeding on small particles where the diet may

include sources that have been modified by nutrient recycling or selective ingestion or assimilation of a food source.

Inter- specific differences.

The observed $\Delta\delta^{15}N$ values revealed that the average values of the mesozooplankton species varied from –1.6 ± 1.1 ‰ for *B. coregoni* to 4.3 ± 0.9 ‰ for *P. acuspes,* relative to seston their assumed food. In general the observed $\Delta\delta^{15}N_{seston}$ of copepods (–0.9±0.9 to 4.3±0.9‰) was higher than the 3 ‰ previously reported by Broman et al. (1992) for the northern Baltic Sea, but close to that observed for the northern Baltic Proper by Hansson et al. (1997), who found that the copepod *Pseudocalanus* sp. had 1 to 2 ‰ higher $\delta^{15}N$ signatures than the other mesozooplankton species (excluding *Sagitta* spp), indicating stronger carnivory in this species. In comparison to the findings in Chesapeake Bay for the copepod *Acartia tonsa* (3.3 to 4.2 ‰ relative to seston) by Montoya et al. (1990, 1991) and for *Temora* spp. (~6 ‰) from the Gulf of Mexico by Checkley & Entzeroth (1985), my data revealed clearly lower enrichments relative to seston for *Acartia* spp (–0.7 ± 0.9 to +2.2 ± 1.2‰) and *T. longicornis* (–0.9 ± 0.9 to 1.9 ± 0.9‰).

Taking the traditional approach of using averages of absolute $\delta^{15}N$ values (Fig. IV.8) and accepting the 3.4‰ enrichment per trophic leads to the following conclusion: The $\delta^{15}N$ values of *T. longicornis* (5.3 ± 0.4 ‰); *Acartia* spp. (5.9 ± 1.0 ‰); *Podon* spp. (6.0 ± 0.9 ‰); *C. hamatus.* (6.1 ± 0.6 ‰) and particularly *P. acuspes* (7.6 ± 1.4 ‰) indicate that these species are not exclusively herbivorous. This is because they feed also on small heterotrophs (flagellates and ciliates) (Rau et al. 1990, Hobson et al. 1995). Between the species, $\delta^{15}N$ averages ranged from 2.8 ± 0.2 ‰ for the herbivorous and endemic brackish water cladoceran *B. coregoni* to 13.7± 1.4 ‰ for the carnivorous chaetognath *Sagitta* spp. These two species form end points of the herbivory–carnivory gradient within the plankton community. The relative position of all other species can be defined relative to them. However, it is important to note that *Sagitta* spp. is a primary carnivore, but can also be a secondary carnivore, since this species shows ^{15}N enrichments greater than one trophic level with respect to the calanoid copepod *P. acuspes*.

B. coregoni is believed to be a primary herbivore, feeding preferably on nanoplankton (DeMott 1982) during the spring and summer bloom, particularly in August when it attains its maximum abundance and when surface temperatures rise above 20°C. Although *E. nordmanni* is also considered a herbivorous species (Kim et al. 1989), its feeding strategy (direct particle capture) differs markedly from that of *B. coregoni*, because *E. nordmanni* feeds rather on microplankton and not only on large diatoms, but also on small heterotrophs. At TL_{Seston} 1.6 ± 0.4 or TL_{EvBo} 2.3 ± 0.3, these results concur with the hypothesis of *T.*

longicornis having an omnivorous diet dominated by herbivory. The similarly high trophic level of the cladoceran *Podon* spp (TL_{Seston} 1.7 ± 0.3 or TL_{EvBo} 2.4 ± 0.3) and of the copepods *Acartia* spp (TL_{Seston} 1.7 ± 0.4 or TL_{EvBo} 2.5 ± 0.4) and *C. hamatus* (TL_{Seston} 1.8 ± 0.3 or TL_{EvBo} 2.5 ± 0.4) supports an omnivorous diet dominated by carnivory, such as for the copepod *Metridia longa* (TL 2.5) in the northern Baffin Bay (Hobson et al. 2002).

Although *Pseudocalanus* spp. is considered an primarily herbivorous copepod (e.g. Schnack 1975, Corkett & McLaren 1978, Cotonnec et al. 2001), their high $\delta^{15}N$ signatures (Fig. IV.3) and apparent trophic level (TL_{Seston} 2.2 ± 0.4 or TL_{EvBo} 3.1 ± 0.4) in this study suggest that this species has an carnivory tendency over most of the year. This finding is consistent with recent fatty acid based studies by Peters et al. (2006), who concludes that *P. acuspes* displays an opportunistic and a more omnivorous feeding behaviour in the Bornholm Basin.

The predator *Sagitta* spp. (Chaetognatha) is known to feed on small prey such as tintinnids and rotifers, barnacle nauplii, appendicalarians, cladocerans, fish larvae, and other chaetognaths (Pearre 1981, Øresland 1987, Baier & Purcell 1997), but the main diet consists of copepods and nauplii (Feigenbaum & Maris 1984, Duró & Saiz 2000, Tönnesson & Tiselius 2005), making it an extremely important link in the transfer of energy from copepods to higher trophic levels (Terazaki 1998). In this study, the $\delta^{15}N$ of *Sagitta* spp. ranged from 13.1 ± 1.0 ‰ to 15.8 ± 1.1 ‰ indicating an voracious behaviour, and therefore occupied the highest trophic level (TL_{Seston} 4.0 ± 0.6; TL_{EvBo} 4.3 ± 0.6) in the planktonic community of Bornholm Basin.

As in most predators, the diet of carnivorous zooplankton such as *Sagitta* spp. varies with size, shape, differential movement pattern or escape capability of prey (Saito & Kiorboe 2001). Although speculative, I believe that the detected differences in the trophic level of *Sagitta* spp., particularly the $\delta^{15}N$- depletion in Nov/Dec. might indicate that *Sagitta* individuals fed only on nauplii and small copepodites. In contrast, the most likely scenario in August was that this chaetognath (probably *S. elegans*) preyed on large copepodites and adult copepods (mainly *P. acuspes* and *O. similis*) below the halocline, while the high inferred trophic level in March likely reflects the predation on congeners of *Sagitta spp.* (probably *S. setosa*), when there was still low copepod abundances in the water column. This intraguild predation is known to be important in chaetognaths (Øresland 1987) and can on average removed 5 to 32% d^{-1} (Kimmerer 1984) or even more (50%, Øresland 1987) of the population of *Sagitta* spp. Unfortunately we did not separate the samples by species (*S. setosa* and *S. elegans*) and by size classes, and no comparative isotopic data exist for *Sagitta* species in the Bornholm Basin. Further study is required to investigate the trophic status and trophodynamics using stable isotope for this predator.

In terms of carbon (Fig. IV.8), the differences among species indicated not only an increase in $\delta^{13}C$ from *E. nordmanni* to *Sagitta* spp, which is similar to the findings in the $\delta^{15}N$ signatures, but also indicated different carbon sources, especially for *E. nordmanni* and *Acartia* spp, which differed significantly from the other species of the Bornholm Basin.

Foraging strategies of mesozooplankton.

The different foraging behaviours of planktonic species lead to different preferences for non-motile and motile prey (Tiselius & Jonsson 1990). It seems plausible, that a higher efficiency in capturing motile prey should enable a copepod to ingest relatively more heterotrophic prey and should, therefore, be reflected in its $\delta^{15}N$ and $\delta^{13}C$ signatures (Sommer et al. 2005). Based on the study by Tiselius & Jonsson (1990), three different foraging modes in calanoid copepods were distinguished: (i) stationary suspension-feeding by *T. longicornis* and *Pseudocalanus* spp., which feed primarily as herbivores, (ii) fast swimming interrupted by sinking periods ('cruise and sink' mode) for *C. hamatus* and (iii) motionless sinking with short jumps ('ambushing') for *Acartia* spp. The latter species may include a higher percentage of motile heterotrophic prey in their diet, since they forage in a hydrodynamically less 'noisy' manner (Sommer et al. 2005).

As expected, *C. hamatus*, a cruising species, such as *Acartia* spp., a ambushing species, showed isotopic increases during this study, particularly in August and Nov/Dec (Fig. IV.3B), suggesting that they were the most efficient species in capturing heterotrophic organisms (e.g. ciliates). The copepod *T. longicornis*, a stationary suspension-feeder, showed a uniform and lower isotopic pattern over the time, which we believe resulted from nutritional stress arising from poor feeding on both ciliates (too fast for ingestion by *T. longicornis*) and nanoflagellates (too small). However, *P. acuspes*, a species equally categorised as a stationary suspension-feeder and therefore traditionally acknowledged as a primary herbivore, showed enrichments in its $\delta^{15}N$, higher to those for *C. hamatus*, over most of the year (Fig. IV.3B). Only the observed $\delta^{15}N$-depletion in March for this species was indicative of a stationary suspension-feeding strategy. In general, the $\delta^{15}N$ signatures of *P. acuspes* may indicate potential switching in its foraging mode, in part, explained by seasonal changes in its vertical position in the water column (Fig. IV.10) associated with its ontogenetic vertical migration or only as result of its vertical position in deeper layers with strong autotrophic food limitations without a change in its foraging mode.

It has been suggested that *Bosmina* spp. classified as filter feeders, can reduce filtering activity and also switch from filtering to searching for individual food items at low levels of food (Bleiwas & Stokes 1985). This is based on the fact that *Bosmina* have lower feeding rates on small than large particles (DeMott 1982). I believe that my data cannot

support this assumption, because the $\delta^{15}N$ of *B. coregoni* were always the lowest and uniform signatures within the Basin during August, indicating that this species fed on a particular seston size fraction, dominated likely by small particles (e.g. from pico- to nanophytoplankton and bacteria) with a lower $\delta^{15}N$ than the rest of the seston. Hence, explanations for a switch from filtering to searching mode in *Bosmina* cannot be confirmed.

In the past decades the *Evadne* sp. and *Podon* spp. have been classified as either raptorial carnivores (Nival & Ravera 1981), or as phytoplankton grazers (Bainbridge 1958). This contradictory classification could be clarified partly with the $\delta^{15}N$ signatures of both species (Fig. IV.3A). The results revealed that *E. nordmanni* was less ^{15}N-enriched (herbivory tendency) than *Podon* spp. This result supports the findings by Kim et al. (1989), that *E. nordmanni* seem to feed largely on discrete autotrophic particles (preferentially centric diatoms), however, the observed isotopic enrichment in Nov/Dec might indicate a switch to raptorial feeding dominated by carnivory. Jagger et al. (1988) reported that *Podon* sp. (e.g. *P. intermedius*) is a raptorially-feeding herbivore, because no animal remains were found in the fecal pellets and because Podon may not be sufficiently fast to capture zooplankters with well-developed escape responses. In contrast, this study suggests that *Podon* spp. fed not only on phytoplankton, but also on small heterotrophs, perhaps on soft-bodies organisms such as naked ciliates. The $\delta^{15}N$ and $\delta^{13}C$ of *Sagitta* spp. are coherent with the assumption that this species as ambush feeders is a zooplankton predators.

$\delta^{15}N$ vertical distribution of seston and mesozooplankton.

The relationships between depth-specific seston $\delta^{15}N$ and environmental factors at the central station no.23, revealed in both months significant associations (p <0.001), suggesting the influence of the thermal stratification, permanent halocline, oxygen, nitrate concentrations (see below), Chl *a*, and phytoplankton composition in August and March, respectively (see Table IV.4).

As expected and according to Checkley & Miller (1989), the variation in $\delta^{15}N$ signatures of seston with respect to the nitrate concentrations showed positive correlations in August (r = 0.53; p <0.01) and in March (r = 0.95; p <0.001), suggesting in general, that seston $\delta^{15}N$ increases in nitrate-replete waters likely due to fractionation during nitrate assimilation by light-limited phytoplankton, while seston $\delta^{15}N$ decreases in nitrate-depleted waters (Fig. IV.9C,D and 10A, B) due to fractionation by heterotrophs (e.g. bacteria, ciliates, cladocerans and copepods) during the metabolism of assimilated nitrogen and due to occurrences of diazotrophic cyanobacteria (*Aphanizomenon* sp, *Nodularia spumigena*) in summer (Fig. IV. 4 and 9E).

Dinitrogen (N_2) fixation by cynobacteria is a significant nitrogen input to the central Baltic Sea (Voss et al. 2005), generating low ^{15}N values of seston in the upper water column of the Bornholm Basin. In both months, the $\delta^{15}N$ of seston increased clearly and rapidly with depth below the mixed layer, a pattern that is commonly observed in oxic marine systems, where it is attributed to the isotopic discrimination associated with microbial decomposition and remineralization (e.g. ammonification and nitrification) of organic nitrogen (Saino & Hattori 1980, Altabet & McCarthy 1986, Montoya et al. 1992, Voss et al. 1997). Despite the formation of ^{15}N-depleted seston by N_2-fixers and low nitrate concentrations at the mixed layer in August, the seston $\delta^{15}N$ values around the thermocline and above the halocline were relatively high, averaging about 4 to 5 ‰ at 15 and 25 m depth, respectively (Fig. IV.10A). Thus, the elevated $\delta^{15}N$ in seston at these depths may partly reflect the effects of (i) increased ciliate biomass relative to phytoplankton (Fig. IV.9E), (ii) concentration of $\delta^{15}N$ enriched particles (i.e. copepods feces) around the themocline, (iii) remineralization processes or (iv) recent nitrate depletion by phytoplankton (e.g diatoms) and minimal nitrogen recycling (Checkley & Miller 1989).

Although under initial spring conditions during March, the depleted $\delta^{15}N$ signatures of seston (Fig. IV.10B), would be a consequence of the relative high nitrate concentration in the mixed layer (Fig. IV.9D) associated with slow phytoplankton growth rates dominated by smaller autotrophs (Fig. IV.9F, mainly cryptophytes) at low temperatures (~3°C, Fig. IV.9B). This isotopic-depletion is similar to the patterns observed in the Southern Ocean (Goericke & Fry 1994), and in the Bering and Chukchi Seas (Schell et al. 1998), where high surface nutrients and dissolved carbon dioxide produce very low $\delta^{13}C$ and $\delta^{15}N$ values in the food webs components. In both months, the high $\delta^{15}N$ signatures around and below the halocline (Fig. IV.10A, B) would be indicative of strong microbial degradation of particulate organic nitrogen (Voss et al. 1997) and may also partly reflect the effects of denitrification at sub-anoxic and anoxic layers in the Bornholm Basin. Denitrification occurs below the oxycline (Brettar & Rheinheimer 1991, 1992) and should result in enriched $\delta^{15}N$ of nitrate (~5‰ in the Baltic Proper, Voss et al. 2005), suggesting in consequence, high $\delta^{15}N$ in seston. Unfortunately, isotopic data for dissolved nitrate in this study are not available to examine isotopic changes in the nitrogen pools of the water column.

The $\delta^{15}N$ vertical distribution of copepods and cladocerans investigated in this study confirmed indirectly their vertical distribution patterns previously displayed by (Dippner et al. 2000, Möllmann et al. 2000, Hansen et al. 2006, Schmidt 2006, Renz et al.2006). In August, the depleted $\delta^{15}N$ values of *B. coregoni* indicated an effective herbivorous condition in the surface layer. The copepods *T. longicornis, Acartia* spp., and *C. hamatus* were found in the

uppermost water layers (Fig. IV.9 and 10), particularly around the thermocline and the chlorophyll maximum (mainly diatoms). Despite the optimal conditions at these depths, the vertical $\delta^{15}N$ signatures of the two *Acartia* species and *C. hamatus* were high (Fig. IV.10C, E), suggesting omnivory dominated by carnivory.

Although speculative, these ^{15}N enrichments might be a result of avoidance of reactive aldehydes produced by diatoms (a non-universal property of diatoms, Paffenhoefer et al. 2005) Therefore, I believe that in accordance with their foraging strategies (e.g. cruising and ambushing), the isotopic enrichment of these copepods was caused by active predation on motile prey (e.g. ciliates), which were dominant around the thermocline. These or closely related species, are know to have high predation on ciliates (Wiackowski et al. 1994, Merrell & Stoecker 1998, Johansson et al. 2004). Indeed, this feeding preference seemed to reduce the importance of diatoms as copepod food. In fact, some copepods previously regarded as 'herbivores' have been shown to readily ingest heterotrophic protists and sustain fecundity on these food taxa (Ohman & Runge 1994).

The utilization of detritus colonized by bacteria (expected ^{15}N-enriched) as food source, is also a possibility of isotopic enrichment, because detritus contains significant amounts of protein, carbohydrate, lipid, and nonprotein nitrogen compounds, and thus may supplement the energy demands of copepods (Roman 1984). Additionally the bacteria that colonize detrital material immobilize ^{15}N-enriched N from the water column, and copepods consuming these bacteria have higher $\delta^{15}N$ signatures (Caraco et al. 1998). It is important to note that the *Acartia* species in August (Fig. IV.10C) showed an isotopic difference of ~ 1.3‰ between *A. bifilosa* (~5.4‰; less enriched, more herbivorous and distributed in the thermocline) and *A. longiremis* (~6.8‰, more enriched, more carnivorous and distributed below the thermocline).

The copepod *P. acuspes,* was recorded in the deeper layer under strong food limitations (Fig. IV.9E), indicating however, the physiological need of high salinity and low temperatures during their reproduction phase (Möllmann et al. 2000). Thus, in contrast to its stationary suspension-feeding and mouth morphology (typical of a herbivore), the higher $\delta^{15}N$ signatures (Fig. IV.10C) and $\Delta\delta^{15}N_{Seston}$ (Fig. IV.10E) might be evidence of predation on heterotrophs (i.e. ciliates and bacteria) and/or may possibly be the result of coprophagy (Gonzalez & Smetacek 1994), since copepods feces are ^{15}N-enriched (Checkley & Entzeroth 1985, Checkley & Miller 1989). This ^{15}N- increase might be also tied to starvation in adults of *P. acuspes* (Adams & Sterner 2000), as well as, to predation on early stages of *Oithona similis*, which dwell at the same depths as *P. acuspes* and/or cannibalism (Sell et al. 2001, Bonnet et al. 2004, Basedow & Tande 2006).

In contrast to August, the observed vertical patterns in mesozooplankton $\delta^{15}N$ signatures in March (Fig. IV.10B), could be due to the strong and deeper mixed layer (Fig. IV.9B) and be indicative of slow phytoplankton growth rates and/or excess nutrient conditions, as stated by Schell et al. (1998). In March, the $\delta^{15}N$ of mesozooplankton is relatively homogenous over depth: if zooplankton do not stay permanently at the same depth but either make vertical migrations or random walks through the water column, they might have eaten more or less the same, whether they have been caught from the shallow or the bigger depths.

Conclusions.

The main conclusion of this study is that significant intra-guild differences in the isotopic signatures of mesozooplankton exist in the Bornholm Basin, where species may be allocated into 4 feeding groups along a gradient of increasing carnivory: herbivorous species (*B. coregoni and E. nordmanni*), omnivorous species dominated by herbivory (*E. nordmanni* and *T. longicornis*), omnivorous species dominated by carnivory (*Acartia* spp., *Podon* spp., *C. hamatus* and *P. acuspes*) and carnivorous species (*Sagitta* spp.). These results are consistent with recent field studies on copepods fatty acids in the Bornholm Basin (J. Peters, pers. comm.), and on natural mesozooplankton $\delta^{15}N$ in the Kiel Fjord, western Baltic Sea (Sommer & Sommer 2004) and in Hopavågen lagoon in the central coast of Norway (Sommer et al. 2005). Additionally, the $\delta^{15}N$ and $\delta^{13}C$ signatures of seston and mesozooplankton, such as the trophic enrichment and their apparent trophic levels varied greatly over time, but not significantly across the basin. A combination of environmental conditions, non- and selective feeding, foraging strategies, lipids (see chapter VII) and vertical feeding positions in a stratified water column influences the $\delta^{15}N$ and $\delta^{13}C$ among mesozooplankton species in the Bornholm Basin. However, this study shows that consumer $\delta^{15}N$ and $\delta^{13}C$ are difficult to interpret, even if potential food sources and aspects of the species' biology are known, and thus emphasises the necessity for further laboratory studies to help better interpret zooplankton $\delta^{15}N$ and $\delta^{13}C$ in the field. Despite the possible drawbacks of stable isotope analysis, the results of this study, provides the first stable isotopes survey of mesozooplankton in the Bornholm Basin and yield a number of insights into the mesozooplankton community structure of the Central Baltic Sea.

Table IV.1. Summary of the two-way ANOVA analysis (with exception [A1W]: one-way ANOVA for *B. c. maritima* and *Sagitta* spp.) of $\delta^{15}N$ and $\delta^{13}C$ for seston, cladocerans, copepods and chaetognaths using sampling station and months as independent variables. Data were Box-Cox -transformed in order to reduce heteroscedasticity in the variances. Statistically significant *p* values are emboldened. df = degrees of freedom, MS = mean square.

			Dependent variable					
			$\delta^{15}N$			$\delta^{13}C$		
	Independent variable	df	MS	*F* ratio	*p*-value	MS	*F* ratio	*p*-value
Seston								
Seston < 100 µm	Month	4	30.88	65.23	**< 0.001**	13.92	235.48	**< 0.001**
	Stat no.	6	1.57	3.31	**< 0.01**	0.30	5.00	**< 0.001**
	Month x Stat no.	21	2.03	4.30	**< 0.001**	0.28	4.68	**< 0.001**
	Error	100	0.47			0.06		
Cladocerans								
Bosmina coregoni maritima [A1W]	Stat no.	6	0.01	1.03	0.449	0.38	4.57	**< 0.01**
	Error	14	0.01			0.08		
Evadne nordmanni	Month	2	41.43	229.62	**< 0.001**	13.36	780.46	**< 0.001**
	Stat no.	6	0.59	3.29	**< 0.05**	0.41	23.90	**< 0.001**
	Month x Stat no.	11	0.85	4.70	**< 0.001**	0.37	21.81	**< 0.001**
	Error	40	0.18			0.02		
Podon spp.	Month	3	2.55	272.29	**< 0.001**	22.00	422.80	**< 0.001**
	Stat no.	6	0.04	4.06	**< 0.01**	0.18	3.49	**< 0.01**
	Month x Stat no.	17	0.04	4.48	**< 0.001**	0.26	5.06	**< 0.001**
	Error	54	0.01			0.05		
Copepods								
Temora longicornis	Month	4	446.42	15.73	**< 0.001**	9.38	282.35	**< 0.001**
	Stat no.	6	19.82	0.70	0.652	0.03	0.84	0.544
	Month x Stat no.	20	63.83	2.25	**< 0.001**	0.07	2.15	**< 0.05**
	Error	62	28.38			0.03		
Acartia spp.	Month	4	2.39	125.81	**< 0.001**	29.19	52.98	**< 0.001**
	Stat no.	6	0.08	4.28	**< 0.01**	0.45	0.81	0.563
	Month x Stat no.	20	0.07	3.58	**< 0.001**	1.28	2.33	**< 0.01**
	Error	62	0.02			0.55		
Centropages hamatus	Month	4	0.01	165.98	**< 0.001**	2.49	213.90	**< 0.001**
	Stat no.	6	0.00	3.32	**< 0.01**	0.03	3.00	**< 0.05**
	Month x Stat no.	20	0.00	6.01	**< 0.001**	0.05	4.24	**< 0.001**
	Error	62	0.00			0.01		
Pseudocalanus acuspes	Month	4	0.02	721.36	**< 0.001**	8.09	77.82	**< 0.001**
	Stat no.	6	0.00	9.96	**< 0.001**	0.44	4.24	**< 0.01**
	Month x Stat no.	20	0.00	3.68	**< 0.001**	0.28	2.69	**< 0.01**
	Error	62	0.00			0.10		
Chaetognaths								
Sagitta spp [A1W]	Month	2	0.00	10.48	**< 0.001**	0.58	1.92	0.166
	Error	26	0.00			0.30		
	Stat no. (Nov/Dec)	6	0.00	13.27	**< 0.001**	0.77	3.88	**< 0.01**
	Error	22	0.00			0.20		

Table IV.2. Summary of the two-way ANOVA analysis of isotopic signatures and trophic enrichment of nitrogen and carbon ($\Delta\,\delta^{15}N$, $\Delta\,\delta^{13}C$) relative to seston and relative to reference cladocerans using month and mesozooplankton species as independent variables. Data were Box-Cox -transformed in order to induce homocedasticity. Statistically significant results are shown in bold. df = degrees of freedom, MS = mean square.

		Dependent variable					
Independent		$\delta^{15}N$			$\delta^{13}C$		
variable	df	MS	*F* ratio	*p*-value	MS	*F* ratio	*p*-value
Month	4	3.3	306.7	**<0.0001**	59.4	361.3	**<0.0001**
Species	6	5.7	535.1	**<0.0001**	15.7	95.7	**<0.0001**
MonthxSpecies	17	0.6	52.2	**<0.0001**	4.5	27.6	**<0.0001**
Error	506	0.01			0.2		
		$\Delta\,\delta^{15}N_{Seston.}$			$\Delta\,\delta^{13}C_{Seston.}$		
Month	4	45.2	104.3	**<0.0001**	178.5	238.1	**<0.0001**
Species	6	51.3	118.4	**<0.0001**	42.1	56.1	**<0.0001**
MonthxSpecies	17	5.2	11.9	**<0.0001**	13.0	17.3	**<0.0001**
Error	506	0.4			0.7		
		$\Delta\,\delta^{15}N_{EvBo.}$			$\Delta\,\delta^{13}C_{EvBo.}$		
Month	3	56.9	675.9	**<0.0001**	126.0	72.2	**<0.0001**
Species	4	31.4	372.5	**<0.0001**	114.4	65.6	**<0.0001**
Month*Species	12	2.5	29.1	**<0.0001**	21.1	12.1	**<0.0001**
Error	385	0.1			1.7		

Table IV.3. Summary of the Tukey post hoc test testing for inter-guild differences in mesozooplankton $\delta^{15}N$ and $\delta^{13}C$ signatures and trophic enrichment ($\Delta\delta^{15}N$, $\Delta\delta 1^{13}C$) relative to seston and cladocerans in the Bornholm Basin. ns = not significant ($p > 0.05$), * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.001$. Bos, Eva, Pod, Tem, Aca, Cen, Pse and Sag = *B. coregoni*, *E. nordmanni*, *Podon* spp, *T. longicornis*, *Acartia* spp, *C. hamatus*, *P. acuspes* and *Sagitta* spp., respectively.

