BIO-C3
Biodiversity changes: causes, consequences and management implications

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BIO-C3 overview

The importance of biodiversity for ecosystems on land has long been acknowledged. In contrast, its role for marine ecosystems has gained less research attention. The overarching aim of BIO-C3 is to address biodiversity changes, their causes, consequences and possible management implications for the Baltic Sea. Scientists from 7 European countries and 13 partner institutes are involved. Project coordinator is the GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, assisted by DTU Aqua, National Institute of Aquatic Resources, Technical University of Denmark.

Why is Biodiversity important?

An estimated 130 animal and plant species go extinct every day. In 1992 the United Nations tried countering this process with the "Biodiversity Convention". It labeled biodiversity as worthy of preservation – at land as well as at sea. Biological variety should not only be preserved for ethical reasons: It also fulfils key ecosystem functions and provides ecosystem services. In the sea this includes healthy fish stocks, clear water without algal blooms but also the absorption of nutrients from agriculture.

Biodiversity and BIO-C3

To assess the role of biodiversity in marine ecosystems, BIO-C3 uses a natural laboratory: the Baltic Sea. The Baltic is perfectly suited since its species composition is very young, with current salt level persisting for only a few thousand years. It is also relatively species poor, and extinctions of residents or invasions of new species is therefore expected to have a more dramatic effect compared to species rich and presumably more stable ecosystems.

Moreover, human impacts on the Baltic ecosystem are larger than in most other sea regions, as this marginal sea is surrounded by densely populated areas. A further BIO-C3 focus is to predict and assess future anthropogenic impacts such as fishing and eutrophication, as well as changes related to global (climate) change using a suite of models.

If talking about biological variety, it is important to consider genetic diversity as well, a largely neglected issue. A central question is whether important organisms such as zooplankton and fish can cope or even adapt on contemporary time scales to changed environmental conditions anticipated under different global change scenarios.

BIO-C3 aims to increase understanding of both temporal changes in biodiversity - on all levels from genetic diversity to ecosystem composition - and of the environmental and anthropogenic pressures driving this change. For this purpose, we are able to exploit numerous long term data sets available from the project partners, including on fish stocks, plankton and benthos organisms as well as abiotic environmental conditions. Data series are extended and expanded through a network of Baltic cruises with the research vessels linked to the consortium, and complemented by extensive experimental, laboratory, and modeling work.

From science to management

The ultimate BIO-C3 goal is to use understanding of what happened in the past to predict what will happen in the future, under different climate projections and management scenarios: essential information for resource managers and politicians to decide on the course of actions to maintain and improve the biodiversity status of the Baltic Sea for future generations.

CONTENT

Executive Summary of BIO-C3 Task 2.2

The Baltic Sea experienced large changes in community composition of consumers, yet the effects of changing top down control are largely unknown. **The objectives of BIO-C3 Task 2.2 is to improve our understanding on the consequences of changing selective predation pressures on biodiversity**, focusing across different trophic levels and across the benthic and pelagic ecosystems.

Riverine input of DOC, determined by hydrology and characteristics of the catchment area, contributes markedly to the DOC concentrations in the Baltic Sea. Most of the DOC inputs are degraded in the Baltic Sea, as only 10–40% of the total DOC input is exported into the North Sea. Loss of DOC is dominated by bacterial degradation. Direct bacterial degradation is estimated to be 2-18% of the total river load, but another source is photochemical degradation (21%) of riverine DOC. The input of total DOM into the Baltic Sea have been predicted to increase, which highlights the need for proper understanding of the role of DOM in the trophic state of the Baltic Sea in present and future climate. This contribution is not a direct BIO-C3 product but is included in the report to demonstrate that the data are now complete and available for other BIO-C3 work packages.

Assessing the relative importance of environmental conditions and community interactions is necessary for evaluating the sensitivity of biological communities to anthropogenic change. Using two long-term time series from the Baltic Sea, we evaluated coastal and offshore phytoplankton patterns over annual and monthly time-scales and assessed their response to environmental drivers and biotic interactions. Overall, coastal phytoplankton responded more strongly to environmental variation than offshore phytoplankton, although the specific environmental driver changed with time scale. A trend indicating a state shift annual biovolume anomalies occurred at both sites and the shift's timing at the coastal site closely tracked other long-term Baltic Sea ecosystem shifts. Biotic interactions within communities were rare and did not overlap between the coastal and offshore sites.

The smallest grazers, microzooplankton, are often neglected in the Baltic Sea food web analysis due to the scarce monitoring data available. We found that microzooplankton, in our case ciliates can be a major grazer group for small phytoplankton. The species composition of protozoan grazers has an effect on grazing effectively and prey selection. Though our material are from local experiments the results can be applied to a variety of Baltic Sea samples if ciliate community structure is known.

We also investigated a potential top-down control of herring larvae on zooplankton community, which allowed us to conclude that such conditions are possible in areas strongly isolated from adjacent waters and when initial extremely high hatching success and high growth rate due to favourable environmental and feeding conditions subsequently caused a very strong predatory pressure on main prey organisms. Especially, older larvae and juveniles may be responsible for the highest pressure on zooplankton because of their relatively high feeding intensity (consumption of wide range of zooplankton size classes and increasing food demand).

We investigated the predation on eggs of Atlantic herring (*Clupea harengus*) by a resident predator community in an important herring spawning area at the southern coast of the Baltic Sea. Existing data sets of several predator exclusion experiments were utilized to estimate the cumulative predation effect of all predators on the survival of herring eggs. During an experiment in May 2012, predators caused a significant egg loss of 19 % within the 4 days the

experiment was conducted. Considering the temperature dependent developmental time of herring eggs, we extrapolated total predation mortalities up to 43%. Additionally, an empirical model was used to estimate the specific contribution of a dominant predator species, the threespine stickleback (*Gasterosteus aculeatus*) to the overall predation effect. Despite high abundances of this species at selected herring spawning beds in May (more than 3 individuals per m²), the specific mortality of herring eggs caused by *G. aculeatus* predation was relatively low (1.4% in total which is less than 10% of the overall predation mortality). We discuss the problems on determining the contribution of particular predators to egg mortality and conclude predator exclusion experiments to be a practical and reliable alternative if a cumulative perspective on the effects of the whole predator community is of interest rather than specific contributions. Our results emphasize the importance of field investigations of the coastal small fish community as a foundation for understanding mortality processes acting on living marine resources and for providing robust, ecosystem-based advice for the management of Baltic aquatic resources in the Baltic Sea and elsewhere.

Addressing the ecological and economic importance of the top predator cod, several lines of research were conducted within this task:

** The current situation for Baltic cod stock shows that the main part of the stock is distributed in the Bornholm basin where the individual condition is characterized by lean and malnourished fish that seldom reach fishable size. Their diet mainly consists of clupeids and benthic invertebrates. We investigated if cod prey consumption as well as benthic biomass and production to evaluate if cod consumption could possibly influence populations of benthic prey species and if declines in benthic biomass can explain the declining condition of cod. Estimated cod consumption of benthos is sometimes larger than the estimated production which indicates that cod could have a top-down control of some benthic prey. However, the biomasses of benthic prey species seem to have increased rather than decreased the latest years when the cod condition has decreased, suggesting that some other factors than food competition of benthic prey may be responsible for the declining individual condition in cod.

** Understanding the feeding ecology of commercial fish species is an essential component of multi-species stock assessments and food web models, and is consequently assessed as part of monitoring programs. Yet even with large investments in studies based on stomach content analysis (SCA), the spatio-temporal resolution that is logistically feasible to obtain is frequently coarse. This is particularly problematic in the environmentally highly dynamic Baltic Sea, as illustrated by recent strong declines in condition in the top predator Baltic cod (*Gadus morhua* L.), which were not predicted by existing models (*“the starving cod problem”*). Here, we used carbon, nitrogen and sulphur stable isotope analysis (SIA), a method now irreplaceable in feeding ecology studies but applied surprisingly little in assessments of commercial fishes, to obtain a complementary dataset to SCA for the key commercial species in the Baltic, cod, herring (*Clupea hargengus* L.) and sprat (*Sprattus sprattus* L.), and six other fish species. Highly resolved spatial sampling (19 sites covering Kiel Bight – ICES SD22, Arkona Basin - SD24, Bornholm Basin - SD25, and Gdansk Deep – SD26 and Southern Gotland Basin – SD26/28) during a cruise with RV Alkor in April 2014 revealed the overall trophic structuring of fish communities and the presence of significant, systematic within and between basin differences in isotopic baselines, allowing insights into within and between basin connectivity of fish populations. Three different case studies then highlighted (1) spatial differences in cod feeding ecology, with different patterns in ontogenetic shift observed between basins of the Baltic Sea; (2) spatially consistent patterns of competitive interaction in herring and sprat that can help to identify size classes most likely to compete; (3) a surprising degree of intraspecific plasticity in several species, including flounder in SD 22 consistent with the presence of different feeding strategies of individuals in the same population. This study demonstrates how SIA can serve to obtain long-term feeding estimates for multiple species and with a spatial resolution that would be logistically challenging to obtain with SCA, and represents a baseline dataset for future studies of temporal variation (e.g., pre- and post inflow situations).

** An analysis of cod stomach contents has been reported in deliverable 2.1. As part of an EU financed tender, the cod stomach database for Eastern Baltic cod has been substantially expanded backwards in time and updated to include recent years. Analyses indicate a bottom up control of benthos, especially Saduria entomon, decreasing after the stagnation of inflow events in the early 1980s. However, the control is dampened by cod utilizing sprat as food earlier in their life history. The cod initiate predation on sprat already at length around 20 cm, whereas in the earlier periods before 1980 cod started to predator on sprat at 30 cm total length. However, cod cannot compensate totally for the lack of benthic food, and a decrease in cod condition can be observed since the mid-1980s. Here, we focused on the population level trophic control mechanisms. Both the work in deliverables 2.1 and 2.2 will form the process knowledge and model input for task 4.2 where we model the complex population dynamics resulting from changes in biodiversity. We modelled the populaiotn dynamics of Eastern Baltic cod, herring and sprat in a stochastic multispecies model and interpreted them in relation to the observed changes especially in cod and sprat distributions. Furthermore, we investigated on a smaller spatial scale, the Bornholm Basin of the Baltic Sea, the impact of the interplay between functional response and predator-prey spatial overlap on the population dynamics of the prey.

** Cod, herring and sprat abundances, predation mortality rate and resulting biomasses have been estimated for the time range 1974-2010. The estimation has been conducted both for the Eastern Baltic Sea stocks (named 'Southern Baltic' in the proposal) text and separately for ICES sub-divisions 25, 26 and 28 in order to allow for basin-scale analyses of the strength of trophic interactions between cod, herring and sprat. Accounting for spatial predator-prey overlap in a selected basin, ICES Sub-division 25 which is the only basin with a relatively constant occurrence of the top predator cod, implied that spatial overlap in connection to the type of functional response probably decreased the trophic pressure on sprat over-proportionally at decreasing cod and slightly increasing sprat abundances.

Along a different line, stable isotope analysis was also used in a different study to increase the understanding of temporal variability in the diet of jellyfish, this time focusing on only one location, Kiel Fjord, but covering the entire period of occurrence of the species *Aurelia aurita* and *Cyanea capillata* for the entire year 2012. This study showed surprisingly strong changes in diet composition within just a few months for *A. aurita*, and yielded new insights into benthic-pelagic coupling in coastal areas.

The grey seal (*Halichoerus grypus*) population in the Baltic Sea has increased considerably during the last decades and the conflict between seals and commercial fisheries has become more intense. Using bioenergetics modelling and grey seal population and diet data, we estimated the magnitude and uncertainty in prey consumption. For the most important commercial species, catches generally exceeded the seal consumption in the entire Baltic Sea but regionally, seal consumption could be more important. The consumption model used constitutes a starting point for further assessments of the predatory role of Baltic grey seals.

Benthic-pelagic coupling is ubiquitous in relatively shallow systems and plays an important role in functions from nutrient cycling to energy transfer in food webs. We illustrate the varied nature of benthic-pelagic coupling processes and their potential sensitivity to climate, nutrient loading, and fishing using the Baltic Sea as case study. We assess inorganic nutrient and organic matter exchanges by a range of physical and biological processes. While quantification of traditional benthic-pelagic coupling processes (e.g. sedimentation of organic matter) occurs to some extent, the magnitude and variability of biological processes, particularly those governed by complex food web feedbacks, are not well quantified. We advocate the use of ecosystem models to evaluate the role of benthic-pelagic coupling coastal and estuarine systems and the effects of projected future anthropogenic change.

Results from Task 2.2 will feed into several upper WP. The evaluation on bacterial utilization of allochthnous dissolved material and the top-down effect of microzooplankton to bacteria and phytoplankton are intended to benefit WP 4. Next, the complementary datasets to traditional stomach content analysis, and the resulting novel insights, from the stable isotope studies in this deliverable will be discussed in depth with partners in WP1 and 2 to strengthen the interpretation of results. Use of the output on benthic vs. pelagic components in the diet of the top predator cod, the strength of competition between herring and sprat, feeding ecology of non-commercial fish species, and on a smaller scale the feeding ecology of the jellyfish species *A. aurita* and *C. capillata*, as well as the resulting individual based information on trophic level and dietary ecology, will then be assessed with partners in BIO-C3 WPs 4 and 5. Further, data on seal consumption will feed into WP5 Tools for adaptive management.

I) Introduction

Changes in community composition of nearly all trophic levels ranging from plankton to fish and benthos have been described in the Baltic Sea {Ojaveer:2010dr}, but the underlying processes by which these changes impact on coastal and pelagic systems are only partly understood. Improved knowledge on how direct and indirect effects propagate through the entire food web is critical in order to predict ecosystem-wide consequences of changing biodiversity under spatio-temporally varying drivers. The goal of deliverable 2.2 is to describe top-down effects of shifts in species dominance on food web composition, functioning and biodiversity using a combination of existing information, field work, experiments, and modelling considering different trophic levels, functional groups and habitats (e.g. pelagic, benthic, coastal, offshore).

We reviewed the availability of allochthonous land derived dissolved matter to bacteria, which is a major source of energy to bacteria at the whole Baltic Sea level. Within the pelagic system, we evaluated coastal and offshore phytoplankton biovolume patterns over annual and monthly time-scales and assessed their response to environmental drivers and biotic interactions. In addition, we investigated how microzooplankton species composition creates selective grazing pressure to phytoplankton communities, specifically the smallest size-classes.

We used video plankton recorder (VPR) data to investigate if copepods (here egg sac carrying *Pseudocalanus acuspes* females in the central Baltic Sea, Bornholm Basin) still show diel vertical migration patterns when the likely trigger for this behaviour (in this case the clupeid swarm-fish sprat – *Sprattus sprattus*) is missing. Our study draws the comparison of copepod behaviour between several years, and comes to the conclusion that no DVM pattern can be observed for ovigerous *P. acuspes* females in the central Baltic Sea in years with low sprat abundances.

To investigate top-down control of fish on prey communities a suit of different studies were conduced: fish larvae top-down control on zooplankton community were carried out in two important herring spawning areas along the southern coast of the Baltic Sea. Different geographical characteristics as well as different larval densities observed provided an opportunity to identify drivers responsible for appearance of top-down control conditions. We also focused on dominant species among the resident small fish community (flounder, threespine stickleback, long-tailed duck) within shallow vegetated zones and their potential to affect herring egg survival by predation.

We also estimated cod prey consumption as well as benthic biomass and production in order to evaluate if cod consumption could possibly influence populations of benthic prey species and if declines in benthic biomass can explain the declining condition of cod. In addition, Baltic cod, hering and sprat population dynamics have been estimated using a stochastic multispiecies model. Growth of herring and sprat has been density-dependent, and growth of cod has, to some extent, been dependent on herring and sprat biomass. the relative distributions of predator (cod) and prey (herring and sprat, possibly juvenile cod) have changed substantially during the last years, and for the time being much herring and sprat are outside the predatory reach of cod. The basin scale predator prey has been analysed for the Bornholm Basin of the Baltic, and consequences for trophic control have been elaborated theoretically.

We conducted the first systematic assessment of the Baltic fish community (plus 2 jellyfish species) with the powerful tool stable isotope analysis, in order to obtain a high spatial resolution dataset on the feeding ecology of these species that complements existing information from traditional stomach content analysis approaches.

In addition, we used bioenergetics modelling and grey seal population and diet data, to estimate the magnitude and uncertainty in prey consumption, and compared the prey consumption to fish catches to get a first assessment of potential resource competition between seals and fisheries.

We also synthesized scientific understanding of benthic-pelagic coupling processes in the Baltic Sea and identified knowledge gaps when it comes to the sensitivity of benthic-pelagic coupling to environmental pressures, with a special focus on effects of changing species composition.

Due to the wealth of new information and detail outputs of the different studies, the key results are highlighted in the core deliverable text and the detailed material and result descriptions are provided as appendices.

II) Core Activities

Focus areas of this task were the Northern Baltic Proper for phytoplankton interaction analysis, in Lithuanian coastal waters for the experimental microzooplankton work, the Southern Baltic Proper and Bornholm Basin for herring top-down control.

The study on *Pseudocalanus* diel vertical migration and on seasonal diet and predation impact of herring and sprat on zooplankton was conducted in the central Baltic Sea.

Allochthonous DOM utilization was calculated for the whole Baltic Sea basin.

The study on cod consumption was conducted in the Bornholm basin. The study on the feeding ecology and connectivity of commercial fishes in the Baltic Sea based on stable isotope analysis comprised a very comprehensive replicated spatial sampling design including most of the deeper Basins of the Baltic Sea (ICES SD22, 24, 25, 26 and 28). The study on seasonal patterns in the feeding ecology of the jellyfish species *A. aurita* and *C. capillata* had a high temporal resolution (bi-weekly sampling), but was focused only on Kiel Fjord (ICES SD22).

The study on grey seal consumption was conducted in the Baltic Proper and Southern Baltic Sea.

Major activities of Task 2.2 'TOP-up control' per partner were as follows:

P1 Obtained a sample set of commercial fish species with a high spatial resolution, conducted stable isotope analysis, and provided the first systematic feeding ecology assessment based on stable isotope analysis (carbon, nitrogen, and a pilot dataset of sulphur data) for these species in the Baltic Sea. Secondly, conducted a study on seasonal patterns in feeding ecology in two jellyfish species in Kiel Fjord, using stable isotope analysis.

P2 analyzed existing data with bi-weekly to monthly time resolution of zooplankton abundance and production and simultaneous measurements of sprat abundance and predation in the Bornholm basin with a focus on the quantification of consumption/predation and consumption biomass ratios.

P3 conducted a separate study to answer why the prey (*Temora longicornis*) is actually dominating the stomach content of sprat, although the vertical overlap is also fairly limited. Here the hypothesis was tested that sprat actually feed on the morning descent to deeper waters in the regions with maximum concentrations. Assisted in the interpretation of stable isotope results from P1 by providing background information on existing knowledge from stomach content analysis.

P7 and **P8** contributed with the analysis of microzooplankton selective grazing on bacteria and phytoplankton based on experimental data. The experiment was designed to reveal how microzooplankton (ciliate) community structure affects top-down grazing pressure. The grazing patterns of different communities can be generalized to Baltic Sea. In addition, bacterial utilization of allochthonous matter via bacteria in Baltic Sea pelagial was quantified in a literature-based review. These contributions were aimed for the further development of our understanding on the role of lowest trophic levels in pelagic systems.

P4 contributed with multivariate autoregressive models (MAR models) on existing historical time series of phytoplankton and abiotic drivers at a coastal and open sea station in the Northern Baltic proper to study drivers and interactions between of the plankton community.

P4 performed analysis of cod prey consumption estimations as well as benthic biomass and production in order to evaluate if cod consumption could possibly influence populations of benthic prey species and if declines in benthic biomass can explain the declining condition of cod.

P4 was also responsible for investigating grey seal top-down control on fish. The magnitude and uncertainty in grey seal prey consumption was estimated based on grey seal diet composition, available grey seal population assessment data and seal bioenergetics modelling. The modelling provides an assessment of potential resource competition between seals and fisheries.

In addition, **P4** synthesised current understanding and knowledge gaps of benthic-pelagic coupling in the Baltic Sea during two workshops in collaboration with other BONUS projects.

P5 was responsible to investigate an impact of fish larvae on zooplankton in two important herring spawning areas to estimate feeding selectivity of larvae (Vistula Lagoon) and potential predation effects on the zooplankton community (Vistula Lagoon and Pomeranian Bay).

P11 utilized Greifswald Bay as a study area for the interaction between resident predators (particularly threespine stickleback *Gasterosteus aculeatus*) and immigrating herring. The lagoon between the Island of Rügen and the German mainland (figure 1) comprises an area of approximately 514 km^2, and is characterized by a mesohaline waterbody (6-8 PSU) with a mean depth of 5.6 m (max approx. 13 m) (Reinicke, 1989; Stigge, 1989).

P11 contributed with investigations on the effects of local resident predators such as the threespine stickleback on the survival of early herring stages and thus on the reproduction success of spring spawning herring within Greifswald Bay. We primarily used existing data sets from earlier investigations (mainly conducted in 2012) to characterize the importance of stickleback predation on herring eggs. The data sets included in-situ observations, field samplings and field experiments. Furthermore, we continued the sampling of the small fish fauna during the spawning season of herring in spring in consecutive years and combined these data to evaluate the spatio-temporal overlap of herring eggs and resident predator species. Additionally, we tried to examine the interannual variations in stickleback abundances using bycatch data of a weekly ichthyoplankton survey, which is conducted in Greifswald Bay between March and July since 1991.

P2 analysed top-down effects by cod on cod, herring and sprat using stomachs content data and a stochastic multispieces model. The importance of predator-prey overlap has been underlined and was analysed further.

III) Scientific highlights

Allochthonous dissolved matter utilization by bacteria (for details see Appendix 1)
Although this review it not a BIO-C3 product, it is included in the report to provide the original data to the usage of BIO-C3 model work for other BIO-C3 work packages. Riverine input of DOC, determined by hydrology and characteristics of the catchment area, contributes markedly to the DOC concentrations in the Baltic Sea. Most of the DOC inputs are degraded in the Baltic Sea, as only 10–40% of the total DOC input is exported into the North Sea. Loss of DOC is dominated by bacterial degradation. DOC concentrations in the Gulf of Finland, Gulf of Riga and Gulf of Gdańsk are higher than those in the Baltic Proper, due to high input of riverine DOC. DOC concentrations in the open-sea of the Gulf of Bothnia do not clearly differ from those in the Baltic Proper. Direct bacterial degradation is estimated to be 2-18% of the total river load, but another source is photochemical degradation (21%) of riverine DOC. The number of studies addressing DOM has increased during the last decade, but the sporadic data still impedes estimation of seasonal and spatial trends in the DOM concentrations in large parts of the Baltic Sea. The input of total DOM into the Baltic Sea have been predicted to increase, which highlights the need for proper understanding of the role of DOM in the trophic state of the Baltic Sea in present and future climate.

Phytoplankton community interactions and environmental sensitivity in coastal and offshore habitats (for details see Appendix 2)
Assessing the relative importance of environmental conditions and community interactions is necessary for evaluating the sensitivity of biological communities to anthropogenic change. Phytoplankton communities have a central role in aquatic food webs and biogeochemical cycles, therefore, consequences of differing community sensitivities may have broad ecosystem effects. Using two long-term time series (28 and 20 years) from the Baltic Sea, we evaluated coastal and offshore phytoplankton class biovolume patterns over annual and monthly time-scales and assessed their response to environmental drivers and biotic interactions. Overall, coastal phytoplankton responded more strongly to environmental variation than offshore phytoplankton, although the specific environmental driver changed with time scale. A trend indicating a state shift annual biovolume anomalies occurred at both sites and the shift's timing at the coastal site closely tracked other long-term Baltic Sea ecosystem shifts. Cyanobacteria and *Mesodinium rubrum* were more strongly related than other classes to this trend with opposing relationships that were consistent across sites. On a monthly scale, biotic interactions within communities were rare and did not overlap between the coastal and offshore sites. Annual scales may be better able to assess general patterns

across habitat types in the Baltic Sea, but monthly community dynamics may differ at relatively small spatial scales and consequently respond differently to future change.

Microzooplankton grazing on phytoplankton (for details see Appendix 3)

Not only the number of grazers but also their species composition affects the selection of prey. This is true also for the smallest grazers such as ciliates. Dilution experiments were performed to estimate phytoplankton growth and microzooplankton grazing rates at two sites: freshwater (Nida) and brackish water (Smiltyne) in the Curonian Lagoon (SE Baltic Sea). We used size-fractionation approach and dilution experiments and found that microzooplankton community was able to remove of up to 78% of nanophytoplankton (2–20 μm) standing stock and up to 130% of total daily primary production in the brackish waters of the lagoon, and up to 83% of standing stock and 78% of primary production of picophytoplankton (0.2–2 μm) in the freshwater part. The observed differences were attributed to the changes in ciliate community size and trophic structure, with larger nano-filterers (30-60 μm) dominating the brackish water assemblages and pico-nano filterers (<20 μm and 20-30 μm) prevailing in the freshwater part of the lagoon. These results, when applied to other areas, enable to develop more realistic view on the carbon flow in the Baltic Sea.

Baltic Sea *Pseudocalanus*: diel vertical migration patterns & escape behavior (for details see Appendix 4)

We used video plankton recorder (VPR) data to investigate if copepods (here egg sac carrying *Pseudocalanus acuspes* females in the central Baltic Sea, Bornholm Basin) still show diel vertical migration patterns (DVM) when the likely trigger for this behaviour (in this case the clupeid swarm-fish sprat – *Sprattus sprattus*) is missing. Baltic *P. acuspes* stay at depths around the halocline, where they find sufficient amounts of food, as well as high salinities favourable for their reproduction. During daytime, sprat migrate from surface waters towards the halocline, to prey on residing copepods. In avoidance of this predation risk, copepods show DVM patterns. They shift to water masses above and below their favourable habitat. In 2012, sprat abundances where notably lower than in 2002 and 2009, when copepod DVM patterns where investigated based on VPR data. Our study draws the comparison of copepod behaviour between those years, and comes to the conclusion that no DVM pattern can be observed for ovigerous *P. acuspes* females in the central Baltic Sea in years with low sprat abundances.

Seasonal variation in the diet and predation impact of herring (*Clupea harengus* L.) and sprat (*Sprattus sprattus* L.) on zooplankton in the central Baltic Sea (for details see Appendix 5)

The quantification of the trophic dynamics between zooplankton and small pelagic fish and the involved top down and bottom up processes are of particular importance in understanding marine pelagic food webs. Consequently, we conducted a temporally resolved comprehensive investigation on the diet, feeding and predation impact of the two dominant planktivorous fish species, sprat (*Sprattus sprattus* L.) and herring (*Clupea harengus* L.) in the southern central Baltic Sea (Bornholm Basin). Herring and sprat were mainly zooplanktivorous, largely feeding on the calanoid copepods *Temora longicornis* and *Pseudocalanus acuspes* and additionally on the cladocerans *Bosmina longispina* maritima and *Podon* spp. in summer. The overall low number of empty stomachs of herring (5%) and sprat (3%), the stomach fullness (summer period: herring 0.2-0.7% body mass, BM, sprat 0.3-0.7% BM; winter: herring 0.09% BM, sprat 0.06% BM) and the resulting daily food intake (herring: 2.2-2.9% BM in summer and 0.5% BM in winter; sprat: 2.6% BM in summer and 0.4% BM) indicated that the Bornholm Basin is also utilized as a feeding ground by both clupeids. We observed a temporally high predation impact of mainly sprat and herring on *T. longicornis* and *P. acuspes* only in April and May, when considerably more than the production of these two copepod species was consumed. If integrated over the year, the utilization of the copepod production by both clupeids is comparatively low, only 13% of the annual production of *T. longicornis* (all stages) and only 19% of the annual production of *P. acuspes* are consumed by both clupeids together, while the production of *Acartia* species is almost completely unused by fish predators. This indicates an overall poor trophic coupling between copepods and pelagic planktivores in the Bornholm basin.

Herring predation effects on the zooplankton community in the Vistula Lagoon (for details see Appendix6)

Based on data collected in the Vistula Lagoon in 2004 – 2005 (three cruises each year, between hatching and metamorphosis of herring), a noticeable difference in herring larvae abundance was observed in the two successive spring seasons. The high, initial survival of larvae in 2004 potentially caused a considerable “top-down” pressure of older larvae on preferred organisms in May and June. Food composition and food selectivity investigations showed that copepod *Eurytemora affinis* (adults and copepodits IV-V) was the most important food component and it was highly selected even when significantly decreased in abundance. In contrast, the abundance and biomass of zooplankton in 2005, when much lower predatory pressure of fish larvae was observed, did not present as dramatic changes during the comparable, spring and early summer season.

High survival of early larvae in 2004 could be explained by coupling of the hatching period with abundance peak of copepod nauplii (match), while in April 2005 the abundance of this preferred food component was ca. tenfold lower (mismatch).

Different larvae abundance trigged density dependent mechanisms, i. e. growth rate and condition differed significantly between both sampling seasons:

- in 2004, high survival of early larvae resulted in a slower growth rate and lower condition factors of survivors due to food limitation;
- in 2005, lower survival of early larvae resulted in a faster growth rate and better condition factors of surviving individuals due to the favourable feeding conditions.

Potential predation impact of herring larvae on zooplankton community in the Pomeranian Bay (for details see Appendix 7)

Although it is not possible to estimate feeding selectivity and intensity of herring larvae in Pomeranian Bay (no stomach content data are available), their potential predation effects on the zooplankton community (top-down control) can be assessed in that geographical area, to some extent, by the analysis of five-year (2007-2011) herring larvae abundance and zooplankton abundance data sets.

There was a significant correlation between herring larvae and zooplankton abundance, suggesting that zooplankton abundance may have an additional (to SSB) effect on herring larvae abundance by influencing their survival. Although the opposite scenario, assuming herring larvae effect on zooplankton abundance by feeding pressure (top-down), cannot be excluded, it seems to be of low probability. This is because of low herring larvae abundance in the Pomeranian Bay (ca. 100-200 spec. 100 m^{-3}), which is for 10 mm SL larvae even 10 times lower than in the Vistula Lagoon. For the size classes above 16 mm, which could be responsible for the highest pressure on zooplankton because of their relatively high feeding intensity (consumption of wide range of zooplankton size classes and increasing food demand), the difference in abundance between Pomeranian Bay and Vistula Lagoon is even higher (i.e., low abundance of larvae in Pomeranian Bay).

Predation on herring eggs (for details see Appendix 8)
With a combination of earlier data from field investigations and predator exclusion field experiments, we were able to demonstrate a significant potential of this species to affect the survival of herring eggs providing that a spatio-temporal overlap is given. Interannual comparisons of beach seine catches conducted in spring (herring spawning period) demonstrated a strong interannual variability of small fish abundances within the study area implicating different predation effects of these predators on herring eggs in distinct years. A clear seasonal trend was revealed by the weekly stickleback catches in a herring spawning bed at the southern coast of Greifswald Bay. While the stickleback abundances are relatively low during the first spawning wave in March, their abundance usually increases significantly during the mid of April. Considering the field experiment results, we conclude that later spawned herring eggs are subject to an increased predation pressure compared to the early spawned ones. The seasonality of stickleback abundance could be confirmed with the analyses of stickleback bycatches during a weekly ichthyoplankton survey conducted within the area each spring since 1992. However, during the data analyses it became evident, that the recording of bycatch data was not performed in a consistent manner throughout the years making it unpromising to investigate the interannual variability of sticklebacks using this particular approach. Our results underline the urgent need for standardized monitoring approaches focusing the Baltic small-fish fauna (not only sticklebacks) within the shallow littoral areas since a noticeable discrepancy exists between the great importance of these organisms for the Baltic ecosystem (including their effect on commercial species such as herring) and the lack of knowledge on their abundances, distributions and ecology.

Interactions between cod and benthos in the Baltic Sea (for details see Appendix 9)
Until recently, the Baltic cod stock was considered recovering from a near history of serious stock depletion reaching a few decades back. The current situation shows that the main part of the stock is distributed in the Bornholm basin where the individual condition is characterized by lean and malnourished fish that seldom reach fishable size of 38 cm. Their diet mainly consists of clupeids and benthic invertebrates. The clupeid stocks are now mainly distributed far from cod, in the northern Baltic proper. Lack of clupeids is one possible explanation to the low individual cod condition, but the importance of the benthic prey resource and the effect of cod consumption on benthic biomass and production is lacking. Estimations of cod prey consumption as well as benthic biomass and production were made in order to evaluate if cod consumption could possibly influence populations of benthic prey species and if declines in benthic biomass can explain the declining condition of cod. Estimated cod consumption of benthos is sometimes larger than the estimated production which indicates that cod could have a top-down control of some benthic prey. However, the biomasses of benthic prey species seem to have increased rather than decreased the latest years when the cod condition has decreased. These results are contradicting and indicate that some other factor than food competition of benthic prey seems to be responsible for the declining individual condition in cod.

Feeding ecology and connectivity of Baltic fish species re-assessed by stable isotope analysis (for details see Appendices 10 and 11)

Understanding the feeding ecology of commercial fish species is an essential component of multi-species stock assessments and food web models, and is consequently assessed as part of monitoring programs. Yet even with large investments in studies based on traditional stomach content analysis (SCA), the spatio-temporal resolution that is logistically feasible to obtain is frequently coarse. Stable isotope analysis may be a tool to improve the spatio-temporal resolution of such estimates and to complement existing SCA-based information. Here, we provided the first systematic assessment of Baltic commercial fishes. Results included the presence of systematic within and between basin differences in isotopic baselines, indicating spatial sub-structure in fish populations even within basins (e.g., for cod). Secondly, the presence of isotopic outliers in several fish populations better reflecting values of neighbouring populations pointed to between basin migrations, e.g., for herring. Three different case studies then highlighted (1) spatial differences in cod feeding ecology, with different patterns in ontogenetic shift observed between basins of the Baltic Sea; (2) spatially consistent patterns of competitive interaction in herring and sprat that can help to identify size classes most likely to compete; (3) a surprising degree of intraspecific plasticity in several species, including flounder in SD 22 consistent with the presence of different feeding strategies of individuals in the same population. This study demonstrates how SIA can serve to obtain long-term feeding estimates for multiple species and with a spatial resolution that would be logistically challenging to obtain with SCA, and represents a baseline dataset for future studies of temporal variation (e.g., pre- and post inflow situations).

A second feeding ecology study based on stable isotopes on temporal patterns in the jellyfish species *A. aurita* and *C. capillata* over their period of occurrence in Kiel Fjord in 2012 showed the presence of a rapid dietary shift in *A. aurita* within just a few months, and the potential importance of benthic material at the base of Kiel Fjord foodwebs during part of the year. this study again highlighted the potential for stable isotope studies in obtaining high resolution (temporal or spatial) feeding ecology datasets.

Multispecies model runs and trophic control (for details see Appendix 12)
Multispecies runs imply that the decrease in cod condition is a consequence of decreasing sprat abundance in the core cod distribution area. However, as already indicated in deliverable 2.1, this is not the whole story. It is actually not the abundance of sprat that is limiting, but the size of sprat. Very small cod have to forage on sprat due to the lack of the ususla benthic food., These small cod only have a limited access to edible sprat which are at the lowets limits of the sprat size distribution. The trophic control of cod and sprat is probably decoupled by the interplay between functional response type and spatial overlap inside a basin. Only a few good sprat recruitments, as observed in two cases in the id 1980s, can release the sprat population massively from predator pressure and henceforth trophic control by cod.

Grey seal top-down control on fish (for details see Appendix 13)
The grey seal (*Halichoerus grypus*) population in the Baltic Sea has increased considerably during the last decades and the conflict between seals and commercial fisheries has become more intense, mainly because of damaged catch and fishing gear, but also because of potential competition for the fish resource. Using bioenergetics modelling and grey seal population and diet data, we estimated the magnitude and uncertainty in prey consumption, and by comparing the prey consumption to fish catches we got a first assessment of potential resource competition between seals and fisheries. For the most important commercial species (cod, herring and sprat), catches generally exceeded the seal consumption in the entire Baltic Sea but regionally, seal consumption could be more important, e.g. cod in ICES subdivision 27-

31. The consumption of common whitefish, salmon, trout and eel was similar or exceeded fish catches regionally, indicating potential competition with fisheries and possible effects on the fish populations that should be investigated further. The length distributions of prey and commercial catch of herring and common whitefish overlapped, strengthening the concerns for competition, while the consumed cod was generally smaller than in catches. The uncertainty in prey consumption is substantial for many prey species. Except for the most common prey, herring, this is mainly explained by uncertain diet data rather than population and bioenergetics data. The consumption model used constitutes a starting point for further assessments of the predatory role of Baltic grey seals.

The importance of benthic-pelagic coupling in a changing world: affecting ecosystem responses to human pressures (for details see Appendix 14)
Coastal and estuarine ecosystem structure and function are strongly affected by anthropogenic pressures but there a large knowledge gaps when it comes to the sensitivity of benthic-pelagic coupling to these pressures. Benthic-pelagic coupling is ubiquitous in relatively shallow systems and plays an important role in functions from nutrient cycling to energy transfer in food webs. We illustrate the varied nature of benthic-pelagic coupling processes and their potential sensitivity to climate, nutrient loading, and fishing using the Baltic Sea as case study. We assess inorganic nutrient and organic matter exchanges by a range of physical and biological processes. While quantification of traditional benthic-pelagic coupling processes (e.g. sedimentation of organic matter) occurs to some extent, the magnitude and variability of biological processes, particularly those governed by complex food web feedbacks, are not well quantified. The sensitivity of biological coupling mechanism to all three anthropogenic pressures, however, is high and variable in space and time. Improved empirical and experimental understanding of benthic-pelagic coupling processes, especially variability in time and space, will improve the robustness of assessments of anthropogenic impacts. We also advocate the use of ecosystem models to evaluate the role of benthic-pelagic coupling coastal and estuarine systems and the effects of projected future anthropogenic change.

IV) Progress and next steps

Studies and work-tasks were performed according to the workplan and original objectives were obtained. The progress and outstanding next steps are as follow:

The study on *Pseudocalanus* diel veritical migration and on the seasonal variation in the diet and predation impact of herring and sprat on zooplankton is being finalized an will be submitted for peer-review.

Bacterial utilization of allochthonous matter in the Baltic Sea pelagic system is estimated from existing literature. It can be applied in WP4 though the review proved our knowledge still to be far from perfect. The selective microzooplankton grazing on bacteria and phytoplankton based on experimental data is estimated, and the next step is the application of results to the wider Baltic Sea data. Both assignments were completed as planned.

The analysis of herring predation effects on the zooplankton community in the Vistula Lagoon has been completed and it is currently being prepared for publication. Potential predation impact of herring larvae on zooplankton community in the Pomeranian Bay will be further investigated which requires also stomach content analyses.

The analysis with multivariate autoregressive models (MAR models) on phytoplankton species interactions is published in Oikos. The study on cod prey consumption estimations and benthic biomass and production has been finalized within a master thesis. The study on grey seal top-down control on fish is being finalized and sent for peer-review. A synthesis of benthic-pelagic coupling processes has been submitted for peer-review in an international journal.

Studies and work-tasks on predation on herring eggs were performed according to the work plan and original objectives were obtained. The progress and outstanding next steps are as follow: The existing experimental and field sampling data have been analyzed and compiled to prepare a manuscript for publication in a peer review journal that will be submitted within the next weeks.

The study on the feeding ecology and connectivity of Baltic commercial fishes assessed by stable isotope analysis is completed. Interpretation of data will be strengthened via discussion with P3, who has strong background on feeding ecology of these species. The manuscript attached as Appendix xxx will be submitted to a peer-reviewed journal in April. The second study on jellyfish feeding ecology assessed with stable isotope analysis (attached as Appendix xxx) has been accepted for publication in the journal Marine Biology.

The bycatch data derived from the ichthyoplankton survey (RHLS) are utilized to investigate the spatio-temporal overlap between herring larvae and their potential predators. These investigations will contribute to task 3.3.

The analyses of trophic interactions between the small fish fauna and Atlantic herring within Greifswald Bay finally resulted in the successful completion of the dissertation of P. Kotterba at the Thünen-Institute, Rostock / University of Hamburg (Kotterba, 2015).

All future outcomes of the task 2.1 will be included in upcoming annual reports.

V) Methods and results

Major results are highlighted in section (*i*), detailed methods and results for each respective study are described in the Appendices. A list of all attached appendices is given in section (*v*).

VI) Recommendations

The energy flow in the Baltic Sea is mainly based on autotrophic production, which is based on the availability of inorganic nutrients. However, the available system energy is supplemented by the riverine (allochthonous) land-based inputs of dissolved organic matter (DOM). Bacteria mediate dissolved organics to the microbial loop, which may fuel a considerable part of system productivity. We collected all available information on allochthonous DOM and its bacterial utilization. Though our knowledge is still rather incomplete, we could estimate a considerable flow of energy through bacteria towards higher trophic levels. The amount of DOM inputs may change because of climate change effects in the Baltic Sea catchment, which makes the data valuable for WP4.

The top-down effects are generally evaluated at the higher trophic levels only. Though these are important in shaping food webs as in case top predators like seals and their prey and e.g. cod and clupeids, the same mechanism works also at the lower trophic levels. One of the neglected grazer groups is microzooplankton. They are microscopic, but still their size ranges at least one order of magnitude and their feeding mode from filtration to direct particle capture. They are an important grazer group with high prey selection capacity. Our study provided information on selection process by two different ciliate communities. The results indicate that lower trophic level prey selection may be important in the Baltic Sea, and that ciliate community structure would be an important parameter to be analysed in the Baltic Sea food webs. The study can be used in further analysis of changing food webs in WP4.

The study on phytoplankton interactions stowed that there is little predictability at the base of the food web as we investigate the monthly scale interactions of these rapidly responding primary producers. Coherent patterns among sites were observed on annual scales reinforcing that temporal scale affects our ability to generalize about taxa and community responses. Furthermore, capturing how complex ecological interactions will alter ecosystem functioning, and in turn services provided to people, is critical. We have taken the first step of evaluating plankton community interactions in a coastal and offshore site in the northern Baltic Sea. Analyses that broadly assess community interactions across the Baltic gradient, evaluate their dynamics over time, and connect them to emergent ecosystem properties are appropriate next steps to improve our understanding of community-ecosystem dynamics.

The study on *Pseudocalanus acuspes* diel vertical migration revealed that while generally the interaction between sprat and *P. acuspes* is referred to as a potential top down control case, the interaction is more complex. Apparently *P. acuspes* have evolved a behavioural response to the peak predation pressure, which occurs mainly in April and May at peak spawning of sprat. The vertical downward escape provides *P. acuspes* with a protection mechanism against predation, ensuring the survival of sufficient numbers of egg producing adults during the main egg production season of *P. acuspes* in spring.

We conducted a highly resolved spatio-temporal investigation on the predatory effect of the main planktivores in the Baltic Sea, herring and sprat, on the dynamics of the key copepods, *P. acuspes*, *T. longicornis* and *Acartia* spp. The analysis of our monthly or bi-monthly coverage of the Bornholm Basin over more than one year allowed us to characterize the seasonal variations in the diet and feeding of herring and sprat, to quantify the interspecific competition for food resources and to assess the predation impact of both clupeids on the calanoid copepods *Temora longicornis*, *Pseudocalanus acuspes* and *Acartia* spp. over an annual cycle by the quantification of the consumption versus production and biomass.

Similar study of potential impact of herring larvae on zooplankton communities carried out in two different herring spawning areas (i.e. open Pomeranian Bay and much more isolated Vistula Lagoon) provided more insight to this prey – predator relationship: top-down control on prey organisms is more probable in the case of areas where observed larvae abundances are much higher and where spawning and nursery areas are characterized by very limited exchange with adjacent Baltic Sea waters. For herring larvae in the Vistula Lagoon, the extremely high hatching success and high growth rate in spring caused by favourable environmental and feeding conditions not necessarily indicates the overall, high survival and good condition of older larvae.

Predation by local predators is a significant factor potentially affecting the survival of herring eggs in the shallow littoral of inshore waters and lagoons of the Baltic Sea. However, data series on the distribution and seasonal succession of the different non-commercial species are rare and investigations on their general effect on the recruitment of commercially important species (e.g. in the framework of predictive models) remain challenging. Considering the high interannual variability of these communities observed at the few data spots that are available, further investigations are strongly encouraged to fill these gaps of knowledge. *In-situ* Predator exclusion experiments have been demonstrated to be a useful tool to investigate the total predation effect on the survival of fish eggs, particularly of those species that spawn demersally. This type of experiment can deliver effective measurements of fish egg predation, if the whole predation effect is of prior interest rather than the specific contribution of particular predators.

Outcome of the studies on the top predator cod revealed the following results: The decreased individual weight in cod cannot be described by the benthic biomass due to the latest period of increased biomass of both Bylgides *sarsi* and *Saduria entomon*. This indicates that benthic biomass is not strongly affected by the presence of cod. At the same time cod consumption of benthos has increased as well as benthic production, and in some cases the cod consumption is higher than the benthic production, at least in the case of *B. sarsi.* In regard to these counteractive events, the results indicate that there are some other factors responsible for the low condition in cod.

This first systematic assessment of Baltic commercial fish species with stable isotope analysis across large spatial scales confirms existing knowledge from SCA based approaches, but also provides new and surprising insights that provide food for thought, and indicate which next steps would be needed to make best use of SIA as complementary method to SCA, and as a future indicator in monitoring studies. Results of interest include the high-spatial resolution view at most of the deep basins of the Baltic Sea at the same time point, the potential of stable isotope analysis to provide individual based insights (e.g., the trophic level, or the degree of benthic feeding of specific individuals), and the potential new insights into connectivity of fish populations between and within basins. In the medium to long range, in our view, SIA values have a high potential to complement existing indicators by assessing changes in the trophic position and dietary composition of key species, the availability and incorporation of benthic food sources in the different basins of the Baltic, and of overall food web structure routinely and with a high spatio-temporal resolution.

The second study on jellyfish feeding ecology based on stable isotope analysis confirms the potential of this method to generate both high resolution spatial as well as temporal datasets that can complement traditional stable isotope analysis results.

The complex population dynamics of Baltic cod, herring and sprat and resulting vital rates are poorly understood if analysed with regression-based methods only. The processes involved include the behavioural aspect of foraging as well as small-scale predator prey overlap. The wide-spread assumption that these processes are more or less white noise and can be modelled using simple symmetrical probability distributions is misleading and might hamper the predictive understanding of important ecosystem processes including the consequences in changes in biodiversity. We found that (i) decrease in predator growth rate in partially decoupled from prey abundance (in concert with deliverable 2.1), and (ii) that whether or not and to what extent the cod fit their search activity to prey density can decoupe the prey from trophic control at low but increasing prey density. Especially this finding is to some extent counter intuitive and deserves further attention. Both dyamics will be investigate further and integrated into the population dynamic modelling in WP4.

We conducted the first comprehensive study of energy requirement and prey consumption of Baltic grey seals. By comparing the seal prey consumption to fish catches we get a first rough assessment of the importance seal predation in relation to the effects of fisheries on different fish populations. Judging from the confidence intervals of estimated consumption levels, the grey seal predation in the Baltic Sea is quite uncertain for several prey species. The contribution to the output variability was largest for the diet composition for most prey (except herring), suggesting that future research should focus on more diet studies. Different input variables of the consumption model vary to a different extent. In the long-term (inter-annual), bioenergetic variables are more likely to be stable, whereas diet and population are more prone to changes. We therefore stress the relevance of using up-to-date estimations of diet composition and size, distribution and structure of the grey seal population to obtain the prey consumption at the present time and to monitor variations in prey consumption in the Baltic Sea.

Using the Baltic Sea to illustrate how benthic-pelagic coupling drives coastal and estuarine systems shows the challenges of understanding the interdependency between pelagic and benthic habitats and the effects of human pressures from eutrophication, fishing and climate change. These pressures have multiple direct and indirect effects on the rates and spatio-temporal dynamics of inorganic nutrient and organic material cycling between these habitats. The extent of low oxygen areas, controlled by both climate and eutrophication in the Baltic Sea, directly regulates the flux of inorganic nutrient dynamics and the potential for biological activity to contribute to inorganic nutrient fluxes. Oxygen availability also governs the spatial and temporal dynamics of biological interactions, which result in organic matter exchange. Given the widespread increase in hypoxia, benthic-pelagic coupling dynamics are probably widely governed by the availability of oxygen.

VII) Appendices

APPENDIX 1: Allochthonous dissolved matter utilization by bacteria

APPENDIX 2: Phytoplankton community interactions and environmental sensitivity in coastal and offshore habitats

APPENDIX 3: Microzooplankton grazing on phytoplankton

APPENDIX 4: Baltic Sea *Pseudocalanus*: diel vertical migration patterns & escape behavior

APPENDIX 5: Seasonal variation in the diet and predation impact of herring (*Clupea harengus* L.) and sprat (*Sprattus sprattus* L.) on zooplankton in the central Baltic Sea (for internal BONUS review only)

APPENDIX 6: Herring predation effects on the zooplankton community in the Vistula Lagoon (for internal BONUS review only)

APPENDIX 7: Potential predation impact of herring larvae on zooplankton community in the Pomeranian Bay (for internal BONUS review only)

APPENDIX 8: Resident evil? - Predation on Atlantic herring *Clupea harengus* eggs in vegetated spawning beds in a Baltic Sea lagoon (for internal BONUS review only)

APPENDIX 9: Interactions between cod and benthos in the Baltic Sea (for internal BONUS review only)

APPENDIX 10: Feeding ecology and connectivity of Baltic commercial fish species assessed by stable isotope analysis (for internal BONUS review only)

APPENDIX 11: Seasonal patterns in jellyfish feeding ecology assessed with stable isotope analysis.

APPENDIX 12: Multispecies model runs and trophic control

APPENDIX 13: Grey seal top-down control on fish (for internal BONUS review only)

APPENDIX 14: The importance of benthic-pelagic coupling in a changing world: affecting ecosystem responses to human pressures (for internal BONUS review only)

Appendix 1

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Dissolved organic matter in the Baltic Sea

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ABSTRACT

Several factors highlight the importance of dissolved organic matter (DOM) in coastal ecosystems such as the Baltic Sea: 1) DOM is the main energy source for heterotrophic bacteria in surface waters, thus contributing to the productivity and trophic state of bodies of water. 2) DOM functions as a nutrient source: in the Baltic Sea, more than one-fourth of the bioavailable nutrients can occur in the dissolved organic form in riverine inputs and in surface water during summer. Thus, DOM also supports primary production, both directly (osmotrophy) and indirectly (via remineralization). 3) Flocculation and subsequent deposition of terrestrial DOM within river estuaries may contribute to production and oxygen consumption in coastal sediments. 4) Chromophoric DOM, which is one of the major absorbers of light entering the Baltic Sea, contributes highly to water color, thus affecting the photosynthetic depth as well as recreational value of the Baltic Sea. Despite its large-scale importance to the Baltic Sea ecosystem, DOM has been of minor interest compared with inorganic nutrient loadings. Information on the concentrations and dynamics of DOM in the Baltic Sea has accumulated since the late 1990s, but it is still sporadic. This review provides a coherent view of the current understanding of DOM dynamics in the Baltic Sea.

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1. Introduction

The Baltic Sea, with its high input of river water from a wide catchment area, receives a substantial proportion of its dissolved organic matter (DOM) from terrestrial sources (Deutsch et al., 2012). Terrestrial DOM (tDOM) transported by streams and rivers represents an important pathway of carbon (C) and nutrients from terrestrial to aquatic ecosystems. The input of DOM has consequences in food web structure, since it is a source of energy and nutrients for bacteria (Sandberg et al., 2004). It may also promote the growth of dinoflagellates (Purina et al., 2004), thus affecting autochthonous production of organic matter. DOM in natural waters is both a natural background source of acidity and a pH buffer in low-alkalinity waters, and thus affects the acid–base balance in surface waters. It also plays an important role in the transport and availability of trace metals and contaminants; a significant, though highly variable, part of nutrient trace metals (e.g. iron (Fe), copper (Cu) and nickel (Ni)) as well as non-nutrient trace metals (e.g. aluminium (Al), mercury (Hg) and lead (Pb)) in the dissolved phase, is bound in organic ligands in coastal and open-sea waters (summarized by Wells, 2002). The binding of trace metals to organic ligands can prevent their adsorption to particles and subsequent sinking, but, on the other hand, flocculation of these complexes may at times be significant and cause a drawdown of the trace metals from the surface water (Wells, 2002). The optical properties of DOM have major implications for ecosystem functioning (Kothawala et al., 2014). The chromophoric DOM (CDOM) compounds that absorb ultraviolet (UV) and visible light play a dominant role in the light regime, allowing less light to penetrate into water (Dupont and Aksnes, 2013). The transparency and heat budgets of surface waters are thus modified and partly controlled by DOM. In this way DOM has an indirect effect on the primary producers. Sandberg et al. (2004) suggested that this contributes to the low phytoplankton production in the Bothnian Bay, where riverine inputs of tDOM are high. Thus, DOM plays a multiple system-wide role in the ecology of the Baltic Sea. This has been recognized by the Helsinki Commission (HELCOM, 2010) as assigning inputs of organic matter from rivers high status regarding their potential pressures on the Baltic Sea.

This review compiles the data published from the Baltic Sea. It begins at the catchment and ends in giving up-to-date budgets on allochthonous and autochthonous DOM. We attempt to summarize our knowledge of all aspects of abiotic and biotic transformation and utilization of DOM and its major elements (C, nitrogen (N) and phosphorus (P)). The second specific question is how the available information can be summarized to describe the specific conditions in different Baltic Sea areas and if any trends in concentrations can be found. We have given an account of recent work, as well as pinpointed the gaps in our knowledge. This review highlights the importance of DOM as one of the major pressures in the Baltic Sea ecosystem management, improving the understanding of DOM sources and its fate in the Baltic Sea with implications for ecosystem modeling and system analysis.

2. Spatial distribution of DOM

2.1. Distribution of DOC

In the Baltic Sea (Fig. 1), studies of dissolved organic carbon (DOC) concentrations were already conducted in the 1970s and early 1980s, but information on DOC concentrations began to accumulate more rapidly only in the 1990s. The number of DOC studies has increased during the last decade, but information on DOC concentrations and dynamics in the Baltic Sea is still sporadic. In the majority of the studies DOC has been measured with high-temperature oxidation, which is the most widely used method in DOC analytics. Few investigations have used persulfate oxidation (Jurkowskis et al., 1976; Kuliński and Pempkowiak, 2008; Kulinski et al., 2011; Pempkowiak et al., 1984), which gave comparable concentrations but higher scatter than high-temperature oxidation in an intercalibration of DOC measurement (Sharp et al., 2002). Two studies reported total organic carbon (TOC) instead of DOC (Table 1; Perttilä and Tervo, 1979; Wedborg et al., 1994). Since DOC concentrations exceed those of particulate organic carbon (POC) by an average of 48-fold in the Baltic Proper (Nausch et al., 2008), and the DOC stock has been modeled to exceed that of POC by 100-fold in the Baltic Sea (Gusstafsson et al., 2014), we presumed that the DOC concentrations are roughly equal to those of TOC.

In the open-sea surface water of the Baltic Sea, concentrations of DOC range from about 260 to about 480 μmol C l^{-1} (Table 1), exceeding those in the surface water (top 100 m) of the Atlantic Ocean approximately 3–6 fold (about 50–80 μmol C l^{-1}; Carlson et al., 2010). In the open ocean, almost all of the DOM ultimately derives from local phytoplankton production, whereas in coastal areas allochthonous loading contributes extensively to DOM concentrations. DOC concentrations of 290–1900 μmol C l^{-1} in the rivers entering the Baltic Sea are clearly higher than those in the Baltic Sea itself (Fleming-Lehtinen et al., 2014; Räike et al., 2012; Stepanauskas et al., 2002). Accordingly, the DOC concentrations in the Baltic Sea are generally higher in areas with high terrestrial influence. Strong temporal variation in DOC concentrations and sporadic data impede examination of spatial and seasonal trends, but some features are nevertheless prominent.

2.1.1. Gulf of Finland

In the open-sea water of the western Gulf of Finland, DOC concentrations vary widely (290–480 μmol C l^{-1}; Fig. 2). The concentrations are generally about 50 μmol C l^{-1} above those in the Baltic Proper (Fig. 2, Table 1), probably due to higher allochthonous inputs. The DOC concentrations in the open-sea area increase eastward from the mouth of the Gulf (Hoikkala et al., 2012), and DOC concentrations in the Neva Bay are over 200 μmol C l^{-1} (50–60%) higher than those in the western Gulf of Finland (Aarnos et al., 2012). The gradient is probably affected by discharge into the Neva Bay, mainly from the Neva River the largest river draining into the Baltic Sea (discharge 2500 m^3 s^{-1}). Due to the anticlockwise circulation of the water mass, the DOC loads from the

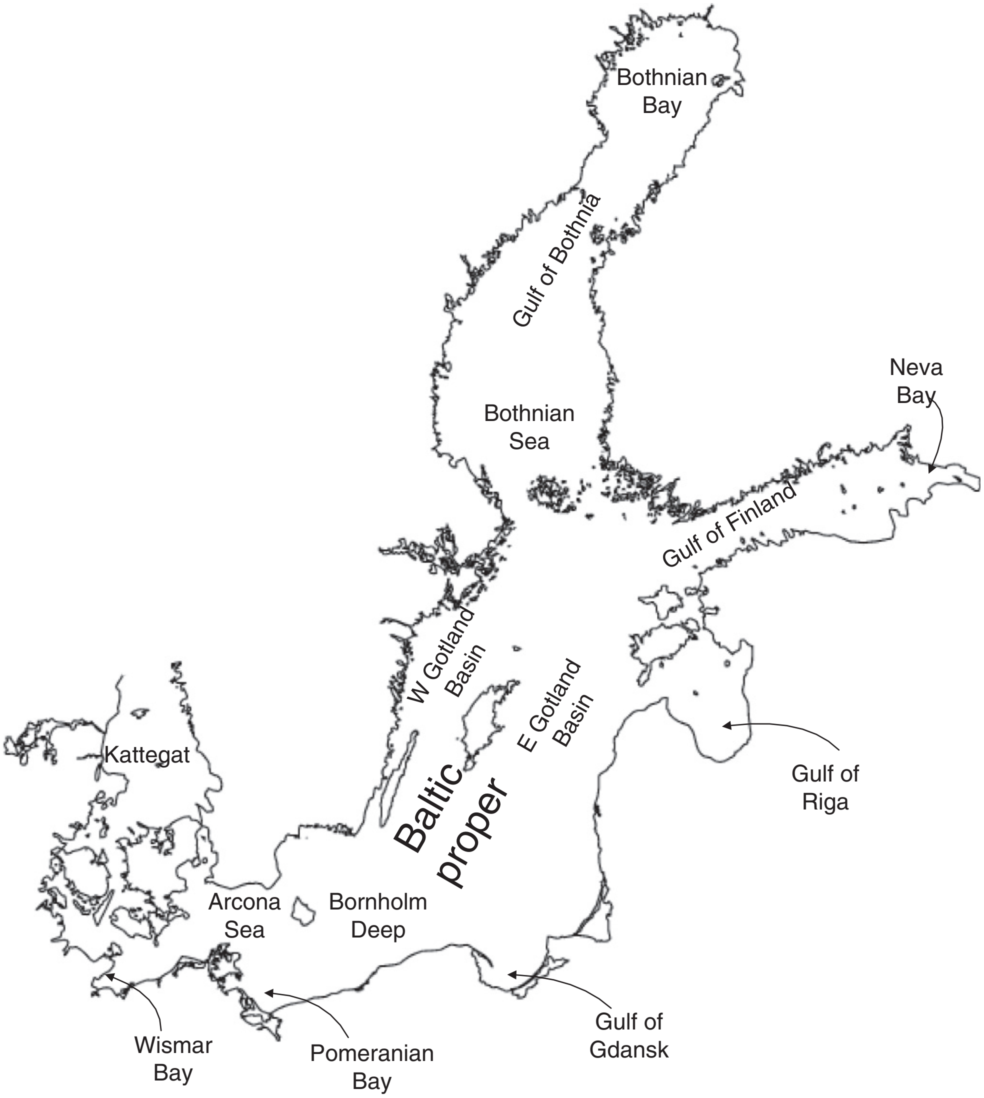

Fig. 1. The Baltic Sea with its subbasins.

Neva cause a decreasing east–west gradient in the DOC concentrations along the coast of Finland (Fleming-Lehtinen et al., 2014).

2.1.2. Gulf of Bothnia

The Gulf of Bothnia receives nearly half of the freshwater inputs in the Baltic Sea, with the highest inputs per volume of the basin in the Bothnian Bay. The DOC concentrations in the rivers draining the peatland-dominated, lake-free Ostrobothnian region are among the highest in the Baltic Sea area (Räike et al., 2012; Stepanauskas et al., 2002). The DOC concentrations could hence be expected to decrease from the Gulf of Bothnia to the Baltic Proper. However, the DOC concentrations in the open-sea water of these areas do not differ (Fig. 2, Table 1). In accordance, the DOC correlates only weakly with salinity in transect from the Bothnian Bay to the Baltic Proper (Deutsch et al., 2012). Despite the freshwater inputs into the Gulf of Bothnia being about twice as high as those into the Gulf of Finland, the average DOC concentrations in the open-sea water of the Gulf of Bothnia were 25–110 μmol C l^{-1} lower than in the Gulf of Finland in the late 1970s (Perttilä and Tervo, 1979). More recent data show the same pattern (Fig. 2, Table 1). Similar salinities in the surface water of the Gulf of Finland and the Gulf of Bothnia suggest that dilution of riverine DOC via physical mixing in the Baltic Sea is not a major driver of the difference.

The discrepancy may be caused by differences in autochthonous DOM (marine DOM; mDOM) production, since the area-specific annual primary productions in the Baltic Proper and the Gulf of Finland exceed those in the Bothnian Bay and Bothnian Sea about 2–6 fold (Hagström et al., 2001). Analysis of δ^{34}S and δ^{13}C isotopes showed that the contribution of terrestrial DOC (tDOC) decreases from 70% to 87% of the total DOC (TDOC) pool in the Bothnian Bay, to 52% to 75% in the Bothnian Sea, and 43% to 67% in the Baltic Proper (Alling et al., 2008; Deutsch et al., 2012). Moreover, a large part of the tDOC seems to be lost in the river estuaries of the Baltic Sea (Fleming-Lehtinen et al., 2014; Markager et al., 2011). Therefore, no gradient occurs between the open-sea waters of the Bothnian Bay and the Baltic Proper.

2.1.3. Gulf of Riga

The Gulf of Riga is highly influenced by terrestrial input (7% of the total freshwater inputs into the Baltic Sea drain into the Gulf of Riga). The DOC concentrations increase rapidly towards the southern part of the Gulf of Riga, where the Daugava River, the fourth largest river in the Baltic Sea, discharges, resulting in coastal concentrations of over 1200 μmol C l^{-1} (Zweifel, 1999). The DOC concentrations in the open-sea area of the Gulf of Riga are higher than those in the open-sea water of the Baltic Proper, being comparable to those in the western Gulf of Finland (Fig. 2, Table 1).

Table 1
Literature values for DOC, DON and DOP concentrations and the C:N:P stoichiometry of DOM in the Baltic Sea.