$\delta^{15}N$

Species	Bos	Eva	Pod	Tem	Aca	Cen	Pse	Sag
Mean ± SD	2.8 ± 0.2	5.3 ± 0.6	6.0 ± 0.9	5.3 ± 0.4	5.9 ± 1	6.1 ± 0.6	7.6 ± 1.4	13.7 ± 1.4
Bos								
Eva	***							
Pod	***	**						
Tem	***	ns	***					
Aca	***	*	ns	**				
Cen	***	***	ns	***	ns			
Pse	***	***	***	***	***	***		
Sag	***	***	***	***	***	***	***	

$\delta^{13}C$

Species	Bos	Eva	Pod	Tem	Aca	Cen	Pse	Sag
	-23.4 ± 0.9	-25.5 ± 0.9	-23.8 ± 1.5	-23.9 ± 0.9	-24.7 ± 0.9	-24 ± 1.1	-23.4 ± 1.1	-21.6 ± 0.7
Bos								
Eva	***							
Pod	ns	***						
Tem	ns	***	ns					
Aca	**	**	***	***				
Cen	ns	***	ns	ns	***			
Pse	ns	***	ns	ns	***	**		
Sag	***	***	***	***	***	***	***	

$\Delta\delta^{15}N_{Seston}$

Species	Bos	Eva	Pod	Tem	Aca	Cen	Pse	Sag
Mean ± SD	-1.6 ± 1.1	-0.5 ± 1.1	0.5 ± 1	0.2 ± 1.2	0.7 ± 1.4	1.0 ± 1.1	2.5 ± 1.4	8.5 ± 1.9
Bos								
Eva	*							
Pod	***	***						
Tem	***	*	ns					
Aca	***	***	ns	*				
Cen	***	***	ns	***	ns			
Pse	***	***	***	***	***	***		
Sag	***	***	***	***	***	***	***	

$\Delta\delta^{13}C_{Seston}$

Species	Bos	Eva	Pod	Tem	Aca	Cen	Pse	Sag
	2.7 ± 0.9	1.2 ± 1.3	2.7 ± 1.4	2.9 ± 1.0	2.1 ± 1.2	2.8 ± 1.1	3.4 ± 1.2	4.2 ± 1.4
Bos								
Eva	**							
Pod	ns	***						
Tem	ns	***	ns					
Aca	ns	**	*	***				
Cen	ns	***	ns	ns	**			
Pse	ns	***	**	ns	***	*		
Sag	***	***	***	***	***	***	***	

$\Delta\delta^{15}N_{EvBo.}$

Species	Pod	Tem	Aca	Cen	Pse	Sag
Mean ± SD	1.3 ± 0.7	0.7 ± 1.1	1.5 ± 1.4	1.6 ± 1.2	3.4 ± 1.5	7.9 ± 2.2
Pod						
Tem	**					
Aca	ns	***				
Cen	ns	***	ns			
Pse	***	***	***	***		
Sag	***	***	***	***	***	

$\Delta\delta^{13}C_{EvBo.}$

Species	Pod	Tem	Aca	Cen	Pse	Sag
	1.1 ± 0.9	1.0 ± 1	0.2 ± 0.8	1.1 ± 0.9	1.9 ± 0.9	3.1 ± 1.4
Pod						
Tem	ns					
Aca	***	***				
Cen	ns	ns	***			
Pse	***	***	***	***		
Sag	***	***	***	***	***	

Table IV.4. Correlations of depth-specific seston $\delta^{15}N$ signatures as a function of environmental parameters at the station no. 23 in August 2003 and March 2004. *r* = coefficient of correlation, r^2 = coefficient of determination. Statistically significant *p* values are emboldened.

Month	Predictor variable (X)	X units	Dependent Variable (Y)	Y units	Correlation coefficients			
					n	*r* (X,Y)	r^2	*p* - value
August 2003	Temperature	° C	Seston $\delta^{15}N$	‰	18	-0.93	0.86	**<0.001**
	Salinity		Seston $\delta^{15}N$	‰	18	0.51	0.26	**<0.01**
	Oxygen	ml L^{-1}	Seston $\delta^{15}N$	‰	18	-0.87	0.75	**<0.001**
	NO_2	μmol L^{-1}	Seston $\delta^{15}N$	‰	18	0.57	0.33	**<0.001**
	NO_3	μmol L^{-1}	Seston $\delta^{15}N$	‰	18	0.53	0.28	**<0.01**
	Chl *a*	μg L^{-1}	Seston $\delta^{15}N$	‰	18	-0.83	0.69	**<0.001**
	Diatoms	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.50	0.25	**<0.01**
	Cryptophytes	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.88	0.77	**<0.001**
	Cyanobacteria	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.88	0.77	**<0.001**
	Dinoflagellates	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.20	0.04	0.24
	Ciliates	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.60	0.37	**<0.001**
March 2004	Temperature	° C	Seston $\delta^{15}N$	‰	18	0.80	0.65	**<0.001**
	Salinity		Seston $\delta^{15}N$	‰	18	0.95	0.90	**<0.001**
	Oxygen	ml L^{-1}	Seston $\delta^{15}N$	‰	18	-0.94	0.89	**<0.001**
	NH_4	μmol L^{-1}	Seston $\delta^{15}N$	‰	18	-0.45	0.20	**<0.01**
	NO_2	μmol L^{-1}	Seston $\delta^{15}N$	‰	18	0.09	0.01	0.59
	NO_3	μmol L^{-1}	Seston $\delta^{15}N$	‰	18	0.95	0.90	**<0.001**
	Chl *a*	μg L^{-1}	Seston $\delta^{15}N$	‰	18	-0.64	0.41	**<0.001**
	Diatoms	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.94	0.88	**<0.001**
	Cryptophytes	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.42	0.18	**<0.05**
	Chlorophytes	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.88	0.77	**<0.001**
	Dinoflagellates	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.88	0.77	**<0.001**
	Ciliates	μg C L^{-1}	Seston $\delta^{15}N$	‰	18	-0.85	0.73	**<0.001**

V. Mesozooplankton trophic levels in the Gdansk Deep and Gotland Basin (Central Baltic Sea).

Abstract

The $\delta^{15}N$ and $\delta^{13}C$ of marine mesozooplankton species was measured at five sampling stations in a transect from Gdansk Deep to Gotland Basin during July 2003. Significant differences ($p < 0.001$) were found between copepods and cladocerans. Seston $\delta^{15}N$ and $\delta^{13}C$ were negatively and positively correlated with the diatom:ciliate biomass ratio, respectively. This indicates that both $\delta^{15}N$ and $\delta^{13}C$ of seston were enriched at higher ciliates and diatoms biomass, respectively. The seston isotopic signatures showed a $\delta^{15}N$-depletion over time caused by blooms of diazotrophic cyanobacteria. Considering the average trophic enrichment relative to *E. nordmanni* $\delta^{15}N$ (i.e. $\Delta\delta^{15}N_{E.nordmanni}$) allowed species to be divided into 5 homogenous groups increasing in the order: *T. longicornis* (0.9 ±1.0, TL=2.3) < *C. hamatus*, *Acartia* spp. (both, 1.5 ±0.8, TL=2.4) < *Podon* spp. (1.9 ±1.1, TL=2.6) < *P. acuspes* (4.6 ± 0., TL=3.4) < *L. macrurus* (5.9 ± 0.2, TL=3.7), suggesting a gradient of herbivory to strong carnivory in the same order. The increase in carbon trophic enrichment (i.e. $\Delta\delta^{13}C_{E.nordmanni}$) resulted again in 5 groups: *Acartia* spp. (1.2±0.8‰) < *T. longicornis* (1.6±1.0‰), *C. hamatus.* (1.8±0.8‰) < *C. hamatus, Podon* spp. (1.9±0.8‰), < *P. acuspes* (3.0±0.5‰) < *L. macrurus* (3.3±0.3‰). These groups indicate different carbon sources, especially for *Acartia* spp. The high *L. macrurus* $\delta^{15}N$ signature as well as its apparent TL are indicative of its known omnivorous feeding mode with a strong tendency towards carnivory. Overall, this study showed that the trophic structure of mesozooplankton using analysis of nitrogen stable isotopes was very similar to the mesozooplankton structure in the Bornholm Basin in the same month. However, the $\delta^{13}C$ signatures were significantly different suggesting that mesozooplankton in both areas used different carbon sources.

Results

The water column was strongly stratified in July 2003 (Fig. V.1A, B) showing a warm (16°C) surface layer (<20 m), an intermediate cold (~4°C) layer (40 to 80 m) and a somewhat warm (old water mass, > 6°C) bottom layer (>90 m). The upper thermocline was well developed in 25 m depth (Fig. V.1A). Below 60 m, salinity (> 8 psu) increased with depth (Fig. V.1B). Seston composition (Fig.V.1C) was dominated by large and relatively constant amounts of detritus across the transect (291 to 358 µg C L^{-1}). Phytoplankton increased slightly at stations no. 101 and 109 in Gotland Basin. Overall, the phytoplankton biomass ranged between 36 and 56 µg C L-1. The biomass of ciliates was low and decreased slightly from Gdansk Deep (6.9 µg C L^{-1}) to Gotland Basin (5.6 µg C L^{-1}). The biomass of major taxonomic groups (Fig.V.1D) showed that diatoms constituted 40 to 48 % of the total carbon biomass, followed in decreasing order by chlorophytes (11 to 26 %), cryptophytes (10 to 22%), ciliates (9 to 16%), dinoflagellates (6 to 11%) and cyanobacteria (3 to 8%).

Significant spatial differences were detected in the $\delta^{15}N$ and $\delta^{13}C$ signatures of seston (Table V.1, Fig. V.2). The post hoc test (Tukey HSD, $p<0.001$) indicated that only the seston $\delta^{15}N$ signatures at station no. 109 (Gotland Basin) were significantly less enriched in comparison to the other sites. While seston $\delta^{13}C$ were more enriched at the station in the Gdansk Deep (stations 65, 74 and 85), isotopic signatures of seston (data not shown) were not correlated with temperature, salinity or chlorophyll *a* concentrations ($p>$ 0.05), respectively. However, seston $\delta^{15}N$ and $\delta^{13}C$ were negatively (seston $\delta^{15}N = -0.92X + 8.1$, $r^2 =0.4$, $p <0.01$) and positively (seston $\delta^{13}C = 0.33X- 27.5$, $r^2 =0.5$, $p <0.001$) correlated with the diatom:ciliate biomass ratio, respectively. This indicates that both $\delta^{15}N$ and $\delta^{13}C$ of seston were enriched at higher ciliates and diatoms biomass, respectively.

Significant correlations of seston isotopic signatures were detected only for $\delta^{13}C$ as a function of the diatom:ciliate biomass ratio (seston $\delta^{13}C= 0.21X – 27.6$ $r^2 =0.85$, $p <0.0001$) and as a function of detritus concentrations (seston $\delta^{13}C= – 16X – 12.1$, $r^2 =0.4$, $p <0.01$). The positive slope of the regression as function of the diatom:ciliate biomass ratio suggests that the $\delta^{13}C$ of diatoms was slightly higher (0.2 to 0.3‰) than that of dinoflagellates, because the $\delta^{13}C$ increased with the higher contribution of diatoms (higher ratio). In turn, the negative slope suggests that small differences in larger amounts of detritus led to seston ^{13}C-depletion.

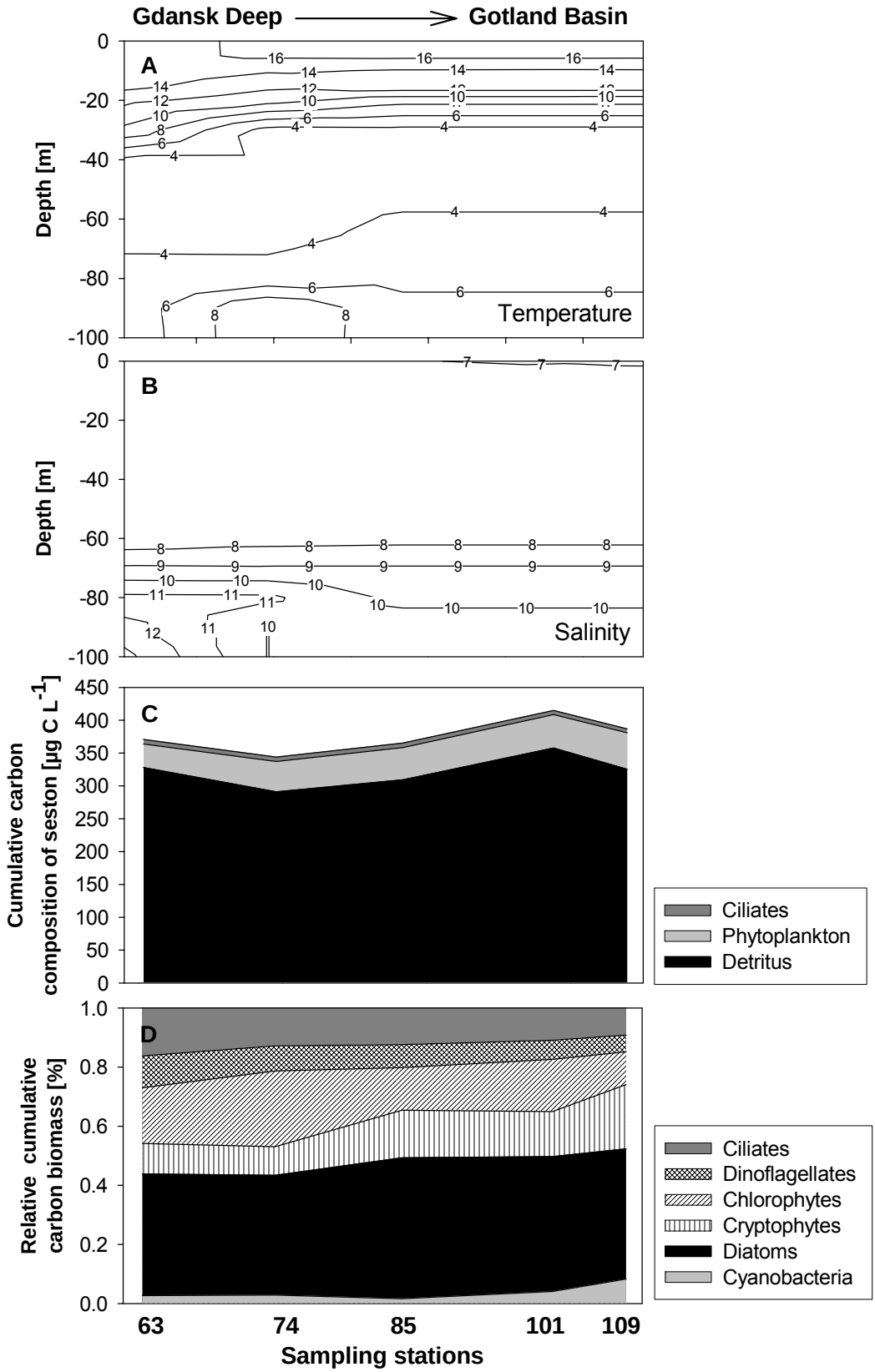

Figure V.1. Vertical section of temperature (A), salinity (B),seston composition (C) and relative carbon biomass of major taxonomic groups (D) in a transect from Gdansk Deep to Gotland Basin during July 2003.

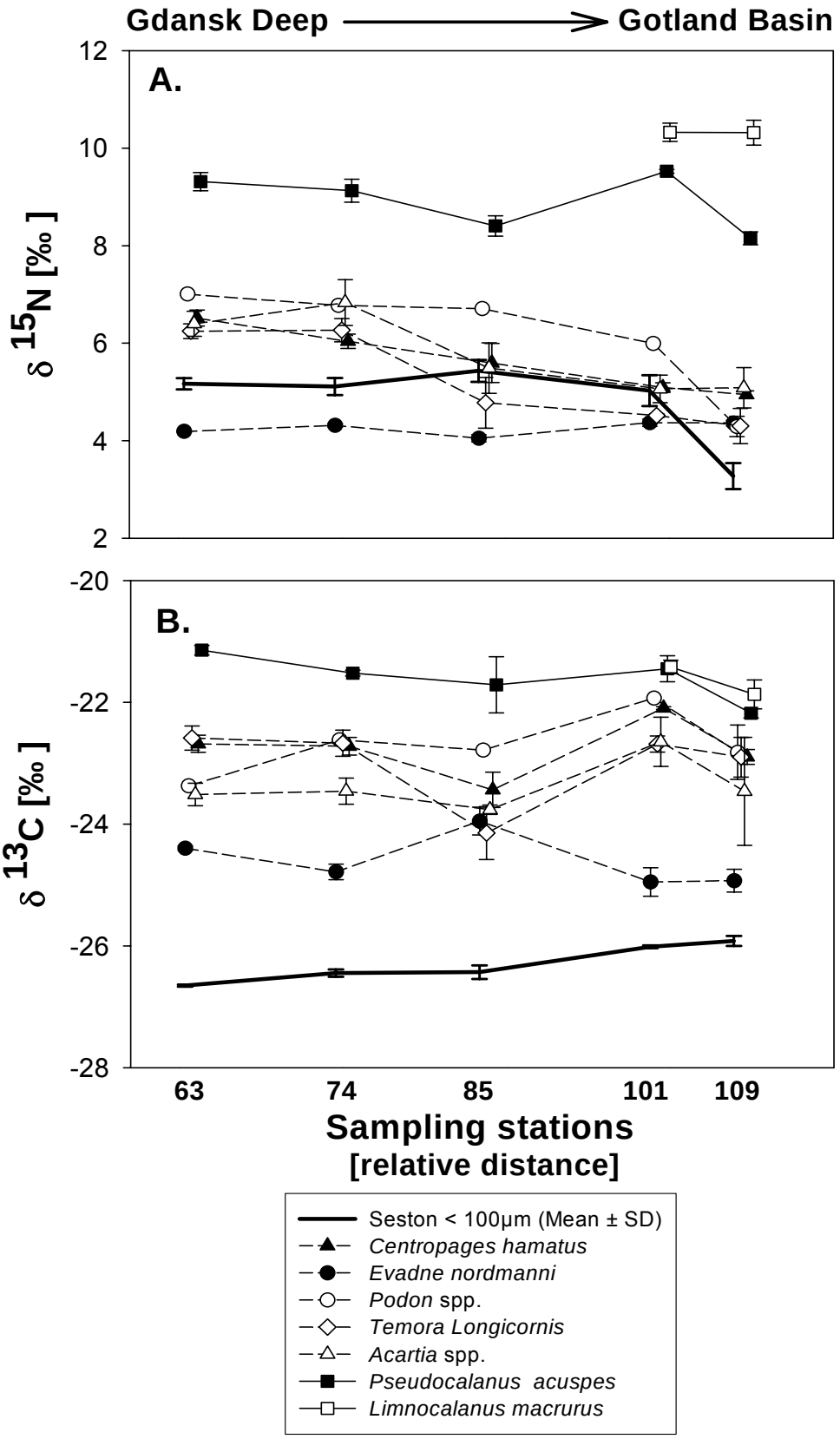

Figure V. 2. Spatial distribution of seston and mesozooplankton δ^{15}N and δ^{13}C in a transect from Gdansk Deep to Gotland Basin in July 2003.

The major spatial variation in seston $\delta^{15}N$ was explained by the occurrence of cyanobacteria at the stations in the Gotland Basin (stations 101 and 109). Seston $\delta^{15}N$ in July were significantly and negatively correlated with the biomass of diazotrophic cyanobacteria (Fig. V.3, Y = 6.0 - 0.52X; n = 15, r = -0.95, r^2 = 0.91, p < 0.001) indicating fixation of isotopically light atmospheric nitrogen.

Two-way analysis of variance with sampling station and species as independent variables revealed significant differences (Table V.1, ANOVA, p < 0.0001) between sites and between mesozooplankton species. However only 14% for $\delta^{15}N$ and 9% for $\delta^{13}C$ of the total variance was attributed to spatial differences (Table V.1), whereas 84% and 87% was accounted for by inter-specific differences (Fig. V 2). The $\delta^{15}N$ and $\delta^{13}C$ of *E nordmanni* were the lowest isotopic signatures of all species and showed the most stable values across the study area. *Podon* spp. showed both the highest $\delta^{15}N$ and $\delta^{13}C$ among the cladocerans and exhibited more enriched values than the copepods *T. longicornis*, *C. hamatus* and *Acartia* spp. (Fig. V.2A, B). With the exception of *E nordmanni*, the $\delta^{15}N$ signatures of all species tended to decrease at the station no. 109 (Gotland Basin), while in terms of carbon tended to increase from st. no. 85 to 109. The copepods *P. acuspes* and *L. macrurus* exhibited the highest isotopic signatures in terms of both its $\delta^{15}N$ and $\delta^{13}C$ (Fig. V.2A,B).

Average mesozooplankton $\delta^{15}N$ signatures (Fig. V.4) showed significant interspecific differences between 6 groups, with increasing enrichment in the order *E. nordmanni.* (4.2±0.1‰) < *T. longicornis* (5.2±0.9‰) < *C. hamatus.* (5.6±0.6‰), *Acartia* spp. (5.7±0.8‰) < *Podon* spp. (6.1±1.0‰), < *P. acuspes* (8.9±0.6‰) < *L. macrurus* (10.3 ±0.2‰). In terms of carbon (Fig. V.4), average $\delta^{13}C$ increased in a somewhat different order between also 6 groups: *E. nordmanni.* (-24.6±0.4 ‰) < *Acartia* spp. (-23.4±0.5‰) < *T. longicornis* (-23.0±0.6‰), *C. hamatus* (-22.8±0.5‰) < *C. hamatus*, *Podon* spp. (-22.7±0.5‰) < *L. macrurus* (-21.7±0.3‰) and *P. acuspes* (-21.6±0.4‰).

Overall, mesozooplankton was enriched by 1.5 ± 2‰ relative to seston. Although using seston as baseline food source is methodologically simple, it is only suitable if the mixture of seston components reflects the $\delta^{15}N$ and $\delta^{13}C$ of the primary food source, and when mesozooplankton species feed non-selectively on seston. In contrast, if mesozooplankton species feed selectively on seston groups and these taxonomic groups are isotopically distinct, using seston could introduce error into the assessments of mesozooplankton trophic levels. This circumstance and time delays in the transmission of rapid changes (>2‰) in seston $\delta^{15}N$ to mesozooplankton complicate the calculation of mesozooplankton trophic levels. In order to obtain an accurate trophic level indication, we used *Evadne nordmanni* (classified as herbivorous) as baseline reference organism.

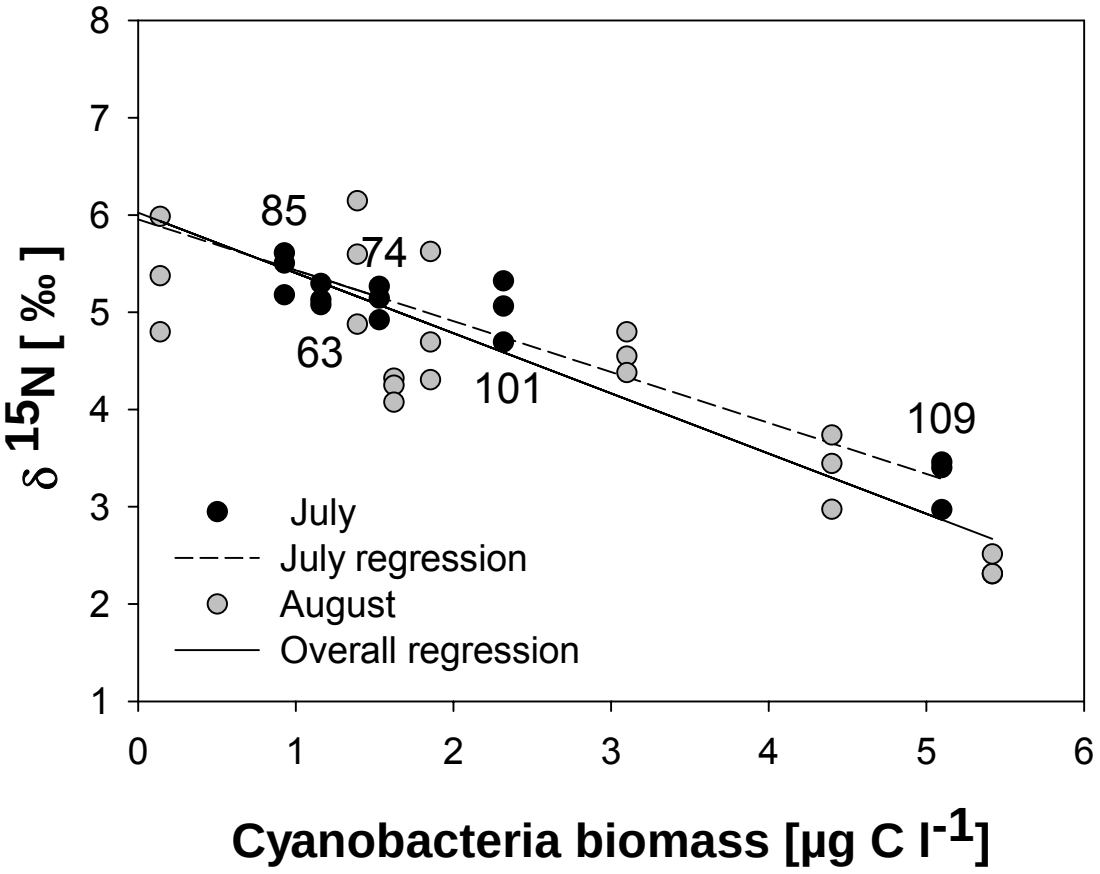

Figure V.3. Depth-Integrated seston $\delta^{15}N$ as a function of total cyanobacterial biomass in July (Gdansk Deep and Gotland Basin) and August 2003 (Bornholm Basin). A linear regression model II (SMA: Standardised major axis test) was fitted to the data of July (Y = 6.0 - 0.52X; n = 15, r = -0.95, r^2 = 0.91, p < 0.001) and including the data of August (Overall: Y = 6.0 - 0.62X; n = 36, r = -0.88, r^2 = 0.78, p < 0.001). Numbers above symbols indicate sampling stations.

Therefore, considering only the average trophic enrichments relative to *E. nordmanni* $\delta^{15}N$ (i.e. $\Delta\delta^{15}N_{E.nordmanni}$) allowed species to be divided into 5 homogenous groups increasing in the order: *T. longicornis* (0.9 ±1.0, TL=2.3) < *C. hamatus*, *Acartia* spp. (both, 1.5 ±0.8, TL=2.4) < *Podon* spp. (1.9 ±1.1, TL=2.6) < *P. acuspes* (4.6 ± 0., TL=3.4) < *L. macrurus* (5.9 ± 0.2, TL=3.7), suggesting an gradient from herbivory to high degree of carnivory in the same order. The increase in carbon trophic enrichment (i.e. $\Delta\delta^{13}C_{E.nordmanni}$) again resulted in 5 groups: *Acartia* spp. (1.2±0.8‰) < *T. longicornis* (1.6±1.0‰), *C. hamatus.* (1.8±0.8‰) < *C. hamatus, Podon* spp. (1.9±0.8‰), < *P. acuspes* (3.0±0.5‰) < *L. macrurus* (3.3±0.3‰). These groups indicated clearly different carbon sources, especially for *Acartia* spp.