Area	Season	Year	DOC	DON	DOP	DOC:DON	DON: DOP	Reference
Gulf of Bothnia								
Whole gulf, OS & coast	Year round	1977–1978	241–483	–	–	–	–	Perttilä and Tervo (1979)
Bothnian Bay, coast	May–early June	1997	390–520	–	–	–	–	Gustafsson et al. (2000)
Bothnian Bay, coast (Oulu)	September	2005	347	7.8	–	28	–	Stedmon et al. (2007)
Bothnian Bay, coast	May–early June	2005	464 ± 2^{a}	–	–	–	–	van Dongen et al. (2008)
Bothnian Bay, coast & OS	August–September	2009	–	14.8 ± 3.0	–	–	–	Korth et al. (2011)
Bothnian Bay, coast	Apr–May, Aug, Oct	2010	393 ± 27	–	–	–	–	Asmala et al. (2013)
Bothnian Bay, OS	May–early June	1997	290^{b}	–	–	–	–	Gustafsson et al. (2000)
Bothnian Bay, OS	May–early June	2005	288–320^{a}					van Dongen et al. (2008)
Bothnian Bay OS	June–July	2008	–	–	0.12 ± 0.03	–	–	Nausch and Nausch (2011)
Bothnian Bay OS and coast (average value)	March, August–September	2009	308	–	–	–	–	Deutsch et al. (2012)
Bothnian Sea OS	May–June	1992	300–325^{c}	–	–	–	–	Wedborg et al. (1994)
Bothnian Sea, OS	Year round	1991–1992	270–335	–	–	–	–	Zweifel et al. (1995)
Bothnian Sea, OS	June–July, February–March	2008–2009	–	–	0.13 ± 0.02 0.18 ± 0.02	–	–	Nausch and Nausch (2011)
Bothnian Sea, OS	March, August–September	2009	316	–	–	–	–	Deutsch et al. (2012)
Bothnian Sea, Coast	Year round	1990–1992	270–400	–	–	–	–	Zweifel et al. (1995)
Bothnian Sea, Coast	Spring–Autumn	1991–1992	320 ± 9	13 ± 3	0.13 ± 0.25	25	100	Zweifel et al. (1993)
Bothnian Sea, Coast	Apr–May, Aug, Oct	2010–2011	417 ± 67	–	–	–	–	Asmala et al. (2013)
Gulf of Finland								
W, coast & OS	Year round	1977–1978	333–475^{c}	–	–	–	–	Perttilä and Tervo (1979)
W, OS	April–September	1979	300–416^{a}	11.5–31.6^{d}	0.06–0.29^{e}	10–26	49–419	Leppänen and Tamelander (1981)
W, OS	October–December	1979	483–690^{a}	20.4–22.8	0.12–0.33	24–34	67–187	Leppänen and Tamelander (1981)
W, OS	July–August	1990	–	18.4–29.0	0.23–0.80	–	25–104	Kononen (1992)
W, OS	June–July	2001	383–483	17.9–22.1	–	20–22	–	Lignell et al. (2008)
W–E, OS	April–September	2002	290–460	8.6–22.8	0.37–0.66	21–26	32–43	Hoikkala et al. (2012)
W, OS	May–August	2003	350–408	15–20	–	20–23	–	Hoikkala et al. (2009)
W, OS	May	2005	449 ± 39	20.2 ± 1.2	0.22 ± 0.04	~21	~92	Vähätalo et al. (2011)
W, OS	Average of three seasons	2006–2007	392–424	15.5–16.1	–	25–26	–	Aarnos et al. (2012)
W–E, OS	July, March	2008–2009	–	–	0.17 ± 0.02 0.25 ± 0.01	–	–	Nausch and Nausch (2011)
W, Pojoviken	April–September	2002	540–710	10.7–38.5	–	22–54	–	Hoikkala et al. (2012)
W, Pojoviken	May–August	2003	575–716	33–36	–	18–22	–	Hoikkala et al. (2009)
W, Pojoviken	March	2006	407–724	–	–	–	–	Kuivikko et al. (2010)
W, coast	Apr–May, Aug, Oct	2010–2011	363 ± 37	–	–	–	–	Asmala et al. (2013)
E, coast (Kotka)	August	2005	574	18.0	–	30	–	Stedmon et al. (2007)
Neva Bay	Average of three seasons	2006–2007	623 ± 46	15.1 ± 0.8	–	41 ± 5	–	Aarnos et al. (2012)
Gulf of Riga								
OS	Spring–Summer	1997	400–570	–	–	–	–	Zweifel (1999)
NW, Koiguste	Year round	1996	420–490	–	–	–	–	Zweifel (1999)
SW, Saulkrasti	Year round	1996	600–1230	–	–	–	–	Zweifel (1999)
SW Coast	Spring–Summer	1997	570–670	–	–	–	–	Zweifel (1999)
NW, Koiguste	May, July	1996	–	15–29	–	–	–	Jørgensen et al. (1999)
SW, Saulkrasti	May–November	1996	–	10–38	–	–	–	Jørgensen et al. (1999)
Coast & OS	May–July	1999	–	12–35	~0.50–0.90	–	20–60	Põder et al. (2003)
Gulf of Gdańsk								
	April and September	1994	438–503	–	–	–	–	Ferrari et al. (1996)
	May	2000	520–640				–	Grzybowski and Pempkowiak (2003)
	October–November	2000	480–520	20–30	–	25	–	Grzybowski (2002)
	May	2006	348–386	–	–	–	–	Kuliński and Pempkowiak (2008)
	March–October	2009–2011	410 ± 118	–	–	–	–	Maciejewska and Pempkowiak (2014)
Baltic Proper								
OS	June–October	1977	308–458	–	–	–	–	Perttilä and Tervo (1979)
OS	May–June	1992	300–325^{c}	–	–	–	–	Wedborg et al. (1994)
OS	May–July	2005	–	–	0.20–0.30			Nausch and Nausch (2007)
OS	June–July, February–March	2008–2009	–	–	0.21 ± 0.05, 0.32 ± 0.04	–	–	Nausch and Nausch (2011)
OS, Baltic Proper–Bothnian Bay	March, August–Septeber	2009	273–351	–	–	–	–	Deutsch et al. (2012)
OS, Northern Baltic	March, August–Septeber	2009	299	–	–	–	–	Deutsch et al. (2012)
OS	August–September	2009	–	15.9 ± 1.2	–	–	–	Korth et al. (2011)
Gotland Basin, OS	Year round	1994–2006	~320 ± 20	~16 ± 2	–	~20	–	Nausch et al. (2008)
E Gotland Basin, OS	May–July	1999	–	12–24	~0.50–0.90	–	20–30	Põder et al. (2003)

Table 1 *(continued)*

Area	Season	Year	DOC	DON	DOP	DOC:DON	DON: DOP	Reference
E Gotland Basin, OS	March–September	2001	330–380	~17.6–203	–	18.7 ± 1.7	–	Schneider et al. (2003)
Gotland Basin	May–July	2001	–	–	0.20–0.52	–	–	Nausch and Nausch (2006)
Gotland Basin	Average of three seasons	2006–2007	371 ± 22	14.5 ± 0.5	–	~26	–	Aarnos et al. (2012)
Gotland Deep	March–October	2009–2011	361 ± 74	–	–	–	–	Maciejewska and Pempkowiak (2014)
Bornholm Deep	April–October	2009–2011	403 ± 74	–	–	–	–	Maciejewska and Pempkowiak (2014)
Arkona Sea, OS	August	2005	316	10.2	–	17	–	Stedmon et al. (2007)
Arkona Sea, OS	Average of three seasons	2006–2007	334 ± 22	14.7 ± 0.4	–	~23	–	Aarnos et al. (2012)
Arkona Sea, OS	August, September, February	2006–2007	297–320	–	–	–	–	Osburn and Stedmon (2011)
Southern Baltic, OS		1970s	267–517	–	–	–	–	Jurkowskis et al. (1976) from Kuliński and Pempkowiak (2008)
Southern Baltic, OS	September	1983	383–592[f]	–	–	–	–	Pempkowiak et al. (1984)
Southern Baltic, OS	April	1994	435 ± 54	–	–	–	–	Ferrari et al. (1996)
Southern Baltic, OS	May	2006	322–341[f]	–	–	–	–	Kuliński and Pempkowiak (2008)
Southern Baltic, OS	Year round	1994–2006	~270–325[g]	~14–17[g]	–	~20	–	Nausch et al. (2008)
Pomeranian Bay, OS	Year round	2009?	259–358[f]	–	–	–	–	Kulinski et al. (2011)
Southern Baltic, Coast	April and September	1994	465–488	–	–	–	–	Ferrari et al. (1996)
Southern Baltic, Coast	Year round	2002–2003	–	–	0.18–0.44	–	–	Nausch and Nausch (2004)
Southern Baltic, Coast	July	2003–2004	270–370	–	–	–	–	Beck et al. (2005)
Wismar Bay	July	2003–2004	260–460	–	–	–	–	Beck et al. (2005)
Pomeranian Bay	September	1994	529 ± 58	–	–	–	–	Ferrari et al. (1996)
Odra river mouth	Year round	1994–2006	~360 ± 30	~20 ± 6	–	–	–	Nausch et al. (2008)
Kattegat and Skagerrak, Coastal Danish sites								
Kattegat	May–June	1992	200[c]	–	–	–	–	Wedborg et al. (1994)
Kattegat	August, September, February	2006	87–261	–	–	–	–	Osburn and Stedmon (2011)
Kattegat and Skagerrak	Summer and winter–early spring	2008–2009	–	–	0.15–0.36	–	–	Nausch and Nausch (2011)
Aarhus Bay	June	1992	–	–	0.48–0.98	–	–	Thingstad et al. (1996)
Horsens Fjord	Year round	2001–2002	175–369 av. 258	10.4–51.6 av. 20.2	0.18–0.77 av. 0.47	9–17	30–52	Markager et al. (2011)
Horsens Fjord	Year round	2004–2005	172–394	12–35	–	11 ± 4	–	Lønborg and Søndergaard (2009)
Dars Sill	Year round	2004–2005	186–324	17–36	–	11 ± 2	–	Lønborg and Søndergaard (2009)

OS = open-sea.

[a] DOC Counted by subtracting POC from TOC (POC 4–13% of TOC).

[b] For data unity, DOC is defined in this review as organic C passing <0.2 μm filter instead of <3 kD used in this study, and the figures presented here contain both DOC (<3 kD) and colloidal organic C (3 kD–0.2 μm) defined in the study.

[c] TOC values.

[d] DON calculated by subtracting PON, NO_3^-–N, NO_2–N and NH_4^+–N from total N (DON > 69% of total N).

[e] DOP calculated by subtracting POP and PO_4^{3-}–P from total P (DOP 7–39% of total P).

[f] Measured with persulfate oxidation.

[g] Range of average concentrations.

2.1.4. Baltic Proper

In the open-sea surface water of the Baltic Proper, DOC concentrations range generally between 270 and 380 μmol C l^{-1}, without any clear north–south gradient (Fig. 2, Table 2; Nausch et al., 2008; Wedborg et al., 1994). The DOC concentrations correlate with salinity and appear to form clusters that separate in the supply of river water and highly saline waters from the Kattegat (Nausch et al., 2008). The DOC concentrations are higher in the proximity of the river mouths (Table 1), due to inputs of tDOC (e.g. Nausch et al., 2008) and to higher primary production combined with release of marine DOC (mDOC) induced by river inputs of inorganic nutrients (e.g. Maciejewska and Pempkowiak, 2014). The subhalocline water originates from the North Sea, where the DOC concentration ranges between 90 and 120 μmol C l^{-1} 1 (Kulinski et al., 2011). It enters the Baltic Proper via the Kattegat, where the North Sea water is mixed with the Baltic Sea water, resulting in DOC concentrations between 90 and 260 μmol C l^{-1} (Osburn and Stedmon, 2011, Wedborg et al., 1994). Consequently, the DOC concentrations in the subhalocline layer of the Baltic Proper are clearly lower than those in the surface layers, ranging between 200 and 330 μmol C l^{-1} 1 (Maciejewska and Pempkowiak, 2014; Nausch et al., 2008). Close to the bottom, the DOC concentrations increase slightly, due to diffusion of DOC from interstitial water or decomposition of POC on the sediment surface (Maciejewska and Pempkowiak, 2014). In the southern Baltic Proper, the mean DOC concentrations for all depths tend to decrease from east to west (Nausch et al., 2008), which could reflect the higher influence of North Sea water in the west. In the coastal site in Denmark near the Dars Sill (southern Baltic Sea), the DOC concentrations vary between 170 and 390 μmol C l^{-1}, being thus clearly lower than those in the coastal areas in the northern parts of the Baltic Sea (Table 1).

2.2. Distribution of DON and DOP and elemental ratios of DOM

Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) concentrations in the Baltic Sea surface water are highly variable, ranging generally between 9 and 23 μmol N l^{-1} and 0.12 and

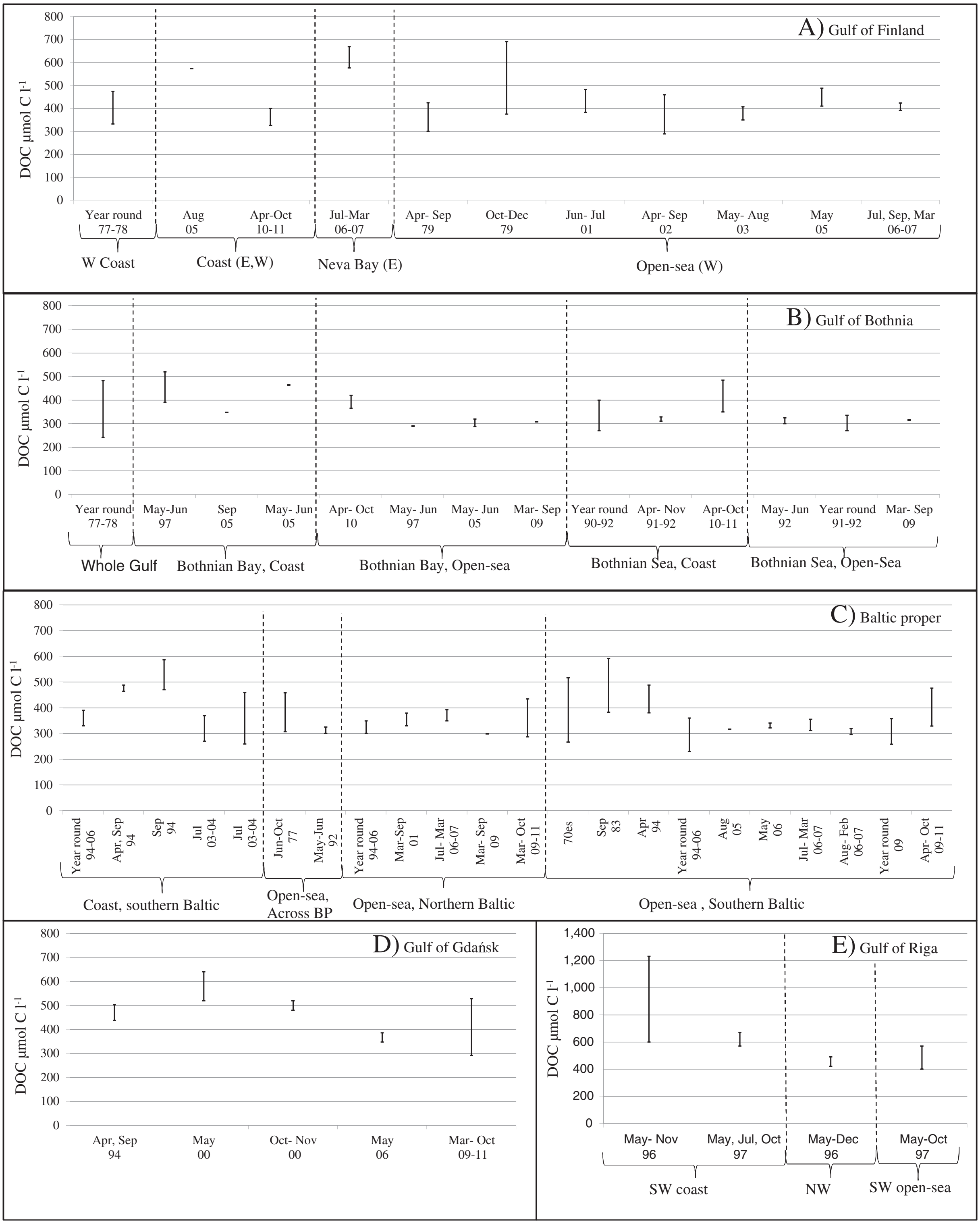

Fig. 2. DOC concentrations in different basins of the Baltic Sea as reported in the literature. Sampling sites and references in Table 1. Note the different scales in E.

0.90 µmol P l^{-1} in the open-sea areas (Table 1). Intrasite variation in sporadically measured DON concentrations masks the possible interbasin variation. An over 10-year dataset of DON concentrations in the Baltic Proper shows no significant basin-scale variation (Nausch et al., 2008). The DOP concentration increases from the Bothnian Bay to the southern Gotland Basin (Nausch and Nausch, 2011). The DOP concentrations in the Gulf of Finland exceed those in the Bothnian Sea, but are lower than those in the Baltic Proper (Nausch and Nausch, 2011). The DOP concentrations obtained have varied several folds over the years, at least in parts of the Gulf of Finland and the Baltic Proper. The wide variation in the DOP concentrations may have resulted from inter-annual changes in internal P loading and

Table 2
Degradability of the DOM pools for heterotrophic bacteria in the Baltic Sea and in rivers draining to the Baltic Sea reported in the literature.

Area	Season	Year	BDOC μmol C l^{-1} (%)	BDON μmol N l^{-1} (%)	BDOP μmol P l^{-1} (%)	C:N:P of BDOM	Incubation time (d)	Reference
Gulf of Bothnia								
Bothnian Sea, coastal	Spring–Autumn	1991–1992	23 ± 3 (1–7)	–	–	–	~5	Zweifel et al. (1993)
Bothnian Sea, coastal and open-sea	Spring–Summer	1992	11–34 (3–10)	–	–	–	4–7	Zweifel et al. (1995)
Bothnian Bay	Summer and Early Spring	2008–2009	–	–	0.01–0.04 (8)	–	5	Nausch and Nausch (2011)
Gulf of Finland								
Open-sea	Summer	2001–2002	4–20 (1–5)	(14–21)	–	C:N; <1–7	14	Lignell et al. (2008)
Open-sea	Spring–Autumn	2002	0–38 (0–9)	0–6.5 (0–41)	–	C:N; 1–12	14	Hoikkala et al. (2012)
Open-sea	Summer and Early Spring	2008–2009	–	–	0.04–0.1 (44–46)	–	5	Nausch and Nausch (2011)
Gulf of Riga								
Coastal	Spring–Summer	1996	21–82 (3–17)	–	–	–	7	Zweifel (1999)
Baltic Proper								
Open-sea	Spring–Summer	2004	–	–	0.03–0.34 (9–65)	–	4–6	Nausch and Nausch (2006)
Open-sea	Spring–Summer	2005	–	–	0.04–0.12 (33–60)	–	6–7	Nausch and Nausch (2007)
Open-sea	Summer and Early Spring	2008–2009	–	–	0.02–0.10 (10–29)	–	5	Nausch and Nausch (2011)
Danish coast								
Horsens Fjord	Year round	2004–2005	8–193 (22 ± 13)	4–17 (43 ± 10)	–	C:N; 6.3 ± 5.7	150	Lønborg and Søndergaard (2009)
Dars Sill	Year round	2004–2005	14–65 (14 ± 5)	3–12 (28 ± 12)	–	C:N; 6.8 ± 5.3	150	Lønborg and Søndergaard (2009)
Rivers								
Rivers draining to the Baltic Sea	Summer	1999	–	3.4–32.4 (8–72 av. 31)	<0.01–0.31 (4–130, av. 75)	N:P; 15–238	14	Stepanauskas et al. (2002)
Kiiminkijoki	November	2011	49–218 (4–18)	–	–	–	55	Hulatt et al. (2014)
3 Finnish river estuaries	Spring–Autumn	2012–2011	53–98 (7–12)	1.2–8.5 (10–22)	–	C:N; 12–52	12–18	Asmala et al. (2013)
Karjaanjoki	Spring–Autumn	2002	10–123 (2–17)	–	–	–	14	Hoikkala et al. (2012)

Concentrations of biologically degradable DOC (BDOC), DON (BDON) and DOP (BDOP) have been measured by incubating the samples with heterotrophic bacteria for 4–150 days. The shares of BDOC, BDON and BDOP as percentages of the total DOC, DON and DOP pools, respectively are in parentheses. In the terminology used in this review, biologically degradable DOM consists of labile DOM (LDOM) in studies where incubation times are ≤2 weeks, and both LDOM and semilabile DOM in studies with >2 week incubation times.

plankton dynamics (e.g. extension and duration of phytoplankton blooms, the primary source of DOM).

DOM transported by rivers contributes to the DON and DOP concentrations in the near-shore areas of the Baltic Sea. In the northern Baltic Sea, most of the N transport is in organic form (Mattsson et al., 2005). Less than a quarter of total phosphorus originated from the catchment area is introduced as DOP (Mattsson et al., 2005). In river estuaries, the DON concentrations can be twice those in the open-sea surface water (Table 1). The loss of DON and DOP in the estuaries may be even higher than that of DOC; in Horsens Fjord (Kattegat) both the DON and DOP concentrations decreased more than 60% from freshwater to the sea (Markager et al., 2011).

The DOC:DON ratio generally ranges from 15 to 30 in the open-sea surface water of the Baltic Sea (Table 1) and averages about 20 across the Baltic Proper (Nausch et al., 2008). Variation in the ratio within the basins of the Baltic Sea exceeds the average variation between the basins, but in the northern parts of the Baltic Sea the ratios tend to be higher at sites with high freshwater influence. tDOM typically has higher C:N ratios than mDOM. In the rivers draining into the Baltic Sea, the average DOC:DON ratio was 31 in summer (Stepanauskas et al., 2002), whereas in oceanic areas, where DOM is mostly of autochthonous origin, the DOC:DON ratio is between 6 and 11 (Hopkinson and Vallino, 2005). Moreover, the catchment characteristics can affect the C:N ratios of riverine DOM. In rivers in Finland flowing into the Baltic Sea, the average C:N ratio of DOM ranged from 15 to 40 and increased with increasing percentage of peatlands in the catchment and decreased with increasing percentage of agricultural land (Mattsson et al., 2005). The C:N ratios of DOM are generally higher in rivers draining into the northern Baltic Sea than in those draining into the southern Baltic Sea (Stepanauskas et al., 2002), reflecting the predominance of forests and peatlands in the north, whereas the coverage of agricultural land increases to the south. Furthermore, higher inorganic nutrient concentrations lead to higher DOM production (with lower C:N:P ratios than tDOM) in rivers draining into the southern Baltic Sea (Markager et al., 2011; Stepanauskas et al., 2002). The northern Baltic Sea receives the major part of its riverine DOM during high flow periods (Lepistö et al., 2008) taking place rather in the dormant than the growing season. In these northern rivers, autochthonous DOM production may be low due to the marginal primary production, and thus, the majority of DOM may be terrestrial.

The DON: DOP ratio varies widely, ranging from 20 to 100, the highest proportion being encountered in the northern Baltic Sea (Gulf of Bothnia and Gulf of Finland; Table 1). Both DOC and DOP concentrations have simultaneously been reported in only a few studies, with wide variation in the ratio ranging from 300 to 2500 (Table 1). Constant DOC concentrations and increasing DOP concentrations suggest decreasing DOC:DOP ratios across the north–south transect, which is supported by the data available (Table 1). The C:N:P stoichiometry values in the Baltic Sea (C:N = 15–30, N:P = 20–200, C:P = 300–2500) are typically higher than those in oceanic surface waters, where average values of C:N = 14–15, N:P = 20–27 and C:P = 300–

374 have been encountered (Benner, 2002; Hopkinson and Vallino, 2005). The high C:N:P ratios in the Baltic Sea are due to the high proportion of tDOM. The average C:N:P ratio of riverine inputs of DOM into the Baltic Sea is 2500:100:1 in summer (Stepanauskas et al., 2002).

3. Temporal variability in DOM concentration

3.1. Seasonal variability

Decoupling of the DOC supply and loss processes can lead to its accumulation, as occurs seasonally in the surface water of many coastal and oceanic areas (Carlson et al., 1994; Copin-Montégut and Avril, 1993). In the Baltic Sea, DOC accumulates in the surface water during summer and autumn, when DOC concentrations reach 20–200 μmol C l^{-1} (from 10% to >100%) higher values than winter levels (Hoikkala et al., 2012; Kulinski et al., 2011; Maciejewska and Pempkowiak, 2014; Markager et al., 2011; Zweifel et al., 1995). In a 10-year dataset, DOC concentrations showed seasonal signals on an average of ~30–100 μmol C l^{-1} across the Baltic Proper (Nausch et al., 2008). Accumulation of mDOC in the surface water contributes to the vertical DOC gradient that is most prominent during the growing season (Hoikkala et al., 2012; Kuliński and Pempkowiak, 2008; Maciejewska and Pempkowiak, 2014).

Both terrestrial sources and marine primary production contribute to DOC accumulation in the Baltic Sea, and their relative importance varies spatially. In the coastal Bothnian Sea, where terrestrial influence is high, DOC that accumulated in surface water during summer (DOC concentration 24–31% over the winter level) was mostly of riverine origin (Zweifel et al., 1995). DOC accumulation in the open-sea area of the western Gulf of Finland (10% over the winter level, ~30% of contemporary primary production) and the Baltic Proper (12–15% over the nongrowing season level) was again attributed to primary production (Hoikkala et al., 2012; Maciejewska and Pempkowiak, 2014). In Horsens Fjord (Denmark), where the main DOM source is presumably primary production, the concentration of DOC increased during spring and summer more than 100% over the winter values (Markager et al., 2011).

Extrapolating the values observed in DOC accumulation (20–200 μmol C l^{-1}) throughout the Baltic Sea (surface area 371000 km^2), seasonal accumulation in the 15-m-deep surface layer would account for 0.11–1.12 Tmol C. The fate of the seasonally accumulating DOC is yet unclear, but it is clearly of great significance for the C budget of the Baltic Sea. A large but varying part (20–100%) of the accumulated DOC is degradable for the indigenous bacterial community (Hoikkala et al., 2012; Zweifel, 1999; Zweifel et al., 1995), suggesting that a considerable proportion of the accumulating DOM is released as CO_2 within weeks.

DON and DOP concentrations show variable seasonal trends. In the Gulf of Finland, DON and DOP may accumulate in surface water during the productive season (Hoikkala et al., 2009, 2012). In the Gulf of Riga, DOC and DON showed contrasting patterns during the productive season in 1996; the DOC accumulated in summer, whereas the DON concentration was highest in spring and during late autumn (Jørgensen et al., 1999; Zweifel, 1999). In the Baltic Proper, the DON concentrations show no clear seasonal trend (Nausch et al., 2008), as was also observed for the DON and DOP concentrations in Horsens Fjord (Markager et al., 2011). In the central Baltic Sea, the DOP concentrations tend to decrease during summer (Nausch and Nausch, 2006, 2007). Accumulation of DOM occurs when DOM inputs are decoupled from DOM sinks, and contrasting trends in DOC compared with DON and DOP concentrations could be related to higher bioavailability of the DON and DOP pools (see Section 5.2.2). Clearly, further simultaneous measurements of DOC, DON and DOP concentrations are needed to improve the overall understanding of the fate of DOM in the Baltic Sea.

3.2. Long-term changes

Numerous studies have shown increasing TOC concentrations in headwater lakes and streams in the northern midlatitudes since the 1990s (e.g. Monteith et al., 2007; Sarkkola et al., 2009). A long-term survey (1975–2011) of TOC concentrations on the coastline of Finland shows a clear increase in TOC concentrations in the coastal area of the Bothnian Bay, the Quark and the eastern Gulf of Finland (Fleming-Lehtinen et al., 2014), presumably due to elevated TOC import from some rivers (Räike et al., 2012). Based on the few available data from the open-sea area of the Baltic Sea and assuming that TOC is equal to DOC, no trend towards increase in DOC concentrations can be found from the 1970s to 2010 (Fig. 2, Table 1). The effective removal of tDOC in the coastal zone has probably contributed to the missing trend in the DOC concentrations in the open sea.

During the past century, Secchi depth-based estimates of water transparency have decreased by 1–4 m (14–44%) in most of the Baltic Sea, with the highest changes in its northeastern parts, where water transparency has continued to decrease during recent decades (Fleming-Lehtinen and Laamanen, 2012). Along with the chlorophyll *a* concentration, this decrease was attributed to CDOM (Fleming-Lehtinen and Laamanen, 2012), which accounts for approximately 50–90% of the absorption of short-wavelength visible light (380–443 nm) in the Baltic Sea (Babin et al., 2003). Furthermore, Fe concentrations have increased in the northeastern coastal zone and are closely linked to the Secchi depth (Fleming-Lehtinen et al., 2014), presumably also contributing to water color. In rivers in Sweden flowing into the northern Baltic Sea, Fe concentrations have increased more than the DOC concentrations (Kritzberg and Ekström, 2012).

In contrast to the role of CDOM in the previously mentioned decrease in water transparency in the Baltic Sea, Højerslev (1989) stated that the concentration of gelbstoff (yellow substances) had not changed for 50 years in the Baltic Proper. This suggests that the contemporary 1–2-m decrease in water transparency in the area (Fleming-Lehtinen and Laamanen, 2012) cannot be attributed to changes in the DOM pool. The effects of long-term increase in tDOM loads and eutrophication on the quantity and quality of the open-sea pools of DOM in the Baltic Sea remain open.

4. Origin of dissolved organic matter in the Baltic Sea

Most of the DOM pool in the Baltic Sea is of terrestrial origin. Analysis of $\delta^{34}S$ and $\delta^{13}C$ isotopes showed that the contribution of tDOC is 70–87% of the total DOC pool in the Bothnian Bay and decreases to 52–75% in the Bothnian Sea and further to 43–67% in the Baltic Proper (Alling et al., 2008; Deutsch et al., 2012). However, the highest annual DOM inputs originate from autochthonous primary production of particulate organic matter (POM) that annually contributes 4.2 Tmol C, or about 90% of the TOC inputs into the Baltic Sea (Elmgren, 1984; Hagström et al., 2001; Sandberg, 2007). Part of the POM produced is subsequently degraded into mDOM in various food web processes, including 'sloppy feeding' and excretion by grazers, extracellular release from phytoplankton, release from bacteria and viral lysis (summarized in Nagata, 2000). A large part of the mDOM released is rapidly consumed by heterotrophic bacteria, the predominant degraders of DOM in the surface waters. Thus, in estimating the flow from autochthonous POC to mDOC, both accumulation of mDOC as well as C consumption by heterotrophic bacteria need to be taken into account. In the Baltic Proper and the Gulf of Finland, where bacterial production is mostly based on autochthonous organic C production, bacterial C demand is 50–70% of the phytoplankton production (Hagström et al., 2001). A seasonal accumulation of mDOC of 0.11–1.12 Tmol yr^{-1} (see Section 3.1) again accounts for 3–27% of the annual primary production. Thus, the annual production of mDOC constitutes more than half of the autochthonous POC production or >2.1 Tmol C yr^{-1}, but a large part of the mDOC produced is rapidly cycled and does not contribute to the ambient DOC pool.

More than half of the autochthonous organic C production (primary production) occurs in the Baltic Proper (Elmgren, 1984; Hagström et al., 2001). In our minimum estimate in which 50% of the primary production in the Baltic Sea is released as mDOC, mDOC production could account for 94% of the total DOC inputs in the Baltic Proper (autochthonous TOC 97% of TOC inputs: Elmgren, 1984). Inputs of mDOC could also dominate the DOC inputs in the Gulf of Finland (mDOC 67%), the Gulf of Riga (mDOC 90%) and the Bothnian Sea (mDOC 87–89%), as estimated from the respective proportions of autochthonous TOC inputs (80% in the Gulf of Finland, 95% in the Gulf of Riga and 93–94% in the Bothnian Sea; Elmgren, 1984; Hagström et al., 2001; Sandberg et al., 2004). In the Bothnian Bay, the proportion of mDOC is smaller, about 43–45% of the total DOC inputs (autochthonous TOC 60–62%; Elmgren, 1984; Hagström et al., 2001).

The riverine inputs (0.25–0.33 Tmol C yr^{-1}) are the second largest source of TOC (Elmgren, 1984; Gusstafsson et al., 2014; Kuliński and Pempkowiak, 2011; Sandberg, 2007), of which over 90% is in the form of DOC (Mattsson et al., 2005). DOC inputs from the North Sea via bottom flux to the Baltic Sea have been estimated as 0.02 Tmol C yr^{-1} (Kuliński and Pempkowiak, 2011; Kulinski et al., 2011) or as 0.19 Tmol C yr^{-1} (Osburn and Stedmon, 2011). Atmospheric sources may contribute 0.04 Tmol organic C yr^{-1} and point sources only 0.003 Tmol organic C yr^{-1} (Kuliński and Pempkowiak, 2011), although for Finland Räike et al. (2012) gave a much higher point-source contribution. The predominance of tDOM in the ambient DOM pools can be explained by less rapid processing of tDOM in the Baltic Sea.

mDOM may predominate over the tDOM inputs, even in small river estuaries, where inorganic nutrient concentrations are higher than the DOM concentrations, as was the case in Horsens Fjord based on the annual DOC inputs from land and estimated level of mDOC production (Kattegat; Markager et al., 2011). In contrast, e.g., in the Öre estuary (Gulf of Bothnia), tDOC contributed more than 80% of the TOC inputs (Sandberg et al., 2004). In river estuaries in the northern Baltic Sea, tDOC probably predominates over the autochthonous DOC due to low inorganic nutrient concentrations and primary production (Stepanauskas et al., 2002).

4.1. Origin of tDOM

Heterogenous land-use cover is typical in the majority of Baltic Sea catchments resulting in tDOM transport through a mixture of forests, peatland, agricultural land and urban areas. The northern watersheds that drain into the Gulf of Bothnia, are generally more sparsely populated and less eutrophic than the watersheds in the south that are dominated by agriculture. In the north, the predominant land cover is coniferous forest and peatland. The eastern watersheds in Finland are peatland dominated, whereas the mean slope of the western watersheds in Sweden is much steeper and the area covered by peatlands is smaller.

In most rivers, organic matter concentrations vary with discharge and season (e.g. Lepistö et al., 2008). Organic C is a typical runoff-dependent variable. DOM input can be highly dynamic; a large fraction of the annual flux may enter during a relatively brief time period. Many of these dynamics are controlled by hydrologic events, such as snowmelt and storms (Sinsabaugh and Findlay, 2003). Half of the annual runoff and leaching from headwater catchments in Finland may occur in spring, although the spring period represented only 10–15% of the entire year (Kortelainen et al., 1997). Numerous studies have shown that upstream lakes decrease downstream DOC and DOM concentrations and fluxes (Mattsson et al., 2005).

In the soil, processes such as organic matter production, decomposition, sorption and solubilization affect the amount of potentially leachable organic matter, whereas the export of DOM is controlled by hydrological mobilization of the sources. High organic matter production and/or low levels of decomposition lead to accumulation of organic matter, and this together with low amounts of sorptive surfaces may lead to high potential for DOM export. Large proportions of wetland or peatland in the catchment area have been associated with elevated concentrations and export of DOC (Kortelainen et al., 1997; Laudon et al., 2004; Mattsson et al., 2005; Räike et al., 2012), but high DOC export has also been reported from forested land, where surficial runoff predominates (Ivarsson and Jansson, 1994). The DOC concentration of the water decreases when percolating through the soil profile, because deeper flow paths enable more contacts between solutes and soil, thus enhancing DOC sorption onto soil particles (Hinton et al., 1998; Hope et al., 1994; McDowell and Wood, 1984; Thurman, 1985). On the other hand, rainfall events and moisture conditions that generate surface runoff flowing through organic-rich soil horizons can be important contributors to DOC export in streams (Hornberger et al., 1994; Mulholland, 1997). The composition of DOM entering aquatic systems also varies according to the discharge and flow path (Ågren et al., 2008; Schiff et al., 1997). The pool delivered through soil is often highly degraded and recalcitrant, whereas fast surface-layer flow carries younger and more labile pools derived from recently produced organic matter (Schiff et al., 1997).

Large drainage basins are composed of numerous subbasins, differing in character and arranged in complicated mosaic patterns. The DOM concentrations in the outlets of large catchments give an average, integrated picture of the DOM concentrations in the subbasins. They reflect differences in climate, soil and vegetation type between different catchments, but are also influenced by internal processes in lakes and streams, such as sedimentation, photooxidation, bacterial uptake and mineralization. Most of the DOM moving downstream is humic matter with a turnover time that exceeds the water retention time of the system through which it passes. The metabolism of small upstream systems is dominated by allochthonous DOM that originates in terrestrial systems. With high inputs and short hydraulic residence times, DOM dynamics often exceed microbial community response times, and all but the most reactive material passes downstream unaltered. In large systems, DOM fluxes become less variable and residence time increases (Sinsabaugh and Findlay, 2003).

5. Transport and fate of DOM in the Baltic Sea

5.1. Flocculation

When river water enters the Baltic Sea, increasing ionic strength can induce flocculation of colloidal DOM molecules. Despite the potential contribution of the flocs and their deposition to transport of riverine nutrients and trace metals, little direct information on flocculation in the Baltic Sea is available. In the Bothnian Bay, investigation of several biogeochemical variables suggested that riverine DOM generally aggregated into colloids and POM in the low-salinity mixing zone (LSZ) of the Kalix River (Gustafsson et al., 2000). Along transects of three estuaries in Finland (Gulfs of Bothnia and Finland), about 10–15% of the riverine DOM flocculated in low salinities up to 2, after which no further flocculation occurred (Asmala et al., in press). The fate of the flocculated riverine DOC remains open in the Baltic Sea. In the LSZ of the Kalix River, particle settling was insignificant and most of the aggregates may have been transported into adjacent areas (Gustafsson et al., 2000). The proportion of flocs that settle probably varies among the estuaries, correlating negatively with the ratio of organic matter to denser detrital mineral loads (cf. Gustafsson et al., 2000).

5.2. Biological degradation of DOM

5.2.1. Bacterial degradation of DOC

In the Baltic Sea, ≥50% of the primary production or ≥2.1 Tmol C yr^{-1} may be released as mDOC and subsequently taken up by bacteria (see Section 4). The turnover times of the most labile DOM compounds in marine waters can range from hours to days (Keil and Kirchman, 1999; Skoog et al., 1999), whereas most (≥70%) of the vast DOM pools are generally refractory to rapid bacterial utilization (within days to weeks; summarized in Hopkinson et al., 2002; Søndergaard and Middelboe, 1995). In the Baltic Sea surface water, the proportion of labile DOC (LDOC, DOC degradable to the bacterial community within days to weeks), ranges between 0% and 17% of the total DOC pools (Table 2). tDOC is exposed to bacterial degradation before it enters the Baltic Sea and is more refractory than mDOC. From 2% to 18% of riverine DOC entering the Baltic Sea is degraded by heterotrophic bacteria within weeks to months (2–8-week incubations; Asmala et al., 2013; Hoikkala et al., 2012; Hulatt et al., 2014).

Bacterial growth efficiency (BGE) determines how much of the biologically degraded DOC is released as CO_2 and how much is bound to bacterial biomass and may thus provide energy to higher trophic levels. It is most affected by the supply and quality of organic substrates and availability of nutrients (del Giorgio and Cole, 1998). In the Baltic Sea, BGE varies widely, ranging from 5% to 60%, with the temporal variation exceeding the spatial variation (Asmala et al., 2013; Donali et al., 1999; Hoikkala et al., 2009; Zweifel, 1999; Zweifel et al., 1993). Degradation of DOC loads carried by rivers into the Baltic Sea may occur, with BGE values ranging from 9% to 38% (Asmala et al., 2013; Hulatt et al., 2014). The effect of inorganic nutrients (N and P) on the BGE at the estuarine and coastal sites in the Gulf of Finland and Gulf of Bothnia appears to be low (Asmala et al., 2013; Zweifel et al., 1993), suggesting that the quality of organic substrates plays an important role in determining the BGE, as was the case in a temperate salt-marsh estuary (Apple and Del Giorgio, 2007). A wide variation in BGE (6–60%) in the Gulf of Riga was attributed to the freshness of the available DOM pool in a modeling study (Donali et al., 1999). Moreover, in river water the predominance of forests and wetlands in the catchment may decrease BGE (Asmala et al., 2013; Berggren et al., 2007).

5.2.2. Bacterial degradation of DON and DOP

The proportions of labile DON (LDON) and DOP (LDOP) of the total DON (TDON) and total DOP (TDOP) pools are generally larger than those of LDOC of the total DOC pools in the Baltic Sea (LDON and LDOP = DON and DOP degradable by the bacterial community within days to weeks; Table 2; Hoikkala et al., 2012; Lignell et al., 2008; Nausch and Nausch, 2011), as it is for DOM that is degradable by the bacterial community within 150 days (Lønborg and Søndergaard, 2009), following the general pattern in marine areas (e.g. Hopkinson et al., 2002). Accordingly, average molar C:N ratios of LDOM and semi-LDOM are clearly lower than those of the total DOM pools, ranging from 6 to 7 (±4–5):1 at the coastal sites in Denmark and in the western Gulf of Finland (Hoikkala et al., 2012; Lignell et al., 2008; Lønborg and Søndergaard, 2009). However, the proportions of LDON and LDOP vary widely. The estimated proportions of LDON in the Gulf of Finland, Gulf of Riga and at the coastal sites in Denmark range from 0% to 53% (Hoikkala et al., 2012; Jørgensen et al., 1999; Lignell et al., 2008; Lønborg and Søndergaard, 2009). In a survey throughout the Baltic Sea, the proportion of LDOP (degradable within 5 days) increased from the P-limited Bothnian Bay (8%) towards the Baltic Proper (LDOP 25–29% of the total DOP; Nausch and Nausch, 2011). The highest proportions of LDOP (on average 46%) were encountered in the Gulf of Finland (Nausch and Nausch, 2011). Temporal variation in the proportions of LDOP is wide. In the Baltic Proper, the proportion of LDOP (degradable within 4–6 days) has ranged from 9% to 65% of the total DOP pool (Nausch and Nausch, 2006, 2007).

In N-limited open-sea areas of the Baltic Sea, the significance of LDON to plankton nutrition can be high. In the Baltic Proper, on average 66% of the total N is in the form of DON, of which most is potentially available for remineralization processes, although the degradation process is slow (Nausch et al., 2008). In the Gulf of Finland, LDON may contribute from 0% to over 90% of the (total dissolved N) that is utilizable by the plankton community or bioavailable (calculated from Hoikkala et al., 2012; Lignell et al., 2008). In the Gulf of Bothnia, part of the Gulf of Finland and part of the northern Baltic Proper, LDOP was again the only bioavailable P source in summer (Nausch and Nausch, 2011). In the southern Baltic Sea (Arkona Basin and Bornholm), where inorganic P concentrations are high, the proportion of LDOP ranged again from 5% to 25% of the total bioavailable P (Nausch and Nausch, 2011).

In rivers draining into the Baltic Sea, the proportions of LDON and LDOP (degradable within 2 weeks) of the total DON and total DOP pools varied widely among the rivers in summer (8–72%, average 25% for DON and 4–100%, average 75% for DOP; Stepanauskas et al., 2002). The proportion of LDON seems to be lowest in the Gulf of Bothnia, with high proportions of forests and wetlands in the catchment (Asmala et al., 2013; Stepanauskas et al., 1999, 2002). The importance of organic nutrient loading to the coastal ecosystem is high: the proportions of LDON and LDOP averaged 25% and 30% of the total bioavailable nutrients in rivers draining into the Baltic Sea, respectively (Stepanauskas et al., 2002). Larger proportions were encountered in the Bothnian Bay and Bothnian Sea, where terrestrial DOP (tDOP) contributes greatly to P-limited primary production (Stepanauskas et al., 2002). At coastal sites in Denmark, LDON accounted for a large proportion (averaging 52–72%) of the bioavailable N (Lønborg and Søndergaard, 2009).

5.2.3. DOM as a nutrient source for autotrophs

LDON and LDOP are potentially valuable nutrient sources for autotrophs (Berg et al., 2001; Korth et al., 2011). Nutrients bound in tDOM may support phytoplankton growth in the Baltic Sea (Jurgensone and Aigars, 2012; Purina et al., 2004), and may thus contribute directly to eutrophication of the Baltic Sea. Primary producers (>1.6-μm size fraction) may even predominate in the uptake of autochthonous DON and total DON, implying that LDON can fuel primary production in the central Baltic Sea (Korth et al., 2011). Phytoplankton also dominated in the uptake of urea in incubation experiments in the western Gulf of Finland, based on size fractionation and parallel dark and light incubations (Tamminen and Irmisch, 1996). Depletion of urea occurred at the same rate as that of ammonium, showing that urea can be a valuable N source for phytoplankton, when sources of new N are scarce (Tamminen and Irmisch, 1996). Loadings of DOM into coastal waters may also affect the phytoplankton community composition. For example, humic-rich DOM may support the growth of dinoflagellates, but not diatoms (Granéli and Moreira, 1990), and cyanobacteria and prasinophytes may correlate positively with uptake of dissolved free amino acids and diatoms and dinoflagellates with uptake of urea (Berg et al., 2001). Of the bloom-forming filamentous N_2-fixing cyanobacteria, *Nodularia spumigena* (Mertens ex Bornet & Flahault) can grow more efficiently with LDOP as the P source than *Aphanizomenon flos-aquae* (Ralfs ex Bornet & Flahault), giving it a competitive advantage when the availability of phosphate is low (Vahtera et al., 2007). Alkaline phosphatase, produced by bacteria and algae, is an important enzyme that hydrolyses DOP. *Nodularia* may have lower substrate half-saturation constants for the alkaline phosphatase activity than does *A. flos-aquae*, suggesting that its affinity for DOP is higher (Degerholm et al., 2006).

Moreover, tDOC loads provide bacteria an additional C source and may thus intensify bacterial competition for P, thus deepening the P limitation of the phytoplankton in the Gulf of Bothnia (Sandberg et al., 2004). In support, in experiments in the coastal waters of Denmark, release of bacterial C limitation by glucose additions enabled the bacterial communities to outcompete the phytoplankton for the available nutrients (Jacquet et al., 2002; Joint et al., 2002).

5.3. Photochemical degradation of DOM

5.3.1. Photochemical degradation of DOC

The part of DOM that absorbs light in the UV and visible parts of the spectrum, or CDOM, mostly originates from terrestrial sources in the

Baltic Sea (Kowalczuk, 1999). Autochthonous production is also an important source of CDOM in the open-sea area (Kowalczuk, 1999), deriving either directly from algal exudation or from microbial processing of mDOM (Stedmon and Markager, 2005). The turnover of CDOM in surface waters is affected by photochemical reactions (Moran and Zepp, 1997; Vähätalo, 2009). Photochemical release of dissolved inorganic C (DIC) in coastal areas may contribute markedly to the loss of tDOC from the oceans (e.g. Miller and Zepp, 1995). Solar alteration of DOM of mostly terrestrial origin from coastal sites in the Bothnian Bay and the eastern Gulf of Finland may lead to a loss of its terrestrial characteristics (fluorescence and molecular-size distribution) and to resemblance to more autochthonous DOM in the southern Baltic Proper, suggesting that photochemical reactions contribute markedly to the transformations of DOM on its route from the northern to southwestern parts of the Baltic Sea (Stedmon et al., 2007). From the Neva Bay (eastern Gulf of Finland) to the Baltic Proper, DOC may photomineralize to DIC with annual rates of 0.13–0.18 Tmol DOC yr^{-1}, based on alteration of particle-free (<0.2 μm) sample water to simulated solar light (Aarnos et al., 2012). This corresponds roughly to 40–70% of the annual river loadings of TOC (0.25–0.33 Tmol C yr^{-1}) to the Baltic Sea.

In addition to direct photomineralization, photochemical reactions may enhance bacterial DOM degradation by releasing labile bacterial substrates from initially refractory DOM compounds (e.g. Benner and Biddanda, 1998; Miller and Moran, 1997). The BGE of labile photoproducts is low and ≥80% of the C utilized is respired to CO_2 (Pullin et al., 2004). In the Baltic Sea, photochemical release of bioavailable DOM may support 0–7% of the daily bacterial production in summer (Aarnos et al., 2012; Hoikkala et al., 2009; Lignell et al., 2008; Vähätalo et al., 2011). Via bacteria, part of the C from the labile photoproducts is transferred to higher trophic levels (Vähätalo et al., 2011). On the other hand, initially labile bacterial substrates may photochemically transform into more refractory compounds and thus decrease bacterial activity (Benner and Biddanda, 1998; Tranvik and Kokalj, 1998).

The part of DOC that is photoreactive and can thus be photochemically mineralized (either directly or via photochemical release of LDOC) ranges between 80% and 97% and 36% and 41% for wetland-derived and lake DOC, respectively (summarized in Aarnos et al., 2012). Assuming that ~50% of riverine DOC inputs into the Baltic Sea are photoreactive, the sum of direct photomineralization and bacterial mineralization of photochemically released LDOC in the Baltic Sea (0.22–0.32 Tmol DOC yr^{-1}) exceeds the annual river inputs of photoreactive DOC, suggesting that mDOC is also photochemically mineralized in the Baltic Sea (Aarnos et al., 2012).

5.3.2. Photochemical degradation of DON

Photochemically released labile bacterial substrates also include N-rich compounds, such as amino acids and ammonia (Bushaw et al., 1996; Bushaw-Newton and Moran, 1999). In the Baltic Sea, the estimated rates of photochemical release of ammonium (photoammonification) range from 7 μmol N m^{-2} d^{-1} to 237 μmol N m^{-2} d^{-1}, with the highest values in the Baltic Proper (Arkona Sea) (Aarnos et al., 2012; Stedmon et al., 2007; Vähätalo and Zepp, 2005). The estimated rates account for 13% to over 100% of the atmospheric N deposition rates in the Baltic Sea (Aarnos et al., 2012; Stedmon et al., 2007; Vähätalo and Zepp, 2005). In 2006–2007, the annual photoammonification in the Baltic Sea was estimated as 2.9–3.6 Gmol N, accounting for 5–9% of annual riverine N loads, but unlike riverine loads that are restricted to coastal areas and are low during summer, it could provide new N to N-limited open-sea areas of the Baltic Sea (e.g. Vähätalo and Zepp, 2005). Photoammonification from biologically refractory DOM is thus a valuable source of new N in the N-limited open-sea areas of the Baltic Sea during the summer months when other new N sources are scarce (e.g. Stedmon et al., 2007; Vähätalo and Zepp, 2005). It appears to be lower in the northern parts of the Baltic Sea than in the Baltic Proper, as is the case for atmospheric N deposition. In the Gulf of Finland, photoammonification was estimated to support from 1% to 4% of the daily N demand of primary production that is based on new N (Vähätalo and Järvinen, 2007; Vähätalo and Zepp, 2005).

5.4. Transport of DOC

5.4.1. Vertical transport

DOC that escapes rapid degradation and accumulates in the surface water is susceptible to vertical transport out of the surface layer via water-mixing events. In oceanic areas, this vertical export of DOC may equal or exceed that of POC, being potentially an important sink of atmospheric CO_2 (Carlson et al., 1994; Emerson et al., 1997). Its extent is dependent on the intensity of annual mixing events (e.g. Carlson et al., 2010). In the Gulf of Finland, the estimated vertical transport of DOC out of the surface layer was 150 mmol C m^{-2} during the summer stratification and 560 mmol C m^{-2} during the autumn overturn, summing up to about 10–25% of the POC sedimentation estimates (Hoikkala et al., 2012).

In the eastern Gotland Sea, the DOC flux via water mixing across the permanent halocline (at depths of about 70 m) was again estimated to account for about 30% of the POC flux (Schneider et al., 2000). The relatively low seasonal export of DOC in the Baltic Sea may partly be explained by its plankton dynamics. The spring bloom is markedly contributed to by diatoms, which sediment rapidly, decreasing the release of mDOM in the surface water (Heiskanen and Kononen, 1994). Moreover, C limitation of heterotrophic bacteria, which occurs in the Gulf of Finland, may lead to a more efficient utilization of LDOC and thus lower accumulation of DOC than would occur if bacterial growth were limited by N or P (Hoikkala et al., 2009, 2012).

Deposition of POC onto sediments provides yet another source of DOC into subsurface layers of the water column. It may range from 0.17 Tmol C yr^{-1} to 1.17 Tmol C yr^{-1}, being a potentially valuable sink of organic C from the surface water (4–28% of primary production; Gusstafsson et al., 2014; Kuliński and Pempkowiak, 2011; Winogradow and Pempkowiak, 2014). The return flux from the sediments to the water column may be considerable (23–55% of the C deposited), with the proportion of DOC in the return flux ranging between 9% and 14% or 0.007–0.008 Tmol C yr^{-1} (Kuliński and Pempkowiak, 2011; Winogradow and Pempkowiak, 2014).

5.4.2. Horizontal transport of DOM to the North Sea

Recent estimates have shown that the net export of DOC from the Baltic Sea to the North Sea is 0.05–0.20 Tmol C yr^{-1} (Kulinski et al., 2011; Osburn and Stedmon, 2011; Thomas et al., 2010), accounting for 22% of the total C export (Kulinski et al., 2011). The annual export of tDOC of 0.09 ± 0.03 Tmol C yr^{-1} out of the Kattegat into the North Sea was estimated from the intensity of a terrestrial humic-like fluorescent component of DOM (Osburn and Stedmon, 2011). The figure obtained accounts for about one-fourth of the estimated annual inputs of 0.33 Tmol C yr^{-1} from rivers draining into the Baltic Sea (Kuliński and Pempkowiak, 2011). In contrast, only a small proportion of the autochthonous organic C appears to be exported out of the Baltic Sea. The autochthonous DOC export out of the Kattegat of about 0.24 Tmol C yr^{-1} (calculated from Osburn and Stedmon, 2011) would account for about 6% of the estimated annual primary production in the Baltic Sea. Annual export of DON and DOP from the Baltic Sea to the North Sea may contribute 85% and 47% of the total N and P export to the North Sea, respectively, accounting for 16–17% of the annual nutrient inputs into the Baltic Sea (Wulff et al., 2001).

6. Annual budgets for terrestrial and marine DOC

In several recent papers, a C budget for the entire Baltic Sea was constructed (Gusstafsson et al., 2014; Kuliński and Pempkowiak, 2011; Thomas et al., 2010). These budgets show that the Baltic Sea may act either as a net source (0.09 Tmol C yr^{-1}; Kuliński and Pempkowiak, 2011) or as a net sink (0.12–0.15 Tmol C yr^{-1} of CO_2; Gusstafsson et al., 2014; Thomas et al., 2010), and the direction of net CO_2 flow may vary interannually (Gusstafsson et al., 2014). The annual inputs of DOC into the Baltic Sea are about 0.33 Tmol C yr^{-1} for tDOC and more than 2.1 Tmol C yr^{-1} for autochthonous DOC (see

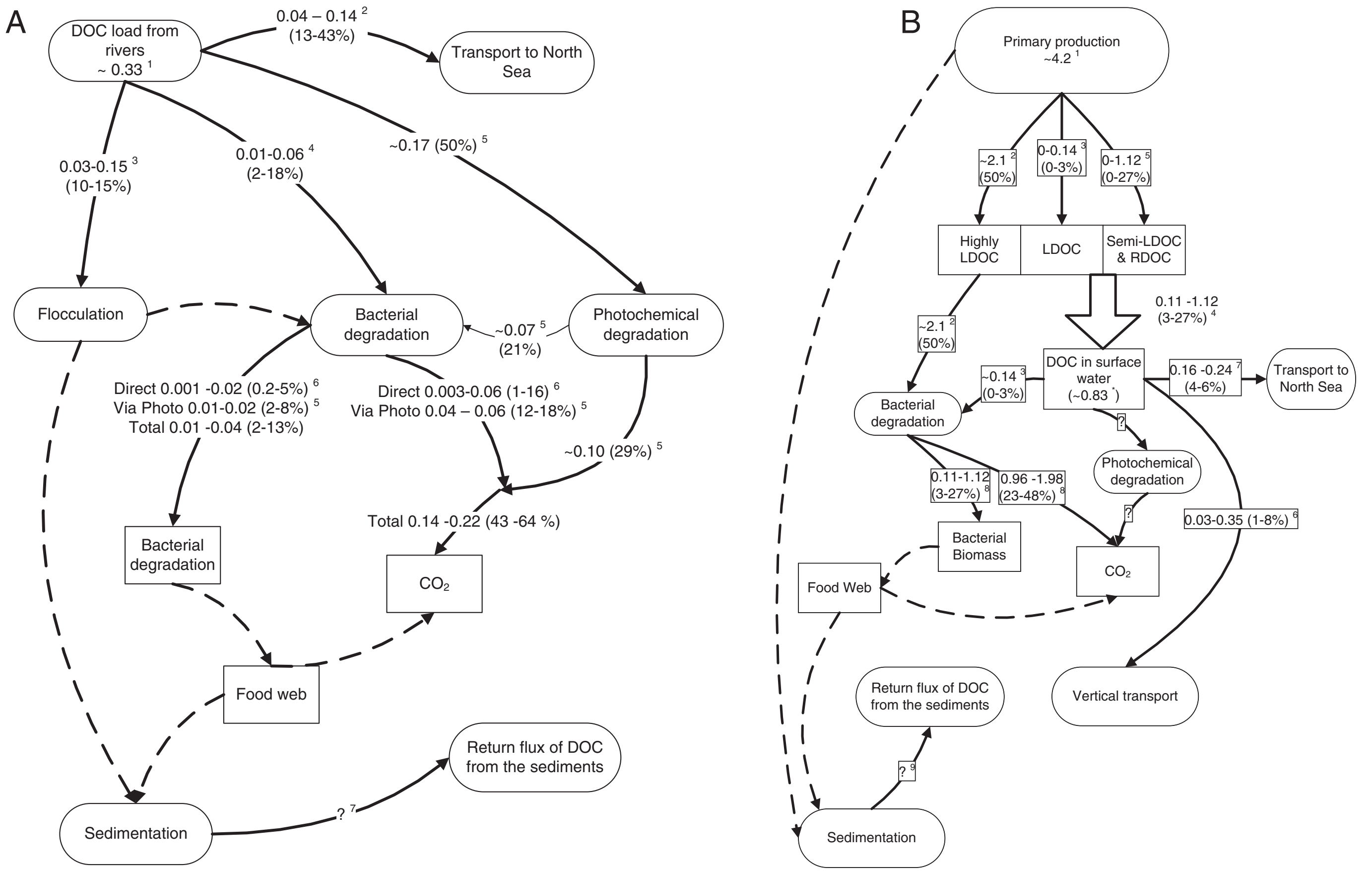

Fig. 3. Conceptual models of the loss terms for A) DOC loading from rivers and B) autochthonous organic C produced in the Baltic Sea. The ovals describe the various processes and the rectangles describe the different C pools. The rates of the C flows are given as Tmol C yr^{-1}. The C flows depicted by dashed lines were out of the scope of this study. In A) the rates of the loss processes were related to the estimated annual DOC loading from rivers (% values in parentheses). Inset (via photo) refers to bacterial degradation of photochemically released labile substrates. In B) both DOC production and DOC loss rates were related to the estimated annual primary production of organic C. Footnotes for A

1 Annual river loads of organic C to the Baltic Sea in Kuliński and Pempkowiak (2011) and Gusstafsson et al. (2014). DOC contributes over 90% of the riverine TOC load (Mattsson et al., 2005), and the TOC load was used as a proxy for the DOC load in this study.
2 Total DOC transport to the North Sea in Osburn and Stedmon (2011), Kuliński and Pempkowiak (2011) and Gusstafsson et al. (2014), multiplied by the proportion of terrestrial DOC in Osburn and Stedmon (2011) and Gusstafsson et al. (2014).
3 Flocculation estimates in Asmala et al. (in press).
4 Proportions of biologically degradable DOC in rivers in Table 2.
5 Values for photochemical degradation of DOC to CO_2 and to labile bacterial substrates in Aarnos et al. (2012). Proportions of the DOC degraded by bacteria that are released as CO_2 and bound in bacterial biomass: BGE range of 9–38% in Asmala et al. (2013) and Hulatt et al. (2014).
6 Proportions of the C degraded by bacteria that are released as CO_2 and bound in bacterial biomass: BGE range of 9–38% in Asmala et al. (2013) and Hulatt et al. (2014).
7 The return flux of DOC from the sediments to the water column 0.007–0.008 Tmol C yr^{-1} (Kuliński and Pempkowiak, 2011; Winogradow and Pempkowiak, 2014). The proportions of tDOC and mDOC in this flux are not known. Footnotes for B

1 Annual primary production in the Baltic Sea in Elmgren (1984), Hagström et al. (2001) and Sandberg (2007).
2 Ducklow and Carlson (1992): Bacteria may process about half of the organic C produced by phytoplankton (Hagström et al., 2001): Bacterial C demand is 50–70% of the phytoplankton production in Baltic Sea basins where bacterial C demand is mostly based on autochthonous organic C production.
3 Proportions of biologically degradable DOC in Table 2 (river values excluded) multiplied by estimated total surface-water pool of autochthonous DOC (*).
4 Calculated from the seasonal increase in DOC concentrations in Hoikkala et al. (2012), Kulinski et al. (2011) and Markager et al. (2011), assuming surface-water depth of 15 m and surface area of 377000 km^2 of the Baltic Sea.
5 Seasonal accumulation of DOC in surface water (4)–LDOC in surface water (3).
6 Schneider et al. (2000) and Hoikkala et al. (2012): Proportion of vertical transport of DOC 12.5–30% of POC sedimentation in the Gulf of Finland.
7 Total DOC transport to the North Sea in Osburn and Stedmon (2011), Kuliński and Pempkowiak (2011) and Gusstafsson et al. (2014), multiplied by the proportion of autochthonous DOC in Osburn and Stedmon (2011) and Gusstafsson et al. (2014).
8 Proportions of the DOC degraded by bacteria that are released as CO_2 and bound in bacterial biomass: BGE range 5–54% in Asmala et al. (2013), Hoikkala et al. (2009), Zweifel (1999) and Zweifel et al. (1993).
9 The return flux of DOC from the sediments to the water column 0.007–0.008 Tmol yr^{-1} (Kuliński and Pempkowiak, 2011; Winogradow and Pempkowiak, 2014). The proportions of tDOC and mDOC in this flux are not known.
* Approximate surface-water pool of DOC, estimated using DOC concentrations from Fig. 2 (300 μmol C l^{-1} for the Bothnian Bay, 400 μmol C l^{-1} for the Gulf of Finland, 350 μmol C l^{-1} for the Baltic Proper and 500 μmol C l^{-1} for the Gulf of Riga). Autochthonous DOC was estimated to account for 50% of the total DOC in the Baltic Proper and the Gulf of Finland, 40% in the Bothnian Sea and 30% in the Bothnian Bay (average values are from Alling et al., 2008; Deutsch et al., 2012). For the Gulf of Riga we used the proportion of autochthonous DOC in the Bothnian Bay. The volume of the ~15-m-deep surface layer in each basin was estimated, using areas in Sandberg (2007).

Section 4), showing the importance of DOC loss processes to the C budget and CO_2 balance of the Baltic Sea. In this study, conceptual models were constructed for the fate of tDOM and autochthonous DOM in the Baltic Sea, using available published data (Fig. 3).

6.1. Terrestrial DOC

In the Baltic Sea basins, the residence times of tDOC can be clearly lower than the water residence times (Alling et al., 2008; Deutsch et al., 2012). A marked loss of tDOC occurs in the river estuaries (Deutsch et al., 2012; Fleming-Lehtinen et al., 2014; Markager et al., 2011). In the open-sea area, the tDOC concentration decreases linearly with increasing salinity, suggesting that water mixing is the predominant process affecting changes in tDOC concentrations and that there occurs no marked removal of tDOC (Deutsch et al., 2012).

In a recent budget for organic C in the Baltic Sea, it was estimated that 56% of the tDOC entering the Baltic Sea is degraded, 36% is exported to the North Sea and about 8% is buried (Gusstafsson et al., 2014). Uncertainties and variation in the estimates for different loss processes are high, but the range of literature values supports the role of degradation (52–68%) and export to the North Sea (13–43%) as the main loss terms for tDOC in the Baltic Sea (Fig. 3A). A direct photochemical mineralization appears to predominate in the degradation process, accounting for about 30% of the tDOC inputs, followed by biological degradation of labile photoproducts (~20%, see Section 5.3.1). It appears that all photoreactive tDOC can be degraded during its passage through the Baltic Sea, but the proportion of photoreactive tDOC out of all tDOC is dependent on the source and varies widely (36–97%; c.f. Aarnos et al., 2012). Direct biological degradation ranges between 2% and 18% (see Section 5.2.1). The direct biological degradation may, however, occur at rates higher than photochemical degradation, since it is not dependent on light. Most of the degraded tDOC is released as CO_2, and consequently about half (43–63%) of the tDOC may end up in the DIC pool in the Baltic Sea. Flocculation of tDOC can also be significant, but the fate of the flocs remains unknown.