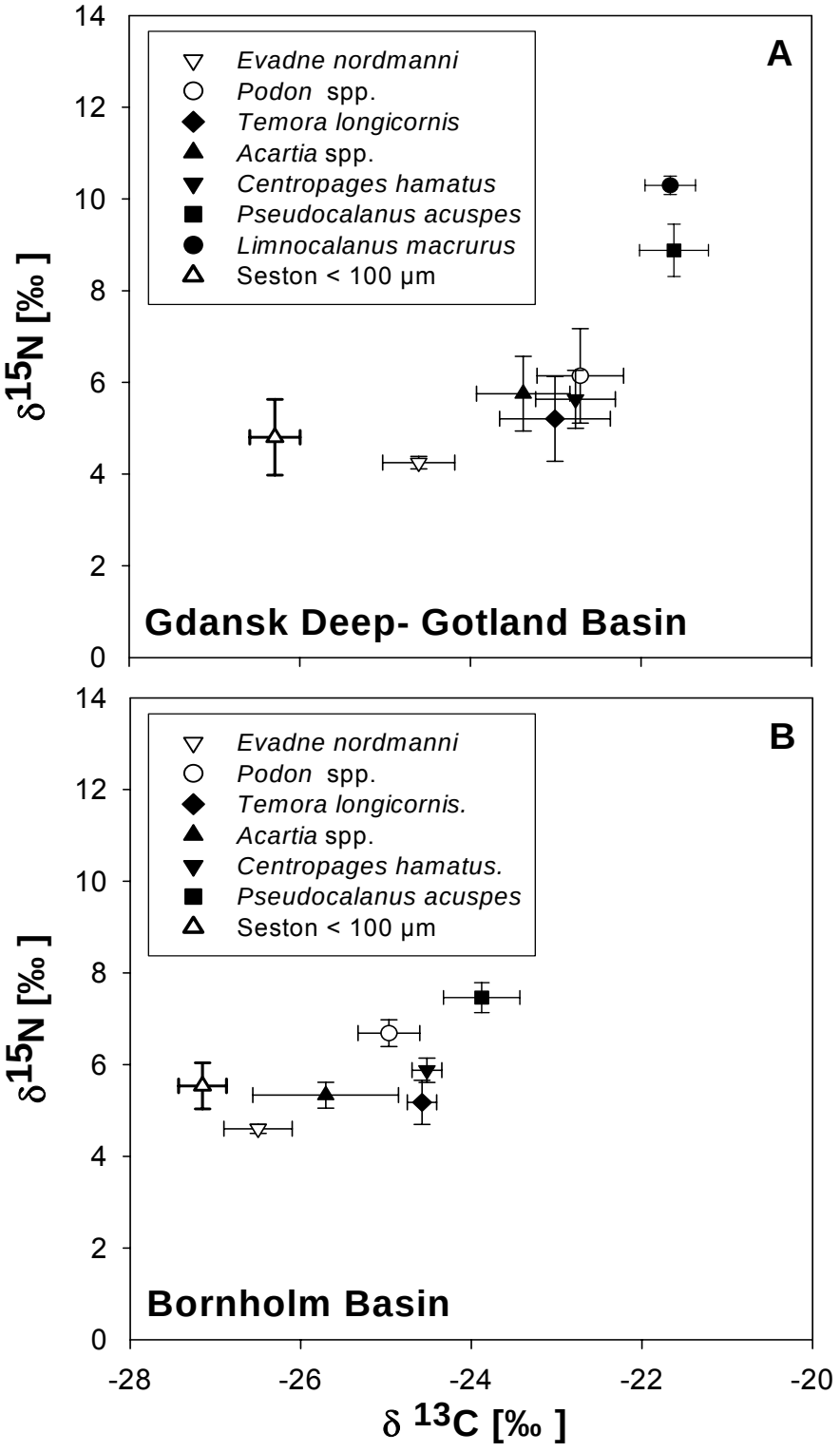

Figure V.4. General dual isotope diagram of depth-integrated (mean ± 1SD) $\delta^{15}N$ and $\delta^{13}C$ signatures of mesozooplankton species in the Gdansk Deep-Gotland Basin (A) and in the Bornholm Basin (B).

Discussion

Several aspects (e.g. foraging strategies, $\delta^{15}N$ vertical distribution, fixation of atmospheric nitrogen by cyanobacteria) that lead to isotope variation in seston and mesozooplankton in the Central Baltic Sea have already been discussed in chapter IV.

Overall, this study showed that the trophic structure of mesozooplankton using stable isotope of nitrogen was very similar to the mesozooplankton structure in the Bornholm Basin in the same month (Fig. V.4). However, $\delta^{15}N$ of seston in the Bornholm Basin was slightly, but significantly more enriched than seston in the Gdansk Deep/Gotland Basin. With the exception of *E. nordmanni* and *P. acuspes* $\delta^{15}N$, the most species showed similarities in $\delta^{15}N$ signatures within species between Gdansk Deep/Gotland Basin and Bornholm Basin (Table V.2). In turn, the $\delta^{13}C$ signatures of seston and of all species were significantly different between both study areas (Table V.2), being more ^{13}C-enriched across the transect Gdansk Deep/Gotland Basin than in the Bornholm Basin. This implies that seston and mesozooplankton used different carbon sources in comparison with those at Bornholm Basin. The carbon enriched values of all component of the planktonic community in the Gdansk Deep/Gotland Basin indicate likely that this area is strongly influenced by allochtonous factors such as terrestrial input (e.g. anthropogenic nutrient load) via freshwater run-off, which may cause significant variation in coastal zones (Rolff 2000, Rolff & Elmgren 2000). These carbon enriched values are consistent with the findings reported by Voss et al. (2000), who found a gradient from eutrophic coastal areas (e.g. Gdansk Deep/Gotland Basin) with higher isotopic signatures to open more oligotrophic waters (e.g. Bornholm Basin) with lower signatures. The switch to lower $\delta^{15}N$ signatures, particularly in seston, but also in some copepods species at the station no. 109 coincided with an increasing abundance of summer blooms of nitrogen fixing cyanobacteria (Fig. V.1D, 2, 3). The highest isotopic signatures of the calanoid copepod *Limnocalanus macrurus* (a large, glacial-relict copepod) in terms of both its $\delta^{15}N$ and $\delta^{13}C$ (Fig. V.2A, B) are consistent with the assumption that this species is an omnivore with a strong carnivorous tendency, as also indicated by its predaceous feeding behaviour (see Warren 1983, 1985, Hirche et al. 2003).

Conclusions.

This study showed clear within-guild differences in the isotopic signatures of mesozooplankton along a transect from Gdansk Deep to Gotland Basin. According to the nitrogen trophic enrichment, species may be allocated along a gradient from herbivory (*E. nordmanni*) to omnivory dominated by carnivory (*P. acuspes* and *L. macrurus*). These results are consistent with the recent field study on mesozooplankton in the Bornholm Basin.

However, they were significantly different in terms of carbon, suggesting that mesozooplankton in both areas used different carbon sources. In general, stable isotope analysis has been proven to be a useful tool in elucidating the food web structure.

Table V.1. Summary of the two-way ANOVA analysis (with exception ^{A1W}: one-way ANOVA for seston) of $\delta^{15}N$, $\delta^{13}C$ and trophic enrichments of nitrogen and carbon relative to *E. nordmanni* as baseline. Sampling station and species were used as independent variables. Data were Log-transformed in order to reduce heteroscedasticity in the variances. Statistically significant *p* values are emboldened. df = degrees of freedom, MS = mean square.

	Independent variable	Dependent variable								
Seston < 100 µm ^{A1W}		$\delta^{15}N$					$\delta^{13}C$			
		df	MS	*F* ratio	*p*-value	% variation	MS	*F* ratio	*p*-value	% variation
Isotopic signatures	Stat no.	4	0.016	45	**0.000**	98	0.000	62	**0.000**	98
	Error	10	0.000			2	0.000			2
Mesozooplankton		$\delta^{15}N$					$\delta^{13}C$			
Isotopic signatures		df	MS	*F* ratio	*p*-value	% variation	MS	*F* ratio	*p*-value	% variation
	Stat no.	4	0.026	104	**0.000**	14	0.000	19	**0.000**	9
	Species	5	0.158	631	**0.000**	84	0.004	190	**0.000**	87
	Stat no. x Species	20	0.004	15	**0.000**	2	0.000	8	**0.000**	4
	Error	60	0.000			0.1	0.000			0.5
		$\Delta\delta^{15}N_{Evadne}$					$\Delta\delta^{13}C_{Evadne}$			
Trophic enrichment		df	MS	*F* ratio	*p*-value	% variation	MS	*F* ratio	*p*-value	% variation
	Stat no.	4	0.223	107	**0.000**	18	0.188	91	**0.000**	29
	Species	5	1.004	483	**0.000**	80	0.452	218	**0.000**	69
	Stat no. x Species	20	0.031	15	**0.000**	2	0.018	8	**0.000**	3
	Error	60	0.002			0.2	0.002			0.3

Table V.2. Comparison of seston and mesozooplankton $\delta^{15}N$ and $\delta^{13}C$ signatures (mean ±1SD) between Gdansk-Gotland Basin and Bornholm Basin using t-test by groups (Basin) or Mann Whitney U-test (mwut, Non-parametric test). Data were Log -transformed in order to reduce heteroscedasticity in the variances. Symbols are: n.s. (not significant, $p > 0.05$), * (0.01<p<0.05), * * (0.001<p<0.01), * * * (p<0.001).

			$\delta^{15}N$					$\delta^{13}C$				
Species	**Basin**	***n***	**Mean**	**SD**	**z- / t-value**	***p*-value**		**Mean**	**SD**	**z- / t-value**	***p*-value**	
Seston	Gdansk-Gotland	15	4.8	0.8	3.3	* *		-26.3	0.3	-8.9	* * *	
	Bornholm	21	5.5	0.5				-27.2	0.3			
Cladocerans												
E. nordmanni	Gdansk-Gotland	15	4.2	0.1	4.8	* *	mwut	-24.6	0.4	-14.1	* * *	
	Bornholm	18	4.6	0.1				-26.5	0.4			
Podon spp.	Gdansk-Gotland	15	6.1	1.0	1.4	n.s	mwut	-22.7	0.5	-14.9	* * *	
	Bornholm	18	6.7	0.3				-25.0	0.4			
Copepods												
T. longicornis	Gdansk-Gotland	15	5.2	0.9	0.3	n.s	mwut	-23.0	0.6	-4.6	* * *	mwut
	Bornholm	18	5.2	0.5				-24.6	0.2			
Acartia spp.	Gdansk-Gotland	15	5.8	0.8	-1.2	n.s	mwut	-23.4	0.5	-9.1	* * *	
	Bornholm	18	5.3	0.3				-25.7	0.9			
C. hamatus	Gdansk-Gotland	15	5.6	0.6	1.4	n.s	mwut	-22.8	0.5	-4.9	* * *	mwut
	Bornholm	18	5.9	0.3				-24.5	0.2			
P. acuspes	Gdansk-Gotland	15	8.9	0.6	-4.9	* * *	mwut	-23.9	0.5	-10.5	* * *	
	Bornholm	18	7.5	0.3				-26.3	0.7			

VI. Mesozooplankton trophic levels in the German Bight (Southern North Sea).

Abstract

This study provides information about the trophic structure of mesozooplankton in the German Bight, Southern North Sea, in 2004. In order to obtain a trophic level indication of mesozooplankton, I analyzed the temporal and spatial variability of stable isotopes signatures ($\delta^{13}C$, $\delta^{15}N$) of mesozooplankton and seston. I found highly significant isotopic differences ($p < 0.001$) between sampling stations and months. Variation in seston $\delta^{15}N$ and $\delta^{13}C$ was negatively and significantly correlated with phyto:microzooplankton biomass ratios and detritus concentrations. Strong within-guild differences were found only in February ($\delta^{15}N$) and in April ($\delta^{13}C$), being less pronounced in the remaining months. The general pattern of nitrogen trophic enrichment relative to seston ($\Delta\delta^{15}N_{seston}$) allowed species to be divided into 3 homogenous groups increasing in the order: *T. longicornis* (3.4±0.9‰), ctenophores (4.4±2.0‰), and *P. elongatus* (4.6±3.4‰) < ctenophores, *P. elongatus*, *C. helgolandicus* (5.3±3.2‰), *C. hamatus* (6.0±3.1‰) and *Acartia* spp. (6.0±3.1‰) < *C. helgolandicus*, *C. hamatus*, *Acartia* spp., *Sagitta* spp. (6.6±3.3‰) and medusae (9.2±0.4‰). In terms of carbon ($\Delta\delta^{13}C_{seston}$), no differences were detected between mesozooplankton species (i.e. 1 large homogenous group). Significant negative non-linear regressions were detected for copepod nitrogen trophic enrichment as a function of phyto:microzooplankton biomass ratios, partially explaining the higher TL of copepods at higher microzooplankton biomass. The $\delta^{15}N$ and $\delta^{13}C$ signatures of copepods deserve special discussion, since there were an unusually large differences in $\delta^{15}N$ (-0.9 to 13‰) and $\delta^{13}C$ (-2 to 9.3‰) between seston and copepods. Several explanations are plausible: (i) There are several intermediate trophic linkages within the microbial loop/microzooplankton food web leading from riverine detrital material to copepods, (ii) bacteria that colonize detrital riverine material or copepods feces immobilize ^{15}N-enriched N from the water column, such that copepods consuming these bacteria have higher $\delta^{15}N$, and (iii) riverine seston is largely terrestrial and refractory to food web use, and the large quantity of this material masks a relatively minor ^{13}C- and ^{15}N-enriched riverine/estuarine/marine phytoplankton component selectively used by copepods. Finally, with the exception of *T. longicornis* (TL= 2.5, somewhat less carnivorous), all calanoid copepods according to their assessed trophic level (TL: 2.8 to 4.2), showed in general omnivorous feeding dominated by carnivory. However this conclusion might be uncertain, considering potential effect of detritus masking “true” phytoplankton isotopic signatures.

Results

Hydrography and seston composition.

The southern North Sea is a temperate shallow shelf sea. In the study area, depth varied from 13 to 50 meters. Near Helgoland Island (station no. 11), surface temperatures and salinity ranged from 4.9 to 18.2°C and 32.2 to 33.6 from February to August 2004, respectively. Figure VI.1 summarises the seasonal and vertical variability of temperature and salinity across the sampling stations from west (offshore) to east (coastal). As a result of strong freshwater discharge of several rivers (e.g. Elbe, Weser, Ems) salinity always showed lower values in the most eastern part (station no.7) than in the western part (station no. 35 and 49). Temperature varied mainly over season but also showed differences across the bight, especially in February. Due to the shallow depths the water column was often completely mixed, but the formation of clines and fronts by river run-off (in winter) and surface warming (in summer) is a commonly observed feature in the coastal areas of the German Bight (Fig.VI.1,2).

Temperature–salinity (TS) plots were calculated using the mean water column temperature and salinity from 0 to 50 m, which includes all of the vertical distribution of the seston and mesozooplankton that we investigated. These plots help to define the characteristics of water masses (Fig.VI.2). TS properties at the sampling stations across the bight illustrate that the different water masses could be distinguished by salinities greater than (western part, offshore) or less than (eastern part, coastal) 33.0 psu. The vertical (Fig.VI.1) and TS profiles (Fig.VI.2) demonstrate that vertical mixing in the different water masses across the bight occurred from February to May, especially in April. During June and August, the TS plots revealed thermal stratification due to surface warming.

With the exception of May, seston composition (Fig.VI.3) was dominated by large amounts of detritus, particularly in February (210 to 1686 µg C L^{-1}) and April (276 to 3448 μg C L^{-1}). The cumulative sums revealed different seasonal (months) and local (stations) trends in the concentrations of phytoplankton, ciliates and detritus across the German Bight. Phytoplankton biomass (Fig.VI.3) was generally dominated by diatoms (mainly *Coscinodiscus* spp., *Skeletonema costatum*, *Thalasiosira* spp., *Chaetoceros* spp. and *Rhizosolenia* spp.), which constituted 22 % to 90% of total carbon biomass during the study period. After the spring bloom of diatoms, high abundances (739 to 1149 µg C L^{-1}) of prymnesiophytes (i.e. *Phaeocystis globosa*) were observed in the eastern part of the bight (st.no. 7, 11 and 22), which made up 53 to 65% of the total biomass in May. The biomass of crytophytes ranged between 0.4 μg C L^{-1} in June (1%, at st.no. 22) to 49.4 μg C L^{-1} in April (33%, at st.no. 7). Large dinoflagellates like *Ceratium* species (e.g. *C. fusus*, *C. furca* and

C.horridum) occurred regularly in June (0.1 to 1.8 μg C L^{-1}) and especially in August (0.5 to 10.1 μg C L^{-1}), varying generally from 1 to 6%. Ciliate biomass ranged from 3% to 16% from February to August (Fig.VI.3).

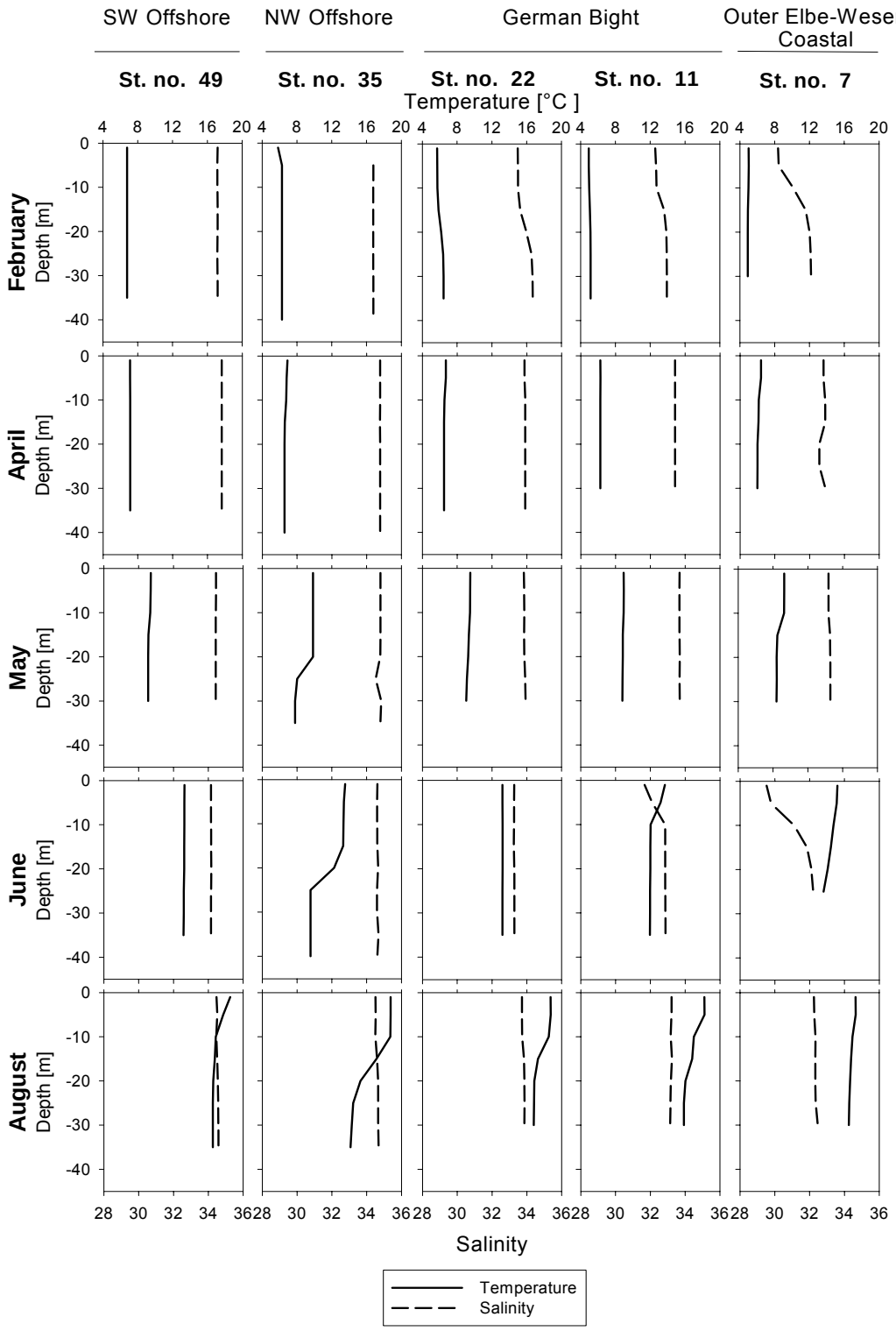

Figure VI.1. Vertical profiles of temperature (solid lines) and salinity (dash lines) from February to August 2004.

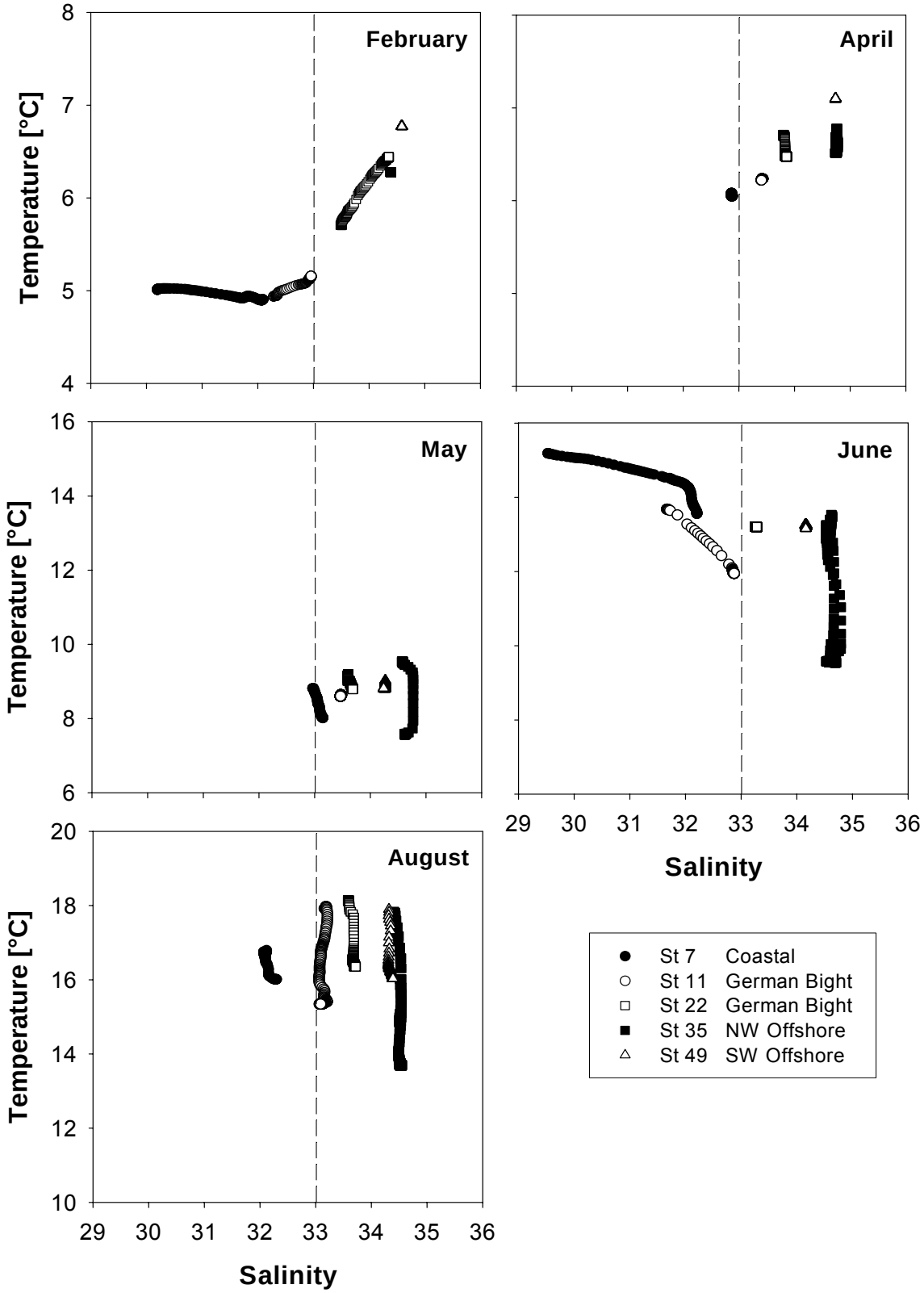

Figure VI.2. TS plots from five sampling station along the Southern North Sea from February to August 2004. Vertical dash lines denote the reference salinity.

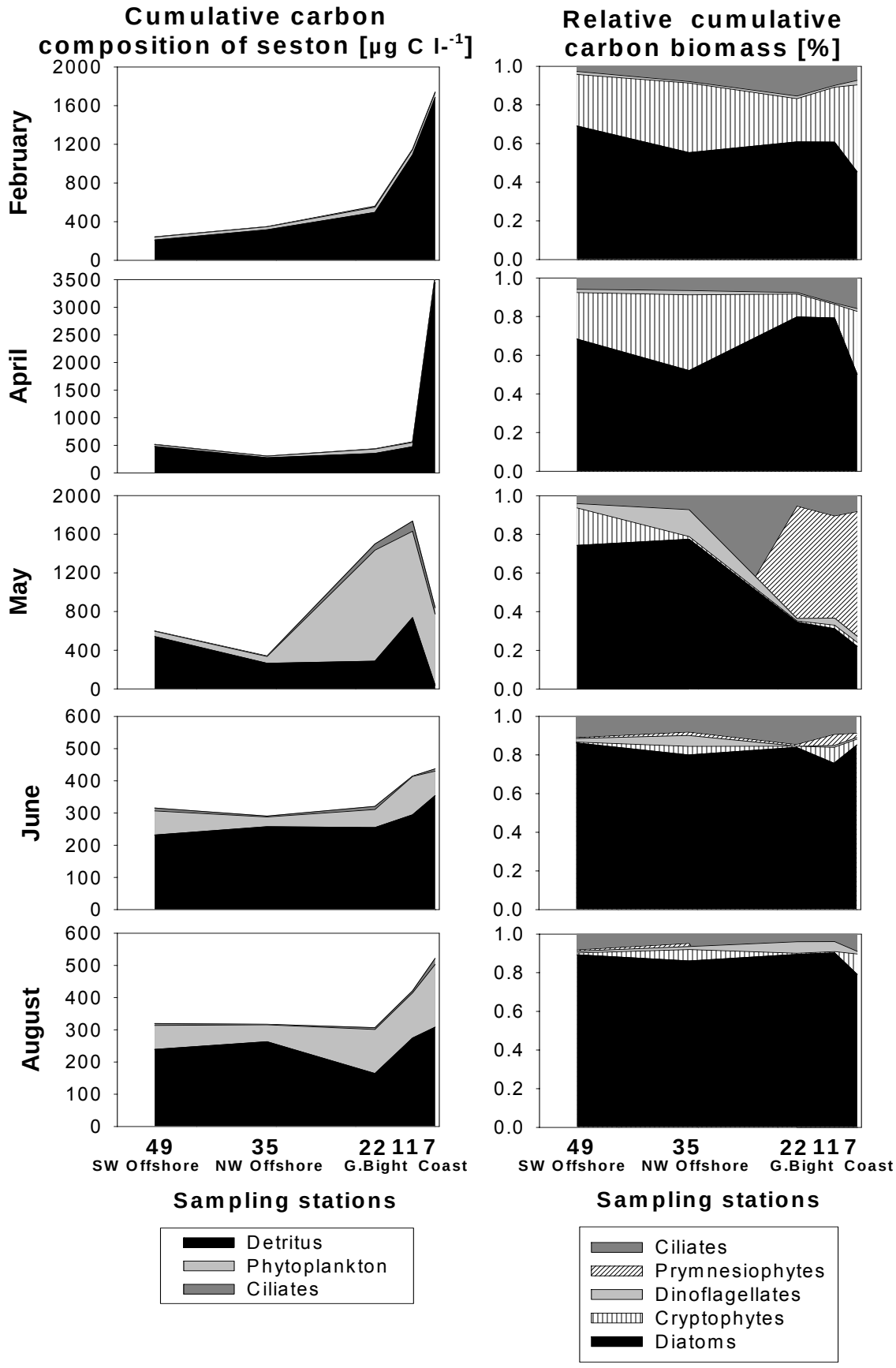

Figure VI.3. Seston composition in the Southern North Sea from February to August 2004.

$\delta^{15}N$ and $\delta^{13}C$ of seston.

The isotopic signatures of seston showed significant horizontal differences (Fig. VI.4,5) between the sampling stations across the bight with respect to both their $\delta^{15}N$ (Table VI.1, ANOVA, $F = 62.5$, $p < 0.001$) and $\delta^{13}C$ ($F = 147.8$, $p < 0.001$) signatures. Strong seasonal fluctuations in both seston isotopic ratios were observed during the study period (Fig.VI.6, Table VI.1). The $\delta^{15}N$ signatures were isotopically less enriched in April (3.8 ± 1.2 ‰) and August (2.7 ± 1.2 ‰), being significantly most enriched in May (7.3 ± 1.4 ‰). The $\delta^{15}N$ signatures in February and June exhibited relatively high averages of 6 and 5‰, respectively (Fig.VI.6A). Seston $\delta^{13}C$ were most enriched in February (-22.4 ± 2.5‰) decreasing drastically in April (-25.9 ±1.5) and increasing again in May (-23.3 ± 1.4‰). No significant differences (Tukey HSD, $p > 0.05$) were detected in the $\delta^{13}C$ signatures of June (-24.4 ± 0.6‰) and August (-24.0 ± 1.4‰).

Seston stable isotope signatures of nitrogen and carbon were separately correlated with salinity, temperature and nitrate concentrations (Table VI.2). The $\delta^{15}N$ of seston values and salinity were significantly and negatively correlated only in February ($r^2 = 0.37$; $p < 0.01$), April ($r^2 = 0.28$; $p < 0.05$) and June ($r^2 = 0.54$; $p < 0.01$), while seston $\delta^{13}C$ showed highly significant and negative correlations in February ($r^2 = 0.89$; $p < 0.001$), June ($r^2 = 0.63$; $p < 0.001$) and August ($r^2 = 0.83$; $p < 0.001$). The overall analysis including all data ($n = 87$) revealed for $\delta^{15}N$ ($r^2 = 0.10$; $p < 0.01$) and for $\delta^{13}C$ ($r^2 = 0.36$; $p < 0.001$) negative and highly significant correlations as a function of salinity, respectively. The relationship with temperature showed a significant negative correlation with $\delta^{15}N$ in February and the expected positive correlations in May and June. However, the overall correlation was negative (Table VI.2, $r^2 = 0.20$; $p < 0.001$). Seston $\delta^{13}C$ exhibited a significant negative correlation with temperature in February and a positive one in August, while the overall correlation was not significant. As expected, significant positive correlations were detected between $\delta^{15}N$ and nitrate (NO_3) concentrations in February, June and including all data ($n = 72$). In contrast, no significant correlations ($p > 0.05$) were found between seston isotopic signatures and ammonium (NH_4) concentrations (data not shown) during this study.

Although all ANCOVA regressions (Fig. VI.7) yielded a weak predictive capability ($r^2 = 0.04 – 0.09$), sestoṇ $\delta^{15}N$ and $\delta^{13}C$ signatures were both significantly (ANCOVA, $0.0001<p<0.05$) and negatively correlated with the phyto:microzooplankton ratios (excluding *Phaeocystis globosa* biomass) (Fig. VI.7A, C) and detritus concentrations (Fig. VI.7B, D) indicating enriched values at higher densities of microzooplankton (e.g. ciliates) and at small amounts of detritus.

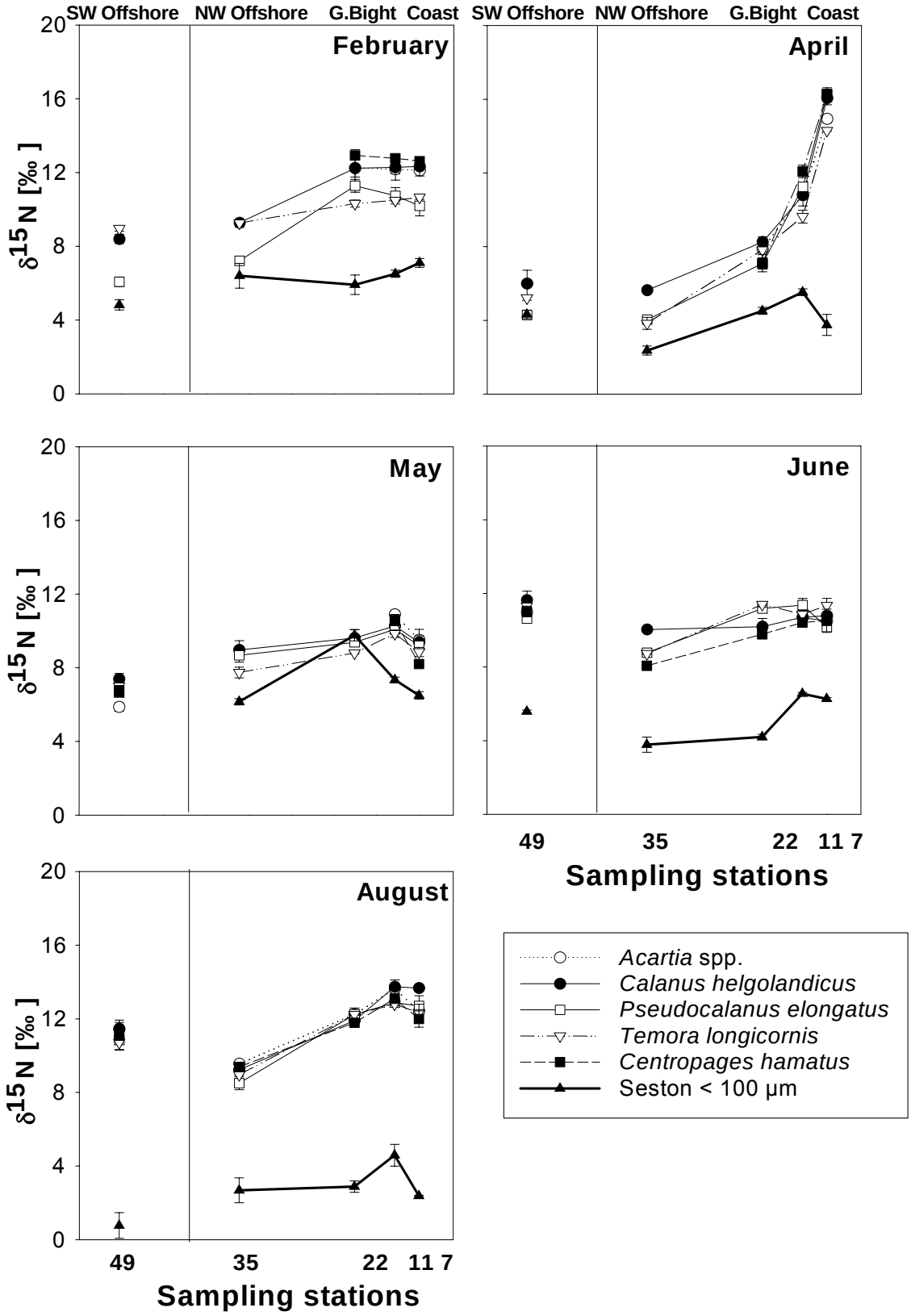

Figure VI. 4. Spatial distribution of seston and mesozooplankton $\delta^{15}N$ in the German Bight in 2004.

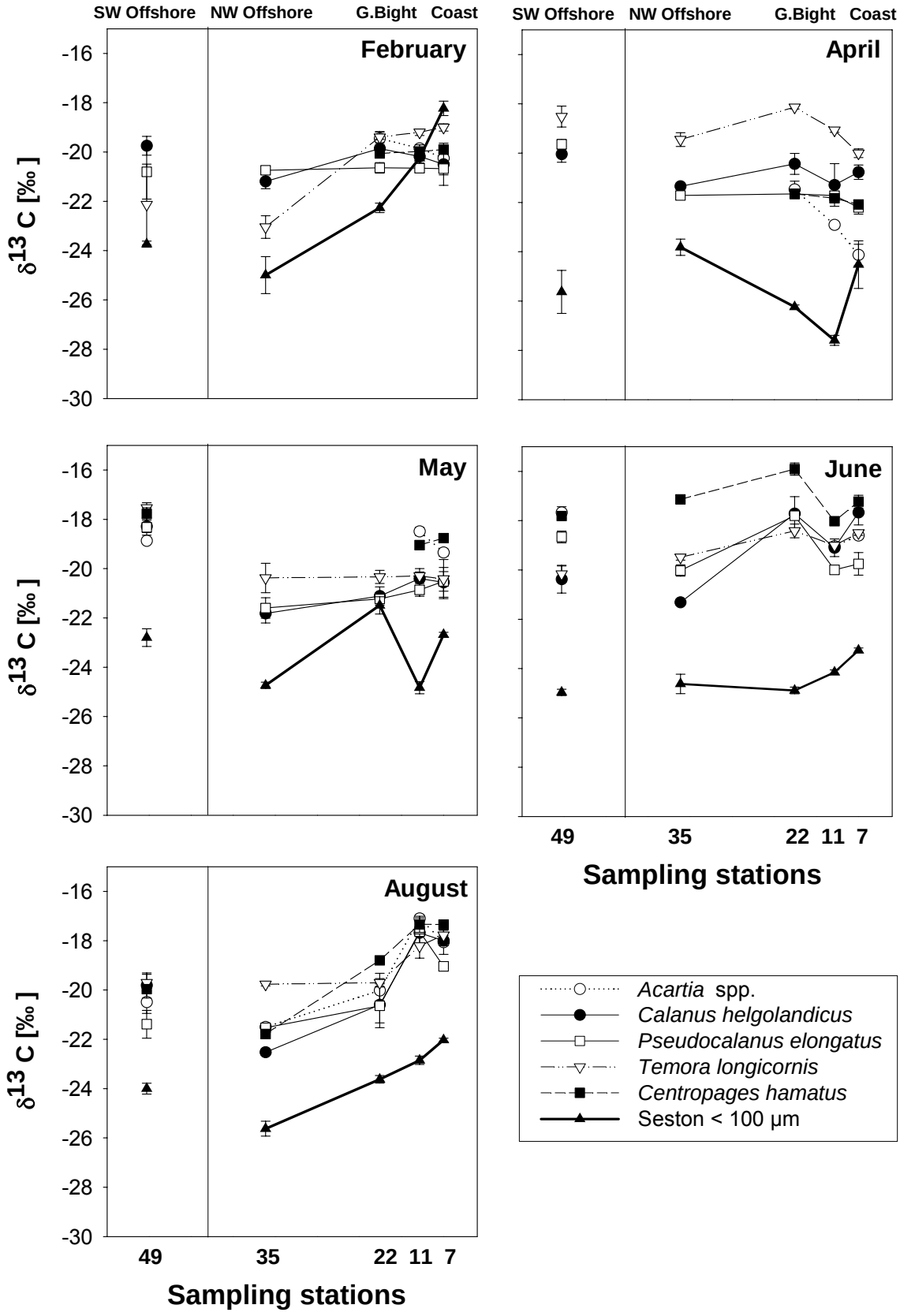

Figure VI. 5. Spatial distribution of seston and mesozooplankton $\delta^{13}C$ in the German Bight in 2004.

Spatial and seasonal variability of mesozooplankton $\delta^{15}N$ and $\delta^{13}C$.

The two-way ANOVA with station and month as independent variables revealed significant differences (Table VI.1) over time (Fig. VI.6) and across the bight (Fig. VI.4,5) for copepods and chaetognaths ($p < 0.001$). The general spatial trend of mesozooplankton $\delta^{15}N$ signatures showed more enriched values at the coast decreasing across the bight to offshore (particularly in April, Fig. VI.4). In terms of $\delta^{13}C$ the spatial trend was more variable, depending on the month (Fig. VI.5).

Depth-integrated isotopic signatures were obtained for 5 calanoid copepod species and 1 chaetognath species on all sampling occasions during 2004. Ctenophores were obtained in April and May and medusae only in August (Fig. IV.6). The copepods *T. longicornis* and *P. elongatus* were the least enriched species with respect to nitrogen, especially in February and April, and was slightly ^{15}N-depleted from May to August in comparison to the other species. With the exception of these two species in February and April, all copepods showed the same seasonal trend, with $\delta^{15}N$ signatures decreasing drastically in May followed by increases in June and August (Fig. IV.6A). The $\delta^{13}C$ signatures of *Acartia* spp. (-22.5±1.2‰) and *C. hamatus* (-21.9±0.2‰) exhibited the lowest values during the study period in April. In general, all copepods species were significantly more enriched in June than in other months (Fig. IV.6C). Sagitta spp. $\delta^{15}N$ and $\delta^{13}C$ showed a very similar seasonal trend to those of the copepods (Fig. IV.6B, D) varying from 9.1±1.3 to 13.1±0.9‰ and from -20.4±0.8 to -17.4±0.9‰, respectively. The ctenophore isotopic signatures (Fig. IV.6B, D) revealed no significant differences (Tukey HSD, $p > 0.05$) for $\delta^{15}N$ between April (11.4±1.8 ‰) and May (10.0±1.4‰). In contrast, a significant difference (Tukey HSD, $p < 0.01$) in terms of the $\delta^{13}C$ was detected between both months, with isotopic values increasing from April (- 21.7±0.4‰) to May (-20.5±0.6‰). On average the $\delta^{15}N$ and $\delta^{13}C$ signatures of medusae in August were 13.0±0.8‰ and -19.1±0.6 ‰, respectively (Fig. IV.6B, D).

No significant correlations (data not shown) were detected between seston $\delta^{15}N$ and copepods $\delta^{15}N$ ($p = 0.06 – 0.75$) and between seston $\delta^{13}C$ and copepod $\delta^{13}C$ ($p = 0.06 – 0.43$). In turn, the $\delta^{15}N$ and $\delta^{13}C$ signatures of the carnivore *Sagitta* spp. were significantly and positively correlated with copepod $\delta^{15}N$ and $\delta^{13}C$ (Table VI.3). However, the slopes for $\delta^{15}N$ (0.8 to 1.0‰) indicated low ^{15}N-enrichment, considering an expected difference of 3.5 ‰ between a carnivore (*Sagitta* spp.) and its prey (e.g. Copepods).

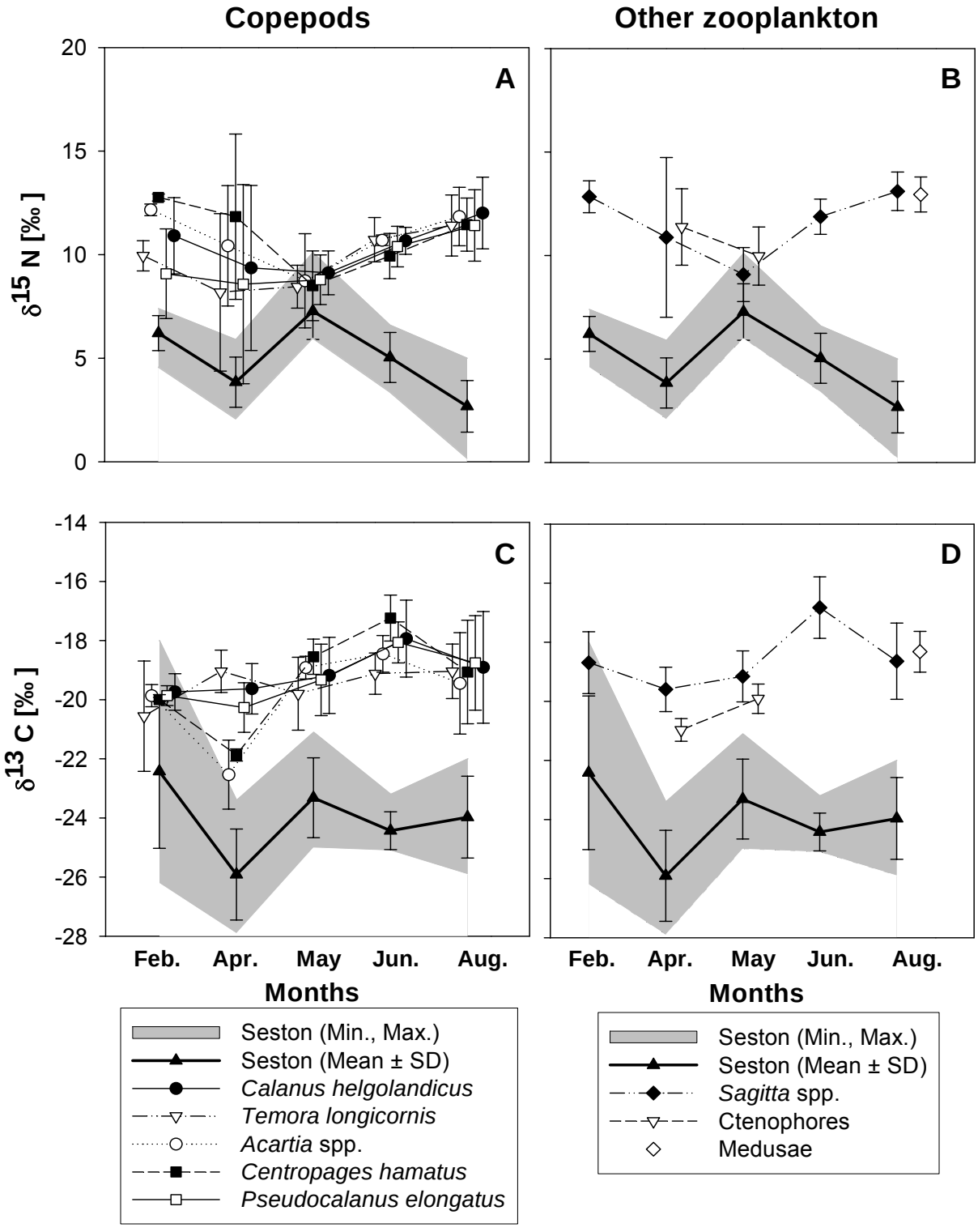

Figure VI.6. Seasonal depth-integrated δ^{15}N (top) and δ^{13}C (bottom) signatures (mean ± 1SD, all sampling stations pooled by month) of copepods (A and C), non-copepods (B and D) and seston <100μm in the German Bight. Shaded region represents minima and maxima of seston δ^{15}N and δ^{13}C. Note that species symbols of a given month are shifted relative to each other to improve readability.

The isotopic signatures of seston and mesozooplankton (Fig. VI.6) revealed unusually large differences (Fig. VI.8, -0.9 to 13‰) between the $\delta^{15}N$ values of seston (0.2 to 10 ‰) and copepods $\delta^{15}N$ (4 to 17 ‰) across the bight (especially at the coast, near to the mouth of the Elbe river). For the $\delta^{13}C$ signatures this discrepancy ranged between -2.1 and 9.3 ‰

Mesozooplankton trophic level (TL).

With few exceptions in May ($\Delta\delta^{15}N_{seston}$) and February ($\Delta\delta^{13}C_{seston}$), the calculation of mesozooplankton trophic levels relative to seston $\delta^{15}N$ and $\delta^{13}C$ resulted in positive averages during the entire sampling period in 2004 (Fig. VI.8). In general, the averages of nitrogen trophic enrichments ($\Delta\delta^{15}N_{seston}$) of copepods ranged from 1.2 (TL = 1.8) to 9.3‰ (TL= 4.2), being remarkably different among species only in February, strongly variable in April, lowest in May (1.2 to 2.0‰) and strongly higher in August (8.7 to 9.3‰). On all occasions, but particularly in February, *T. longicornis* and *P. elongatus* showed lower trophic enrichment than the other copepod species. However, the general pattern showed that from April to August, the $\Delta\delta^{15}N_{seston}$ among copepods were very similar (Fig. VI.8). Copepod trophic enrichment using seston $\delta^{13}C$ as baseline ($\Delta\delta^{13}C_{seston}$) revealed a more variable pattern between species in April and between months than the $\Delta\delta^{15}N_{seston}$ values. On averages, the copepod $\Delta\delta^{13}C_{seston}$ increased gradually from February to August, varying in general between 0.3 and 7.2‰.

Trophic enrichment averages of the chaetognath *Sagitta* spp. (data not shown) relative to the seston baseline ranged from 1.6±1.6 (TL=2) to 10±1.9‰ (TL=4.4) and from 2.5±1.8 to 6.9±0.9‰ in terms of nitrogen and carbon, respectively. Ctenophores (data not shown) nitrogen enrichments decreased from April to May (6.3±1.3‰ and 3.4±1.3‰, respectively), while carbon enrichment was similar in both months (3.3±1.2‰ and 3.1±1.1‰, respectively).In August, the enrichment of medusae (data not shown) was 9.2±0.4‰ (TL= 4.2) and 4.2±0.2‰ for the $\Delta\delta^{15}N_{seston}$ and $\Delta\delta^{13}C_{seston,}$ respectively. In general, the trophic enrichment and apparent trophic levels (TL) of these species were similar to those of the copepods.

Significant negative non-linear regressions ($p < 0.05$, $Y=Y_0+ae^{-bX}$) were detected for the nitrogen trophic enrichment (indicative of TL) as a function of phyto:microzooplankton biomass ratio (excluding *P. globosa* biomass) for each copepod species in all months (Table VI.4, Fig. VI.9). Only *T. longicornis* in February, *Acartia* spp. in June and *C. helgolandicus* and *P. elongatus* in August exhibited no significant non-linear correlations. However the data of the latter species in June and August, were significantly and negatively correlated with a

linear model ($Y = Y_0 \pm bX$). When using seston signatures as baseline, trophic enrichment in nitrogen ($\Delta\delta^{15}N_{seston}$) and carbon ($\Delta\delta^{13}C_{seston}$) were insignificantly and positively correlated for each mesozooplankton species ($0.2 < p < 0.7$) and including all data ($p = 0.7$, Fig. VI.10B).

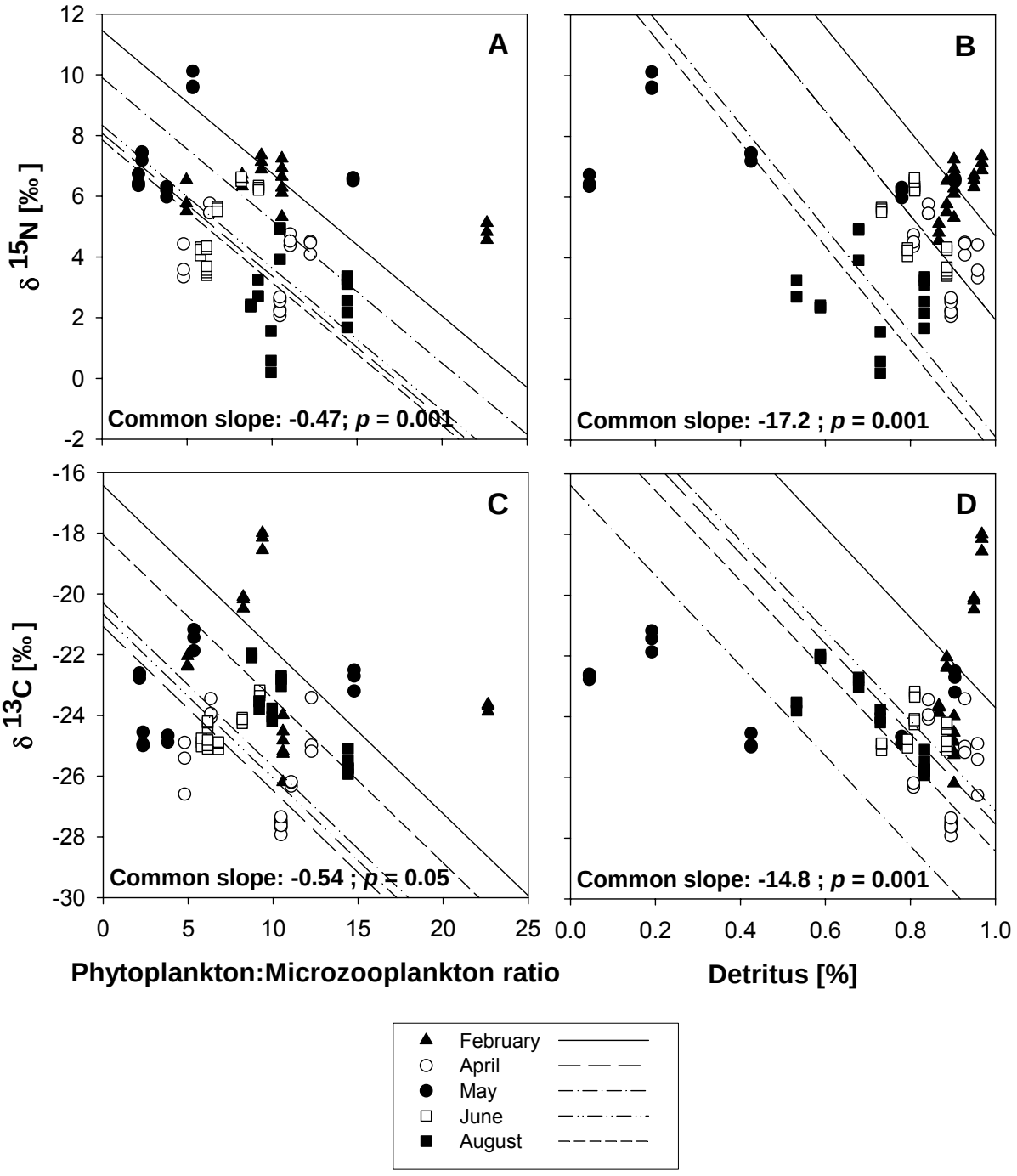

Figure VI.7. ANCOVA regressions of depth-integrated $\delta^{15}N$ (top) and $\delta^{13}C$ (bottom) signatures of seston as a function of phytoplankton:microzooplankton ratio (A and C, *P. globosa* biomass was excluded) and detritus concentrations (B and D).