6.2. Marine DOC

Bacterial degradation appears to predominate in the loss of mDOC in the Baltic Sea. Based on ratios of bacterial production to primary production, ≥50% of the primary production is released as mDOC and taken up by bacteria (see Section 4). Accordingly, we estimated that 50% of the primary production was released as DOC and subsequently utilized by bacteria in our model (highly labile DOC in Fig. 3B). Total mDOC inputs in the model (2.2–3.2 Tmol yr^{-1}) consist of the estimated flow of mDOC to heterotrophic bacteria (~2.1 Tmol yr^{-1}) and the measured accumulation of mDOC in the surface water (see Section 4; Fig. 3B; 20–200 μmol C l^{-1}, or 0.11–1.12 Tmol yr^{-1}, extrapolated over the entire Baltic Sea; Hoikkala et al., 2012; Maciejewska and Pempkowiak, 2014; Markager et al., 2011). The proportions of LDOC out of the total DOC pools in the Baltic Sea surface water (0–17%; see Section 5.2.1) were used as the range for degradability of the surface-water mDOC pool in the model (Fig. 3B), but may be an underestimate. The gulfs of the Baltic Sea have residence times ranging from 2 to 7 years, and thus seasonally accumulating LDOC is probably also degraded within the gulfs (e.g. Hoikkala et al., 2012). Photochemical degradability of mDOC has not been estimated in the Baltic Sea, but is probably of minor importance, since algal-derived DOM has low photochemical degradability and is mainly degraded by bacteria (Thomas and Lara, 1995). Vertical transport of mDOC via water-mixing events may seasonally remove up to 8% of the annual autochthonous organic C production from the surface water. Estimates of its export to the North Sea are in the same range (4–6%) as that of annual primary production.

7. Conclusions

Riverine inputs of DOC, determined by the hydrology and characteristics of the catchment area, contribute markedly to the DOC concentrations in the Baltic Sea. Primary production appears to predominate in the annual DOC inputs into the Baltic Sea, but a large part of the mDOC is recycled rapidly and does not contribute to the ambient DOC pools. Most of the DOC inputs are degraded in the Baltic Sea, since only 10–40% of the tDOC input and 4–6% of the autochthonous organic C is exported into the North Sea. A large proportion (43–63%) of the tDOC probably ends up in the DIC pool via photochemical and biological degradation, which illustrates the importance of combining the DOC and DIC budgets. Loss of mDOC is dominated by bacterial degradation.

The DOC concentrations in the Gulf of Finland, Gulf of Riga and Gulf of Gdańsk are higher than those in the Baltic Proper, due to high input of riverine DOC. The DOC concentrations in the open-sea area of the Gulf of Bothnia do not clearly differ from those in the Baltic Proper, due probably to the lower primary production and release of mDOC in the area.

DON and DOP contribute substantially to the riverine nutrient inputs in the Baltic Sea. In the northern Baltic Sea, the riverine nutrient input of N is dominated by organic compounds and nearly half of the dissolved P is in organic form. The biological availabilities of both DON and DOP are higher than those of DOC, but vary widely. In the open-sea areas of the Baltic Sea, LDON and LDOP may at times predominate as bioavailable nutrients.

The number of studies addressing DOM has increased during the last decade, but sporadic data still impede estimation of seasonal and spatial trends in the DOM concentrations in large parts of the Baltic Sea. In particular, information on the seasonal and interannual variability in C:N:P stoichiometric ratios is scarce. There are still large gaps in the knowledge of DOM loss processes. Few investigations have examined flocculation and the fate of flocs, proportion of photoreactive DOC, biological degradability of riverine DOC or growth efficiency of bacteria degrading riverine DOC, and further research on these subjects is needed to obtain a more elaborate picture of the fate of DOM inputs in the Baltic Sea. Moreover, the uncertainty involved in the loss of mDOC into the DIC pool is high (33–99% of the annual mDOC input), due to wide variation in both the seasonal accumulation of mDOC and in the growth efficiency of DOM-degrading bacteria and the limited number of studies focusing on these subjects. More information on the degradability of river-derived DON and DOP is needed to estimate their effects on productivity in the Baltic Sea. The input of tDOM into the Baltic Sea has been predicted to increase, which highlights the need for proper understanding of the role of DOM in the trophic state of the Baltic Sea in present and future climates.

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Appendix 2

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Phytoplankton community interactions and environmental sensitivity in coastal and offshore habitats

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Assessing the relative importance of environmental conditions and community interactions is necessary for evaluating the sensitivity of biological communities to anthropogenic change. Phytoplankton communities have a central role in aquatic food webs and biogeochemical cycles, therefore, consequences of differing community sensitivities may have broad ecosystem effects. Using two long-term time series (28 and 20 years) from the Baltic Sea, we evaluated coastal and offshore major phytoplankton taxonomic group biovolume patterns over annual and monthly time-scales and assessed their response to environmental drivers and biotic interactions. Overall, coastal phytoplankton responded more strongly to environmental anomalies than offshore phytoplankton, although the specific environmental driver changed with time scale. A trend indicating a state shift in annual biovolume anomalies occurred at both sites and the shift's timing at the coastal site closely tracked other long-term Baltic Sea ecosystem shifts. Cyanobacteria and the autotrophic ciliate *Mesodinium rubrum* were more strongly related than other groups to this trend with opposing relationships that were consistent across sites. On a monthly scale, biotic interactions within communities were rare and did not overlap between the coastal and offshore sites. Annual scales may be better able to assess general patterns across habitat types in the Baltic Sea, but monthly community dynamics may differ at relatively small spatial scales and consequently respond differently to future change.

Communities are shaped by the interactions among organisms that vary across environmental gradients (Thompson 1988). Recent syntheses point to strong context dependency for all forms of interactions between species (Chamberlain et al. 2014), which poses a challenge for understanding how the combination of multiple pairwise interactions affect the structure of the entire community (Tylianakis et al. 2008). Understanding community interactions is suggested to be critical to anticipating effects of changing environmental conditions, especially climate change (Walther 2010), for species and ecosystems. One aspect of this question lies in determining the spatial scale and abiotic gradients that encompass coherent interactions across communities. In aquatic ecosystems, it is especially important to unravel spatial and temporal dynamics of organisms at the base of the food web that have high-frequency dynamics and respond rapidly to environmental conditions.

Phytoplankton communities play a central role in aquatic food webs and biogeochemical cycles. Phytoplankton are the source of ~50% of net global primary productivity (Field et al. 1998) and, therefore, integral to the transfer of energy through the food web. Their role is not limited to the flux of carbon as they are also a source of essential nutrients for higher trophic level organisms (Brett et al. 2000). As production rates, edibility, and food quality vary among phytoplankton taxa, the composition of phytoplankton communities affects upper trophic levels. Moreover, through diverse strategies for nutrient uptake, modification, and sequestration, phytoplankton biomass and composition affect rates and fluxes of elements in the ecosystem (Falkowski et al. 1998). While we know phytoplankton communities are regulated by both bottom–up and top–down processes (Alpine and Cloern 1992), interactions among phytoplankton taxa likely also affect community structure and function but their importance is not well understood.

Phytoplankton have evolved diverse strategies to succeed within aquatic environments which are relatively conserved at coarse taxonomic levels (e.g. class, Litchman and Klausmeier 2008). Environmental attributes that are particularly important for phytoplankton include light, nutrients, temperature, and physical structure (e.g. water column stability). Traits reflecting adaptation to environment conditions include different photosynthetic pigments, growth strategies, nitrogen fixation, nutrient storage capacity, mixotrophy, motility and buoyancy control (reviewed by Litchman and Klausmeier 2008). A taxon's specific functional traits defines its relative competitiveness across environmental and seasonal gradients (Tilman et al. 1982). In this way, the classical view of seasonal phytoplankton succession was developed from observations of phytoplankton tracking changing environmental conditions from spring bloom diatoms to late summer flagellates and cyanobacteria. However, these taxa also overlap within seasons suggesting substantial interaction potential. Additional traits, including toxicity and allelopathic chemical production, can also govern negative and positive interactions among phytoplankton taxa (Smayda 1997), while traits such as nitrogen fixation may not only benefit the fixer but also facilitate other taxa through leakage (Ploug et al. 2010).

Coastal and estuarine systems – of which phytoplankton are integral components – are highly productive systems and provide a wide array of ecosystem services despite substantial anthropogenic modifications and pressure (Lotze et al. 2006). Such systems constitute real-life laboratories in which to investigate phytoplankton dynamics and interactions under a gradient of environmental conditions. Additionally, the same abiotic attributes affecting phytoplankton communities are also sensitive to further local (e.g. nutrients, sediment load) and global (e.g. climate) anthropogenic impacts. Therefore, assessing current community interactions and sensitivity to environmental conditions is an important step in evaluating consequences of future changes for communities and ecosystems.

As natural resource management is becoming increasingly holistic, with the focus shifting from individual species to whole ecosystems (Pikitch et al. 2004), there is substantial need to understand the relative importance of biotic interactions for the function and stability of ecosystems (Ives and Carpenter 2007). Given the fundamental role of phytoplankton in aquatic systems, there is a need to better represent phytoplankton dynamics and variability in current food web models and other ecosystem model approaches. A first step in this direction is to characterize interactions within the phytoplankton community while accounting for environmental conditions to identify the relative importance of direct environmental effects, density dependent processes and inter-taxon interactions.

We used time series analysis techniques to evaluate both long-term trends and community interactions in phytoplankton in the northern Baltic Sea and assessed the coherence of these patterns among coastal and offshore communities. Specifically, we addressed the following questions:

1. What is the sensitivity of major phytoplankton taxonomic group biovolumes to environmental conditions at monthly and annual scales?
2. Are there shared trends among annual major phytoplankton taxonomic group biovolume time series?
3. Do time series data reveal interactions within the phytoplankton community on a monthly scale and how wide-spread are interactions within the community?

Methods

Baltic Sea

The Baltic Sea is one of the largest brackish water areas on the planet (373 000 km^2) draining a watershed area of 1.6×10^6 km^2 (Elmgren and Larsson 2001). It is comprised of a series of basins separated by shallow sills and covers a surface salinity gradient from 2–10. Here we focus on data collected at two long-term monitoring stations in the northwest Baltic Proper, a coastal site (58°48′N, 17°38′E) and an offshore site (58°35′N, 18°14′E). Salinity at these sites is relatively low with little variability in the upper 20 m (monthly mean: 6–7.8 coastal site, 5.9–7.3 offshore site). The monthly mean 0–20 m water temperature ranges over the year from –0.4–19.6°C at the coastal site and 0–19.4°C at the offshore site. Summer mixed layer depths typically range from 10–30 m. Winter ice cover is variable at the coastal site and rare at the off-shore site.

Phytoplankton data

Phytoplankton samples were collected and analysed according to the Baltic Sea monitoring guidelines (HELCOM 1988, updated at: <www.helcom.fi/Documents/Action%20areas/Monitoring%20and%20assessment/Manuals%20and%20Guidelines/Manual%20for%20Marine%20Monitoring%20in%20the%20COMBINE%20Programme%20of%20HELCOM_PartC_AnnexC6.pdf> in Part C, Annex 6). Sampling frequency was two to four times per month in spring, summer, and fall and once per month in winter (November–February). Phytoplankton were collected as integrated samples from 0 to 20 m with a plastic hose (inner diameter 19 mm) and phytoplankton > 2 µm were counted. Prior to 1992, only species contributing to approximately 90% of the biovolume in a given sample were enumerated while after 1992 all species were counted. For consistency, we only included species in each sample that would have been counted under the earlier counting protocol. All individuals were categorized by size class at the species or genus level and their biovolume was calculated from abundance and size-specific cell volumes according to Olenina et al. (2006) and the HELCOM biovolume table (<www.ices.dk/marine-data/Documents/ENV/PEG_BVOL.zip>).

Abiotic data

Local environmental variables were measured during phytoplankton sampling and integrated from 0–20 m including water temperature, salinity, total nitrogen (TN), inorganic nitrogen (measured as nitrate + nitrite, NO_x) , total phosphorus (TP), phosphate (PO_4) and silica (SiO_4). Mixed layer depth was defined as the depth at which the water density was 0.15 kg·m^{-3} higher than at 1.5 m depth with density calculated as a function of temperature, salinity and pressure (depth). Irradiance data were downloaded from the Swedish Meteorological and Hydrological Institute (SMHI, <www.smhi.se/>) for Norrköping, Sweden. We obtained monthly and winter (December–March) North Atlantic Oscillation (NAO) index values from Hurrell et al. (last modified December 2013).

Statistical approach

Data were aggregated to major taxonomic groups because functional traits can be generalized at relatively coarse taxonomic resolution (Litchman and Klausmeier 2008), to make parameter estimation feasible. We included the six most common taxonomic classes in our analyses (names in parentheses are used in remainder of manuscript): Cryptophyceae (cryptophytes), Diatomophyceae (diatoms), Dinophyceae (dinoflagellates), Litostomatea (autotrophic ciliates, single species *Mesodinium rubrum*), Nostocophyceae (cyanobacteria) and Prasinophyceae (prasinophytes). Dinoflagellate heterotrophs (HT) were separated from auto- and mixotrophs (AU-MX). See Table 1 for an overview of taxonomic groups and associated traits.

Annual trends in phytoplankton biovolume

We used dynamic factor analysis (DFA, Zuur et al. 2003a, b) to assess whether or not there were shared trends in the time series of annual mean phytoplankton group biovolume at each site. DFA is a dimension-reduction technique for multivariate analysis that explicitly accounts for the time-ordered nature of time series data. The linear combination of shared trends, covariates, and an error term are used to model variation in the time series (Zuur et al. 200b). The unknown, shared trends among the time series are estimated as random walks and loadings describe the relationship of each time series to each trend. We fit separate models for the coastal site (1984–2011) and for the offshore site (1994–2011). We evaluated model structures, including one to three trends and difference variance–covariance structures including a shared variance parameter, a unique variance parameter per time series, as well as a shared variance and covariance parameter (one diagonal and one off-diagonal parameter in the variance–covariance matrix).

For all combinations of trends and variance structures, we fit the model without a covariate or with a single covariate. A unique coefficient for each phytoplankton time series was estimated for the covariate in the model. Phytoplankton taxonomic groups differ in their seasonal biomass peaks and we attempted to select environmental measures that characterize overall annual conditions, however the summer metrics are likely only to reflect conditions for groups peaking in summer. The annual environmental variables we evaluated were seasonal 0–20m mean water temperature (Jan–Feb, Mar–Apr, Jul–Aug), winter ice cover (days from previous Dec–Mar with ice cover), winter NAO, winter (Jan–Feb) TN, winter NO_x, summer (Jul–Aug) PO_4, and summer NO_x:PO_4 ratio. After fitting all combinations of the model components described above, we selected the model with the most support from the data using Akaike's information criteria for small sample sizes (AICc, Burnham and Anderson 2002) as suggested by Zuur et al. (2003b). A Monte Carlo initial condition search was implemented to ensure estimates were not stuck on local maxima. Models with delta AIC < 15 are shown in the Supplementary material Appendix 1 Table A1. Phytoplankton group annual mean biovolume were ln-transformed and z-scored while covariates were z-scored. Model residuals were examined for normality, autocorrelation and homogeneity. The relationship of each time series to any shared trends was assessed by the factor loadings while the magnitude and direction of covariate effects were inferred from the estimated coefficients. For clarity in comparing the offshore trend to the coastal trend, the coastal trend and loading values were inverted for display in Fig. 1 and discussed as such in the results. Overall model fit for each time series was assessed by calculating the r^2.

Phytoplankton taxonomic group interactions

We used multivariate autoregressive (MAR) models to simultaneously identify effects of environmental drivers, autoregression, and community interactions on phytoplankton group biovolume at each site. MAR models are a tool for analyzing time series data that explicitly quantify interaction strengths as well as the sensitivity of individual taxa to

Table 1. Phytoplankton taxonomic groups and general traits based on Litchman and Klausmeier (2008), Lindholm and Mörk (1990) and Brett et al. (2000).

Major taxa group	Name in text	Traits
Cryptophyceae	Cryptophytes	Mobile Low-light adapted Poor competitor for inorganic nutrients Mixotrophy High-quality food for consumers
Diatomophyceae	Diatoms	High nitrogen uptake rates, high nitrogen mininum Rapid growth High sinking rate High quality food for consumers
Dinophyceae auto-mixotrophs	Dinoflagellate AU-MX	Mobile Mixotrophy Relatively lower nitrogen uptake Relatively lower maximum growth rates
Dinophyceae heterotrophs	Dinoflagellate HT	Mobile Heterotrophic
Litostomatea	*Mesodinium rubrum*	Mobile - deep and rapid migration Low-light adapted Turbid systems Obtains organelles from cryptophytes
Nostocophyceae	Cyanobacteria	Nitrogen-fixation Buoyancy control Higher temperature optima
Prasinophyceae	Prasinophytes	Mobile

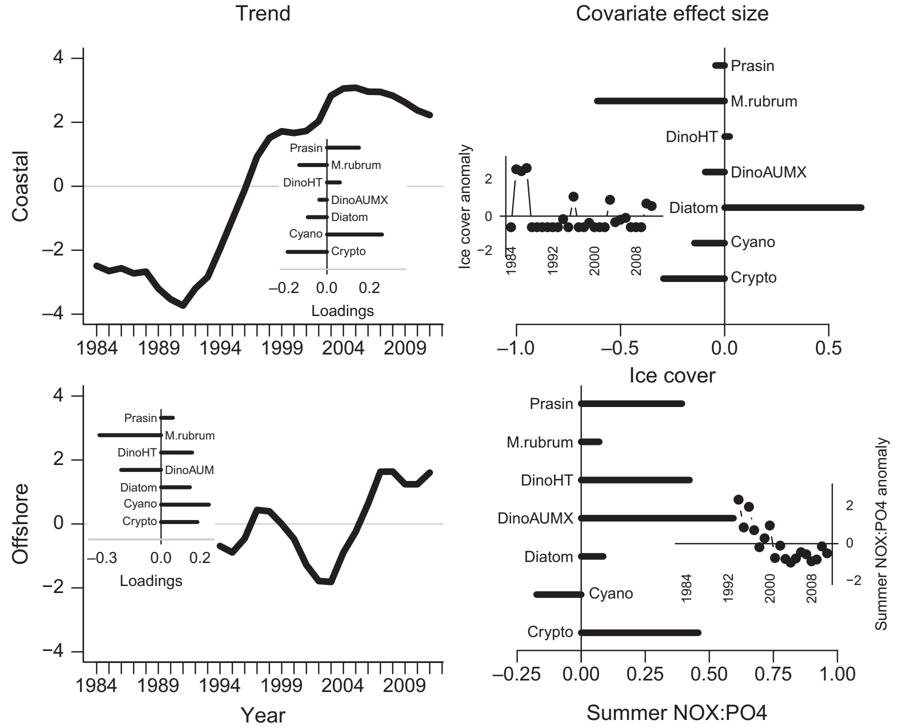

Figure 1. Trend shared among annual major phytoplankton taxonomic group biovolume time series (left panel) and the effect of environmental covariates on group biovolume (right panel) from DFA models with greatest support for each site. Top row is the coastal site (1984–2011) and bottom row is the offshore site (1994–2011). Inset figures in the left panel show the phytoplankton group loadings onto the trend. Inset figures in the right panel show the time series of annual anomalies for the environmental covariate with the greatest explanatory power identified for each site. The coastal site DFA trend values and loading vectors (upper left plot) have been multiplied by −1 for visualization purposes.

environmental conditions (Ives et al. 2003). A state–space extension of the model, MARSS (Holmes et al. 2012), allows for both process and observation error to be quantified and is robust to missing data. These models are described in depth in Ives et al. (2003) and Holmes et al. (2012, 2014) and we provide a brief explanation here.

MARSS models describe the change in log biovolume through time using equations for the state (process model, unknown true biovolume) and for the observations. The state equation in matrix form is as follows:

$$\boldsymbol{x}_t = \boldsymbol{B} \times \boldsymbol{x}_{t-1} + \boldsymbol{u} + \boldsymbol{C} \times \boldsymbol{c}_t + \boldsymbol{w}_t \quad \boldsymbol{w}_t \sim MVN(0, \boldsymbol{Q})$$

The true biovolume of each taxonomic group, *x*, at time *t* are modeled as a function of the biovolume at the previous time step, x_{t-1}, modified by a matrix, *B*, autoregressive coefficients and taxa group interactions. The autoregressive coefficients on the *B* diagonal described how strongly biovolume at time *t* predicts biovolume at $t-1$ and are interpreted as weak density dependence when close to one and strong density dependence near zero (see discussion in Ives et al. 2003 on the Gompertz model). The off-diagonal *B* values are interpreted as the effect of species *i* on species *j*'s per capita growth rate. Population growth rate, *u*, is fixed to zero as we assume the underlying process is at equilibrium and the data are standardized to a mean of zero. Effects of covariates, c_t (seasonality, environmental drivers), on the taxa biovolume are estimated in the *C* matrix. Finally, the process error, w_t, is multivariate normally distributed with a variance–covariance matrix *Q* and different structures of *Q* can be used to test hypotheses regarding process errors.

The observation model links observed data to the unobserved process and is written as:

$$\boldsymbol{y}_t = \boldsymbol{Z} \times \boldsymbol{x}_t + \boldsymbol{v}_t \quad \boldsymbol{v}_t \sim MVN(0, \boldsymbol{R})$$

The matrix of observations *y*, of each taxa group at time *t*, are related in a 1:1 relationship via the *Z* matrix to the true state biovolumes, x_t (from state equation). The observation error, v_t, is also multivariate normally distributed with a variance–covariance matrix *R*. If the community is modeled without observation error (e.g. $R = 0$) then $y_t = x_t$ and the model is the same as the MAR(1) model described in Ives et al. (2003).

We fit models for 1984–2011 at the coastal site and 1992–2011 (Jul–Dec 1992 and all of 1993 not sampled) at the offshore site. All average monthly phytoplankton group biovolumes were ln-transformed and z-scored (Supplementary material Appendix 1 Fig. A1). Zeros were replaced with a small random value between zero and one half the minimum value of the time series (Hampton et al. 2006). We included each month as a covariate to account for seasonality with separate coefficients estimated for each group. Monthly anomalies of environmental variables were also included to assess the effect of above/below average conditions on phytoplankton biovolume. These covariates included 0–20 m mean water temperature, NO_x, PO_4, NO_x:PO_4 ratio, SiO_4, depth of the mixed layer, extinction coefficient (coastal site only), global irradiance and NAO. The model structure did not accommodate missing covariate values so we decomposed all the environmental time-series into annual and monthly components, which were used to replace the missing months. Environmental variables were z-scored by month to de-season the data since seasonality was accounted for by explicitly including month.

We evaluated alternative models with a bootstrapped AIC (AICb) to avoid biases associated with AICc for state space models (Holmes et al. 2014). All candidate models are listed in the Supplementary material Appendix 1 Table A2. The B matrix structure always contained unique diagonal parameters for each time series and unique off-diagonals (the matrix is not symmetrical such that the effect of *i* on *j* differs from that of *j* on *i*). We fit models with different covariate structures including only seasonal effects, seasonal effects and one environmental effect, seasonal effects and a select group of two environmental effects. The models with two environmental effects were determined a priori and the correlation coefficients between the z-scored environmental covariates was not greater than $|0.2|$. In our estimates of process error (Q), we assumed process variance to be unique to each phytoplankton group and process covariance to be unique to each pair of time series (i.e. an 'unconstrained' matrix).

We also tested whether or not estimating observation error (R) improved model performance. When including observation error, we fit a model including the same observation variance for all groups and no covariance among groups (e.g. $R =$ 'diagonal and equal'). Preliminary analyses indicated difficulties in estimating parameters for both the R matrix and the B matrix (interactions). Previous MAR models for plankton community interactions have primarily assumed observation error to be minor and did not estimate it (Hampton et al. 2006) although marine systems have been suggested to have greater observation error due to oceanographic processes and sampling resolution (Scheef et al. 2012). Our data were sampled at frequent intervals according to standardized methods and species identification conducted by the same individual for the entirety of the time series and so in the analyses discussed here we also assumed observation error to be zero. We continue to use the state–space framework, however, because it accommodates missing data in the observation time series.

The models with the greatest support were examined for normality and auto-correlation in the residuals. We obtained confidence intervals for the interaction and covariate parameter estimates through parametric bootstrapping. If the confidence interval of an estimated parameter crossed zero it was determined to be non-significant. We fit a final model only estimating the parameters that were significant under the bootstrapping analysis (e.g. if NO_x was a covariate it was retained in the model but a parameter was only estimated for the time series which had a significant estimate under the full model bootstrap). We evaluated the final model's ability to explain variation in the data using both the total and conditional r^2 (Ives et al. 2003). The conditional r^2 described the variation explained by the model in the change in log biovolume from time $t-1$ to time t (Ives et al. 2003).

We performed all analyses in R (<www.r-project.org>) using the MARSS package (Holmes et al. 2012), reshape package (Wickham 2007), and wq package (Jassby and Cloern 2015). The code for DFA and MARSS analyses is included in the Supplementary material Appendix 2.

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.8hj8t> (Griffiths et al. 2015).

Results

Annual trends in phytoplankton biovolume

Coastal

The DFA model with the greatest support contained a single shared trend in phytoplankton group time series from 1984–2011. In addition, model selection supported the inclusion of ice cover days as an explanatory variable. The shared trend showed below-average annual biovolume prior to 1995 and higher than average levels afterwards (Fig. 1). Factor loadings identified a positive association between this trend and cyanobacteria as well as prasinophytes, while cryptophytes, diatoms and *Mesodinium rubrum* were negatively associated with the trend. Factor loadings of dinoflagellate AU-MX and HT were weak and opposite of each other (negative and positive, respectively). Ice cover explained substantial variation in diatom biovolume (positive effect) and in *M. rubrum* and cryptophytes (negative effects).

The model explained more than 30% of the variation for cryptophytes ($r^2 = 0.34$), diatoms ($r^2 = 0.56$), *M. rubrum* ($r^2 = 0.45$), and cyanobacteria ($r^2 = 0.65$) time series. The model poorly described the variation in the time series of the other groups (dinoflagellate AU-MX $r^2 = 0.02$, dinoflagellate HT $r^2 = 0.03$, prasinophytes $r^2 = 0.21$) leading to an overall r^2 of 0.32. A single error term was estimated for all groups ($R = 0.68$). The relative support for alternative models is shown in the Supplementary material Appendix 1 Table A1.

Offshore

The model with the greatest support from AICc included a single shared trend and no environmental covariate. A model differing by 2.5 AICc units included one trend and the summer NO_x:PO_4 ratio as a covariate and explained more variation in the data (Supplementary material Appendix 1 Table A1). In this model, from the start of the time series (1994) the trend showed abrupt oscillations up to the mean state until a transition in the early 2000s to above mean state (Fig. 1). Factor loadings show that cyanobacteria (positive), dinoflagellate AU-MX (negative), and *M. rubrum* (negative) were most strongly associated with the trend. Positive, weaker loadings to the trend were observed for dinoflagellate HT, cryptophytes, and diatoms with little relationship apparent for prasinophytes. The summer NO_x:PO_4 ratio had a strong positive effect on dinoflagellate AU-MX, dinoflagellate HT, cryptophytes and prasinophytes. A weak negative effect was estimated for cyanobacteria while there was little evidence for an effect on diatoms or *M. rubrum*.

The model including the summer NO_x:PO_4 ratio explained substantial annual variation in dinoflagellate AU-MX ($r^2 = 0.55$). Moderate variation was explained in *M. rubrum* ($r^2 = 0.32$), cryptophytes ($r^2 = 0.27$), cyanobacteria ($r^2 = 0.24$), and dinoflagellate HT ($r^2 = 0.22$) biovolume time series. It performed poorly in explaining variation in diatoms ($r^2 = 0.07$), and prasinophytes ($r^2 = 0.15$) with an overall r^2 of 0.26. A single error term was estimated for all groups ($R = 0.73$).

Visual inspection of coastal and offshore model residuals indicated no concerns with the assumptions of normality and homogeneity and no autocorrelation.

Phytoplankton taxonomic group interactions

Coastal

We identified one environmental variable and a limited set of group interactions that explained variation in monthly phytoplankton biovolume at the coastal site between 1984 and 2011 (model selection in Supplementary material Appendix 1 Table A2). Given the model structure, effects of either environmental or biotic drivers must be consistent across seasons for the model to detect the effect. Monthly factors used to describe seasonality in the time series had strong effect sizes overall on all groups and showed the common differences in seasonal patterns among these phytoplankton groups in the Baltic Sea (Supplementary material Appendix 1 Fig. A2). The NO_x concentration had significant negative effects on five of the phytoplankton groups: dinoflagellate AU-MX, dinoflagellate HT, *M. rubrum*, cyanobacteria, and prasinophytes. While there were 42 potential interactions among groups, only four interactions were significant (Fig. 2, Supplementary material Appendix 1 Table A3). Dinoflagellate AU-MX negatively affected cryptophytes and diatoms, while *M. rubrum* had a negative effect on dinoflagellate HT. In addition, cryptophytes had a weak positive effect on *M. rubrum*. Autoregressive coefficients were significant for five taxa, with stronger density dependence indicated for cryptophytes, dinoflagellate HT, cyanobacteria, and diatoms than for *M. rubrum* (Fig. 2, Supplementary material Appendix 1 Table A3). The model effectively explained variation in the changes in log biovolume for many but not all groups with conditional r^2-value ranging from 0.04–0.47 (Supplementary material Appendix 1 Table A4, poor explanatory power for cryptophytes and cyanobacteria).

To assess if the shorter times series used for the offshore site was long enough to support the inclusion of environmental variables we fit the coastal MARSS model using data only from 1992–2011 (results not shown). While there were some differences in the best model structure, the inclusion of environmental variables was consistently supported by the data.

Offshore

During the period 1992–2011, variations in the monthly phytoplankton group biovolume were best explained by

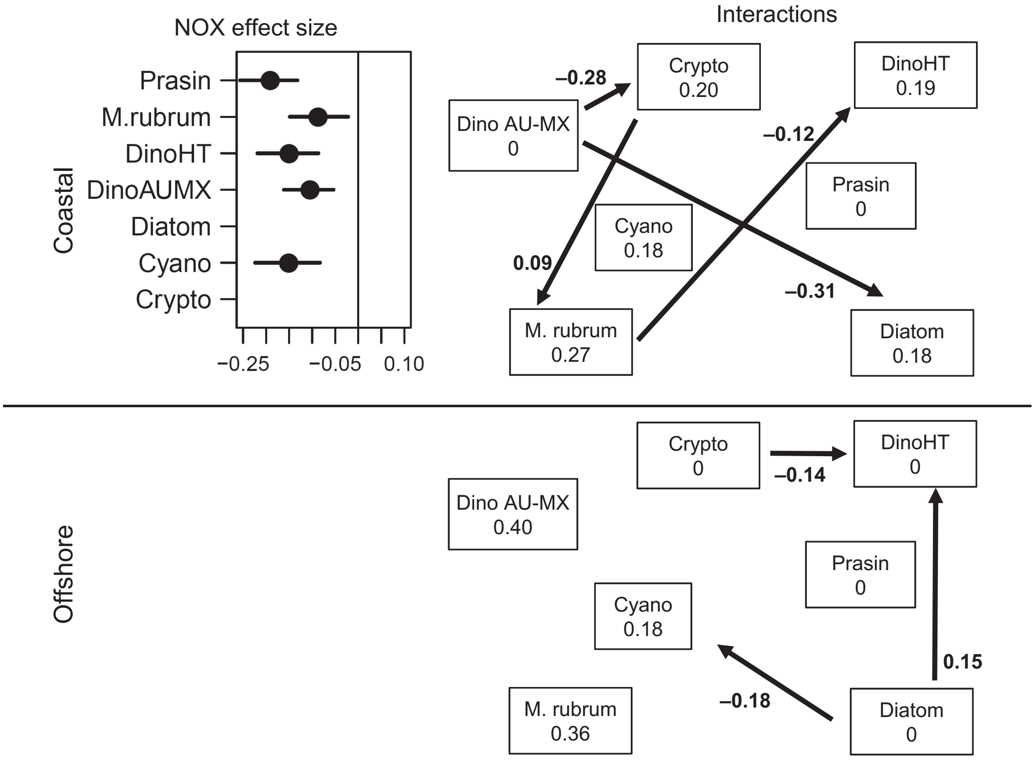

Figure 2. Covariate effect sizes (left panel) and major phytoplankton taxonomic group interaction webs (right panel) for the coastal (1984–2011, top row) and offshore (1992–2011, bottom row) sites from the model with the greatest AICb support. The covariate effect sizes are the ML estimate with 95% confidence intervals obtained through parametric bootstrapping. Maximum likelihood values obtained through parametric bootstrapping are shown for interactions and autoregressive coefficients. Arrows show significant interactions with the arrow head indicated the recipient taxa while significant autoregressive coefficients are included inside the group boxes. Autoregressive coefficients shown as zero were fixed at zero after bootstrapping produced values spanning zero.

interactions and monthly effects. No additional covariates were selected using AICb model selection although models containing either salinity or mixed layer depth showed minimal differences in AICb values indicating some weak support (Supplementary material Appendix 1 Table A2). Again, seasonality, included as monthly factors, had a large effect (Supplementary material Appendix 1 Fig. A2) and reflect common seasonal patterns for Baltic Sea phytoplankton groups. At this site only three significant interactions were found, two of which included diatoms (Fig. 2, Supplementary material Appendix 1 Table A3). Diatoms negatively affected cyanobacteria and positively affected dinoflagellate HT. Cryptophytes also had a negative effect on dinoflagellate HT. Significant autoregressive coefficients were observed for three groups as in the coastal site with stronger density dependent effects for *M. rubrum* and cyanobacteria than for dinoflagellate AU-MX (Fig. 2, Supplementary material Appendix 1 Table A3). Conditional r^2 for the groups ranged from 0–0.47 with five groups' variation in the change log biovolume well explained (Supplementary material Appendix 1 Table A4, poor explanatory power for Dinoflagellate AUMX and cyanobacteria).