Interspecific differences.

A two-way ANOVA indicated significant differences in the nitrogen ($\Delta\delta^{15}N$: F = 9.7 to 82; $p < 0.001$) and carbon ($\Delta\delta^{13}C$: F = 7.2 to 221; $p < 0.001$) trophic enrichment relative to seston between species and between sampling station by months (Table VI.5). Nevertheless, with the exception of February for $\Delta\delta^{15}N_{seston}$ (F = 82; $p < 0.001$) and April for $\Delta\delta^{13}C_{seston}$ (F = 221; $p < 0.001$), the within-guild differences in the remaining months, were strongly restricted to small differences at particular sampling stations, explaining only 2 to 8% of the total variation. In June, such within-guild differences were somewhat higher (10 and 28% of variation) for both trophic enrichment calculations. Despite these significant spatial and temporal differences (Table VI.5) in the mesozooplankton community, I pooled all values by species in order to summarize and determine a general pattern of within-guild trophic enrichment.

The general pattern of average mesozooplankton $\delta^{15}N$ signatures (Fig. VI.10A) showed significant interspecific differences between 2 groups, with increasing enrichment in the order: *P. elongatus* (9.7±2.7‰), *T. longicornis* (9.8±2.3‰), *C. helgolandicus* (10.4±2.4‰), ctenophores (10.5±1.7‰), *C. hamatus.* (10.9±2.3‰) and *Acartia* spp. (11.1±2.1‰) < ctenophores, *C. hamatus, Acartia* spp., *Sagitta* spp. (11.7 ±2.4‰) and medusae (13.0 ±0.8‰). In terms of carbon (Fig. VI.10A), 2 homogenous groups were also detected, however increasing in a somewhat different order: ctenophores (-21.0±0.8‰), *Acartia* spp. (-19.8±1.8‰) and *T. longicornis* (-19.5±1.3‰) < *Acartia* spp., *T. longicornis*, *Sagitta* spp. (-19.3 ±1.4‰), *P. elongatus* (-19.2±1.3‰), *C. helgolandicus* (-19.1±1.4‰), *C. hamatus* (-19.1±1.8‰) and medusae (-19.1 ±0.6‰).

Mesozooplankton trophic enrichment with seston $\delta^{15}N$ as baseline (i.e. $\Delta\delta^{15}N_{seston}$) allowed species to be divided into 3 homogenous groups (Fig. VI.10B) increasing in the order: *T. longicornis* (3.4±0.9‰), ctenophores (4.4±2.0‰), and *P. elongatus* (4.6±3.4‰) < ctenophores, *P. elongatus*, *C. helgolandicus* (5.3±3.2‰), *C. hamatus* (6.0±3.1‰) and *Acartia* spp. (6.0±3.1‰) < *C. helgolandicus*, *C. hamatus*, *Acartia* spp., *Sagitta* spp. (6.6±3.3‰) and medusae (9.2±0.4‰). In turn, no differences were detected in the carbon trophic enrichment (i.e. $\Delta\delta^{13}C_{seston}$) between mesozooplankton species (1 large homogenous group).

$\delta^{15}N$ vertical distribution of seston and mesozooplankton.

The vertical distribution of seston and mesozooplankton exhibited homogenous and stable $\delta^{15}N$ and $\delta^{13}C$ signatures throughout the water column (Fig. VI.11), since the water column was often completely mixed. Vertical seston $\delta^{15}N$ and $\delta^{13}C$ signatures were somewhat more variable than mesozooplankton isotopic signatures. The vertical pattern of

seston and mesozooplankton varied mainly over season (Fig. VI.11, st.no. 35), but revealed also differences across the bight, e.g. the station no. 11, 22 and 35 in August (Fig. VI.11). The within-guild differences throughout the water column were clearly more pronounced in February than in the remaining months, which showed similar signatures among the species by depth. As in the depth-integrated isotopic signatures, these depth-specific showed an unusually large difference in the $\delta^{15}N$ (2 to 8 ‰) and $\delta^{13}C$ (2 to 6 ‰) signatures between seston and mesozooplankton.

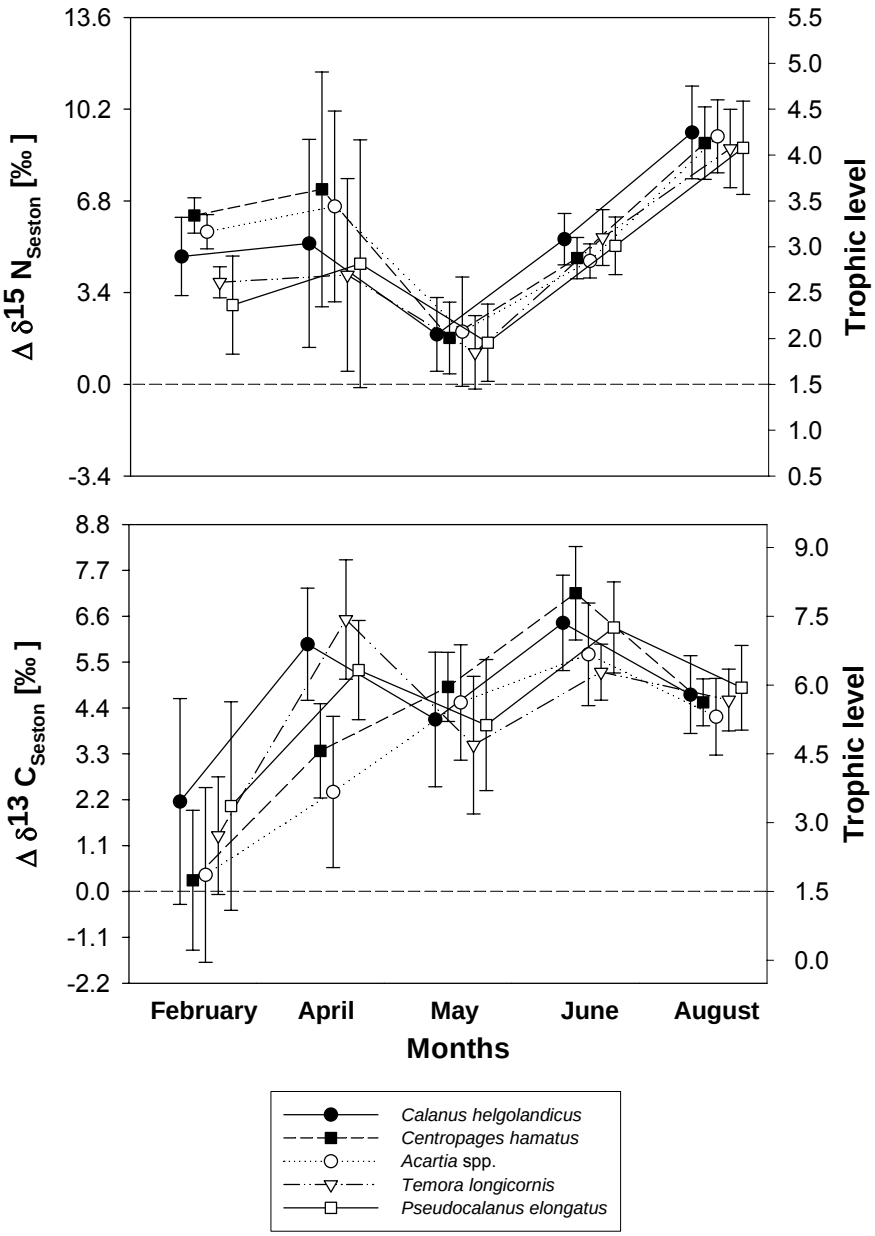

Figure VI. 8. Copepods trophic enrichment (left axis) in terms of nitrogen ($\Delta\delta^{15}N$, top) and carbon ($\Delta\delta^{13}C$, bottom) relative to seston and apparent trophic level (right axis). Dash horizontal lines indicate zero enrichment.

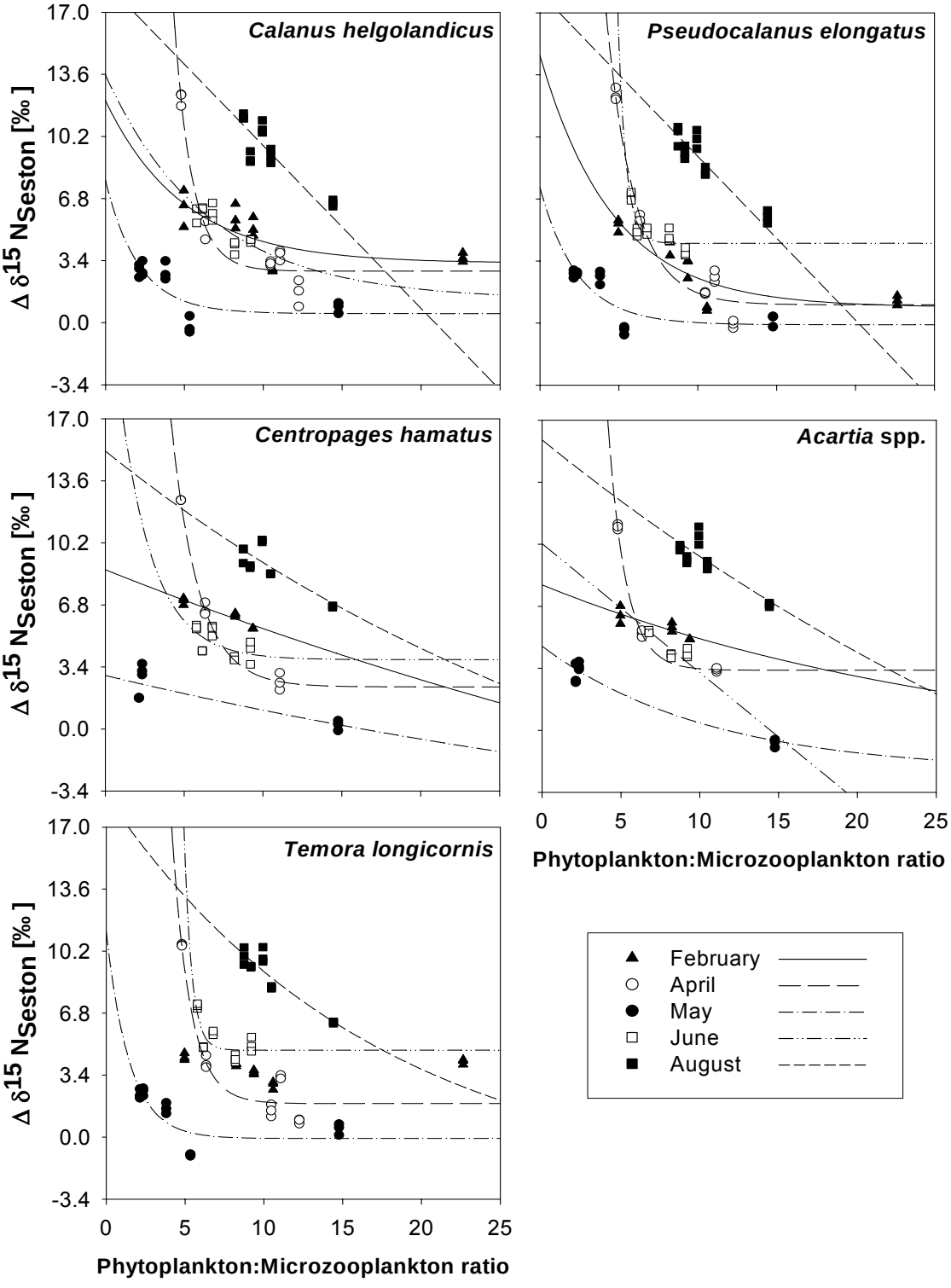

Figure VI. 9. Nitrogen trophic enrichment of copepod species ($\Delta\delta^{15}N$) relative to seston as a function of phytoplankton:microzooplankton ratio (excluding biomass of *P. globosa*), according to the model $Y = Y_0+ae^{-bX}$. Note that a linear model ($Y = Y_0 \pm bX$) was fitted for C. *helgolandicus* and P. *elongatus* in August and for *Acartia* spp. in June. Only significant (p<0.05) correlations are shown.

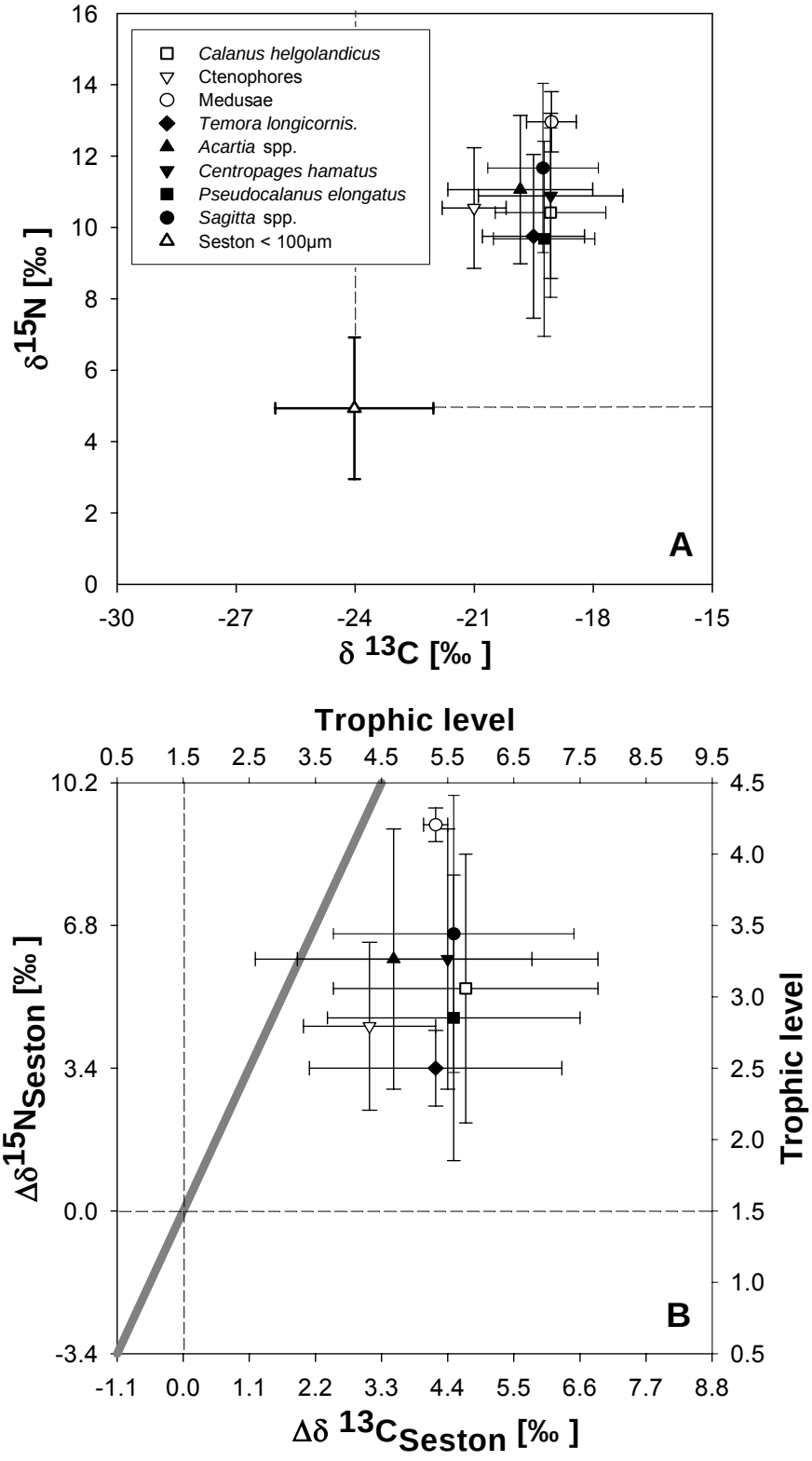

Figure IV.10. General dual isotope diagram of depth-integrated (mean ± 1SD) $\delta^{15}N$ and $\delta^{13}C$ signatures (A) and trophic enrichment of carbon and nitrogen relative to seston (B) of mesozooplankton species in the German Bight. Dash lines in diagram B, indicate zero enrichment. Solid gray line represents a 1:3.4 ‰ trophic enrichment in carbon and nitrogen per trophic level.

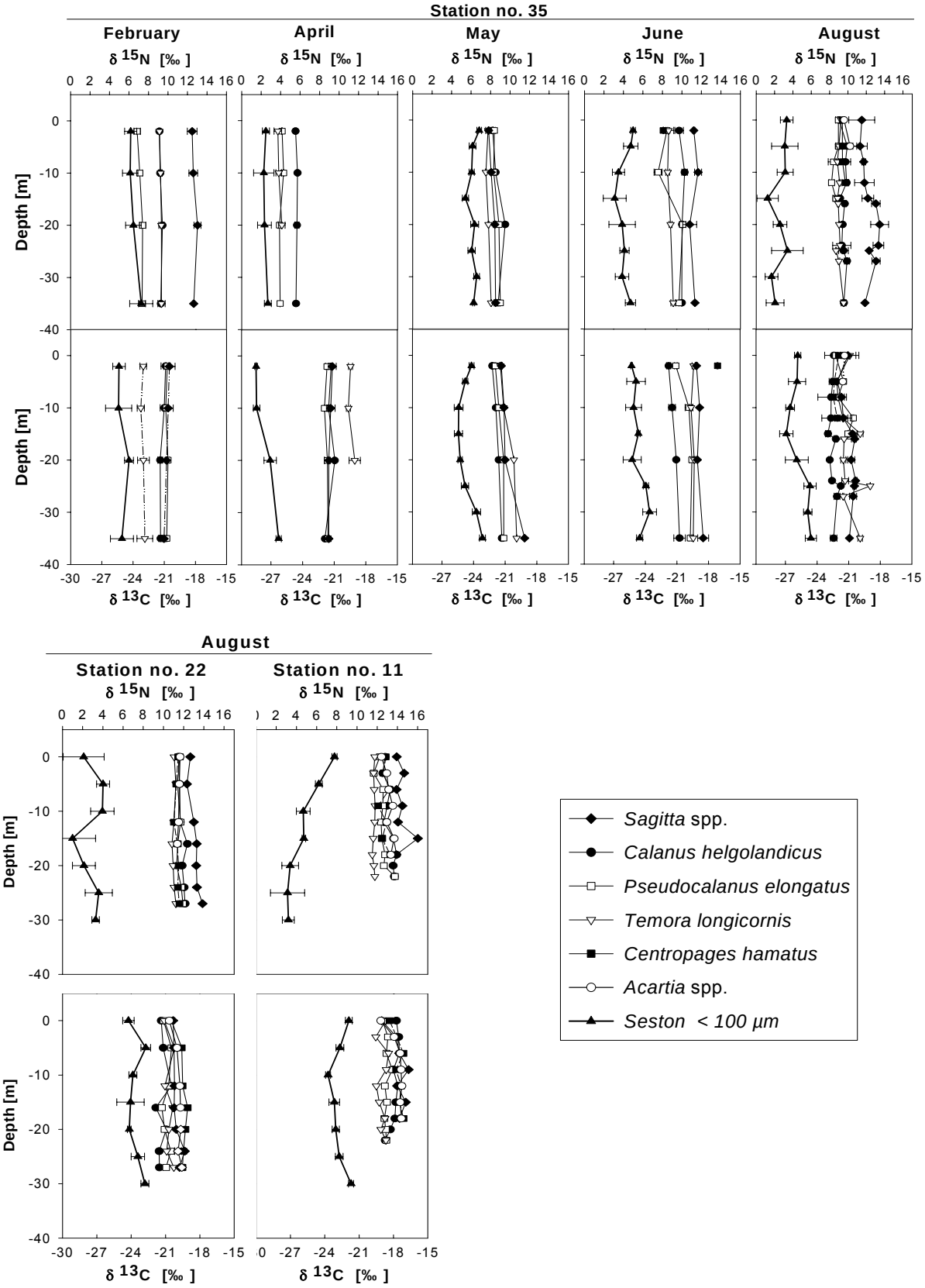

Figure VI.11. $\delta^{15}N$ and $\delta^{13}C$ (mean ± 1SD) vertical distribution of seston and mesozooplankton at the NW offshore station no. 35 during the study period (top) and in the Bight at st. no. 11 and 22 in August 2004 (bottom).

Discussion

Stable isotopic measurements of seston and mesozooplankton showed spatial and seasonal differences in samples from the German Bight, Southern North Sea (Table VI.1). These significant differences could be related to differences in hydrology, nutrient loading, food composition and detritus concentrations.

Seston $\delta^{15}N$ and $\delta^{13}C$.

My data demonstrate that there were differences in the seston stable isotope ratios of nitrogen and carbon across the German Bight. Hydrologic factors (e.g. salinity) were more evident in the $\delta^{13}C$ variability, while nutrient concentrations were (e.g. nitrate) more evident in the $\delta^{15}N$ data (Table VI.2). This study covered a cross-shelf spatial scale of 1 - 353 km, where the $\delta^{15}N$ and $\delta^{13}C$ signatures of seston varied markedly (mainly decreasing) from coastal to offshore sites, according to the month (Fig. VI.4,5).

I do not mean to imply, however, that salinity is the cause of the spatial variation of seston isotopic signatures; rather, I use salinity as an index for coastal and offshore water masses. An important question concerns the reasons for the observed variability in seston isotopic signatures in this study area. Previous studies of phytoplankton suggest that nitrogen and carbon fractionation depends on a number of factors relating to phytoplankton growth rates, nutrient (including CO_2) supply, nutrient uptake mechanisms, cell size, and species composition (Zohary et al. 1994, Perry et al. 1999, Pel et al. 2003). Isotope fractionation can be affected by factors such as photoperiod (less negative or more enriched $\delta^{13}C$ under conditions of increasing frequency of light–dark alternations, Leboulanger et al. 1995), temperature (Rau et al. 1997), and salinity (e.g. somewhat more negative or less enriched $\delta^{13}C$ when grown at 32 psu compared with 35 psu, Leboulanger et al. 1995). In contrast to this finding, we found significant negative correlations of seston $\delta^{15}N$ and $\delta^{13}C$ as a function of salinity (Table VI.2), supporting the contrary notion that in coastal areas with low salinities (29-33 psu due to freshwater discharge of several rivers) there were higher isotopic signatures (more positive $\delta^{15}N$ and less negative $\delta^{13}C$) than in the offshore areas with higher salinities (34 –35 psu). The $\delta^{15}N$ and $\delta^{13}C$ of phytoplankton has been found to be also positively related to temperature (Rau et al. 1997), growth rate and nutrient supply (Laws et al. 1995, Bidigare et al. 1997). In this point, my data were not completely consistent with these findings, since the $\delta^{15}N$ and $\delta^{13}C$ of seston were more negative or not correlated with temperature, respectively. Maybe, the observed negative correlation with temperature was a by-product of a seasonal trend. Nevertheless, I found positive and significant correlations as

a function of nitrate (NO_3) concentrations (Table VI.2). Fry & Wainright (1991) found enriched $\delta^{13}C$ values (in the range of -15 to -19‰) in fast growing spring diatom populations on Georges Bank compared with more depleted values of -21 to -25‰ in other (less fast growing) phytoplankton in the area. Mesozooplankton that were feeding on these fast-growing diatoms were also enriched in ^{13}C. Although speculative, it seems plausible that the gradual enrichment in carbon (from February to August, Fig. VI.8) relative to seston of mesozooplankton in this study is related to increases in the velocity of phytoplankton growth rates during spring-summer conditions.

It is important to note that seston is a mixture of autotrophs, heterotrophic organisms and detritus, each having their own isotopic signature and occupying different trophic levels. Therefore, in order to resolve the mixing effect and to explain the seston $\delta^{15}N$ and $\delta^{13}C$ variation, I tested seston isotopic signatures as a function of phyto:microzooplankton biomass ratios and of detritus concentrations. My results (Fig. VI.7) suggest that both $\delta^{15}N$ and $\delta^{13}C$ signatures of seston were negatively and significantly correlated with both predictor variables, i.e. seston $\delta^{15}N$ and $\delta^{13}C$ were lower at lower microzooplankton (e.g. ciliates) biomass and at larger amount of detritus (probably terrestrial in origin).

Mesozooplankton $\delta^{15}N$ and $\delta^{13}C$.

I found strong spatial and seasonal differences in both mesozooplankton $\delta^{15}N$ and $\delta^{13}C$ (Table VI.1, Fig VI.4,5,6). The horizontal spatial differences explained 26 to 43% ($\delta^{15}N$) and 9 to 39% ($\delta^{13}C$), respectively, of the entire variation by mesozooplankton species (Table VI.1). This variability was high considering the spatial differences by month (Table VI.5), explaining 49 to 94% for $\delta^{15}N$ and 42 to 84% for $\delta^{13}C$ signatures. Spatial isotopic variation in mesozooplankton has been discussed in previous studies (Montoya et al. 1990 , Schell et al. 1998, Kline 1999, Harvey & Kitchell 2000, Rolff & Elmgren 2000, Davenport & Bax 2002, Montoya et al. 2002, Schmidt et al. 2003, Syväranta et al. 2006).

Many mechanisms underlie spatially explicit isotopic signatures. Two key factors that lead to such spatial variation are (i) localized variations in baseline isotope values and (ii) the existence of spatially discrete populations of organisms. Variations in isotopic baselines may result from biogeochemical mechanisms such as nutrient contributions from nearby urban sources, river basins (e.g. Elbe river), or freshwater and terrestrial runoff (Cabana & Rasmussen 1996, Hansson et al. 1997) or possibly from biotic factors such as total primary production or dependence on recycled nutrients (Schindler et al. 1997). Spatially discrete populations of mesozooplankton may then express these localized differences in isotopic

baseline, as opposed to highly migratory species that integrate the baselines of many different areas (e.g. fishes and birds, Hansson et al. 1997).

Spatially discrete populations include small organisms or too immotile to move substantially relative to the scale of the study area (e.g. copepods). Local variations in size distributions, trophic ontogeny and changes in the foraging behaviours of mesozooplankton from the German Bight also contribute to spatial variations in isotopic signatures. Furthermore, tissue turnover rates of different species may confound spatial analysis. For example, the isotopic signatures of seston, copepods, chaetognaths, ctenophores and medusae varied considerably across the bight. Small organisms such as copepods experience faster tissue turnover than larger organisms, such as chaetognaths and medusae, and therefore have greater temporal variability in isotope signatures (Cabana & Rasmussen 1996). This temporal variation is thus superimposed upon any spatial differences that may exist among copepod species and, of course, upon the differences owing to water mass dynamics (e.g. temperature and salinity).