Visual inspection of coastal and offshore model residuals indicated no concerns with the assumption of normality and no autocorrelation.

Discussion

Coastal and offshore time series of northern Baltic Proper phytoplankton each demonstrated evidence of a trend shared among groups within the site but differed in their sensitivity to environmental conditions on both annual and monthly time scales. While interactions among phytoplankton groups were rare overall, different group interactions were detected in coastal and offshore communities. These differences in interactions were observed despite strong similarities between sites in temperature and salinity, which are typically used to describe differences in community composition and taxa performance in the Baltic Sea. Some but not all taxonomic groups had significant autoregressive effects, with a similar range of magnitude at both sites. Our analyses indicated these two pelagic phytoplankton communities, from coastal and offshore habitats, differ in their relative sensitivity to environmental anomalies. When coupled to a difference in interaction patterns, this suggests that these communities will respond in unique ways to future environmental change. It also underscores the challenge of inferring a more general understanding of community dynamics from single sites especially in highly variable and patchily distributed organisms.

Dynamic factor analysis identified an underlying process (i.e. shared trend) which explained variation in annual biovolume of several phytoplankton groups in both coastal and offshore sites in addition to included environmental factors. Indeed, at both sites the trend transitioned in mean state although the shape and timing of the transition differed among sites. The coastal site had smooth transition initiated in the early-mid 1990s, while the offshore site trend had fluctuations from the mid-1990s until an upward shift in mean state the early 2000s. This transition took place during a period previously described as having seen abrupt regime or ecosystem state shifts in the Baltic Sea and particularly in the central Baltic (Alheit et al. 2005). Analyses of summer phytoplankton communities have also noted shifts in community composition (1966–2008) over time, although the authors inferred a more gradual transition than in the ecosystem studies (Olli et al. 2011). While the timing and proposed cause of the ecosystem shifts differs among studies, all observed shifts in both the abiotic and biotic characteristics of the pelagic Baltic Sea ecosystem included changes in the dominance of key zooplankton and fish species. The shared trend in our data may reflect the sensitivity of the phytoplankton groups strongly related to the trend to these broad scale ecosystem shifts.

Cyanobacteria and *Mesodinium rubrum* showed a consistent relationship (based on loadings, Fig. 1) to the shared trend at both sites. Here cyanobacteria showed a positive relationship to an increased mean state, while other Baltic Sea studies have demonstrated both increasing (Suikkanen et al. 2007) and decreasing trends in cyanobacteria (Wasmund et al. 2011). More broadly across the Baltic Sea, satellite data show increased cyanobacteria accumulation extent associated with the latter half of our analysis period although this is thought to reflect decadal scale variability (Kahru and Elmgren 2014). Opposite of cyanobacteria, *M. rubrum* showed a negative relationship to the shared trend (i.e. shift from higher to lower biovolume state) over time in our analysis. *M. rubrum* is present throughout the year and highly abundant during and after the spring bloom (Hajdu 2002). Along with dinoflagellates, *M. rubrum* is hypothesized to be a key nutrient transporter due to its rapid and deep migrations (Lindholm and Mörk 1990) and therefore declining biovolume could reduce the access of other taxa to these deep layer nutrients. Diatoms had a relatively weak relationship to the trends and the direction of the loadings differed among sites. Higher diatom species diversity may play a role in the relative weakness and inconsistent responses of the group to the shared trends as different species may show opposing responses to the underlying process. Species diversity may also play a role for dinoflagellates which had much stronger relationships to the trend at the offshore site than the coastal site but with auto-mixotrophs and heterotrophs had opposing loadings. Overall, the ecological process underlying these annual shifts appears to be less related to (or less consistent for) the key spring bloom taxa, diatoms and dinoflagellates, but more to cyanobacteria and *M. rubrum.*

Biovolume sensitivity to environmental factors differed among sites, groups, and temporal scale but overall it was stronger at the coastal site. Ice cover explained substantial annual variation in biovolume at the coastal site, especially for diatoms (positive relationship) and *M. rubrum* (negative relationship). Deep mixing following cold, high ice winters is thought to benefit diatoms, while rapid stratification following mild winters may favor mobile taxa (Wasmund et al. 1998), such as *M. rubrum.* Mobility and nitrogen fixation are mechanisms that increase the competiveness of organisms when surface waters are depleted in nitrogen. Therefore, we expected that groups with these traits would have higher than average biovolume when NO_x is relatively depleted while groups lacking these strategies would perform more poorly. This expectation was moderately consistent with the monthly effects at the coastal site. Mobile groups such as dinoflagellates, *M. rubrum*, and prasinophytes showed a negative relationship to NO_x anomalies. Additionally, cyanobacteria, which are nitrogen fixers, also had a negative NO_x coefficient (e.g. above average NOx conditions decreased cyanobacteria biovolume).

Offshore there was weak support for environmental drivers at the annual scale while at the monthly scale only seasonality had significant explanatory power. At the annual scale, there was weak support for summer nutrient ratios which had effects that were expected for cyanobacteria based on the nutrient acquisition traits discussed above, but had the opposite response for the other mobile taxa. A possible explanation is that in summer, the availability of NO_x is generally limiting and therefore any positive anomaly promotes increases in phytoplankton biovolume, while this would not be the case if NO_x for the entire year was considered. In addition, ammonium was not included in the NO_x concentrations and is likely important for summer production rates. As summer environmental variable was selected, the lack of effect on diatoms may in part be related to their biomass dominance in spring which would likely mask any response by autumn diatoms. Lack of monthly scale environmental drivers could reflect either the overriding importance of seasonality or a lack of coherent responses among months to environmental variables. Thus, while coastal communities show clear sensitivity to environmental anomalies, responses in the offshore community appear to be more difficult to detect.

Seasonality encompasses a suite of variables (light, temperature, water column stability) that influence the dynamics of phytoplankton. Given the strong importance of monthly factors in describing phytoplankton time series variation, it is important to consider how changes in seasonality might affect the composition of communities and their interactions. For example, diatoms and dinoflagellates are key groups in the spring bloom which is indicated by the strong positive spring month anomalies. Warmer springs and reduced ice cover (BACC II Author Team 2015) may shift the phenology of spring taxa and work by Hjerne et al. (unpubl.) suggests that diatom and dinoflagellate phenology is shifted earlier under these conditions. This has implications both for the potential of species interactions and their detection within the MAR/MARSS framework. Interactions detected within the MAR/MARSS framework depend on the patterns of biovolume anomalies among groups, therefore interaction detection and strength would depend on the synchrony of phenology shifts. Changing species composition within a major taxonomic group may account for phenology shifts (Walters et al. 2013), thus interactions among functional groups may be driven by different species. Phenological shifts may occur independently from or in concert with changes in environmental extremes which may further enhance or dampen interactions.

Significant interactions among groups were rare in our MARSS analyses. We expected similar interactions among groups in both the coastal and offshore site because groups also represent functional groups. There were no shared group interactions among sites, however. While in general the diatom to dinoflagellate ratio is much higher at the coastal site (Hjerne et al. unpubl.), the majority of species occur, with similarities in dominance, at both sites (Griffiths et al. unpubl.). One conclusion that could be drawn from this is that interactions are not trait-based but instead reflect random processes. On the other hand, it may be that the physical and chemical environment determines what types of trait-based interactions shape communities and that these were sufficiently different between sites (depth, topography, hydrologic processes) to alter detectable interactions using time series data. A recent study by Vasseur et al. (2014) found that compensatory dynamics in zooplankton communities were relatively rare and the scale of their occurrence was not generalizable across lake systems. In contrast, they found that synchronized dynamics dominated on seasonal time scales. Thus, detecting rare compensatory dynamics when considering only one time scale (as in our analyses) maybe challenging. Finally, if the effect (positive or negative) of an interaction is not consistent among seasons (e.g. in all months species *i* negatively effects species *j*) it may not be detected using our approach.

Interactions in MAR/MARSS models describe the effect of a change biovolume of group *i* on the biovolume of group *j*, but they have the limitation that they cannot describe the mechanism by which species interact. Therefore, interactions detected by the model must be subjected to ecological scrutiny (Ives et al. 2003). Some implementations of MAR models have limited the interactions among taxa a priori based on knowledge of the system (Hampton et al. 2006), however we felt it was more robust in this case to allow all potential interactions to be estimated.

We interpret the interactions characterized using MARSS models through the lens of competition and facilitation in phytoplankton communities. Negative interactions may result from competition, allelopathy or toxicity. Competitive interactions include both exploitative (indirect) competition (e.g. for nutrients) or interference (direct) competition (e.g. light shading). Positive interactions, on the other hand, result from facilitation via provisioning of nutrients or other allelopathic responses. These modes of interactions have been observed in a wide range of laboratory and field studies although they are likely context dependent (Chamberlain et al. 2014). Our analyses suggested that groups differ in their importance to interaction pathways among sites. Cryptophytes at the coastal site both received negative interactions from mobile competitors (dinoflagellates) and had a weak positive effect on *M. rubrum*, which have been shown to take up crytophyte organells (Gustafson et al. 2000). Dinoflagellate AU-MX also imposed negative effects on diatoms at the coastal site, where they are thought to compete primarily in spring. At the offshore site, on the other hand, diatoms were the drivers of two of the three significant interactions while *M. rubrum* and dinoflagellate AU-MX were not included in any significant interactions. Many interactions estimated by the MARSS model have ecological explanations, such as a negative effect between potential competitors (e.g. dinoflagellates on cryptophytes and diatoms). Other interactions should be viewed with caution as we lack an ecological hypothesis (e.g. a positive effect of diatoms on dinoflagellate HT).

Zooplankton, key consumers in the pelagic community, were not included in our models of phytoplankton interactions. This could lead to the detection of spurious interactions due to apparent competition (sensu Holt 1977), whereby what appears as an interaction of two phytoplankton groups is really two independent taxa responding to a consumer (apparent facilitation would similarly be detected). This potential effect on our analysis is unlikely to be equal throughout the year. Zooplankton have low abundances in winter and throughout the spring (Johansson 1992) and have little ability to reduce phytoplankton biovolume during the spring bloom. Their abundances also substantially decline in autumn months due to predation pressure (Adrian et al. 1999). However, during summer months, zooplankton grazing rates can be high (Johansson et al. 2004). Zooplankton could preferentially reduce the biovolumes of specific groups and therefore cause our models to detect interactions among groups when in fact there were none. On the other hand, grazing pressure is highly seasonal and we accounted for seasonal changes in biovolume using monthly covariates. No large shifts in composition have been reported for the northern Baltic Proper where zooplankton community is dominated by the copepods *Arcartia* spp., *Eurytemora affinis*, and several rotifer species (Johansson 1992). In the central Baltic, however, the last several decades have seen a decline of *Pseudocalanus elongatus*, once the dominant copepod, and an increase of *Acartia* spp. (Möllmann et al. 2000). Less well studied over the long term is the role of heterotrophic ciliates as both phytoplankton grazers and as alternative prey for zooplankton.

The heterogeneous environment of the Baltic Sea poses ecological and management challenges. Monitoring data, however, provide a unique opportunity to evaluate communities and their environment across habitats and temporal scales. Here we demonstrate that we can detect some similarities in annual trends among a coastal and an offshore phytoplankton community but little commonality in monthly scale biotic interactions and environmental drivers beyond the relative importance of seasonal variation. Since global and regional anthropogenic drivers affect many abiotic Baltic Sea properties generally important to phytoplankton (salinity, temperature, water column stability, nutrients), it is of great importance to determine community sensitivity to these properties. Our analyses suggest that sensitivity to abiotic conditions depends both on the temporal scale considered and proximity to the coast. Furthermore, biotic interactions also differed substantially between our two sites. A limitation of our study is that we only have data for a single site in each habitat type, and therefore we cannot extend these results to generalize about coastal and offshore communities. Responses in the Baltic Sea are often strongest at the basin scale (Klais et al. 2011), however our results indicate the potential for substantial within basin variation. The strong role of ice cover in describing the dynamics at the coastal site in our dataset suggests that distance from the coast is a major factor differentiating the responses of these two sites. Given the patchy and stochastic characteristics of phytoplankton communities, comparing only two communities may lead to observing these differences by chance not because of habitat type. We hypothesize that if community interactions were characterized across many stations, offshore communities might have more coherent responses across the Baltic Sea, due to the open environment, than coastal communities that tend to be more isolated and may therefore show little coherence within and among regions.

In many aspects the Baltic Sea can serve as a case study for the world's estuaries due to its abiotic gradients and the many anthropogenic challenges facing it (e.g. eutrophication, fishing, invasive species). Yet we find that there is little predictability at the base of the food web as we investigate the monthly scale interactions of these rapidly responding primary producers. More coherent patterns among sites were observed on annual scales reinforcing that temporal scale affects our ability to generalize about taxa and community responses. Furthermore, capturing how complex ecological interactions will alter ecosystem functioning, and in turn services provided to people, is critical (Walther 2010). Here we have taken the first step of evaluating plankton community interactions in a coastal and offshore site in the northern Baltic Sea. Analyses that broadly assess community interactions across the Baltic gradient, evaluate their dynamics over time, and connect them to emergent ecosystem properties are appropriate next steps to improve our understanding of community–ecosystem dynamics.

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Supplementary material (available online as Appendix oik.02405 at <www.oikosjournal.org/readers/appendix>). Appendix 1–2.

Appendix 3

Submitted to Oceanologia

Size-selective microzooplankton grazing on the phytoplankton in the Curonian Lagoon (SE Baltic Sea)

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Summary

Dilution experiments were performed to estimate phytoplankton growth and microzooplankton grazing rates at two sites: freshwater (Nida) and brackish water (Smiltyne) in the Curonian Lagoon (SE Baltic Sea). We used size-fractionation approach and dilution experiments and found that microzooplankton community was able to remove of up to 78% of nanophytoplankton (2–20 µm) standing stock and up to 130% of total daily primary production in the brackish waters of the lagoon, and up to 83% of standing stock and 78% of primary production of picophytoplankton (0.2–2 µm) in the freshwater part. The observed differences were attributed to the changes in ciliate community size and trophic structure, with larger nano-filterers (30-60 µm) dominating the brackish water assemblages and pico-nano filterers (<20 µm and 20-30 µm) prevailing in the freshwater part of the lagoon.

1. Introduction

Microzooplankton (size category 20 to 200 µm) grazers, usually dominated by protists, are considered as one of the most important phytoplankton mortality factors in aquatic systems. They can remove up to 60–75 % (about 2/3) of daily primary production (PP) with the remaining 1/3 part being chanelled directly through mesozooplankton or lost by viral lysis, settling and advection processes (Landry and Calbet, 2004, Calbet, 2008; Schmoker et al., 2013). Due to the high metabolic rate and short generation time, microzooplankton may play a pivotal role in determining the overall rates of grazing, nutrient regeneration and secondary production, especially during periods when they are most abundant (Weisse et al*.,* 1990).

Ciliates tend to dominate microzooplankton communities in estuaries and reach very high abundances (up to 72800 cells/L) (Gallegos, 1998; Quinlan et al., 2009). Despite their preferred prey falls within 5 to 25 µm size-class, ciliates can feed even on the smallest phytoplankton i.e. pico-fraction (<2 µm) (Hansen et al., 1994). Thus ciliates may be an important link in the incorporation of carbon from picophytoplankton to higher trophic levels (Quinlan et al., 2009). In addition, a number of nano-sized (2 to 20 µm) ciliates exists widening the effect of microzooplankton towards smaller prey size.

Size selective grazing by ciliates have important implications for the food-web structure and nutrient cycling, especially in coastal regions, where efficient grazing on small sized phytoplankton, called Fast-Growing-Low-Biomass, is observed (Sun et al., 2007). Moreover, different size groups of phytoplankton commnity also have specific responses to grazing by ciliates.

However, in dilution experiments grazing impact on phytoplankton is frequently masked by the abundant large phytoplankton fraction, not suitable for grazers, which is frequently dominant in coastal eutrophic waters (Galegos et al., 1996). Therefore, the size-fractioning is suggested in coastal and estuarine areas, where less abundant small phytoplankton fraction can have high turnover rates and contribute significantly to the secondary production of microzooplankton (Galegos et al., 1996).

The information available on the trophic role of ciliate as grazers in the transitory ecosystems with changing riverine discharges and salinity regimes is limited, especially in the Baltic Sea region. The dilution method for microzooplankton grazing estimation has been used only in a few Baltic Sea studies (Reckermann, 1996; Lignell et al., 2003; Moigis and Gocke, 2003; Aberle et al., 2006). Setälä and Kivi (2003) used field data combined with experimentally derived species-specific clearance rate information to assess ciliate community grazing in the open Baltic Sea. Reckermannn (1996) estimated microzooplankton carbon consumption rates exceeded mesozooplankton grazing in Gotland Sea by 10 times and in the estuarine Pomeranian Bay by 25 to 30 times.

The Curonian Lagoon is one of the most heavily eutrophicated coastal areas of the Baltic Sea. This transitory ecosystem is characterized by high primary production and the domination of toxic cyanobacteria during summer/autumn (Gasiūnaitė et al., 2005; Krevš et al., 2007; Šulčius et al., 2015). In the estuarine part the overall phytoplankton biomass markedly decreases with increasing salinity (Gasiūnaitė et al., 2008). An important feature of this system is the heterogeneity of the pelagic communities induced by non-stable salinity gradient. The detailed ciliate taxonomical composition of the Curonian Lagoon was described by Mažeikaitė (1978, 2003) and revised including the brackish water ciliate assemblage by Grinienė et al. (2011). Recent observations show significant differences in the community structure of ciliated protozoan between the brackish water and freshwater parts of the lagoon (Grinienė, 2013). It was demonstrated that very small nano-ciliates (< 20 μm) compose more that 60% of total freshwater ciliate assemblage, while in brackish water community the share of nano-ciliates is only 15 % of total abundance. Larger size fraction (20 to 60 μm) dominates the brackish water ciliate assemblage (Grinienė, 2013). From 67 to 84% of total abundance is composed of marine/brackish water phytoplankton in brackish water part of the lagoon (Olenina, 1997).

In this study we applied dilution experiments and phytoplankton size-fractionation to experimentally evaluate the differences in microzooplankton and phytoplankton community structures, grazing and growth rates between freshwater and brackish water parts of the lagoon.

2. Material and methods

2.1. Study area

The Curonian Lagoon (SE Baltic Sea) is a shallow (mean depth 3.8 m) eutrophic water basin connected to the Baltic Sea by the narrow Klaipeda strait. The southern and central parts of the lagoon contain fresh water due to discharge from the Nemunas River. The salinity in the northern part varies from 0 till 7 (PSU) due to seawater intrusions, which are usually restricted to the northern part of the lagoon, rarely propagating more than 40 km (Dailidienė and Davulienė, 2008). Seawater inflows with a residence time of 1 to 6 days are most common (Gasiūnaitė, 2000). In terms of hydraulic regime-based zonation the northern part of the lagoon and Nemunas River avandelta are classified as transitory, while the central part – as stagnant and intermediate (Ferrarin et al., 2008).

2.2. Dilution experiment setup and sample analysis

Water samples for the experiments were collected from two sites: freshwater (Nida) on 29 August and brackish water part (Smiltyne) on 10 October, 2009. Water was collected from 0.5 m depth into two 50 L carboys, and transported to the laboratory.

The particle free water (**FW**) was prepared by filtering lagoon water sequentially through a 20 μm pore size plankton mesh, intermediate 2 μm and 0.7 μm GF/F filters and finally a 0.2 μm Millipore filter under slight air pressure. The length of the filtration process depends on the concentration of phytoplankton and suspended solids and took 20 and 5 hours in Nida and Smiltyne sites, respectively. The whole lagoon water (**WW**) was collected the next day in Nida and the same day in Smiltyne experiment and was gently poured through a 150 μm mesh to remove mesozooplankton. Visual observation before experiments was done to assure that 150 μm size mesh removed mesozooplankton and the filtration through the mesh did not have negative effect on the vitality of ciliates, especially aloricated forms. The **WW** was diluted by **FW** to four target dilutions in ratios of 1:0 (no dilution), 3:1, 1:1 and 1:3 (dilution factor or decimal fraction of **WW**: 1; 0.75; 0.5; 0.25, respectively) in 3 L transparent plastic bottles. The incubation volume was 3 L and all treatments were carried out in triplicates. All bottles were incubated *in situ* at 0.5 m depth for 24 h. During the experiment on 10 October 2009 altogether 6 bottles from 12 were lost during the night time storm.

At the start and at the end of both experiments, 500 ml from each experimental bottle were sampled for nutrient (nitrate, nitrite, ammonium, phosphate and silicate) analysis, 25-30 ml for nano- and picofractions of chlorophyll *a* and 300 ml for microzooplankton counts.

Sample for nanophytoplankton (2 to 20 µm) chlorophyll *a* was filtered through a 20 µm mesh and concentrated onto 2 µm Millipore polycarbonate filter. The remaining filtrate was concentrated on a 0.2 µm Millipore polycarbonate filter for picophytoplankton (0 to 2 µm) chlorophyll *a* measurement. All filters were maintained frozen at -20 ^{o}C and analyzed within two months.

Total chlorophyll *a* concentration in the initial water samples was determined fluorimetrically (FluorProbe II). Pigments of nano- and picofractions were measured by high-performance liquid chromatography (HPLC) at the Baltic Sea Research Institute, Warnemünde, Germany. Samples were analyzed according to Barlow et al. (1997). Pigments were detected by absorbance at 440 nm using a Biotek (545V) diode array detector and identified by retention time and online visible spectra (350 to 750 nm) obtained from scans by the diode array detector. Chlorophylls were further detected by Jasco (FP-1520) fluorescence detector (440 and 660 nm excitation and detection wavelengths, respectively). The chromatograms are processed using the Biotek Kroma 3000 software. Pigment concentrations were calculated by the peak area.

Nutrients were analyzed at the Baltic Sea Research Institute (Warnemünde, Germany) according standard methods (Grashoff, 1983).

Ciliate counts were performed in Lugol fixed samples by Utermöhl's (1958) method. Volumes of 10 to 25 ml were settled for at least 24 h in Utermöhl's chambers. Ciliates were counted, measured and identified with an inverted microscope at 200× magnification. The entire content of each Utermöhl's chamber was surveyed and an additional subsample was counted if the total number was <150 organisms.

Rotifers and copepod nauplii counted using a microscope at 40× magnification in Bogorov chamber. The number of metazoans was very low; they composed 1% of total microzooplankton abundance at both experimental sites.

Ciliate size groups (<20 µm, 20 to 30 µm, 30 to 60 µm and >60 µm) and trophic groups (pico-filterers, nano-filterers, pico/nano-filterers, omnivorous feeding on heterotrophic flagellates, algae or ciliates, and predators feeding on other ciliates) were distinguished according to Mironova et al. (2012). *Myrionecta rubra* (*Mesodinium rubrum*) was observed in Smiltyne site experiment, but not included to the total ciliate abundance counts, because it appears to function mostly as an autotroph.

2.3. Data analysis

Dilution experiment data analysis was performed according to Landry and Hassett (1982). The apparent growth rate of prey (*AGR*) was estimated using the function (1):

$$(1)\quad AGR(d^{-1}) = (\ln(Chla_t / Chla_o)/t),$$

Where $Chla_t$, $Chla_o$ are the final and initial concentration of chlorophyll *a* (µg L^{-1}) and t = time of incubation (d). *AGR* was estimated for both pico- and nanosize fractions.

The rates of growth and grazing mortality were calculated by the linear regression of *AGR* versus actual dilution factor. The absolute value of the slope of the regression is the grazing rate by microzooplankton (g, d^{-1}) and ordinal intercept (y-intercept) of the regression is the growth rate of phytoplankton in the absence of grazing (k, d^{-1}).

Significant negative slope (one-tailed t-test, $p<0.05$) suggests a measurable grazer effect on phytoplankton growth. In the cases of a statistically non-significant regression, grazing rates were not determined and the phytoplankton growth rates were obtained from averaged *AGR* among all dilution treatments (Twiss and Smith, 2011).

The standing stock of phytoplankton biomass (as chlorophyll *a*, $\mu g\ L^{-1}$) removed daily (P_i, % d^{-1}) and phytoplankton potential production grazed daily (P_p, % d^{-1}) were calculated using equations (2) and (3) presented in James and Hall (1998):

$$(2)\quad P_i = 1 - e^{-g};$$

$$(3)\quad P_p = (e^k - e^{k-g})/(e^k - 1),$$

Where k = growth rate of phytoplankton and g = grazing rate of microzooplankton estimated from the linear regression.

For total ciliate community and different trophic groups a biovolume-dependent equation (4) to determine clearance rates (y), established for the Baltic Sea (Setälä and Kivi, 2003) was applied:

$$(4)\quad y = 0.1493 \times x^{0.906},$$

Where x = estimated spherical diameter (ESD) of the ciliate.

3. Results

3.1. Environmental parameters and nutrient concentrations

The differences environmental parameters, nutrient concentration and microzooplankton abundances are given in Table 1. Due to the high initial concentrations of both inorganic nitrogen (nitrate/nitrite+ammonium) and phosphorus (phosphate) as well as silicate no nutrient limitation happened during the incubations (Table 1). The lowest end values were above 1 µmol L^{-1} and 0.5 µmol L^{-1} for inorganic nitrogen and phosphorus, respectively (Table 1).

3.2. Phytoplankton community structure

We used high-performance liquid chromatography (HPLC) estimations of phytoplankton pigment signatures to determine community structure of phytoplankton fractions. Total chorophyll *a* concentration was 6 times higher in freshwater (30.3 µg L^{-1}) than in brackish water site (4.7 µg L^{-1}) (Table 1). The relative abundance of different phytoplankton size groups within the community, represented by the chlorophyll *a* concentrations, differed between freshwater and brackish water areas. In freshwater site the share of of >20 µm, nano- (2 to 20 µm) and picofraction (0 to 2 µm) was 47.7, 46.4 and 5.9% of total chlorophyll *a* concentration, respectively. Nanofraction of chlorophyll *a* dominated the brackish water site with 59.8% of total chlorophyll *a*, while the share of >20 µm fraction was 38.2% and that of picofraction only 1.9% of total.

Pigment composition gives an indication of systematic composition of phytoplanton, but it cannot be considered quantitative. At both sites picofraction of phytoplankton was represented only by chlorophyll *a*, whereas nanofraction of phytoplankton contained additional pigments and varied between sites (Fig. 1). Lutein (green algae), alloxanthin (cryptophytes), β carotene (for all phytoplankton taxonomic groups), divinyl chlorophyll *a* (cyanobacteria) were found in the nanofraction at freshwater (Nida) site, while at brackish water (Smiltyne) site 19’hexanoyloxyfucoxanthin (prymnesiophytes) and zeaxanthin (cyanobacteria) were recorded. In addition to chlorophyll *a* fucoxanthin (diatoms) and was detected in the nanofraction at both sites. Phytoplankton *AGR* calculations were performed using only chlorophyll *a* (as indicator of whole phytoplankton community) data. Other pigments were detected only in undiluted water (dilution factor 1) or weakly diluted treatment (dilution factor 0.75), and they could not be used in *AGR* calculations. However, the pigment results indicate that the autotrophic communities remained stable during the experiments.

3.3. Microzooplankton community stucture

At both experimental sites microzooplankton was dominated by ciliates (99% of total abundance) (Table 1). In brackish water site nano-filterers feeding on nanosized phytoplankton were dominated by medium size (30 to 60 µm) tintinnid *Tintinnopsis* sp., large naked oligotrich *Strombidium conicum, Strombilidium gyrans* and *Lohmaniella* sp. and large (>60 µm) ciliates (*Codonella relicta*, *Tintinnopsis kofoidi*): they shared 48% of total ciliate abundance (Fig. 2). Small ciliates (<20 and 20 to 30 µm) were composed by *Mesodinium* cf. *acarus*, *Strobilidium* spp., *Urotricha* sp. and *Lohmaniella oviformis.* In freshwater site small size (<20 and 20 to 30 µm) pico/nano-filterers (*Strobilidium* spp., *Tintinnopsis* cf. *nana*, *Halteria* sp.) and pico-filterers (*Cyclidium* spp., *Vorticella* spp.) prevailed. These functional groups composed together 77% of total abundance (Fig. 2). Medium-sized ciliates (30 to 60 µm) were represented mainly by tintinnids *Tintinnidium pusillum*, *Tintinnopsis tubulosa* and *Codonella cratera*, and they composed 23% of total ciliate abundance. Predators represented by *Didinium nasutum*, were found only in brackish water site, shared 4% of total ciliate abundance. These could affect experiments by selectively preying on other ciliates, but at this low number the effect is considered to be minor.

3.4. Growth and grazing rates of phytoplankton

In freshwater site the grazing rate (g = 1.8 d^{-1}) on picofraction of phytoplankton community exceeded prey growth rate (k = 1.3 d^{-1}) (Table 2), indicating high microzooplankton pressure on this size class. The microzooplankton grazing pressure on picoalgae expressed by the percentage of grazed biomass as standing stock (P_i) and percentage of grazed potential production (P_p) was 83% and 76%, respectively.

The grazing rate of nanophytoplankton was not estimated, because no significant linear relationship was observed between apparent growth rate (*AGR*) of this fraction and dilution factor, i. e. the slope (microzooplankton grazing rate, *g*) did not differed significantly from zero (Fig. 3; Table 2). However, the growth rate of nanofraction of phytoplankton can be calculated as average of apparent growth rates among all dilution treatments (average ± SE) and replicates (N=10) and it was near zero (-0.02 ± 0.08 d^{-1}).

AGR of the picofraction increased linearly with dilution factor at brackish water site and regression analysis resulted in a positive slope, which did not differed statistically from zero (Fig. 3; Table 2), therefore the microzooplankton grazing rate (*g*) is not interpretable. However, the growth rate (0.28 ± 0.3 d^{-1}) was only less than ¼ of the growth rate calculated in the freshwater site, indicating significant differences in the activity of picosize fraction.

The growth rate of nanoalgae at the brackish water site was 0.9 d^{-1} and largely exceeded nanophytoplankton growth rate in the freshwater site. The grazing rate (1.5 d^{-1}) was higher than the growth of prey community, however, the actual values were lower (Table 2; Fig. 3). In brackish water site microzooplankton grazed 78% of the nanophytoplankton standing stock per day and 130% of potential daily production.

4. Discussion

Dilution experiments have been performed over the past three decades to examine the grazing impact of microzooplankton, ranging from the open sea to coastal zone and estuaries (data reviewed by Landry and Calbet, 2004 and Shmocker et al., 2013). This relatively simple and standard technique is useful for comparative microzooplankton grazing rate studies among the geographic regions as well as revealing the role of microzooplankton in time series of ecological processes (Gallegos, 1989).

However, for estimation of microzooplankton grazing on phytoplankton in the Baltic Sea dilution technique has been applied to a less extent (Reckermann, 1996; Lignell et al., 2003; Moigis and Gocke, 2003; Aberle et al., 2006). Moigis and Gocke (2003) used dilution method as alternative method for ^{14}C and O_2 methods for primary production estimation, they did not take into account grazers community. Grazing rate by microzooplankton varied from 0.21 to 0.41 d^{-1} in Kiel Fjord.

Reckermann (1996) found high microzooplankton grazing rates on ultraphytoplankton (<5 µm) both in Gotland Sea and Pomeranian Bay. In the Gotland Sea 1994, microzooplankton (<200 µm) grazing pressure on *Synechococcus* was higher than on eukaryotic pico- and nanophytoplankton. Generally, microzooplankton grazing on *Synechococcus* was over 100% of gross production grazed per day and pico-and nanoeukaryotic production was not completely grazed. In Pomeranian Bay microzooplankton grazing on ultraphytoplankton varied from exceeding daily growth considerably to rather low values (176 to 51%). In the study by Lignell et al. (2003) microzooplankton grazing rate on the whole phytoplankton community varied between 0.05 and 0.30 d^{-1}. However, in both studies total phytoplankton community rather than different size classes were measured which may mask the effect of the size selective microprotozoa grazing or even genus/species level as is evidenced by Aberle et al., (2006) in their mesoscosm study.

The significant estimates of ciliate grazing rates of phytoplankton pico- and nano fractions were obtained at freshwater (Nida) and brackish water (Smiltynė) sites, respectively. Grazing rates exceeded growth rate of phytoplankton fractions ($g > k$), suggesting that phytoplankton production and biomass accumulation is controlled by microzooplankton, as it was frequently observed by other authors (Burkill et al., 1987; McManus and Ederinger-Cantrell, 1992; Verity et al., 1993; Landry et al., 1995; Lehrter et al., 1999).

The grazing rate of pico-fraction at freshwater site is in the range reported in the other regions (Table 3). Ciliates consumed 76% of potential picophytoplankton production at this freshwater site. The dominance of small pico- and pico/nano-filterers in the freshwater site suggests that predation on the picophytoplankton fraction can be high, but is could be tested visually by observing autotrophic pico-fraction cells via epifluorescence microscopy or flow cytometry. The calculated clearance rate as the daily clearance percentages (% of the water volume cleared in 24 h) by pico/nano-filterers in this site was very similar (70%). This finding it is in good agreement with Rassoulzadegan et al. (1988) study, they found that small ciliates (< 30 μm) take 72% picoplankton and 28% nanoplankton.

In contrast, the dilution experiment provided no statistically significant estimates of grazing rate (*g*) for phytoplankton nano-fraction at freshwater site. The *AGR* of nano-fraction was very similar in all dilution treatments (Fig. 3), which indicate the absence of microzooplakton grazing. This is supported by low number of nano-filterers in initial water at the beginnig of experiment (Fig. 2). Low average value of *AGR* (- 0.02 ± 0.08) indirectly points at a slowly growing nanophytoplankton community, which can be a result of viral lysis, presence of toxic or other unknown inhibitory metabolites that could be released during preparation of the filtered water (Stoecker et al., 2015).

The grazing rate of nano-fraction at brackish water (Smiltyne) site exceeded grazing rates in other estuarine ecosystems by 2–3 folds (Table 3). Ciliates consumed 130% of nanophytoplankton production at brackish water site. Calculated total ciliate community clearance rate as daily percentage was lower – 71% , but 41% was due to nano-filterers. This is not surprising as nanophytoplankton chlorophyll *a* concentration was 30 fold (Table 1) higher than picophytoplankton chlorophyll *a* and ciliate assemblage was dominated by medium sized ciliates (Fig. 2), composed by naked oligotrichs *Strombilidium gyrans, Strombidium conicum* and tintinnid taxa *Tintinnopsis* sp.; all of them prefer to feed on small nano-sized algae (Apendix, Table A.1.). Gallegos et al. (1996) used dilution technique combined with size fractioning and found, that the highest grazing rates of phytoplankton fraction of 5–22 μm coincided with tintinnid abundance increase in ciliate assemblage. The tendency of higher consumption rates is usually reported in dilution experiments where nutrients are not added (Landry and Hassett, 1982). Adding of nutrients is recommended at the start of the experiment to keep the phytoplankton growth unlimited (Gallegos, 1998; Landry et al., 1995). In this study, nutrient was not added, assuming high rates of N and P loading in the Curonian Lagoon during autumn, when experiments were conducted and to avoid increased mortality of delicate protists during experiments (Landry and Hassett, 1982; Gilfford, 1988).

In Smiltyne site, *AGR* of the pico-fraction increased linearly with dilution factor (theoreticaly impossible case), with highest *AGR* values at nondiluted treatment (Fig. 3), similar results were reported previuosly (Gallegos 1989; Lignell et al. 2003; Modigh and Franzè, 2009). Positive slopes usully are atributed to the complex cycling of nutrients between internal and external pools, mixotrophy or filtration contamination and trophic cascade effect (review by Calbet and Saiz, 2013). The last explantion could be the reason for positive *AGR* of pico- fraction trend along dilution factor in our data, suggesting that nano-filterers, which dominated in brackish water site (Fig. 2) intensively grazed not only autotrophic nanofraction of phytoplankton, but also heterotrophic flagellates, which belong to the same size spectra (2-20 μm) and are one of the main pico-fraction feeders, so realeased phytoplankton picofraction from predator control. Unfortunatelly, the number of heterotrophic flagellates was not estimated in this study. Similar food web effect was suggested to affect the dilution experiments in mesocosms (Lignell et al., 2003), but it was not found in the experiments conducted in the Baltic Sea by Reckermann (1996).

5. Conclusion

Dilution experiment approach revealed significant ciliate grazing effect on nano-fraction of phytoplankton in the brackish water, and pico-fraction in the freshwater community. This pattern is related to the differences in ciliate community size structure: larger nano-filterers dominate in the brackish water assemblages, whereas pico/nano-filterers prevail in the freshwaters. Thus it is important to monitor the species composition and/or size class division of specifically ciliate communities to estimate their size-selective grazing effect. This is also important to constructing of more detailed carbon flow models in the Baltic Sea ecosystem.

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Table 1 Environmental parameters and microzooplankton abundance at initial whole lagoon water (WW) at two research sites.

Parameters	Freshwater site	Brackish water site
Temperature [^oC]	18.6	11
Salinity [PSU]	0	6.2
Dissolved oxygen [mgO_2 L^{-1}]	16.6	10.1
Nitrate [μmol L^{-1}]	0.09	7.02
Nitrite [μmol L^{-1}]	0.03	0.31
Silicate [μmol L^{-1}]	1.95	11.81
Ammonium [μmol L^{-1}]	3.37	5.15
Phosphate [μmol L^{-1}]	1.88	0.98
Total chlorophyll *a* [μg L^{-1}]	30.3	4.7
Pico-fraction chlorophyll *a* [μg L^{-1}]	1.8	0.09
Nano-fraction chlorophyll *a* [μg L^{-1}]	14.1	2.8
Microzooplankton abundance:		
Ciliates [ind. L^{-1}]	30667	9800
Copepod nauplii [ind. L^{-1}]	115	24
Rotifers [ind. L^{-1}]	75	-

Table 2 Growth rates of the phytoplankton pico- and nano- fractions [k±SE, day^{-1}] and microzooplankton grazing rates [g±SE, day^{-1}] based on chlorophyll *a*. R^2 – coefficient of determination; N – number of observations. The significance level of regression (i.e. slope, g, was significantly differed from zero, $p<0.05$) is indicated by p-value; n.s. – non significant.

Site	Fraction [μm]	k	g	R^2	p-value	N
Freshwater	0.2-2	1.33±0.36	-1.83±0.53	0.55	<0.01	12
	2-20	0.19±0.19	-0.35±0.29	0.15	n.s.	10
Brackish water	0.2-2	-1.09±0.60	2.19±0.90	0.59	n.s.	6
	2-20	0.92±0.28	-1.52±0.42	0.77	<0.05	6

Table 3 Published results of microzooplankton grazing in other regions. Growth rates of the phytoplankton pico- and nano- fractions [k, day^{-1}] and microzooplankton grazing rates [g, day^{-1}], P_p – potential consumption of primary production [%], N – number of dilution experiments.

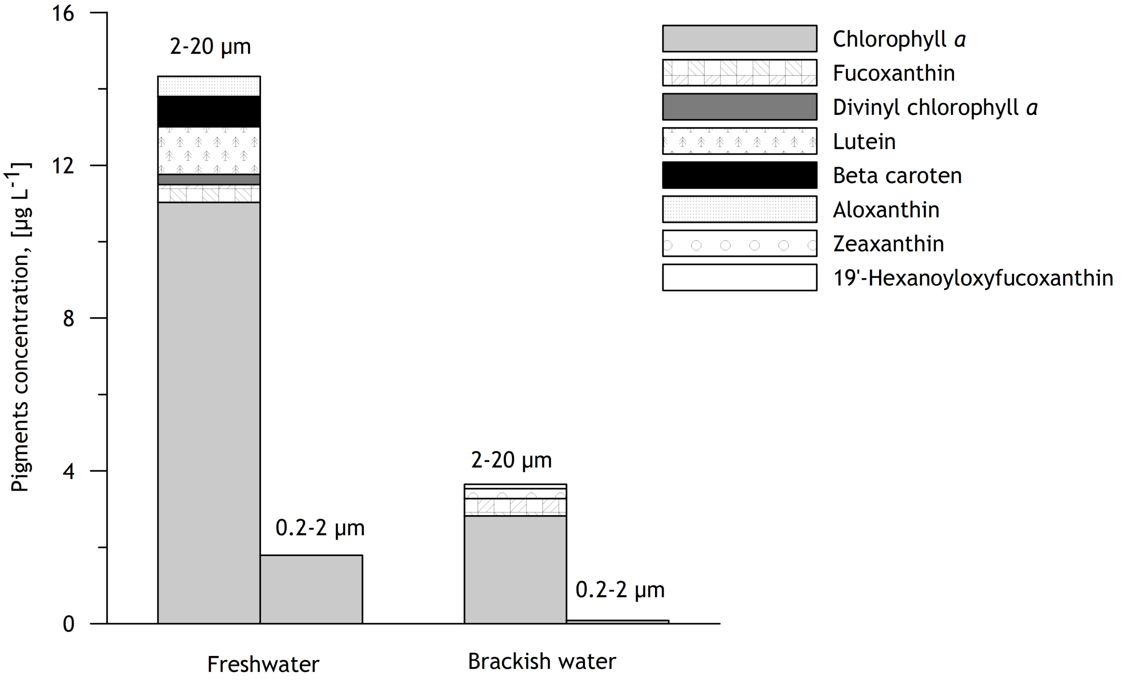
Location	Salinity [PSU]	Fraction [μm]	k	g	P_p	N	Reference
Curonian Lagoon	0	0.2–2	1.33	1.83	76	1	This study
	6.2	2–20	0.92	1.52	130	1	
Chesapeake Bay	20	0.2–2	2.10	1.92	97	1	
		2–20	0.61	0.41	73	1	
Delaware Inland Bay	15	0.2–2	2.05	0.7	58	1	Sun et al. (2007)
		2–20	0.81	0.77	97	1	
Delaware Bay	16	0.2–2	1.83	1.78	99	1	
		2–20	0.84	0.32	48	1	
Gulf of Alaska	-	<5	0.42	0.48 (0.02-1.07)	102 (±29)	39	Strom et al. (2007)
		5–20	0.34	0.39 (0.05–0.92)	102 (±32)		
Manukau estuary (New Zeland)	28–33	<5	0.2–1.8	0.3–1.3	30-230	12	Gallegos et al. (1996)
		5–22	0.2–1.8	0–0.8	0-98		
Upper St. Lawrence River (US)	-	0.2–2	0.2–1.8	0–1.1	-	12–38	Twiss and Smith (2011)
		2–20	0.1–1.3	0–1.2	-		

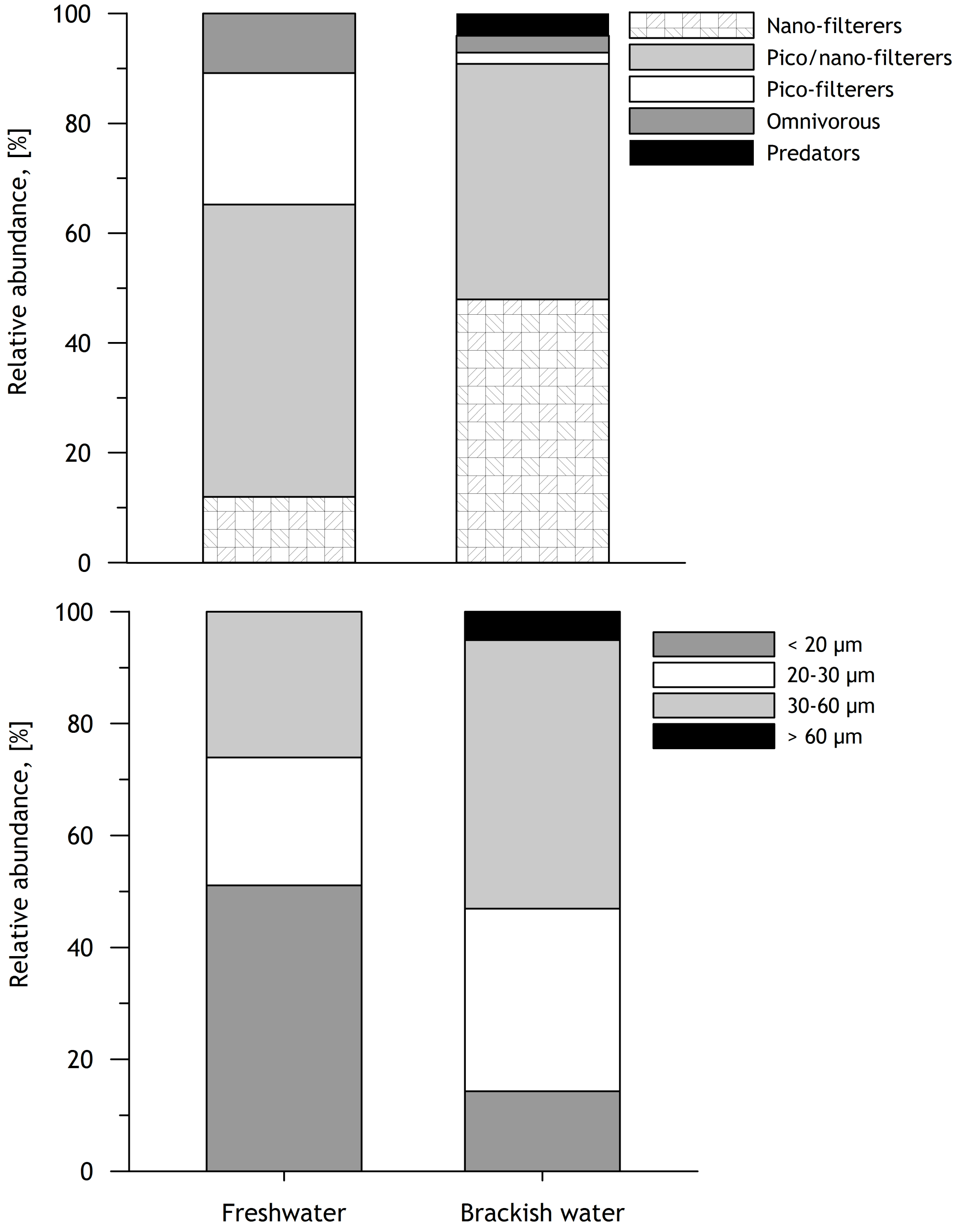
Figure captions

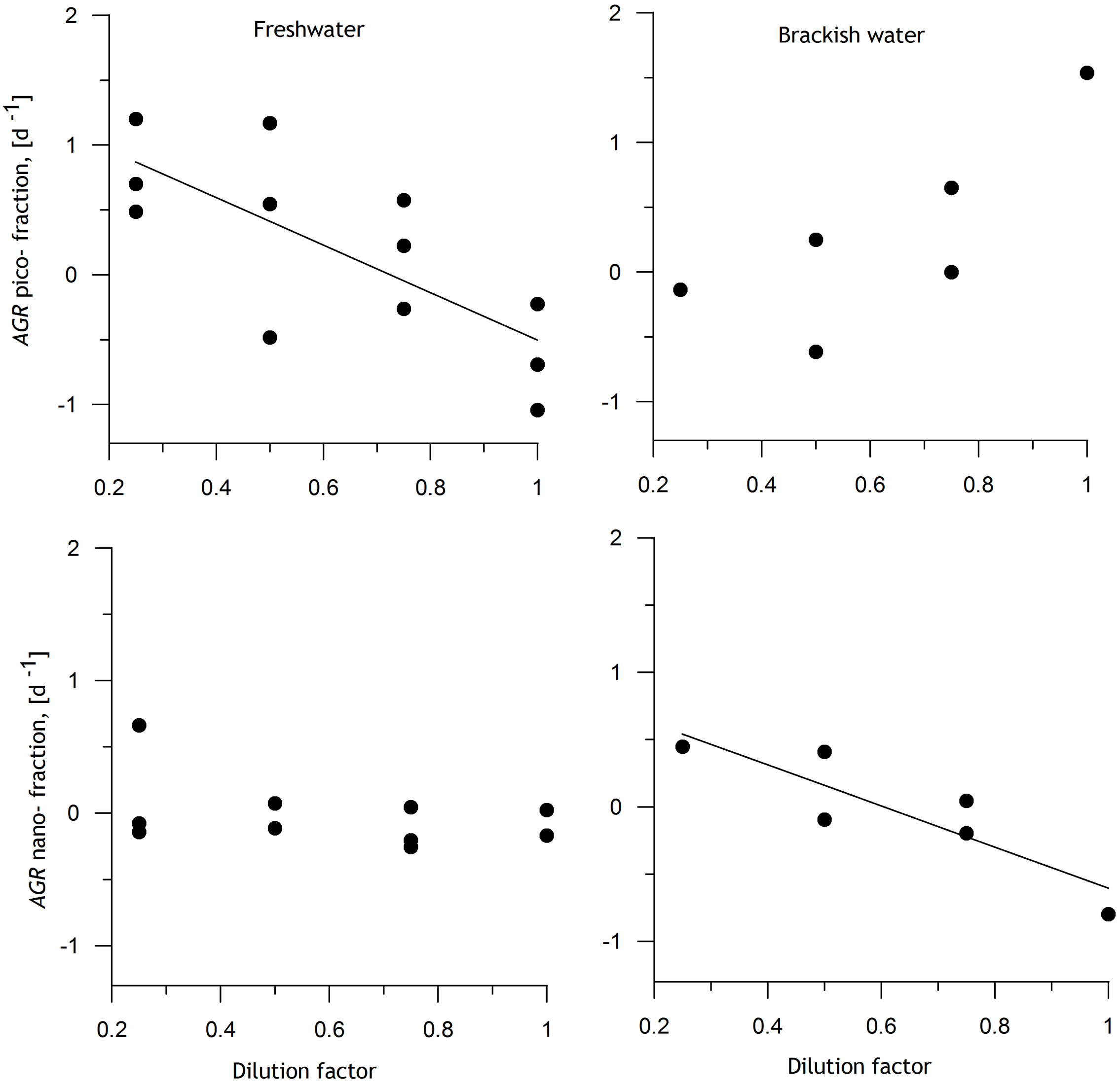
Figure 1 Pigment concentrations of pico- and nanophytoplankton at experimental sites.

Figure 2 Relative abundance of ciliate functional groups and size classes at experimental sites.

Figure 3 Relationship between dilution factor and apparent growth rate (*AGR*) of chlorophyll *a* of pico- and nano- fractions at both sites. Only significant slopes are presented in the graph.


16
12
8
4
0
Pigments concentration, [µg L-1]
2-20 µm
0.2-2 µm
2-20 µm
0.2-2 µm
Freshwater
Brackish water
Chlorophyll a
Fucoxanthin
Divinyl chlorophyll a
Lutein
Beta caroten
Aloxanthin
Zeaxanthin
19'-Hexanoyloxyfucoxanthin


Relative abundance, [%]
0
20
40
60
80
100
Nano-filterers
Pico/nano-filterers
Pico-filterers
Omnivorous
Predators
Relative abundance, [%]
0
20
40
60
80
100
< 20 µm
20-30 µm
30-60 µm
> 60 µm
Freshwater
Brackish water


Freshwater
Brackish water
AGR pico- fraction, [d -1]
AGR nano- fraction, [d -1]
2
1
0
-1
0.2
0.4
0.6
0.8
1
Dilution factor
Dilution factor

APPENDIX 4

Baltic Sea *Pseudocalanus*: diel vertical migration patterns & escape behaviour

Kristin Hänselmann, Jens-Peter Herrmann & Axel Temming

University of Hamburg

We used video plankton recorder (VPR) data to investigate if copepods (here egg sac carrying *Pseudocalanus acuspes* females in the central Baltic Sea, Bornholm Basin) still show diel vertical migration patterns (DVM – as described in Möller, 2013) when the likely trigger for this behaviour (in this case the clupeid swarm-fish sprat – *Sprattus sprattus*) is missing. Baltic *P. acuspes* stay at depths around the halocline, where they find sufficient amounts of food, as well as high salinities favourable for their reproduction. During daytime, sprat migrate from surface waters towards the halocline, to prey on residing copepods. In avoidance of this predation risk, copepods show DVM patterns. They shift to water masses above and below their favourable habitat (Möller, 2013). In 2012, sprat abundances where notably lower than in 2002 and 2009, when copepod DVM patterns where investigated by Möller and colleagues based on VPR data. Our study draws the comparison of copepod behaviour between those years, and comes to the conclusion that no DVM pattern can be observed for ovigerous *P. acuspes* females in the central Baltic Sea in years with low sprat abundances.

1. Methods

1.1 Sampling area

The copepod data analysed in this study was derived from a cruise with RV Alkor in July 2012 at Bornholm Basin, located in the central Baltic Sea (figure 1). Eight VPR tows were conducted between 29.07.2012, 14 pm and 30.7.2012, 12 pm. Acquired data was analysed with respect to diel vertical migration patterns of ovigerous *Pseudocalanus* copepods. The Baltic Sea shows a strong stratification during summer months, with distinct thermo- as well as haloclines. Therefore, this sampling area is predestined for studying vertical migration patterns of planktonic organisms.

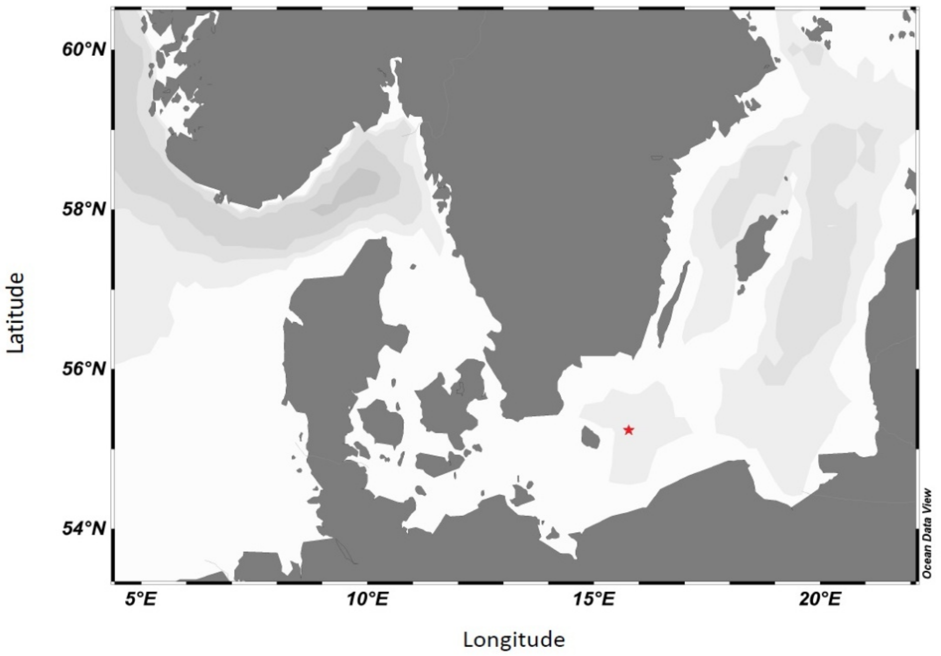


Figure 1. Sampling area (red star) of cruise AL398 in July 2012, located in the central Baltic Sea (Bornholm Basin).

1.2 VPR

The VPR (Seascan) is an underwater video microscope system, used for quantification of planktonic organisms. This optical sampling method has the advantage to be non-invasive and enables scientists to get a glimpse of planktonic behaviour in the field. For the 2012 data, the VPR was attached under a V-fin and towed with 3 kn ships speed undulating through the water column. A total sampling volume of 28423 L was examined.

1.2.1 Technical settings

Technical settings of the VPR include a 1 mega pixel colour camera (Bayer filter, Uniq model UC-1830CL, Pentax 12.5-75 mm F1.8 Lens) with a resolution of 1024 x 1024 pixels, and a mean frame rate of 14 per second, as well as a Xenon strobe (Seascan, maximum rate of 30 flashes per second, 1 joule energy per flash). The applied camera setting was a field of view of 24 x 24 mm with a calibrated image volume of 34.39 mL. Accessory sensors on the VPR included a FastCat 49 CTD (Sea-Bird), as well as an ECO Puck FLNTU fluorometer and turbidity sensor (WetLabs).

1.2.2 VPR data

In contrast to Möller, 2013, we used a VPR system that runs autonomous. There is no real time transmission of image data to an onboard unit, but image data is recorded internally and written to a USB key immediately after the tow. Image processing and classification was conducted as described in Möller, 2013. VPR data from 2012 was compared to data derived by Möller in 2002 and 2009 (Möller, 2013).

1.3 Fishery data

To illustrate that the sprat abundance in 2012 was insufficient to serve as a trigger for vertical migration of copepods, we compared catch data from the respective cruises in 2002 and 2012. This data contained information about haul duration as well as total weight and numbers of caught sprat. We calculated a mean weight and a mean number of caught sprat per 30 minute fishing activity. Fishing gear were pelagic trawl nets: a kombi trawl (KT) with a mesh size of 10 mm (2002 & 2009) (Stepputtis, 2006), and a young fish trawl (YFT) with a mesh size of 5 mm (2012). Sampling was performed in depths between 20 and 65 m (2002), 20 and 78 m (2009) as well as 15 and 79 m (2012) during daytime, corresponding to the diurnal feeding time of sprat.

Due to the fact, that fishing with a kombi trawl results in approximately four times higher catches than with a young fish trawl (based on the information about “spread between doors”: KT 111 m, YFT 24.7 m), the calculated kombi trawl results have been divided by this factor.

Because of the differing sampling gear, the calculated values can only serve as a rough indication for higher sprat abundance in 2002 than in 2012. For 2009, echosounder data shown in figure 3 (displayed as nautical area scattering coefficient, “NASC values”) indicate high sprat abundance. Further publications on this topic will feature echosounder data of the cruise in 2012 for comparison. This data is not available yet.

1.4 Day/Night classification

To compare the frequency distribution of copepod data from 2002, 2009 and 2012, we divided the available data in day and night samples. Daytime was hereby defined as 04:00-20:00h, and nighttime as 20:00-04:00h. This definition was based on information about sunrise and sunset in July 2002, 2009 and 2012 at the Bornholm Basin, central Baltic Sea (available at http://jekophoto.de/tools/daemmerungsrechner-blaue-stunde-goldene-stunde/index.php).

2. Results & Discussion

2.1 Copepod & Fishery data

Comparing the occurrence of ovigerous *Pseudocalanus* females over time, data of 2002 and 2009 show a distinct gap in depths around 60 m, from approximately 4 am to 5 pm (figure 2). This is probably due to migrating sprat, which feed in those depths during daytime. The upper dots might represent individuals showing an escape behaviour, while the lower dots most likely represent individuals conducting a vertical downward shift to avoid spatial overlap with sprat (Möller, 2013). Figure 3 shows high NASC values around 60 m, indicating high abundance of fish filling the “copepod gap”.

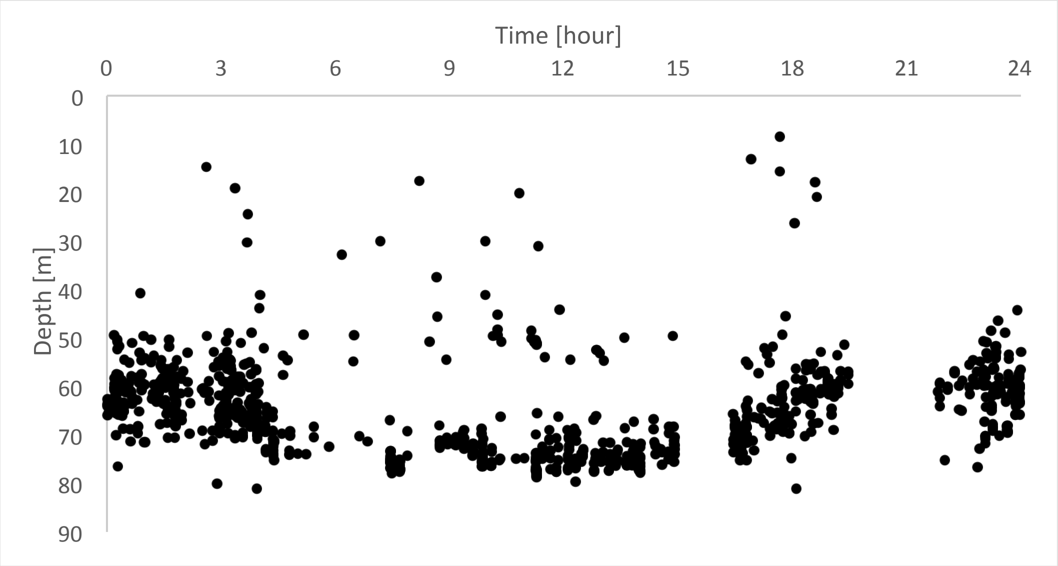


Figure 2. Ovigerous Pseudocalanus females, data obtained from cruises in April 2002 & May 2009, Baltic Sea, Bornholm Basin; 912 copepods sampled; sprat abundance sufficient to serve as vertical migration trigger.

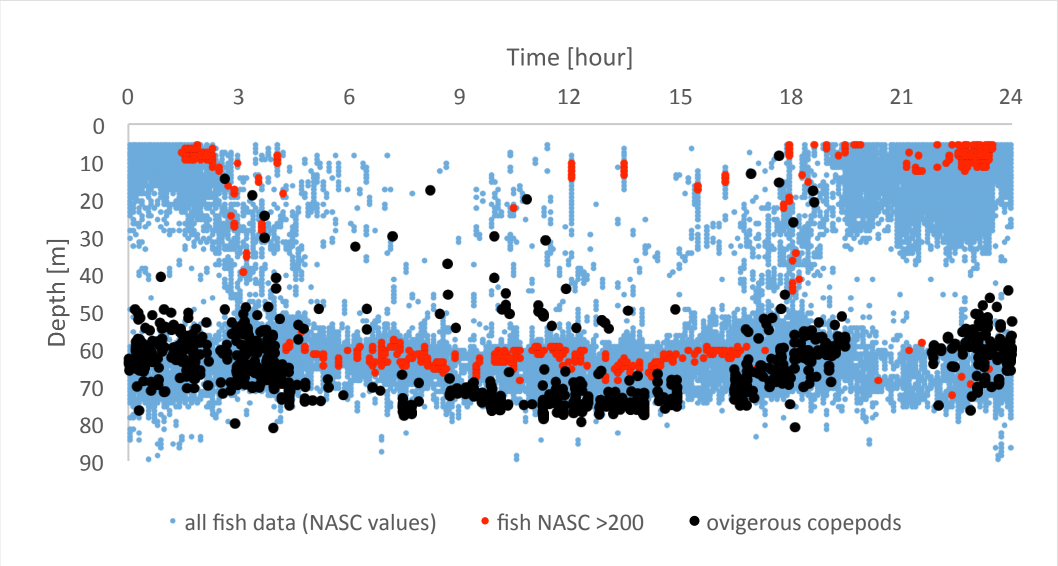


Figure 3. Copepod (April 2002 & May 2009) and fish (2009) distribution, Baltic Sea, Bornholm Basin; blue dots - all fish data (NASC values), red dots – fish data with NASC values >200, black dots - ovigerous copepods (Pseudocalanus).