In this respect, this study also showed that seasonal differences constituted the main factor of isotopic variation in mesozooplankton, explaining 12 to 74% for $\delta^{13}C$, though less (16 to 37%) for $\delta^{15}N$. This seasonal variability is related with the primary production regime, phytoplankton composition and heterotrophic biomass (Fig. VI.3, 9), relative to physical forces such as wind stress and freshwater inflows, which form mixed water column and temporal fronts in the bight. Hydro-climatic conditions (e.g. storms) has also been suggested for seasonal variation in plankton communities from the North Sea (Beaugrand 2004, Beaugrand & Ibanez 2004, Bonnet et al. 2005).

The lowest mesozooplankton $\delta^{15}N$ signatures were observed in May in conjunction with a strong diatom-prymnesiophytes (the latter in coastal areas) bloom and lower amounts of detritus (Fig. VI.3, 6). It is generally assumed (but still controversial) that copepods avoid *P. globosa* as a food source and prey preferentially upon ciliates and dinoflagellates during *Phaeocystis* blooms (Tang et al. 2001). Thus, the main effect of this bloom was probably more evident on seston than on copepod $\delta^{15}N$ and $\delta^{13}C$ signatures. Lower $\delta^{13}C$ signatures were found in February and for some species such as *Acartia* spp. and *C. hamatus* in April (Fig. VI.6), when stormy conditions prevailed in the German Bight. Storms may have introduced ^{13}C-depleted DIC into the water column from freshwarer runoff or from porewater released during sediment resuspension events, with lower $DI^{13}C$ values leading to the observed lower seston and copepods $\delta^{13}C$ signatures (Canuel et al. 1995, Fry 1999). Unfortunately, isotopic data for dissolved inorganic carbon (DIC) are not available in this study in order to examine isotopic changes in the carbon pools of the water column.

Mesozooplankton (excluding carnivore species) $\delta^{15}N$ signatures (3.5 to 17‰) were similar to those reported by Knotz (2006) near to Helgoland Island in the German Bight, but are high relative to the 2.2 – 9.7‰ signatures from the Bornholm Basin, Central Baltic Sea (see chapter IV) and to the 0 – 8‰ $\delta^{15}N$ signatures observed for primary consumers in other aquatic systems (Minagawa & Wada 1984, Fry 1988, 1991, Meili et al. 1993, Hansson et al. 1997). These higher signatures likely indicate an important role for anthropogenic N loading in the German Bight. Several studies show high $\delta^{15}N$ signatures of sediments, algae and invertebrates in diverse aquatic systems (e.g. lakes, estuaries, coastal seas) where inputs of wastewater N are large (Cabana & Rasmussen 1996, Hansson et al. 1997, McClelland et al. 1997, Voss & Struck 1997, Voss et al. 2000). McClelland & Valiela (1998) stated that high $\delta^{15}N$ signatures in ammonium and nitrate from wastewater sources seem responsible for $\delta^{15}N$ signatures of estuaries. Unfortunately, in this study isotopic compositions of ammonium and nitrate were not measured across in study area to verify this assumption. However, I speculate that wastewater loading to German Bight was probably strong and the very high 3.5 –17‰ $\delta^{15}N$ signatures for copepods in this area probably reflect this strong local loading.

The $\delta^{15}N$ and $\delta^{13}C$ of mesozooplankton were temporally decoupled from the seston $\delta^{15}N$ and $\delta^{13}C$ signatures. This is because the rate of isotopic change in seston is faster than the tissue turnover of the mesozooplankton species (Grey et al. 2001). Because wind stress affects the water column structure and freshwater inflows affect salinity and mixing ratios of marine and freshwater in the frontal zone, vertical distribution of mesozooplankton $\delta^{15}N$ and $\delta^{13}C$ signatures recorded hydrologic mixing dynamics across this shallow shelf sea (Fig. VI.1, 11)

Unusual high isotopic difference between seston and copepods.

The $\delta^{15}N$ and $\delta^{13}C$ signatures of copepods in the German Bight deserve special discussion (Fig. VI.6,8), since there was an unusually large difference in $\delta^{15}N$ (-0.9 to 13‰) and $\delta^{13}C$ (-2 to 9.3‰) between isotopic signatures of seston ($\delta^{15}N$: 0.2 to 10‰, $\delta^{13}C$: -28 to -18‰) and copepods ($\delta^{15}N$: 3.5 to 17 ‰, $\delta^{13}C$: -24 to -16‰). These differences were particularly higher in August for $\delta^{15}N$ and April for $\delta^{13}C$ across the bight (especially at the coast, near to the mouth of the Elbe river, Fig. VI. 4,5). Similar large differences between mesozooplankton and the baseline were previously reported and discussedby Rast & Sutton (1988), Fry (1999) in the San Francisco Bay and Grey et al. (2000) in 24 United Kingdom lakes. There are several possible explanations for the unusually large differences between copepod species and seston ^{15}N and $\delta^{13}C$ signatures: (*i*) there are several intermediate

trophic linkages within the microbial loop/microzooplankton food web leading from riverine detrital material to copepods, thus potentially leading to increased ^{15}N and ^{13}C enrichment due to the extra trophic steps (Meili et al. 1993, Fry & Quinones 1994, Grey et al. 2000), (*ii*) bacteria that colonize detrital riverine material immobilize ^{15}N-enriched N from the water column (Caraco et al. 1998), and copepods consuming these bacteria have higher $\delta^{15}N$ signatures, and (*iii*) riverine seston is largely terrestrial and refractory to food web use (Canuel et al. 1995, Cloern et al. 2002), but the large quantity of this material masks a relatively minor ^{13}C- and ^{15}N-enriched riverine/estuarine/marine phytoplankton component selectively used by the copepods species in the German Bight. It is difficult to distinguish between these alternative explanations without direct isotopic determinations of nutrients, phytoplankton, bacteria, and microzooplankton and in amino acids. Isotopic methodology is advancing so that measuring isotopic compositions of these N pools may become more feasible in the future (Coffin et al. 1994, Sigman et al. 1997, Sachs et al. 1999, McClelland & Montoya 2002, McClelland et al. 2003). Future research utilizing these techniques may more clearly show how changes in foods result in the observed seston and mesozooplankton isotope signatures.

Within-guild trophic level differences.

Within-guild differences were detected only in February and in April for $\delta^{15}N$ and $\delta^{13}C$, respectively (Fig. VI.8). In the remaining months, only 2 – 10% of the total variance was attributed to among species $\Delta\delta^{15}N_{seston}$ variability, whereas 2 – 28% was accounted for by within-guild differences in $\Delta\delta^{13}C_{seston}$. This study shown that the nitrogen trophic enrichment of copepods was negatively and significantly correlated (Fig. VI.9) with the phytoplankton:microzooplankton biomass ratio (phytoplankton was mainly diatoms), suggesting that at lower ratios, i.e. higher microzooplankton biomass, there were higher nitrogen trophic enrichments and therefore higher trophic level of copepods. In turn, at higher ratios, i.e. higher phytoplankton biomass, there were stable and lower trophic enrichments close to trophic level 2 (i.e. pure herbivory).

Overall, the observed $\Delta\delta^{15}N$ relative to seston values (Fig. VI.10B) revealed that the averages of the mesozooplankton species varied from 3.4±0.9‰ (TL = 2.5) for *T. longicornis* to 9.2±0.4‰ (TL = 4.2) for medusae. These higher apparent trophic levels indicate an omnivorous diet dominated by carnivory and carnivory. However, this conclusion might be uncertain, considering the isotopic mask effect of detritus on phytoplankton (see above). In general the observed $\Delta\delta^{15}N_{seston}$ for copepods (4.6±3.4 ‰ to 6.0±3.1‰) were higher than the values from Bornholm Basin (see chapter IV). In particular, *Acartia* spp (6.0±3.1‰) was more enriched than *Acartia tonsa* (3.3 to 4.2 ‰) in the Chesapeake Bay (Montoya et al. 1990,

1991) and *Temora longicornis* (3.4±0.9‰) was clearly less enriched than *Temora* spp. (~6 ‰) in the Gulf of Mexico (Checkley & Entzeroth 1985).

Ctenophores $\Delta\delta^{15}N_{seston}$ (4.4±2.0‰, TL=2.8) were similar to *P. elongatus* (4.6±3.4‰, TL= 2.9), but clearly lower than *C. helgolandicus* (5.3±3.2‰, TL= 3.1), *C. hamatus* (6.0±3.1‰, TL= 3.3) and *Acartia* spp. (6.0±3.1, TL= 3.3). This pattern was unexpected, and may reflect: (i) the consumption of significant amounts of seston by the ctenophores, which would lead to a lower $\delta^{15}N$ than a purely carnivorous diet; (ii) carnivorous feeding by these copepods, which would lead to a higher $\delta^{15}N$ than a strictly herbivorous diet or (iii) a fundamental change in the magnitude of the isotopic fractionation effect in either the ctenophores or the copepods. Although isotopic signatures alone do not clearly indicate which of these possible explanations may be important, Montoya et al. (1990) have shown that ctenophores of the genus *Mnemiopsis leidyi* was clearly less enriched (5‰) than *Acartia tonsa*. Additionally, the somewhat smaller range of isotopic signatures in comparison to those of copepods and the relative constancy of the difference in $\delta^{15}N$ between the ctenophores and seston both suggest that the change is in copepod feeding or isotopic fractionation patterns rather than in the behaviour or physiology of the ctenophores. Lonsdale et al. (1979) have documented carnivorous feeding in adult *A. tonsa,* which is classified as an ambushing species (Tiselius & Jonsson 1990) and a greater dependence on heterotrophic food in the German Bight could lead to a significant enrichment in $\delta^{15}N$ signatures.

The Chaetognath *Sagitta* spp. (6.6±3.3‰, TL= 3.4) was about 0.8 to 1.0‰ more enriched than copepods $\delta^{15}N$, an unexpected small difference, considering the predatory feeding behavior of chaetognaths. The trophic enrichment of *C. hamatus* and *Acartia* spp. were very similar to that of the chaetognaths (Fig. VI.6, 10), suggesting that these copepods may also be feeding on crustacean zooplankton (or on similarly enriched microzooplankton). The large scyphozoan medusae collected in August 2004 covered trophic enrichment (9.2±0.4‰, TL = 4.2) similar to that of the chaetognath *Sagitta* spp. (10.4±1‰, TL= 4.5 in August), suggesting that they feed at the same average trophic level.

Conclusions

This study emphasises the major impact of microzooplankton (e.g. ciliates) on the trophic level of copepods. This could indicate intermediate trophic linkages in a microbial loop/microzooplankton food web leading from riverine detrital material to copepods. Multiple pathways of nitrogen and carbon flow through the food web (i.e. multiple food chains) could explain the divergent seasonal isotopic patterns between mesozooplankton and seston in the study area. I speculate that the German Bight has multiple food chains (i.e. algal particularly

in May and microbial in remaining months) and that mesozooplankton species differentially rely on alternate pathways on nitrogen and carbon transfer from basal resources. Another important conclusion is the impact of detritus amounts on the baseline, since detritus may mask the $\delta^{15}N$ and $\delta^{13}C$ signatures of the autotrophic component of seston. This could incur error in the trophic level calculation of mesozooplankton. In conclusion, stable isotope can provide a great deal of information about foodweb structure and functioning, but interpreting such data without complementary approaches may be problematic. Thus, some of the uncertainties involved in isotope fractionation in the baseline (seston) and mesozooplankton may be resolved by measurements of isotopic composition in DIC, nitrate and ammonium, and by the use of essential fatty acids as natural tracer.

Table VI.1. Summary of the two-way ANOVA analysis of $\delta^{15}N$ and $\delta^{13}C$ for seston, copepods and chaetognaths using sampling station and months as independent variables. Data were Box-Cox -transformed in order to reduce heteroscedasticity in the variances. Statistically significant *p* values are emboldened. df = degrees of freedom, MS = mean square.

			Dependent variable							
	Independent		**$\delta^{15}N$**				**$\delta^{13}C$**			
	variable	df	MS	*F* ratio	*p*-value	% variation	MS	*F* ratio	*p*-value	% variation
Seston										
Seston < 100 µm	Month	4	54	316.9	**<0.001**	64	6	157.6	**<0.001**	36
	Stat no.	4	11	62.5	**<0.001**	13	5	147.8	**<0.001**	34
	Month x Stat no.	16	4	25.1	**<0.001**	20	1	28.2	**<0.001**	26
	Error	62	0			3	0.04			4
Copepods										
Temora longicornis	Month	4	1621	484.6	**<0.001**	28	16	33.0	**<0.001**	20
	Stat no.	4	2134	638.0	**<0.001**	37	11	23.2	**<0.001**	14
	Month x Stat no.	16	485	145.0	**<0.001**	34	12	23.8	**<0.001**	58
	Error	50	3			1	0.5			8
Acartia spp.	Month	4	2783	257.3	**<0.001**	35	29	320.4	**<0.001**	65
	Stat no.	4	2066	191.0	**<0.001**	26	5	59.7	**<0.001**	12
	Month x Stat no.	8	1485	137.3	**<0.001**	38	5	52.2	**<0.001**	21
	Error	34	11			1	0.1			2
Centropages hamatus	Month	4	29	401.5	**<0.001**	37	16	1819.2	**<0.001**	74
	Stat no.	4	20	270.5	**<0.001**	25	2	217.8	**<0.001**	9
	Month x Stat no.	10	12	164.9	**<0.001**	38	1	170.2	**<0.001**	17
	Error	38	0			1	0.01			0.4
Pseudocalanus elongatus	Month	4	24	215.0	**<0.001**	16	1	46.4	**<0.001**	34
	Stat no.	4	63	574.0	**<0.001**	43	0	14.0	**<0.001**	10
	Month x Stat no.	16	15	135.8	**<0.001**	40	0	15.5	**<0.001**	46
	Error	50	0			1	0.02			9
Calanus helgolandicus	Month	4	11	113.3	**<0.001**	20	1	18.0	**<0.001**	12
	Stat no.	4	20	212.3	**<0.001**	38	2	58.4	**<0.001**	39
	Month x Stat no.	16	5	53.8	**<0.001**	39	1	15.0	**<0.001**	40
	Error	50	0			2	0.03			8
Chaetognaths										
Sagitta spp.	Month	4	94962	65.9	**<0.001**	28	19	95.5	**<0.001**	52
	Stat no.	4	123858	86.0	**<0.001**	36	8	39.5	**<0.001**	22
	Month x Stat no.	15	27818	19.3	**<0.001**	31	2	9.1	**<0.001**	19
	Error	48	1441			5	0.2			7

Table VI.2. Results from model II regressions of seston $\delta^{15}N$ and $\delta^{13}C$ as a function of salinity, temperature and nitrate, respectively, according to the model Y = a ± bX , where a = intercept, b = slope and r^2 = coefficient of determination. Symbols are: n.s. (not significant, $p > 0.05$), * (0.01<p<0.05), * * (0.001<p<0.01), * * * (p<0.001).

			Seston $\delta^{15}N$				Seston $\delta^{13}C$			
Independent variable	**Month**	***n***	***r²***	**b**	**a**	***p***	***r²***	**b**	**a**	***p***
Salinity	February	18	0.37	-0.7	30.0	* *	0.89	-2.2	51.0	* * *
	April	18	0.28	-1.5	55.4	*	0.20	-1.9	39.4	n.s.
	May	15	0.05	-2.4	87.9	n.s.	0.11	-2.3	56.3	n.s.
	June	18	0.54	-0.9	36.3	* *	0.63	-0.5	-8.2	* * *
	August	18	0.10	-1.4	50.6	n.s.	0.83	-1.6	29.2	* * *
	Overall	87	0.10	-2.0	71.4	* *	0.36	-2.0	42.8	* * *
Temparature	Feb	18	0.52	-1.3	13.7	* *	0.71	-3.9	0.8	* * *
	April	18	0.02	-4.1	30.9	n.s.	0.00	-5.2	8.4	n.s.
	May	15	0.28	7.1	-57.0	*	0.19	7.0	-86.8	n.s.
	June	18	0.34	1.1	-8.7	*	0.20	0.6	-31.5	n.s.
	August	18	0.01	-2.3	41.3	n.s.	0.36	2.5	-66.7	* *
	Overall	87	0.20	-0.5	9.8	* * *	0.00	-0.5	-19.2	n.s.
Nitrate (NO_3)	February	15	0.27	0.1	5.1	*				
	April	15	0.14	0.1	2.1	n.s.				
	May	12	0.29	-0.5	9.5	n.s.				
	June	15	0.52	0.3	3.8	* *				
	August	15	0.01	1.2	1.4	n.s.				
	Overall	72	0.09	0.3	2.9	* *				

Table VI.3. Results from overall model II regressions of *Sagitta* spp. $\delta^{15}N$ and $\delta^{13}C$ as a function of isotopic signatures of copepod species in the German Bight, according to the model Y = a ± bX , where a = intercept, b = slope and r^2 = coefficient of determination. Symbols are: *** * *** (p<0.0001).

Dependent variable	Predictor variable	*n*	r^2	b	a	*p* - value
Sagitta spp. $\delta^{15}N$	*Acartia* spp. $\delta^{15}N$	48	0.68	0.9	2.6	* * *
Sagitta spp. $\delta^{13}C$	*Acartia* spp. $\delta^{13}C$	48	0.51	0.7	-5.0	* * *
Sagitta spp. $\delta^{15}N$	*C. hamatus* $\delta^{15}N$	54	0.73	0.8	3.7	* * *
Sagitta spp. $\delta^{13}C$	*C. hamatus* $\delta^{13}C$	54	0.63	0.7	-5.1	* * *
Sagitta spp. $\delta^{15}N$	*T. longicornis* $\delta^{15}N$	72	0.62	1.0	1.7	* * *
Sagitta spp. $\delta^{13}C$	*T. longicornis* $\delta^{13}C$	72	0.24	1.1	1.8	* * *
Sagitta spp. $\delta^{15}N$	*P. elongatus* $\delta^{15}N$	72	0.56	0.9	3.4	* * *
Sagitta spp. $\delta^{13}C$	*P. elongatus* $\delta^{13}C$	72	0.55	1.1	1.4	* * *
Sagitta spp. $\delta^{15}N$	*C. helgolandius* $\delta^{15}N$	72	0.63	1.0	1.4	* * *
Sagitta spp. $\delta^{13}C$	*C. helgolandius* $\delta^{13}C$	72	0.56	1.0	-0.6	* * *

Table VI.4. Non-linear regressions of nitrogen trophic enrichment ($\Delta\delta^{15}N_{seston}$) relative to seston as a function of phytoplankton:microzooplankton ratio of copepod species in the German Bight, according to the model ($Y=Y_0+ae^{-bX}$), where Y_0 = intercept, a = constant, b = slope and r^2 = coefficient of determination. Symbols are: n.s. (not significant, $p > 0.05$), * ($0.01<p<0.05$), * * ($0.001<p<0.01$), * * * ($p<0.001$). * * * ($p<0.0001$) and $^{++}$ linear model ($Y = a \pm bX$).

Species	Month	*n*	r^2	Y_0	a	b	*p*
C. helgolandicus	February	15	0.47	3.3	8.9	-0.2	* *
	April	15	0.95	2.8	619	-0.9	* * *
	May	15	0.53	0.5	7.3	-0.5	* *
	June	15	0.67	1.2	12.3	-0.2	* *
	August	15	0.00	7.3	15.9	-2.6	n.s.
	August^{++}	15	0.69	18.6	----	-0.9	* * *
P. elongatus	February	15	0.70	0.9	13.8	-0.2	* * *
	April	15	0.96	1.0	163	-0.6	* * *
	May	15	0.66	-0.1	7.5	-0.4	* *
	June	15	0.76	4.3	202462	-2.0	* * *
	August	15	0.00	7.3	58.0	-1.6	n.s.
	August^{++}	15	0.81	18.1	----	-0.9	* * *
C. hamatus	February	9	0.87	-16.1	24.9	-0.01	* *
	April	9	0.99	2.3	159	-0.6	* * *
	May	9	0.61	-8.6	11.5	-0.02	*
	June	15	0.27	3.8	22.6	-0.5	*
	August	15	0.67	-11.29	26.5	-0.03	* *
Acartia spp.	February	9	0.65	-2.0	9.9	-0.04	*
	April	9	0.99	3.3	552	-0.9	* * *
	May	9	0.93	-2.3	6.9	-0.1	* * *
	June	9	0.00	4.6	6.0	-4.8	n.s.
	June^{++}	9	0.64	10.2	----	-0.7	* *
	August	15	0.72	-20.4	36.3	-0.02	* * *
T. longicornis	February	15	0.00	3.8	-97.5	-529	n.s.
	April	15	0.93	1.8	568	-0.9	* * *
	May	15	0.64	-0.1	11.3	-0.7	* *
	June	15	0.60	4.8	392690	-2.1	* *
	August	15	0.83	-3.0	21.7	-0.06	* * *

Table VI.5. Summary of the two-way ANOVA analysis of trophic enrichment of nitrogen and carbon ($\Delta\delta^{15}N$, $\Delta\delta^{13}C$) relative to seston by month using sampling stations and mesozooplankton species as independent variables. Data were Box-Cox -transformed in order to induce homocedasticity. Statistically significant results are shown in bold. df = degrees of freedom, MS = mean square.

			Dependent variable							
	Independent		$\Delta\delta^{15}N_{Seston.}$				$\Delta\delta^{13}C_{Seston.}$			
Month	variable	df	MS	*F* ratio	*p*-value	% variation	MS	*F* ratio	*p*-value	% variation
February	Stat no.	4	10	120.4	**<0.001**	49	56	273.3	**<0.001**	84
	Species	4	7	82.3	**<0.001**	33	2	9.0	**<0.001**	3
	Stat no. x Species	12	1	11.0	**<0.001**	13	2	11.0	**<0.001**	10
	Error	42	0.08			4	0.2			3
April	Stat no.	4	15	1208.7	**<0.001**	94	23.5	232.3	**<0.001**	47
	Species	4	0.3	26.5	**<0.001**	2	22.4	221.0	**<0.001**	45
	Stat no. x Species	12	0.2	14.8	**<0.001**	3	1.0	9.8	**<0.001**	6
	Error	42	0.01			1	0.1			2
May	Stat no.	4	20	301.4	**<0.001**	87	21	36.4	**<0.001**	62
	Species	4	1	9.7	**<0.001**	3	1	1.9	0.136	3
	Stat no. x Species	12	1	7.8	**<0.001**	7	2	3.3	**<0.01**	17
	Error	42	0.1			3	0.6			18
June	Stat no.	4	0.4	113.5	**<0.001**	67	12	287.6	**<0.01**	50
	Species	4	0.1	16.2	**<0.001**	10	7	161.5	**<0.01**	28
	Stat no. x Species	14	0.03	8.4	**<0.001**	17	1	32.8	**<0.01**	20
	Error	46	0.003			7	0.04			2
August	Stat no.	4	1961	337.1	**<0.001**	89	6	40.3	**<0.001**	42
	Species	4	68	11.6	**<0.001**	3	1	7.2	**<0.001**	8
	Stat no. x Species	16	25	4.3	**<0.001**	5	1	8.9	**<0.001**	37
	Error	49	6			3	0.1			13

VII. Mesozooplankton lipid correction of $\delta^{13}C$.

Abstract

For an accurate interpretation of mesozooplankton structure and trophodynamics from carbon stable isotope data it is necessary to correct for lipids effect. This is because the lipid content varies within and among tissues in both space and time, and because lipids are ^{13}C-depleted relative to proteins. However, lipid extraction may affect $\delta^{15}N$ and thus require a costly and time-consuming separation of $\delta^{13}C$ and $\delta^{15}N$ analyses. These problems have prompted the development of arithmetic correction approaches for $\delta^{13}C$, but these corrective procedures and their underlying assumptions have not been systematically tested. This study investigated the relationship between C:N ratios as lipid indicators and mesozooplankton $\delta^{13}C$ and compared the effects of arithmetic correction approaches on $\delta^{13}C$. I found highly significant correlations ($p < 0.001$) of $\delta^{13}C$ as a function of C:N ratios only for *P. acuspes* from the Central Baltic Sea and *P. elongatus* and *C. helgolandicus* from the Southern North Sea. In these three copepod species, seasonal variation in C:N ratio explained more of the variation in $\delta^{13}C$ than did the $\delta^{13}C$ of seston (see chapters IV and VI). This suggest that variation in the lipid content of mesozooplankton can strongly influence temporal variation of $\delta^{13}C$ of some species (e.g. *P. acuspes* , *P. elongatus* and *C. helgolandicus*), but not in all species (like *T. longicornis*). Using these data, I evaluate arithmetic correction procedures that estimate the $\delta^{13}C$ of only the non-lipid component of mesozooplankton in order to analyse the consumer's protein and carbohydrate content. In contrast to the mass balance approach proposed by Fry et al. (2003) and the ANCOVA regressions, the traditional lipid-normalization technique developed by McConnaughey & McRoy (1979) gave coherent $\delta^{13}C$ values in accordance with my expectations. However, if lipids are primarily dietary in origin, than normalizing $\delta^{13}C$ based on C:N ratios will exclude a major dietary source, and therefore may be inappropriate. Despite some drawbacks, I recommend the corrective procedure proposed by McConnaughey & McRoy (1979). The application of this arithmetic correction can lead to significant time and cost savings, because the majority of the $\delta^{13}C$ and $\delta^{15}N$ analyses would not need to be run separately. I conclude that temporal variation in mesozooplankton lipid content can significantly influence the temporal variation of $\delta^{13}C$ signatures of some mesozooplankton species from the Baltic Sea and North Sea.

Results

Lipids have not been extracted from the samples in this study. However, it has been shown, first, that lipids in an organism normally possess lower ^{13}C:^{12}C ratios (lighter $\delta^{13}C$) than proteins and carbohydrates (McConnaughey & McRoy 1979) and, second, that the lipid content of a sample can be predicted accurately from its C:N ratio (Lesage et al. 2001). Thus, since lipids have lighter $\delta^{13}C$ than other body constituents, organisms rich in lipid content could be erroneously interpreted as indicative of use of different carbon sources. Therefore, in order to avoid such underestimates of the carbon isotopic ratios, I applied three correction approaches using the C:N ratios as lipid-indicator: (i) the lipid normalization technique of McConnaughey & McRoy (1979). (ii) a mass balance approach proposed by Fry et al. (2003) and (iii) the analysis of covariance (ANCOVA) of the original $\delta^{13}C$ of sampling stations and months as a function of C:N ratios.

All $\delta^{13}C$ values of *Pseudocalanus acuspes* from the Central Baltic Sea, *Pseudocalanus elongatus* and *Calanus helgolandicus* from the Southern North Sea given in this study are normalized according to McConnaughey & McRoy (1979). The differences of averages between the normalized $\delta^{13}C$ values (i.e. δ less negative) and the original values, exhibited clear increases in ^{13}C from 1.6 ± 0.5 ‰ to 2.4 ± 0.4‰ for *P. acuspes* (Fig. VII.1A). The differences in the $\delta^{13}C$ values of *P. elongatus* and *C. helgolandicus* varied for both species from 0.6 ± 0.1 ‰ to 1.3 ± 0.4‰ (Fig. VII.1B, C), during the study period.

The lipid correction of $\delta^{13}C$ values based on the mass balance after Fry et al. (2003) generally resulted in remarkably more strongly enriched $\delta^{13}C$ values (δ less negative) in comparison to the another corrective procedures. This corrective approach showed high differences between the corrected $\delta^{13}C$ and original values, which varied on averages from 1.1 ± 0.1 ‰ to 3.4 ± 0.5‰ for *P. acuspes* and from 1.1 ± 0.1 ‰ to 2.0 ± 0.5‰ for both *P. elongatus* and *C. helgolandicus* (see Fig. VII.1).

ANCOVA regressions of $\delta^{13}C$ on C:N ratio was fitted to each mesozooplankton species. The results were highly significant only for *P. acuspes*, *P. elongatus* and *C. helgolandicus* (Table VII.1, F = 206, 51.9 and 15.2, respectively, all $p < 0.001$). The covariant model explained 77 %, 52 % and 24 % of the within-group variation (sampling station and month) in the data of *P. acuspes*, *P. elongatus* and *C. helgolandicus*, respectively. A negative common slope for each of these species was found (-0.6±0.04‰, -0.4±0.05‰, -0.5±0.12‰, respectively) confirming the expected dependence of $\delta^{13}C$ as a function of C:N ratios (Fig. VII.2). The corrected $\delta^{13}C$ of *P. acuspes* by ANCOVA (Fig. VII.1A) were relatively small with respect to the original $\delta^{13}C$ in May ($\delta^{13}C - \delta^{13}C_{corr}$ = 0.2 ± 0.4 ‰), August (0.1 ± 0.8 ‰) and March (0.6 ± 0.02 ‰), while in July (1.5 ± 0.1 ‰) and Nov/Dec. (1.1 ± 0.1 ‰) the

differences were some higher. Nevertheless, for both species from the North Sea, the corrected $\delta^{13}C$ were similar to the original $\delta^{13}C$ values (Fig. VII.1B, C).

In general, all of the previous corrective approaches confirmed the expected isotopic depletion caused by the lipid content on $\delta\ ^{13}C$ of the analyzed species (Fig. VII.1).

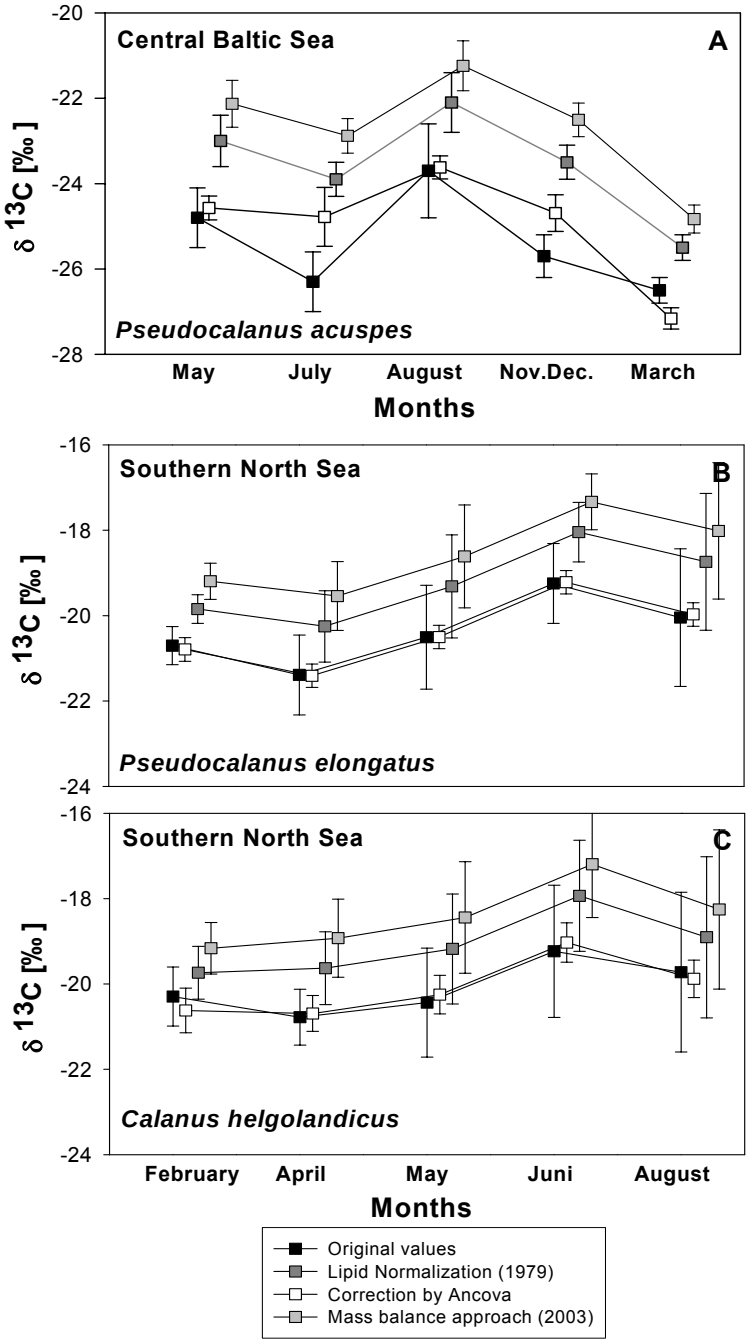

Figure VII.1. Depth-integrated (mean ± 1SD) $\delta^{13}C$ (black solid symbols), lipid-corrected $\delta^{13}C$ after Ancova regressions on C:N ratios (open symbols), according to McConnaughey & McRoy (1979) (dark gray solid symbols) and after Fry et. al. (2003) (light gray solid symbols) of *P. acuspes* (A), *P. elongatus* (B) and *C. helgolandicus* (C). The x axis is not continuous (to enhance visibility of the data for one month are shifted in x direction).

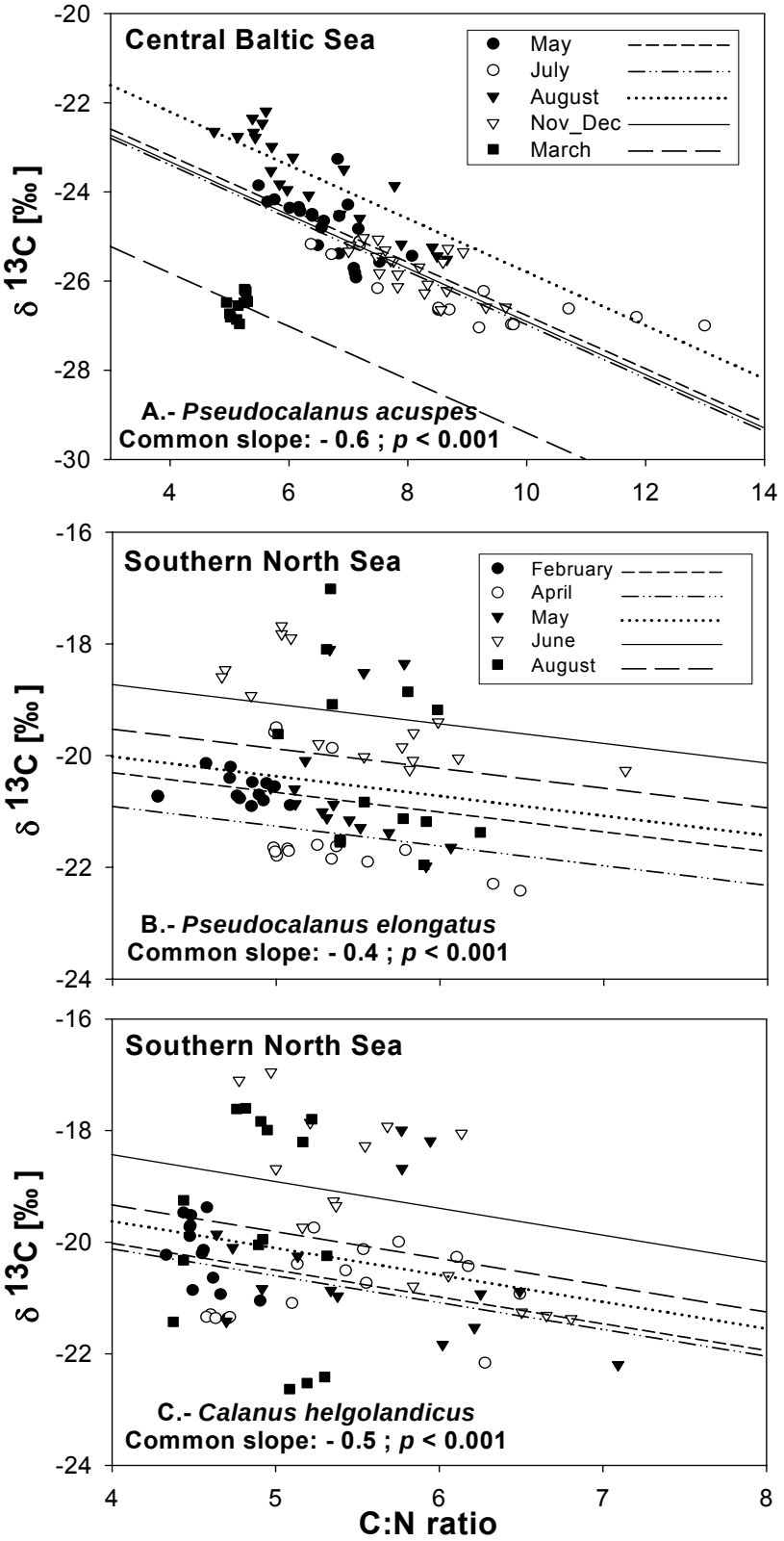

Figure VII.2 $\delta^{13}C$ (‰) as a function of C:N ratios (Ancova regressions of stations and months) of *P. acuspes* (A.- common slope = -0.6, n = 93 , r^2 = 0.77, p < 0.001)*, P. elongatus* (B.- common slope = -0.4, n = 75 , r^2 = 0.52, p < 0.001) and *C. helgolandicus* (C.- common slope = -0.5, n = 75 , r^2 = 0.24, p < 0.001).

Discussion

Mesozooplankton (mainly copepods) can store large volumes of lipids, depending on the time of year. This lipid content varies with species, nutritional status and reproductive cycle (Arts & Wainman 1998). Since the C:N ratio is positively related to lipid content (Lesage 1999, Schmidt et al. 2003) and the lipids normally posses lighter $\delta^{13}C$ signatures than protein and carbohydrate in an organisms, lipid-rich organisms tend to be isotopically light compared to non lipid-rich ones and given the whole-body $\delta^{13}C$ variations that complicates interpretation of other causes of variation of carbon (Rolff & Elmgren 2000).

Many studies have reported the lipid effect on $\delta^{13}C$ signatures (McConnaughey & McRoy 1979, Peterson & Fry 1987, Kling et al. 1992, Rau et al. 1992, Wainright & Fry 1994, Leggett et al. 1999, Rolff & Elmgren 2000, Matthews & Mazumder 2005, Bodin et al. 2007), but some studies show that lipids do not significantly affect the $\delta^{13}C$ of zooplankton, (e.g. Zohary et al. 1994, France 1995, Campbell et al. 2000). The findings by Zohary et al. (1994) suggest that lipid accumulation is insufficient to account for zooplankton $\delta^{13}C$ depletion, thus, the effect of lipids on $\delta^{13}C$ of different organisms is relative unclear in the current literature.

Assuming a lipid effect on $\delta^{13}C$, some corrective approaches have been suggested, e.g. lipid extraction from organisms prior to $\delta^{13}C$ analysis, lipid normalization equations and regression models. Most of these corrections have drawbacks. Entire lipid extraction is difficult or impossible for small organisms (e.g. cladocerans and copepods), because the choice of the solvent mixture affects the selectivity and completeness of extraction (Rolff & Elmgren 2000) and lipid extraction removes not only lipids, but also N-containing compounds that may alter the $\delta^{15}N$ signatures of a sample (Murry et al. 2006). Moreover, this procedure is costly, time-consuming and involves extensive preparatory work. These are disadvantageous because most ecological studies require extensive replication in space and time and time and funding are always limited (Sweeting et al. 2006).

The lipid normalization technique proposed by McConnaughey & McRoy (1979) (Eq. III.2) was recently used in numerous ecological studies (see Satterfield IV & Finney 2002, Schmidt et al. 2003, Perga & Gerdeaux 2005, Ruus et al. 2006, Bodin et al. 2007). This technique assumes that (i) there is a positive nonlinear relationship between lipid content and C:N ratio and (ii) there is a constant difference between the $\delta^{13}C$ of lipids and proteins/carbohydrates in the body of an organism (parameter D = 6‰, see McConnaughey 1978). Although there is some empirical support (Lesage 1999, Schmidt et al. 2003), the first assumption, is not well established (Matthews & Mazumder 2005). It is often assumed that temporal variation in lipid content can increase intraspecific or interstage variability of C:N because of the high C:N of lipids (Villar-Argaiz et al. 2002). The second assumption may also

have a potential drawback, because the normalization of $\delta^{13}C$ signatures is very sensitive to the assumption that the average difference between the $\delta^{13}C$ signatures of lipids and proteins is 6 ‰. Some studies showed that this parameter (*D*) is variable.

Four decades ago, Parker (1964) found highly variable isotopic differences between the $\delta\ ^{13}C$ of lipids and the bulk organism (0.5 to 15 ‰). Current studies have also indicated that lipid biosynthesis discriminates against ^{13}C (Monson & Hayes 2002), and contributes to isotopic variability among different fatty acids (van Dongen et al. 2002). A recent study by Matthews & Mazumder (2005), suggests that, if this normalization approach is used, researchers should independently measure the *D* parameter, and verify the relationship between lipid and body composition (C:N ratio) of an organism. However, the $\delta\ ^{13}C$ lipid normalization developed by Mcconnaughey & Mcroy (1979), only estimates the $\delta\ ^{13}C$ of the non-lipid fraction of a consumer, and therefore excludes any dietary acquisition and storage of lipid (Goulden et al. 1998).

In spite of the mentioned difficulties, I used the latter normalization approach assuming the traditional constant *D* value of 6 ‰, which is based on several literature values and has been successfully applied in the past and recent food webs studies. I believe that this approach gives coherent $\delta^{13}C$ values (Fig. VII.1) in accordance with the expected trophic enrichment of the copepods *P. acuspes*, *P. elongatus* and *C. helgolandicus* in the Baltic Sea and North Sea (see chapters IV, V and VI). Moreover, as lipids are typically dietary in zooplankton, both the $\delta^{13}C$ of the lipids and the lipid-free component of zooplankton are useful for dietary analyses. In organisms where changes in lipid content reflect changes in synthesis (Chamberlain et al. 2004), rather than accumulation from diet, it is more reasonable to normalize $\delta^{13}C$ values based on C:N ratios (Matthews & Mazumder 2005). In general, lipid normalization is more suitable if one is interested in a dietary analysis of a consumer's proteins and carbohydrates (i.e. in tracing the non-lipid component of a consumer's diet).

Applying the mass balance proposed by Fry et al. (2003) (Eq. III.4) to our data resulted in marked $\delta^{13}C$ increases with respect to the original values and to those obtained by other approaches (Fig. VII.1). Therefore, I could incur error and overestimate the lipid effect on $\delta^{13}C$. I believe that these corrected $\delta^{13}C$ values are too high and can lead to significant difficulties in the interpretation of the trophic level of the copepod species, considering that the fractionation factor (frequently cited as rough "rule-of-thumb") for $\delta\ ^{13}C$ enrichment per trophic level varies from 0.1 to 1 ‰ (Rau et al. 1983, Fry & Sherr 1984, Peterson & Fry 1987, Hobson & Welch 1992). This mass balance correction depends on $C:N_{\ protein}$ which varies among species (Sweeting et al. 2006). Hence, Bodin et al. (2007)

proposed lipid-normalization equations where the corrected $\delta^{13}C$ are inferred from the original $\delta^{13}C$ value and C:N ratio (Eq. VII.1) or lipid content (Eq. VII.2):

$$\delta^{13}C_{corr} = [(\delta^{13}C_{original} + 0.322) \times (C{:}N_{original} - 1.175)] \quad \text{(VII.1)}$$

and

$$\delta^{13}C_{corr} = [(\delta^{13}C_{original} + 0.0588) \times (\%\ lipid)] \quad \text{(VII.2)}$$

These mathematical equations have the advantage of not requiring assumptions of δ_{lipid} and of $C{:}N_{protein}$. However, as nequation III.4 (see chapter III), these equations have been successfully applied only to large organisms (e.g. decapod crustacean).

An alternative to lipid extraction and normalization approaches is the use of a model II ANCOVA regression between $\delta^{13}C$ and C:N ratios as estimator of lipid content. I applied this analysis and fitted a pooled slope between $\delta^{13}C$ and C:N ratio for all groups (sampling station and month), and tested for the existence of a common slope. By fitting independent intercepts in each group, confusion of the lipid effect with the main effects (station and month) can be avoided, and the $\delta^{13}C$ means can be adjusted for differences caused by lipid content (Snedecor & Cochran 1980). This method was previously used by Rolff & Elmgren (2000), with significant results. Therefore, in this study, I applied this analysis for all mesozooplankton species, however only *P. acuspes*, *P. elongatus* and *C. helgolandicus* showed a highly significant and negative relationships between $\delta^{13}C$ and C:N ratio (Table VII.1, Fig. VII.2). This significant result and the high explained regression (76% only for *P. acuspes*, while for *P. elongatus* and *C. helgolandicus,* the relationships were weaker) suggests that ANCOVA regression could be an efficient approach for removal of lipid biases in copepod $\delta^{13}C$, and shows that ANCOVA is better than a single linear regression model, because the latter model may involve an incorrect transformation by not separating the data into relevant subgroups, and may cause the lipid effect to be confounded with the main effect of the factor analyzed (Rolff & Elmgren 2000). Despite these advantages, the main problem is that ANCOVA linear regression assumes an infinite $\delta^{13}C$-depletion as a function of C:N ratios. Logistic regressions of C:N and $\delta^{13}C$ have been also applied (Matthews & Mazumder 2005), but it is only effective when there is a negative nonlinear relationship between both variables. My data did not support this condition.

In general, it is important to note that detecting a negative relationship between C:N and $\delta^{13}C$ for a single mesozooplankton species may be challenging because the range of zooplankton C:N is often small at any one time. However, if was found large variation in $\delta^{13}C$ concurrently with low variability in C:N ratios such as *Temora longicornis* in the Baltic Sea (Table VII.2) or *Acartia* spp. in the North Sea (Table VII.3), this would indicate a limited impact of lipids on $\delta^{13}C$ signatures. The negative relationship between C:N and $\delta^{13}C$ among

mesozooplankton species is also consistent with the hypothesis that zooplankton with a high C:N have higher concentrations of lipids that have low $\delta^{13}C$ signatures (Fig. VII.2). There are important implications of this relationship for interpreting the $\delta^{13}C$ signatures of mesozooplankton. For example, an organism with a low C:N ratio (e.g. *T. longicornis*) can increase its lipid concentration (~10% by weight) without a substantial change in whole body C:N (see Matthews & Mazumder 2005). A similar increase in lipid in an organism with an already high C:N (e.g. *Pseudocalanus* spp.) will result in a greater increase in C:N. This is because lipids have a higher concentration of carbon by weight than either proteins or carbohydrates, and the relationship between lipid concentration and C:N ratios is an increasing non-linear function (McConnaughey 1978). Therefore, a consumer with a low C:N (i.e. *T. longicornis* or *Acartia* spp.) may experience large changes in $\delta^{13}C$ despite small seasonal changes in C:N. The exact form of the relationship between mesozooplankton $\delta^{13}C$ and C:N will depend on the $\delta^{13}C$ of lipids, and the relationship between lipid and C:N ratios.

Conclusions.

Isotopic differences between species can be related to differences in feeding behaviour and differences in body composition (C:N ratios), yet the latter is rarely considered when interpreting the $\delta^{13}C$ of mesozooplankton. Considering the seasonal and interspecific variation in mesozooplankton lipid content, may help explain to the seasonal variation in the mesozooplankton $\delta^{13}C$ signatures (Fig. VII.1) and variation of mesozooplankton $\delta^{13}C$ among sites (see chapter VI). My data support the hypothesis that temporal change in body composition (C:N ratio) can significantly affect the $\delta^{13}C$ of some mesozooplankton species (e.g. *P. acuspes*, *P. elongatus* and *C. helgolandicus*), but not in all species (e.g. *T. longicornis*, *Acartia* spp.). As lipids are primarily dietary in mesozooplankton, dietary studies of mesozooplankton using $\delta^{13}C$ would benefit from a more detailed consideration of lipids. Hence, mesozooplankton lipid content has an important influence on $\delta^{13}C$ values and must be taken into account in ecological studies on mesozooplankton (mainly copepods) using a stable isotope approach. Some researchers recommend the isotopic analysis of carbon and nitrogen separately on lipid-free ($\delta^{13}C$) and bulk materials ($\delta^{15}N$) when lipid extraction will be applied. However, lipid extraction may result in the loss of non-lipid compounds that may alter $\delta^{15}N$ and separation of C and N isotope analyses are costly, time consuming and involve extensive preparatory work. Because of the direct correlations between lipid content, C:N ratios and $\delta^{13}C$ signatures, mathematical equations could be used both to estimate the lipid content of a sample and to lipid-normalize $\delta^{13}C$ values. General equations found in the literature must be used with caution because of the difference in tissue biochemical

composition and lipid isotopic depletion of organisms. In this work, arithmetic correction approaches for estimated lipid content and lipid-normalized $\delta^{13}C$ results were proposed for some copepods, although further investigations should be done to support them. In conclusion, this study demonstrates that researchers should carefully consider the consequences of dietary lipids in the interpretation of consumer $\delta^{13}C$.

Table VII.1. Results of the $\delta^{13}C$ ANCOVA analysis of sampling stations and months on C:N ratios of *P. acuspes, P. elongatus* and *C. helgolandicus*. df = degrees of freedom, MS = adjusted mean square and, r^2 = coefficient of determination. Significant *p* values ($p < 0.001$) are shown in bold.

Species	Source of variation	Dependent variable: $\delta^{13}C$				
Central Baltic Sea		df	MS	*F* ratio	*p*-value	r^2
Pseudocalanus acuspes	Corrected model	31	4.66	63.75	**< 0.001**	0.95
	Covariant (C:N ratio)	1	15.08	206.12	**< 0.001**	0.77
	Month	4	23.26	317.94	**< 0.001**	
	Station no	6	0.64	8.81	**< 0.001**	
	Month x Station no	20	0.70	9.55	**< 0.001**	
	Error	61	0.07			
	Covariant	Slope	SE	$t_{(61)}$	*p*-value	r^2
	C:N ratio	**-0.6**	**0.04**	**-14.36**	**< 0.001**	**0.77**
	Adjusted intercepts	May	July	August	Nov-Dec	March
		-20.8	-21.0	-19.8	-20.9	-23.4
Southern North Sea		df	MS	*F* ratio	*p*-value	r^2
Pseudocalanus elongatus	Corrected model	25	4.75	63.63	**< 0.001**	0.95
	Covariant (C:N ratio)	1	3.88	51.94	**< 0.001**	0.52
	Month	4	10.15	135.85	**< 0.001**	
	Station no	64	4.22	56.45	**< 0.001**	
	Month x Station no	16	3.41	45.7	**< 0.001**	
	Error	49	0.07			
	Covariant	Slope	SE	$t_{(49)}$	*p*-value	r^2
	C:N ratio	**-0.4**	**0.05**	**-7.21**	**< 0.001**	**0.52**
	Adjusted intercepts	February	April	May	June	August
		-18.9	-19.5	-18.6	-17.3	-18.1
		df	MS	*F* ratio	*p*-value	r^2
Calanus helgolandicus	Corrected model	25	5.29	31.24	**< 0.001**	0.91
	Covariant (C:N ratio)	1	2.56	15.15	**< 0.001**	0.24
	Month	4	6.15	36.3	**< 0.001**	
	Station no	64	10.16	59.94	**< 0.001**	
	Month x Station no	16	3.68	21.73	**< 0.001**	
	Error	49	0.17			
	Covariant	Slope	SE	$t_{(49)}$	*p*-value	r^2
	C:N ratio	**-0.5**	**0.12**	**-3.89**	**< 0.001**	**0.24**
	Adjusted intercepts	February	April	May	June	August
		-18.1	-18.2	-17.7	-16.5	-17.4

Table VII.2. Means ± 1SD, minimum and maximum of mesozooplankton C:N ratios and original $\delta^{13}C$ (‰) in the Bornholm Basin, Central Baltic Sea.

	Month		C:N ratio			original $\delta^{13}C$ (‰)		
		n	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
Cladocerans								
Bosmina coregoni maritima	August	21	5.7 ± 0.5	4.0	6.3	-23.4 ± 0.7	-25.0	-20.2
Evadne nordmanni	May	21	6.9 ± 1.0	5.7	8.6	-25.0 ± 0.7	-26.2	-23.4
	July	18	7.8 ± 0.7	5.2	8.2	-26.5 ± 0.4	-27.2	-23.8
	Nov/Dec.	21	6.3 ± 0.8	4.8	8.1	-25.0 ± 0.4	-25.5	-24.2
Podon spp.	May	21	5.8 ± 0.4	5.2	6.7	-24.9 ± 0.5	-25.9	-23.4
	July	18	6.0 ± 0.4	5.3	6.3	-25.0 ± 0.4	-25.4	-24.5
	August	21	5.2 ± 0.6	3.8	5.7	-21.6 ± 0.7	-22.4	-20.2
	Nov/Dec.	21	5.1 ± 0.3	4.7	5.7	-24.1 ± 0.3	-25.0	-23.4
Copepods								
Temora longicornis	May	21	5.5 ± 0.4	5.0	6.4	-25.0 ± 0.2	-25.4	-24.6
	July	18	5.4 ± 0.3	5.1	6.4	-24.6 ± 0.2	-25.0	-24.3
	August	21	4.9 ± 0.4	3.4	5.4	-22.7 ± 0.3	-23.5	-22.1
	Nov/Dec.	21	5.7 ± 0.5	3.9	7.0	-23.3 ± 0.4	-24.4	-22.9
	March	12	4.7 ± 0.3	4.4	5.1	-23.9 ± 0.2	-24.2	-23.5
Acartia spp.	May	21	5.7 ± 0.3	5.1	6.3	-25.0 ± 0.4	-25.7	-24.0
	July	18	7.4 ± 2.1	5.2	12.6	-25.7 ± 0.9	-27.2	-24.4
	August	21	6.0 ± 0.4	5.3	6.6	-23.6 ± 0.6	-24.6	-22.2
	Nov/Dec.	21	6.2 ± 3.0	3.9	18.9	-24.6 ± 0.4	-25.1	-23.5
	March	12	4.4 ± 0.4	3.8	5.1	-24.6 ± 0.5	-25.2	-23.7
Centropages hamatus	May	21	5.2 ± 0.3	4.8	5.7	-24.6 ± 0.4	-25.1	-23.6
	July	18	5.1 ± 0.2	4.7	5.5	-24.5 ± 0.2	-24.8	-24.3
	August	21	4.8 ± 0.4	3.2	5.3	-22.2 ± 0.7	-24.2	-21.2
	Nov/Dec.	21	5.0 ± 0.5	4.2	5.8	-23.9 ± 0.4	-25.1	-23.4
	March	12	4.5 ± 0.2	4.3	4.9	-25.2 ± 0.3	-25.6	-24.9
Pseudocalanus acuspes	May	21	6.6 ± 0.6	5.5	8.1	-24.8 ± 0.7	-25.9	-23.3
	July	18	8.9 ± 1.8	6.4	13.0	-26.3 ± 0.7	-27.0	-25.1
	August	21	6.4 ± 1.2	4.7	8.7	-23.7 ± 1.0	-25.5	-22.2
	Nov/Dec.	21	8.0 ± 0.7	7.0	9.6	-25.7 ± 0.5	-26.7	-25.0
	March	12	5.2 ± 0.1	4.9	5.3	-26.5 ± 0.3	-27.0	-26.2
Chaetognaths								
Sagitta spp.	August	3	4.4 ± 0.1	4.4	4.5	-22.1 ± 0.6	-22.6	-21.5
	Nov/Dec.	21	5.1 ± 0.7	4.0	6.5	-21.5 ± 0.8	-22.9	-20.2
	March	5	5.2 ± 0.2	4.9	5.3	-22.1 ± 0.7	-22.6	-21.1

Table VII.3. Means ± 1SD, minimum and maximum of mesozooplankton C:N ratios and original $\delta^{13}C$ (‰) in the German Bight, Southern North Sea.

	Month		C:N ratio			original $\delta^{13}C$ (‰)		
		n	Mean ± SD	Min.	Max.	Mean ± SD	Min.	Max.
Copepods								
Temora longicornis	February	15	4.4 ± 0.1	4.0	4.6	-20.6± 1.9	-23.5	-18.9
	April	15	4.6 ± 0.5	3.1	5.1	-19.0± 0.7	-20.2	-18.0
	May	15	4.7 ± 0.2	4.5	5.5	-19.8± 1.2	-21.2	-17.4
	June	15	4.8 ± 0.2	4.5	5.2	-19.1± 0.7	-20.5	-18.2
	August	15	4.8 ± 0.2	4.5	5.3	-19.0± 0.9	-19.9	-17.5
Acartia spp.	February	9	4.7 ± 0.1	4.6	4.8	-19.9± 0.4	-20.3	-19.2
	April	9	4.8 ± 0.4	4.3	5.5	-22.8± 1.2	-24.4	-21.2
	May	9	4.7 ± 0.2	4.4	5.0	-18.9± 0.4	-19.5	-18.3
	June	9	5.0 ± 0.3	4.6	5.4	-18.4± 0.6	-19.1	-17.5
	August	15	5.3 ± 0.3	4.8	5.9	-19.4± 1.7	-21.5	-16.9
Centropages hamatus	February	9	4.4 ± 0.1	4.4	4.4	-20.0± 0.1	-20.1	-19.9
	April	9	5.2 ± 0.3	4.8	5.8	-21.9± 0.2	-22.1	-21.6
	May	9	4.5 ± 0.1	4.3	4.6	-18.5± 0.6	-19.2	-17.5
	June	15	4.7 ± 0.4	4.4	5.7	-17.2± 0.8	-18.2	-15.6
	August	15	4.8 ± 0.3	4.4	5.6	-19.0± 1.8	-21.8	-17.0
Pseudocalanus elongatus	February	15	5.2 ± 1.6	3.4	11.0	-20.7± 0.4	-22.1	-20.1
	April	15	5.4 ± 0.5	5.0	6.5	-21.4± 0.9	-22.4	-19.5
	May	15	5.4 ± 0.3	6.3	10.1	-20.5± 1.2	-21.9	-18.1
	June	15	5.5 ± 0.7	4.7	7.1	-19.2± 0.9	-20.3	-17.7
	August	15	5.6 ± 0.4	5.0	6.2	-20.0± 1.6	-21.9	-17.0
Calanus helgolandicus	February	15	4.6 ± 0.1	4.3	4.9	-20.3± 0.7	-21.4	-19.4
	April	15	5.4 ± 0.6	4.6	6.5	-20.8± 0.7	-22.1	-19.7
	May	15	5.6 ± 0.7	4.6	7.1	-20.4± 1.3	-22.2	-17.9
	June	15	5.7 ± 0.6	4.8	6.8	-19.2± 1.5	-21.4	-16.9
	August	15	4.9 ± 0.3	4.4	5.3	-19.7± 1.9	-22.6	-17.6
Chaetognaths								
Sagitta spp.	February	15	4.8 ± 0.2	4.5	5.0	-19.4± 0.9	-20.9	-18.4
	April	15	5.0 ± 0.7	4.5	7.3	-20.4± 0.8	-21.8	-19.3
	May	12	4.7 ± 0.3	4.3	5.4	-19.8± 0.9	-21.1	-18.5
	June	15	4.6 ± 0.2	4.4	4.9	-17.4± 0.9	-19.0	-16.6
	August	15	4.7 ± 0.1	4.6	5.0	-19.3± 1.3	-20.9	-17.7
Ctenophores	April	6	4.8 ± 0.3	4.6	5.4	-21.7± 0.4	-22.1	-20.9
	May	9	4.6 ± 0.2	4.5	5.1	-20.5± 0.6	-21.2	-19.8
Medusae	August	6	4.8 ± 0.2	4.5	5.1	-19.1± 0.6	-19.7	-18.4

VIII. General discussion

A comparative analysis.

Nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope of analysis revealed strong seston and within-guilds isotopic differences (Fig. VIII.1) between the Bornholm Basin (Central Baltic Sea) and German Bight (Southern North Sea).

I decided to standardize the isotopic signatures of congener copepod species using the less ^{15}N-enriched copepod *Temora longicornis* as reference organism in order to compare the trophic structure (trophic levels) between both marine ecosystems. This is based on the following reasons: (i) seston usually does not represent a pure autotrophic baseline and (ii) mesozooplankton species frequently feed selectively on different seston groups (e.g. phytoplankton, ciliates, bacteria), which are isotopically distinct (see Chapter IV, V, VI). Finally, (iii) the absence of herbivorous cladocerans (e.g. *Evadne nordmanni* and *Bosmina coregoni*) excluded their use as baseline reference organism in the German Bight. It is important to note that my intention was the search for a reference organism to compare the isotopic signatures of mesozooplankton in both marine systems and not to give an absolute measure of the trophic level of these copepods species using *T. longicornis* $\delta^{15}N$ as baseline. The latter is true because in this study, *T. longicornis* was found to be an omnivorous species with a tendency to carnivory despite its stationary suspension-feeding (see chapter VI) and thus, omnivory as a variable might complicate the calculation and interpretation of the trophic levels of other copepod species.

Figure VIII.2. shows the seasonal pattern of standardized mesozooplankton trophic enrichment in terms of nitrogen ($\Delta\delta^{15}N$) and carbon ($\Delta\delta^{13}C$) of three congeneric copepod species from the Central Baltic Sea and Southern North Sea using *T. longicornis* as reference organism. In general, no similarities in the seasonal isotopic pattern and trophic enrichments were found for the compared species between the Bornholm Basin and German Bight (Fig. VIII. 2). In addition it is obvious that interspecific difference in ^{15}N were much more pronounced in the Bornholm Basin than in the German Bight.

Why did the mesozooplankton community of the Bornholm Basin show significant within-guild differences whereas no differences were apparent in the German Bight? Is this related to different feeding conditions in both seas? I tested this hypothesis by pooling trophic level data standardized to the isotopic signatures of *T. longicornis* and comparing congeneric species (*Acartia, Centropages, Pseudocalanus*) in both marine systems for the stratified period May to August, since data for this season were available from both systems. *T. longicornis*- standardized trophic enrichments were then related to chlorophyll *a*

concentrations using a non linear regression model (Fig. VIII.3). The resulting regressions indicated that standardized nitrogen trophic enrichment of copepod species were significantly ($p < 0.05$, Fig. VIII.3) and negatively correlated with $\log_{10}$-converted chlorophyll *a* concentrations, suggesting that copepods were in general more herbivorous (with small or no within-guild differences) at higher phytoplankton biomass (mainly copepods of the German Bight) and more carnivorous (with significant within-guild differences) at lower phytoplankton biomass (Baltic Sea). This may indicate but not necessarily imply, changes in the foraging strategies of these copepods, i.e. a switch from an ambushing mode at lower phytoplankton biomass to stationary suspension-feeding at higher phytoplankton biomass.

In conclusion, these findings suggests that the regime of primary production plays an important role in the trophic structure of mesozooplankton, since the annual primary productivity in both systems differs markedly ranging from 80 to 140 *g* C m^{-2} yr^{-1}(Lenz 1995, Horstmann & Hübel 1996) and ~430 *g* C m^{-2} yr^{-1} (Rick et al. 2006) in the Bornholm Basin and German Bight, respectively.

Outlook.

In spite of a number of successful applications in field studies (Rau et al. 1983, Fry & Sherr 1984, Peterson & Fry 1987, Hobson & Welch 1992, France & Peters 1997, Post 2002), isotopic results must be interpreted with care, especially since the amount of isotopic increase per trophic level is not in all cases precisely known. For example, there are some problems with using $\delta^{13}C$ as a trophic level indicator: The increase in $\delta^{13}C$ with trophic level is small (0.1 – 1.1‰) relative to overall sampling errors, and it has been proposed that $\delta^{13}C$ changes in food webs may more strongly reflect the importance of different carbon sources rather than trophic level (Fry & Wainright 1991). Hence, there is more confidence in the use of $\delta^{15}N$ as a trophic level indicator, because the isotopic changes per trophic level are larger (average of 3.4‰) and have been repeatedly documented in aquatic systems (Minagawa & Wada 1984, Fry 1988, Montoya et al. 1990, Kling et al. 1992, Michener & Schell 1994, Post 2002). However, methodological difficulties have so far prevented the measurement of $\delta^{15}N$ signatures of pure phytoplankton and microbial loop organisms separately from $\delta^{15}N$ of bulk seston, and there is few information about isotopic enrichment in the microbial food chain, an important route of matter and energy transfer in planktonic food webs. Additionally, there are indications that the 3.4% per trophic level may not apply in this portion of the planktonic food web (Post 2002), where bacterial processing of detritus (allochthonous or autochthonous in origin) is of fundamental importance (Meili et al. 1993, Meili et al. 1996).

Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) can provide a great deal of information about foodweb structure and functioning, yet interpreting such data without complementary approaches can be problematic. Possibly some of the uncertainties involved in isotope fractionation in the baseline organisms and mesozooplankton may be resolved by measurements of isotopic composition in DIC, nitrate, ammonium and amino acids, by measurements of sulfur stable isotope ($\delta^{34}S$) and/or by the use of essential fatty acids as natural tracer in field studies. The proper interpretation of the inferences that can be generated from these field data demands that we conduct comparative laboratory experiments. These experiments will provide the firm foundation needed to set the limits to what can be deduced from stable isotope data gathered in the field.

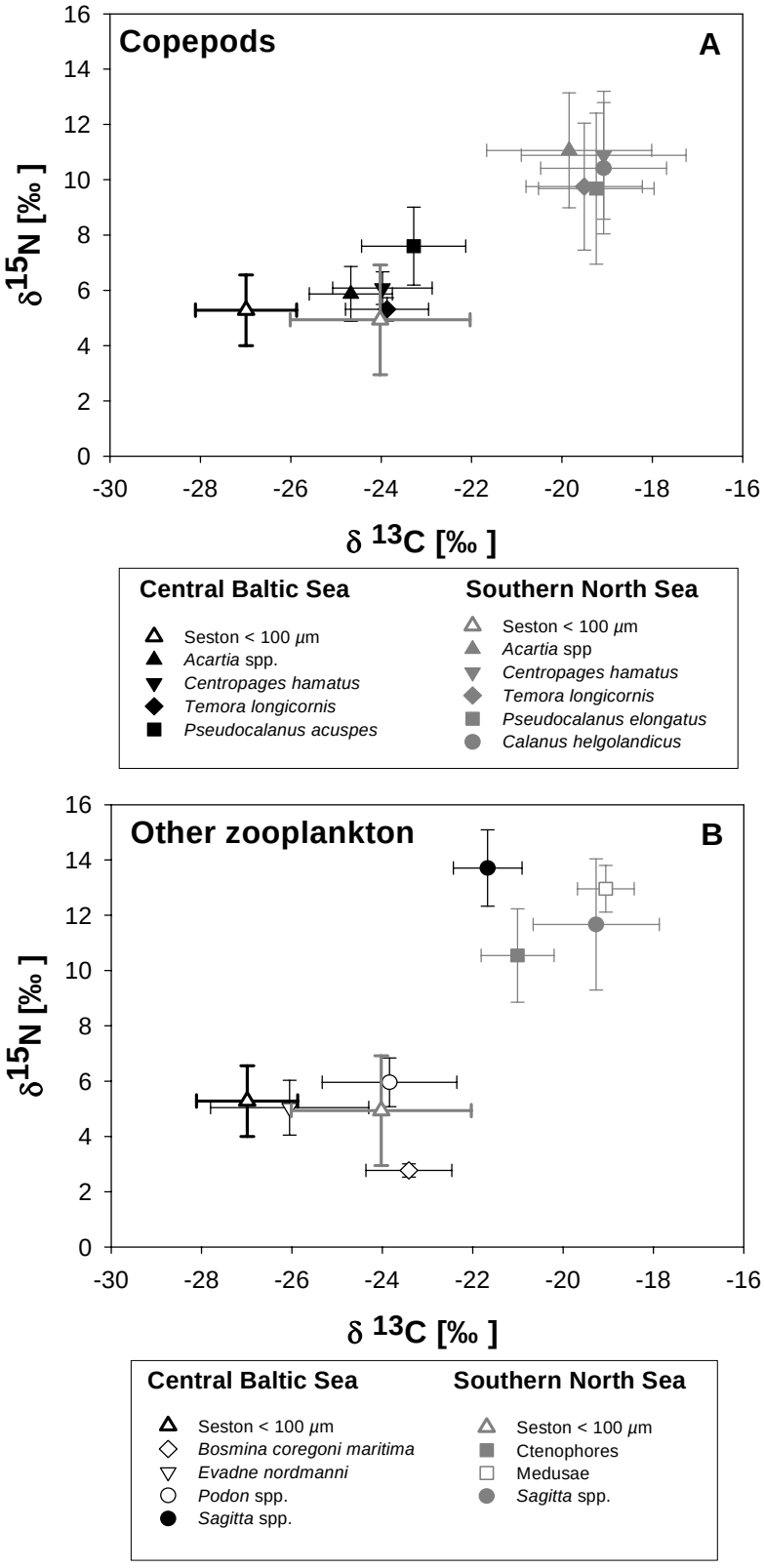

Figure VIII.1. General dual isotope diagram of depth-integrated (mean ± 1SD) $\delta^{15}N$ and $\delta^{13}C$ signatures of seston, copepod (A) and non-copepod species (A) from the Central Baltic Sea (black symbols) and from the Southern North Sea (grey symbols).

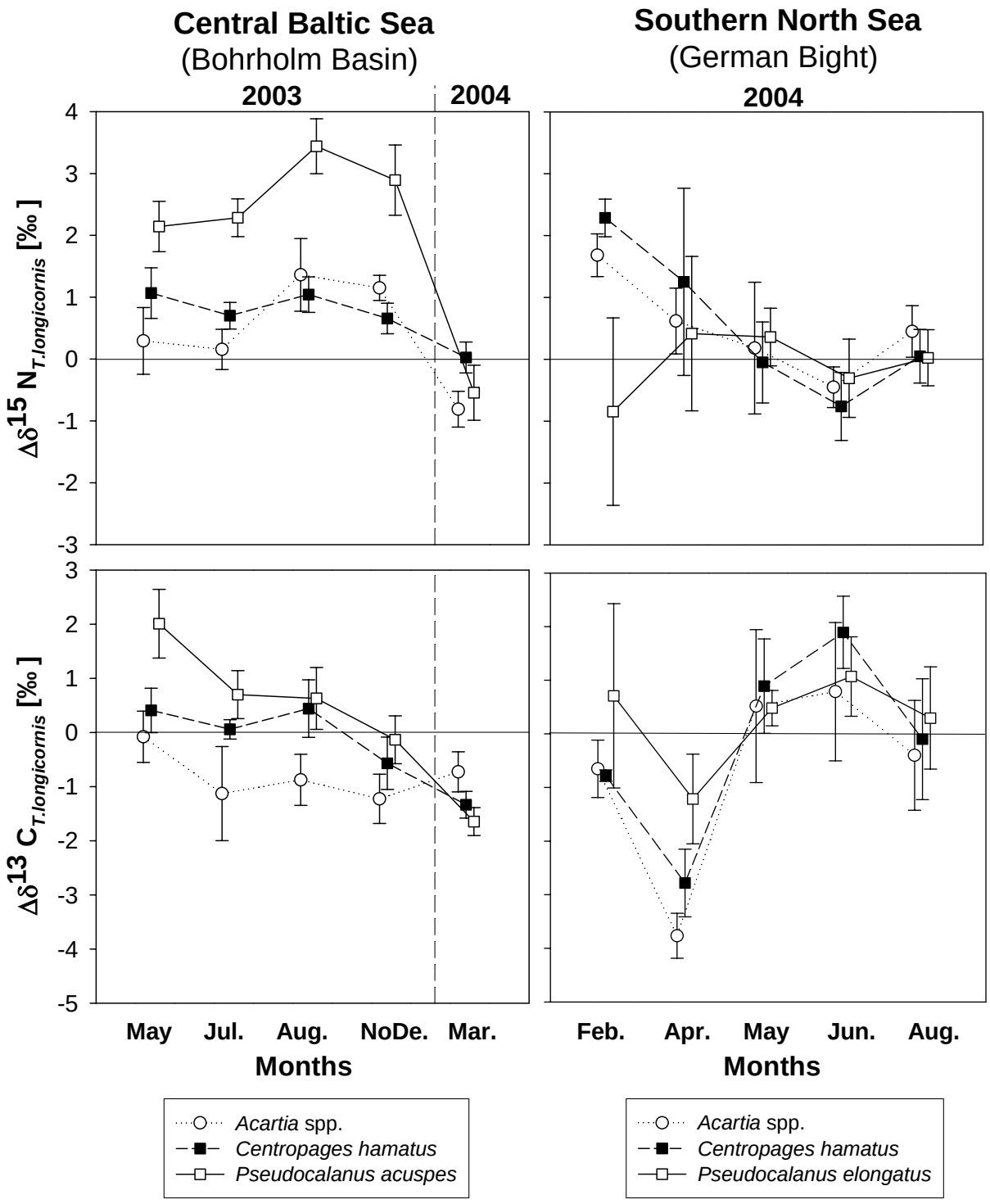

Figure VIII. 2. Seasonal standardized mesozooplankton trophic enrichment (mean ± 1SD, all sampling stations pooled by month) in terms of nitrogen ($\Delta\delta^{15}N$, top) and carbon ($\Delta\delta^{13}C$, bottom) of congener copepod species from the Central Baltic Sea (left axis) and Southern North Sea (right axis) using *Temora longicornis* as reference organism. Note that species symbols of a given month are shifted relative to each other to improve readability. Solid horizontal lines indicate zero enrichment.

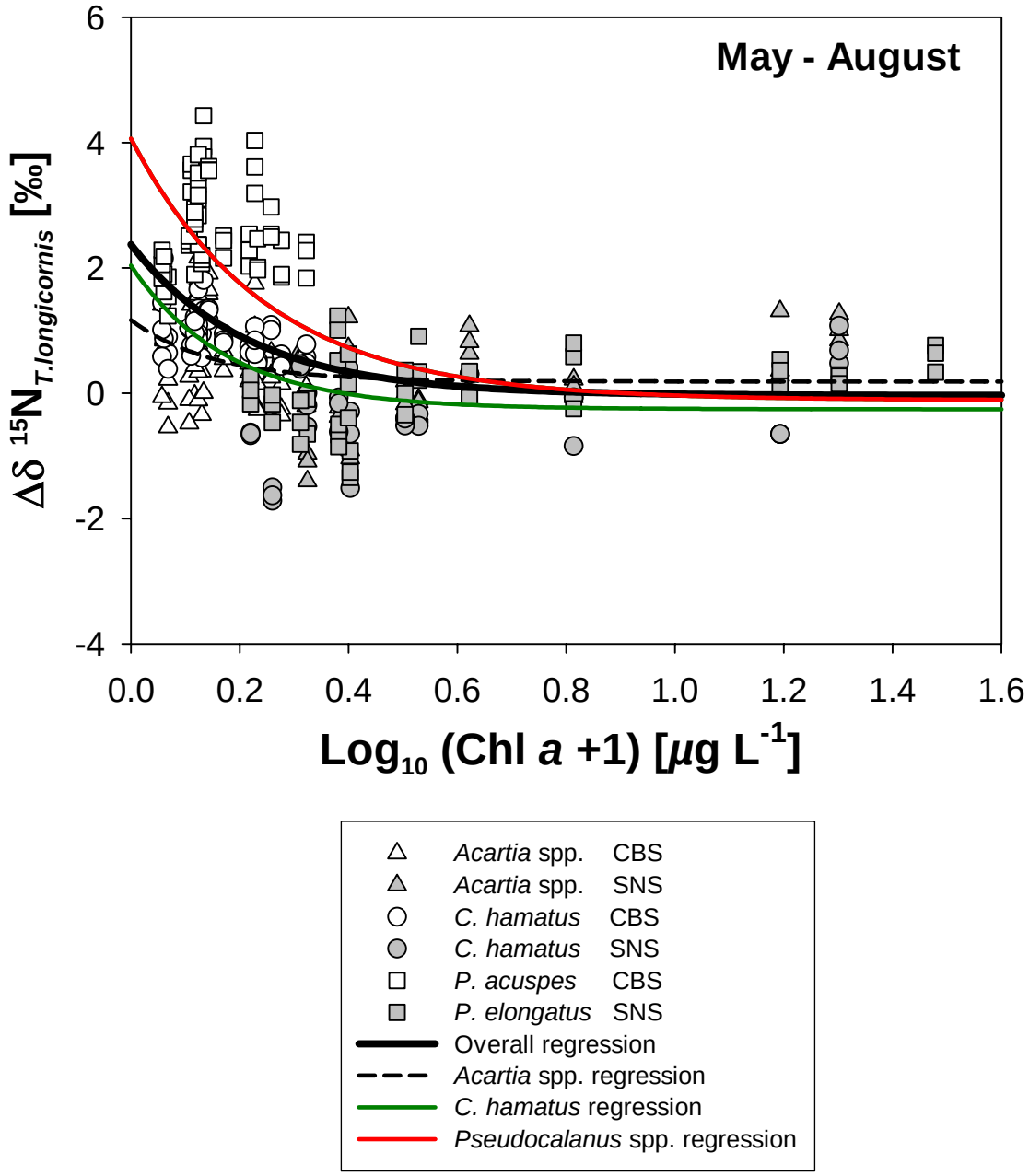

Figure VIII. 3. Standardized nitrogen trophic enrichment ($\Delta\delta^{15}N$) relative to *T. longicornis* of congener copepod species from the Central Baltic Sea (CBS: open symbols) and Southern North Sea (SNS: solid symbols) as a function of Log_{10}-converted Chlorophyll *a* concentrations according to the model $Y = Y_0+ae^{-bX}$. Regression are, Overall ($Y= -0.04+2.4e^{-4.6X}$, r =0.47, r^2=0.22, $p < 0.0001$), *Acartia* spp. ($Y= 0.19+1e^{-6.6X}$, r =0.27, r^2=0.07, $p < 0.05$), *C. hamatus* ($Y= -0.25+2.3e^{-5.6X}$, r =0.64, r^2=0.41, $p < 0.0001$) and *Pseudocalanus spp*. ($Y= 0.19+1e^{-6.6X}$, r =0.27, r^2=0.07, $p < 0.05$)

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List of abbreviations and symbols

Abbreviation	Long spelling	Remarks
ppm	parts per million	unit in mass spectrometry
‰	per mil	unit in mass spectrometry
δ ($\delta^{13}C$, $\delta^{15}N$)	-----	the ratio of two stable isotopes in relation to a standard
C	carbon	-----
^{12}C, ^{13}C, ^{14}N, ^{15}N	-----	stable isotopes of C and N, respectively
chl a	chlorophyll a	-----
$\Delta\delta^{15}N$	nitrogen trophic enrichment	-----
$\Delta\delta^{13}C$	carbon trophic enrichment	-----
TL	Trophic level	-----
DIN	dissolved inorganic N	comprises NO_3, NO_2 and NH_4
DIC	dissolved inorganic C	-----
N	nitrogen	-----
N_2	binitrogen	-----
NH_4	ammonium	correctly: NH_4^+
NO_2	nitrite	correctly: NO_2^{2-}
NO_3	nitrate	correctly: NO_3^{2-}
POM	particulate organic matter	here: equivalent to the term 'seston'
st.	sampling station	-----
no.	number	-----
n	-----	statistics: sample size
F	-----	statistics: *F* ratio
t	-----	statistics: *t* value
p	-----	statistics: probability of an observation
r^2	-----	statistics: strength of a fitted regression
r	-----	Coeficient of correlation
SD	standard deviation	statistics: measure of variability
SE	standard error	statistics: measure of variability
µg L^{-1}	micrograms per liter	-----
mol L^{-1}	mol per liter	-----
µm	micrometer	-----

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Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation - abgesehen von der Beratung durch meine akademischen Lehrer - nach Inhalt und Form meine eigene Arbeit ist. Sie wurde keiner anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Dies ist mein bisher einziges und erstes Promotionsverfahren. Des Weiteren erkläre ich , dass ich Zuhörer bei der Disputation zulasse. Die Promotion soll im Fach Biologische Meereskunde erfolgen.

Kiel, den 30 May 2007

Cristian A. Agurto Munoz