This distribution picture changes, when looking at the copepod data of 2012. The favourable habitat for *Pseudocalanus* lies in depths around 60 m (figure 4), where a quite evenly distribution of ovigerous copepods can be observed during day- as well as nighttime (figure 5). An example for the missing vertical migration behaviour could lie in the lower sprat abundance of that year. Fishery data of the 2012 cruise show a mean weight of 5 kg and 373 individuals of sprat caught per 30 minutes of fishing activity. For the comparative cruise in 2002, mean values of 57 kg and 5599 individuals of sprat per 30 minutes of fishery hauls were calculated. Echosounder data of 2009 (figure 3 – blue and red dots) confirm the high abundance values in 2009. Lower abundance of sprat implies a lower predation risk for the copepods. Hence, ovigerous *Pseudocalanus* females have no reason to leave their favourable habitat during daytime in years with lower sprat abundance.

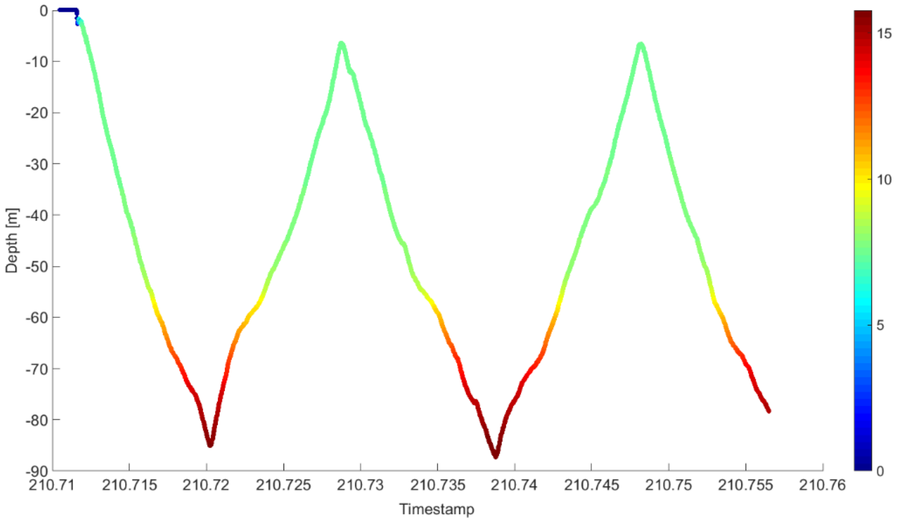


Figure 4. Example for the salinity conditions present during all VPR tows of cruise AL398 in 2012 (due to strong stratification of the Baltic Sea during summer). This situation here occurred during a VPR tow on 29.07.2012, 17:04-18:25h at Bornholm Basin, central Baltic Sea. Colour bar shows salinity values in PSU.

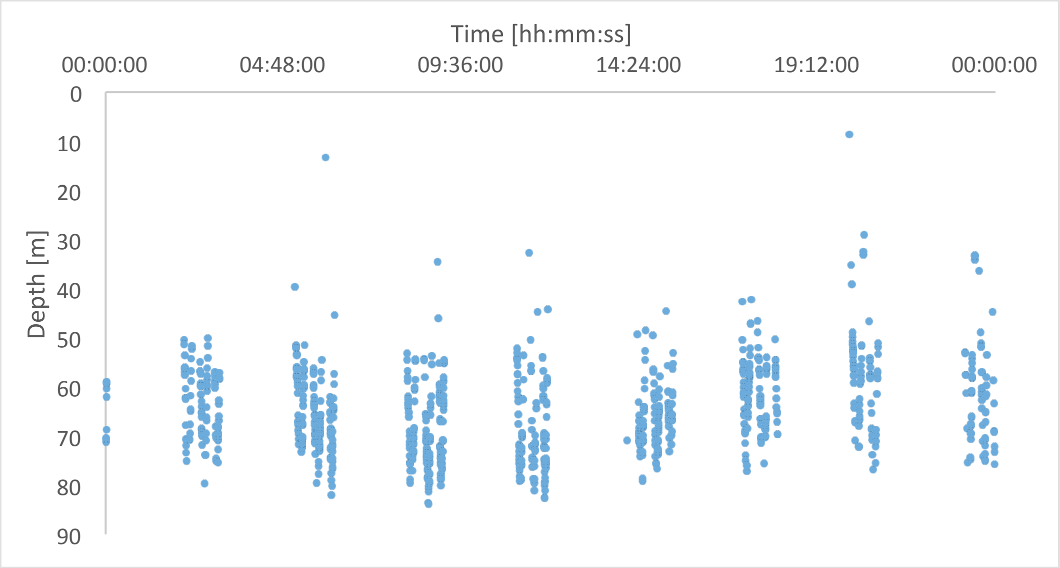


Figure 5. Ovigerous Pseudocalanus females, data obtained from cruise AL398 in July 2012, Baltic Sea, Bornholm Basin; 891 copepods sampled; sprat abundance insufficient to serve as vertical migration trigger.

2.2 Day & Night distribution

Available copepod data from 2002, 2009 and 2012 was divided into day and night samples, to produce depth frequency distributions. Day samples (04:00-20:00h) from 2002 and 2009 display the “copepod gap” in depths around 63 m (figure 6, black line). The difference to figure 2 (no copepods visible at all in depths around 60 m) is due to the fact, that figure 6 takes all copepod data between 4 am and 8 pm into account, whereas the gap in figure 2 does end already at about 5 pm. After this time, copepods have migrated back towards 60 m and by that appear in the data of figure 6. There is no “copepod gap” in the 2012 data (figure 6, grey line). That year featured lower sprat abundance than 2002 and 2009, and by that offered no trigger for copepod vertical migration.

To our understanding, the copepods that reside in water masses above 60 m during daytime in 2012, do not necessarily show an escape behaviour (as described in Möller, 2013), but have already been inhabiting these depths during night. In contrast, the individuals that were sitting between 60 and 70 m during night migrate towards deeper waters during day to reduce feeding pressure by sprat in years with high densities of sprat. This behaviour is reflected in the two peaks in figure 6 (black line).

Night samples from 2002 and 2009 show a rather unimodal distribution (figure 7, black line) – sprat reside within surface waters during night, and by that there is no feeding pressure on copepods in the preferred depth of P. acuspes. Copepod numbers in the 2012 data slightly decline around 63 m (figure 7, grey line). This pattern might be an artefact resulting from a combination of the undulating sampling strategy and the patchy distribution of planktonic organisms. Towing the VPR in an undulating fashion through the water column, delivers spatially isolated data points for a defined water depth. Since copepods aggregate in irregular patches the encounter of such patches becomes a game of pure chance.

Conclusion:

While generally the interaction between sprat and P. acuspes is referred to as a potential top down control case, these data reveal, that the interaction is more complex. Apparently P. acuspes have evolved a behavioural response to the peak predation pressure, which occurs mainly in April and May at peak spawning of sprat. The vertical downward escape provides P. acuspes with a protection mechanism against predation, ensuring the survival of suffient numbers of egg producing adults during the main egg production season of P. acuspes in spring.

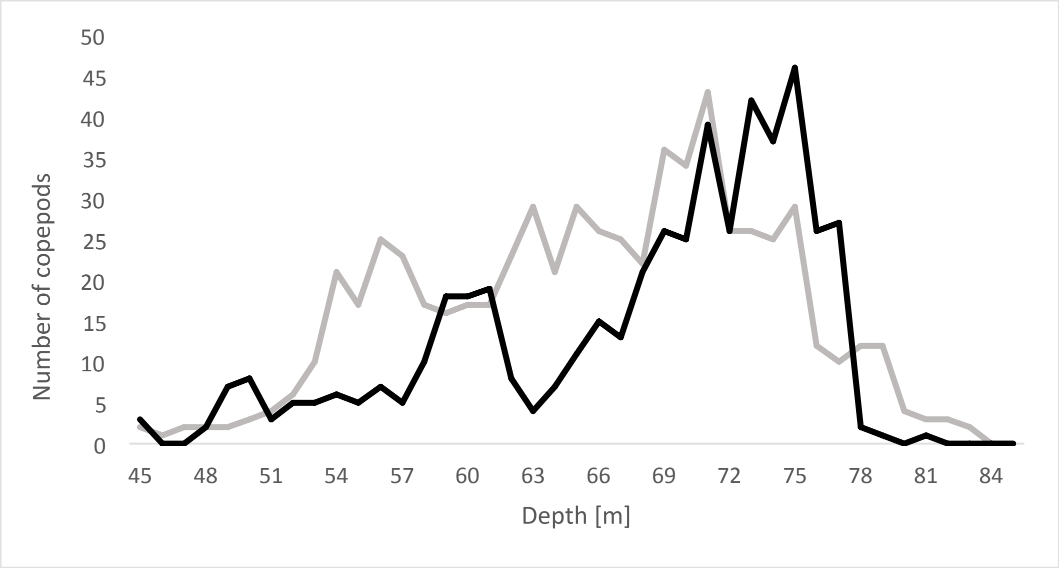


*Figure 6. Ovigerous Pseudocalanus females, frequency distribution 04:00-20:00h, “**Day**” – black line: data obtained from cruises in April 2002 & May 2009, Baltic Sea, Bornholm Basin; 912 copepods sampled; sprat abundance sufficient to serve as vertical migration trigger – grey line: data obtained from cruise AL398 in July*

2012, Baltic Sea, Bornholm Basin; 891 copepods sampled; sprat abundance insufficient to serve as vertical migration trigger. For reasons of clarity, only depths around the halocline (45-85 m) are shown.

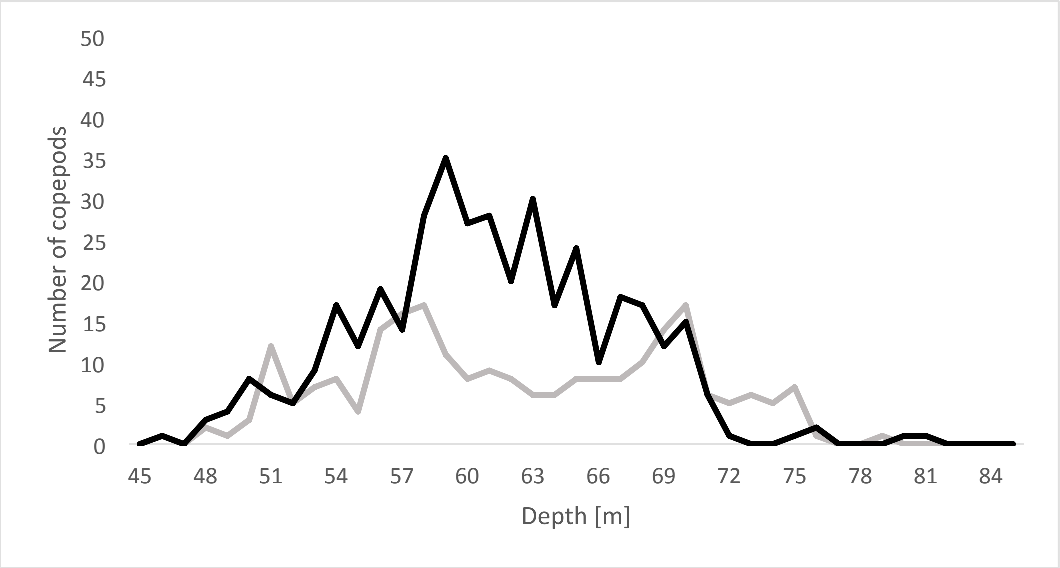


*Figure 7. Ovigerous Pseudocalanus females, frequency distribution 20:00-04:00h, "**Night**" – black line: data obtained from cruises in April 2002 & May 2009, Baltic Sea, Bornholm Basin; 912 copepods sampled; sprat abundance sufficient to serve as vertical migration trigger – grey line: data obtained from cruise AL398 in July 2012, Baltic Sea, Bornholm Basin; 891 copepods sampled; sprat abundance insufficient to serve as vertical migration trigger. For reasons of clarity, only depths around the halocline (45-85 m) are shown.*

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Stepputtis, D., 2006. Distribution patterns of Baltic sprat (Sprattus *sprattus* L.) – causes and consequences. PhD Dissertation, Mathematisch-Naturwissenschaftliche Fakultät der Christian-Albrechts-Universität zu Kiel.

APPENDIX 11: Seasonal patterns in jellyfish feeding ecology assessed with stable isotope analysis.

Accepted for publication in the journal Marine Biology. Text included here will be replaced with the journal formatted text once it is avaiable

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Temporal dietary shift in jellyfish revealed by stable isotope analysis

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Abstract

A temporal change in the stable isotope (SI) composition of jellyfish in the Kiel Fjord, Western Baltic Sea, was documented by analyzing $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of bell tissue of *Aurelia aurita* and *Cyanea capillata* in the period between June and October 2011. A strong and significant temporal change in all SI values of *A. aurita* was found, including an increase of ~3‰ in $\delta^{13}C$, a decrease of ~4‰ in $\delta^{15}N$ and sharp decline of ~7‰ in $\delta^{34}S$. While knowledge gaps in jellyfish isotope ecology, in particular the lack of reliable trophic enrichment factors (TEFs), call for a conservative interpretation of our data, observed changes in particular in $\delta^{34}S$, as indicated by means of a MixSIR mixing model, would be consistent with a temporal dietary shift in *A. aurita* from mesozooplankton (> 150µm) to microplankton and small re-suspended particles (0.8-20 µm) from the benthos. Presence of a hitherto unidentified food source not included in the model could also contribute to the shift. During the two-month occurrence of *C. capillata*, its isotope composition remained stable and was consistent with a mainly mesozooplanktonic diet. Mixing model output, mainly driven by $\delta^{34}S$ values, indicated a lower proportion of *A. aurita* in the diet of *C. capillata* than previously reported, and thus to a potentially lesser importance of intraguild predation among jellyfish in the Kiel Fjord. Overall, our results clearly highlighted the potential for substantial intraspecific isotopic seasonal variation in jellyfish, which should be taken into account in future feeding ecology studies on this group.

Keywords: Jellyfish, Scyphomedusae, Trophic level, Food web, Baltic Sea

Introduction

Global awareness has been drawn to the increase of jellyfish blooms due to their possible negative impacts on ecosystem goods and services, such as interference with tourism, aquaculture, fishing operations and coastal industrial intakes (Richardson et al. 2009, Condon et al. 2012). Population outbreaks of carnivorous jellyfish account for severe impacts on marine food webs, driven by a rapid population growth rate in combination with a highly successful competition for food sources (Hay 2006, Gibbons and Richardson 2013). Populations of *Aurelia aurita* medusae have been known to consume roughly two-thirds of daily secondary production (mainly copepods) and thus compete with fish larvae for resources in the Kiel Bight, Baltic Sea (Behrends and Schneider 1995; Schneider 1989). In order to determine the ecological role and impact of jellyfish on marine food webs, it is important to gain a thorough understanding of their trophic ecology by comprehending both the formation and structure of their blooms, as well as their likely role in the transfer of carbon and energy in the marine food web (Pitt et al. 2009).

In recent years, there has been a rapid rise in the use of stable isotope (hereafter SI) analysis as a tool for studying trophic ecology, which led to a better understanding of origin, pathways and fate of organic matter (Robinson 2001, Michener and Kaufman 2007). By comparing SI values of a consumer over time, information on trophic transfer, carbon and energy flux, and contribution of food sources to the diet of an organism can be gained (Kling et al. 1992, Cabana and Rasmussen 1996a, Ponsard and Arditi 2000). $\delta^{13}C$ and $\delta^{15}N$ have been most commonly used to address ecological questions (review by Grey 2006), since carbon (C) isotopes are well suited to identify the primary carbon sources at the base of a food web (Peterson 1999) and nitrogen (N) isotopes are a good tracer of the trophic position of an organism (Cabana and Rasmussen 1996b). The use of additional elements has increased recently, e.g. sulphur (S) isotopes can reveal whether a food web is driven by benthic or pelagic primary production (Hansen et al. 2009; Jaschinski et al. 2008).

For many groups of animals, information on temporal SI changes is already available (Carlier et al. 2007); however, despite their ecological importance, to date, this information is lacking for most species of jellyfish, leading to misinterpretation of trophic ecology of gelatinous taxa (Fleming et al. 2015; Pauly et al. 2009). At the same time, recent work by Fleming et al. (2015) highlights that such variation in jellyfish can be substantial. Here, we were interested in the strength and patterns in intraspecific seasonal variation in SI values of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ of the pelagic jellyfish species *A. aurita* and *C. capillata* during their bloom period (June – October 2011) in Kiel Fjord, western Baltic Sea. Secondly, we interpreted these values in the context of isotope composition of dietary sources to assess potential temporal changes in diet composition of these two species.

Materials and Methods

Study location- Kiel Fjord constitutes a small and shallow extension of the Kiel Bight in the Belt Sea (Fig. 1) with a mean depth of about 13 m (Javidpour et al. 2009). During most of the investigation period, the water column was well-mixed except during a short period of less than 15 days from July to August, where a weakly thermal stratification was detected.

Sampling- Weekly sampling in the Kiel Fjord was carried out during the annual occurrence of jellyfish from June to November 2011.During this period, *A. aurita* occurred from June to the beginning of October and *C capillata* from the beginning of October to the end of November. A WP3 net with 1 mm mesh size was used to capture jellyfish by means of integrated vertical sampling between depths of 0 and 15 m. At each sampling event, five individuals per species were chosen from the collected material, and bell diameter (inter-rhopalia) was recorded. Specimens were kept in filtered sea water for 2 hours at 20°C, after which no remaining prey items were observed in the guts, indicating that this period was sufficient to ensure complete gut evacuation (FitzGeorge-Balfour et al. 2013). Total wet mass of each individual was then measured to the nearest 0.01 g.

Prior to preparation for stable isotope analysis the specimens were washed with filtered seawater (0.2µm filter). Bell tissue of each individual, the most suitable body part for SI measurements in *A. aurita* (D'Ambra et al. 2014), was dissected, rinsed using milli -Q water, dried to constant dry weight at 50-60 °C and ground to a fine powder using mortar and pestle. Subsamples of 4 ± 0.05 mg, found to yield optimum results in initial analyses, were then weighed out and sealed in tin cups.

Stable isotope data of potential food sources for the same time period including seston and mesozooplankton were obtained from Mittermayr et al. (2014a). Seston samples were sieved through a 20µm mesh to separate zooplankton, and were then filtered on 0.8µm cellulose acetate filters (Sartorius) and carefully scraped off into distilled water with plastic cell scrapers before being desiccated in small watch glasses. Since phytoplankton cannot be reliably separated from similar sized heterotrophic or detrital POM for stable isotope analysis, seston samples were treated as proxy for mixed microplankton food sources. A study by Sommer and Sommer (2004) supports this procedure as they were not able to find a clear connection between seston size fractions and their SI values. In the inner Kiel Fjord, seston can represent a mixture of phytoplankton and protozoans as well as re-suspended particles from benthos. Mesozooplankton samples were collected using a150-µm mesh size plankton net. As spatial variation within the south and central Baltic Sea area only accounts for 0.4% of the total variance in mesozooplankton isotopic values (Agurto 2007), the use of Mittermayr et al. (2014a) data was deemed plausible for comparative purposes in this investigation considering that sampling sites are only ~7 km apart.

Stable Isotope Analysis- Analysis of samples was conducted with a continuous-flow isotope-ratio mass spectrometer (Europa Scientific ANCA-NT 20-20 Stable isotope analyzer with ANCA-NT Solid/Liquid Preparation Module) at the University of California at Davis’ stable isotope facility. Delta notation was used as follows:

$$\delta X(‰) = [(R_{sample}/R_{standard}) - 1] * 1000$$

where X = ^{15}N, ^{13}C or ^{34}S and R= $^{15}N/^{14}N$, $^{13}C/^{12}C$ or $^{34}S/^{32}S$. Reference materials for the calculation of δ-values were atmospheric N_2 for N, Vienna Pee Dee Belemnite for C and SO_2 for S. During analysis, samples were interspersed with replicates of two internal laboratory standards, nylon and bovine liver, previously calibrated against International Atomic Agency reference materials (IAEA-N1, -N2, -S-1, -S-2, -S-3 and USGS-40), in order to correct for drift. The long term standard deviation was 0.2 ‰ for $\delta^{13}C$ and $\delta^{34}S$, 0.3 ‰ for $\delta^{15}N$ and 0.4‰ for $\delta^{34}S$.

Lipid content might severely affect $\delta^{13}C$ values, resulting in ^{13}C depleted values in correspondence with high lipid content and is therefore an important issue to address (DeNiro and Epstein 1977, Post et al. 2007). Post et al. (2007) advises to conduct lipid correction on $\delta^{13}C$ values for aquatic animals if lipid content is higher than 5% of the biomass, or if C: N ratios are higher than 3.5. Since this was the case for C:N ratios of both *A. aurita* and *C. capillata* (see Table 1), $\delta^{13}C$ values were corrected for lipid content based on the methods of Post et al. (2007) and D'Ambra et al. (2014). Both methods led to relatively small shifts in $\delta^{13}C$ and very similar patterns over time compared to our original values. However, while the Post et al. correction slightly decreased variability in our dataset, the D’Ambra et al. correction introduced additional noise into the data set and increased the variability especially at the beginning of the season (*supplementary Fig.* S.1). Therefore, we decided to apply the correction by Post et al. to our original $\delta^{13}C$ data set.

Calculation of Dietary Composition Based on MixSIR- To determine potential contributions of different food sources to the diet of the collected jellyfish, a mixing model (MixSIR) based on Bayesian probability was applied. MixSIR is a graphical user interface (GUI) built on MATLAB that employs an algorithm based on a Bayesian framework to determine the probability distributions for proportional contributions of each food source to the diet mix of a consumer (Semmens and Moore 2008). This model allows for allocation of different fractionation factors ± standard deviation (SD) for each element and source respectively and accounts for uncertainty in isotope values when estimating contributions of sources.

Fractionation values of 0.5 ± 0.5‰ for δ^{13}C (France and Peters 1997, Jaschinski et al. 2011) and 0 ± 0.2‰ for δ^{34}S (Michener and Kaufman 2007) were chosen for all trophic level transfers; for δ^{15}N, 2.4 ± 1.1‰ and 3.4 ± 1.1‰ were chosen for the first and following trophic level transfers respectively (Currin et al. 1995, Vanderklift and Ponsard 2003; Zanden and Rasmussen 2001). MixSIR was run with δ^{13}C, δ^{15}N and δ^{34}S values of *A. aurita* and *C. capillata* on a bi-weekly basis. To account for turnover rates as reported by D'Ambra et al. (2014), where bell tissue of *A. aurita* reached SI steady state with laboratory diet after 18-20 days, a lag time of two weeks between stable isotope values of jellyfish and stable isotope values of their potential food sources was used in the model.

*Statistical Analy*sis- Our initial data exploration for *A. aurita*, zooplankton and seston was carried out with the response variables δ^{13}C, δ^{15}N and δ^{34}S and time (date) as explanatory variable following the protocol described in Zuur et al. 2010. The non-linear relationship between response (SI) and explanatory variables (time) warranted the application of a generalized additive model (GAM) to δ^{13}C, δ^{15}N and δ^{34}S. Data on *C. capillata* were analysed by applying one-way ANOVA for each stable isotope value to determine differences among sampling time points as well as for comparison of SI values of *A. aurita* and *C. capillata.* All statistical assumptions such as normality and constant variances were checked for any analysis and were checked for outliers. Statistical analyses were performed in the software R 3.0.3 (Development Core Team, 2011).

Data management- raw data of the stable isotopes of jellyfish species underlying this paper are available at PANGAEA (http://doi.pangaea.de/10.1594/PANGAEA.858057).

Results

Seasonal changes in jellyfish occurrence, size and C: N ratios- A. aurita was present in all samples from June to September, whereas, *C. capillata* was found only on four occasions in September and October. Biometric measurements of *A. aurita* indicated a significant increase in mean (±SD) diameter over time ($F_{(3,50)}$ =3.8, $p = 0.01$) with a steep increase from June (16.5 ± 3.6 cm) to July (22.8 ± 6.9 cm), followed by a decrease in individual mean size in August and September (19.8 ± 5.3 cm and 17.0 ± 3.1 cm respectively). Maximum mean (±SD) wet mass was recorded in July (531 ± 355 g ind^{-1}). Total carbon (µg) and nitrogen (µg) per 4 mg dry mass showed a peak in August, with 72.7 ± 42.7 µg and 18.9 ± 10.9 µg, respectively (mean ± SD). On average C:N ratios decreased from spring to summer and stayed constant until fall. Maximum C:N values were observed in June (5.4 ± 0.9), whereas the ratio was significantly lower in September (4.7 ± 0.7) (GAM, F = 59.1, $p < 0.01$).

In contrast, during the period of its occurrence (September – October), *C. capillata* showed neither evidence of growth nor change in wet mass or total carbon and nitrogen values (Table 1). C:N ratios also remained constant (3.7 ± 0.4 in Sep. and 3.6 ± 0.2 in Oct.).

Temporal variability in jellyfish stable isotope values - Strong directional temporal changes in all three isotopic markers occurred in *A. aurita* (Fig. 2 a, and Table 2). *A. aurita* δ^{13}C values ranged from -23.9 ± 0.6‰ (mean ±SD) in June, to -21.3 ± 0.4‰ in September with a significant linear increase (GAM, F = 68.6, $p < 0.001$) towards the end of the season. While seston δ^{13}C values were increasing significantly (GAM, F = 39.7, $p < 0.001$) from June (-26.0 ± 1.4) to September (-18.7 ± 1.1), zooplankton δ^{13}C values increased from June (-25.2 ± 2.6) to the beginning of August (-21.8 ± 0.2), before decreasing from mid-August onward (GAM, edf = 3.7, F = 41.4, $p < 0.001$, Fig. 2a).

Maximum δ^{15}N values of *A. aurita* were measured in June with 14.8 ± 2.3‰. These values then rapidly decreased to 10.9 ± 2.3‰ in early July (GAM, F = 15.4, $p < 0.01$), followed by a slight increase until the end of the period of occurrence in September (11.8 ± 0.7‰) (Fig. 2b). δ^{15}N values of seston and zooplankton changed little over the observation period (GAM, F =

1.8, $p = 0.5$; F = 1.2, $p = 0.3$, respectively), ranging from 6.3 ± 1.3‰ and 6.6 ± 1.1‰ in early June to 4.0 ± 1.0‰ and 6.1 ± 1.1‰ in late July and 5.0 ± 0.9 ‰ and 6.5 ± 1.3 ‰ in late September, respectively (Table 2).

Temporal variation in δ^{34}S of *A. aurita* was particularly pronounced, with a high in June and July (on average 17.4 ± 1.8‰ and 17.6 ± 2.0‰ respectively), followed by a steady decline (GAM, F = 45.3, $p < 0.01$) of more than 7‰ until late September (9.8 ± 0.7‰) (Fig. 2c). In contrast, δ^{34}S of zooplankton decreased from 20.9 ± 1.0‰ in early June to 18.2 ± 1.1‰ in early July, followed by a slight increase to 19.1 ± 2.4‰ in September (GAM, F = 29.8, $p >$ 0.01). Seston changed from 11.9 ± 3.2 in June to 12.4 ± 0.9 in late September. Temporal variation was significant (GAM, F = 5.2, $p < 0.01$), but of much lower magnitude than for *A. aurita*. To better illustrate temporal changes in SI composition of *A. aurita*, biplots of δ^{13}C - δ^{15}N and δ^{15}N - δ^{34}S with respect to sampling date are provided in Fig 3.

In contrast to *A. aurita, C. capillata* showed fewer changes over the period of its occurrence in Kiel Fjord (Table 2, Fig. 4). There was a significant increase (ANOVA, $F_{(1,18)} = 6.9$, $p = 0.01$) in δ^{13}C of *C. capillata* from September (-21.1 ± 0.6‰) to October (-20.5 ± 0.5‰), but no significant changes in δ^{15}N or $\delta^{34}S$ were measured. During the short period of species co-occurrence in September, the mean δ^{13}C values of *C. capillata* were not significantly different from *A. aurita* ($F_{(1,18)} = 1.6$, $p = 0.2$), but δ^{15}N ($F_{(1,17)} = 8.1$, $p = 0.01$) and δ^{34}S ($F_{(1,18)} = 632.7$, $p < 0.001$) showed highly significant differences, and no evidence for an approximation of values over time.

Contribution of prey sources to the diets of jellyfish - Regarding the analysis of potential contributions of different prey sources to the dietary mix of *A. aurita*, and assuming that all potential food sources were included, the MixSIR mixing models indicated a drastic shift from a mesozooplankton based diet (96.6 ± 0.8% of total possible food sources) to a seston based diet (99.8 ± 0.2%) at the end of the growing season in September (Fig 5a).

In contrast, the MixSIR mixing model for *C. capillata* indicated that this species fed mainly on mesozooplankton prey items over the limited period of observation in Kiel Fjord, whereas *A. aurita* comprised less than 15% of its diet, and seston was nearly absent from its diet (Fig. 5b).

Discussion

The pronounced shifts of ~3‰ in δ^{13}C, ~4‰ in δ^{15}N and the sharp decline of ~ 7‰ in δ^{34}S within the same population of the jellyfish species *A. aurita* over a period of four months highlighted the potential for substantial intraspecific isotopic seasonal variation in jellyfish populations in their natural environment. This underscores the importance to account for such changes in SI feeding ecology studies on this group to avoid misinterpretation of datasets. Because the temporal changes of the SI values of potential prey items, in particular for δ^{34}S, were much lower it seems most likely that the dietary composition of *A. aurita* changed significantly over time. This interpretation was strengthened by the shift in *A. aurita* δ^{13}C values which again differed from the pattern of the shifts in SI values of the potential prey.

δ^{34}S of POM in Kiel Fjord was recorded at ~ 21‰, whereas sediment δ^{34}S was at ~1‰ (Hansen et al. 2009). These two extremes represent the isotopic endpoints of potential food sources at the base of the local food webs, i.e., δ^{34}S isotopic values of all components in Kiel Fjord food webs generally fall within this range. δ^{34}S has therefore been used in previous studies as indicator of benthic versus pelagic dietary sources (see e.g., Jaschinski et al. 2011; Mittermayr et al. 2014a). While δ^{34}S fractionation rates of jellyfish has not been reported so far, the strong shift to lower δ^{34}S values of *A. aurita* over time may suggest a dietary shift from strictly pelagic to benthic food sources. We were unable to analyze gut contents to support this hypothesis, however, our mixing model results would be consistent with a switch from pelagic mesozooplankton as the main carbon source to benthic microplankton (e.g. protozoan) and/or resuspended organic particles from the benthos over the course of 4 months. In this context, considering the brief (two week) duration and the weak nature of stratification during the study period, hydrographical changes probably were not a driver of the observed changes in SI values.

The changes observed in *A. aurita* SI values during its growing season in Kiel Fjord have important implications. Firstly, there is an ongoing debate in the field of isotope ecology regarding the need to account for species- specific temporal variation in isotopic values (Fleming et al. 2015; Jennings et al. 2008). Our finding confirm recent results by Fleming et al (2015) with respect to substantial temporal variation in C and N values of jellyfish, and in addition highlighted particularly strong variation in S SI values over time that has not been previously assessed. The pronounced and rapid temporal changes observed here strongly underscore that SI feeding ecology studies in particular of jellyfish that do not account for this variation can result in misinterpretation of datasets. This point is illustrated by the fact that conclusions regarding the feeding ecology of *A. aurita* would be diametrically opposite when choosing only one isolated sampling point in June vs. a point in September. Secondly, bentho-pelagic coupling is a key ecosystem process (Marcus 1998). Our data and the resulting mixing model suggest that in contrast to the exclusively planktonic feeding ecology commonly assumed for this species (Behrends and Schneider 1995, Hansson et al. 2005, Moller and Riisgard 2007), it may also depend on benthic food sources at the base of its food web (see also Pitt et al. 2008). This would have consequences for assessments of the ecological role and impact of jellyfish, and should be considered in the parameterization of food web models.

While the period of overlap of *A. aurita* with *C. capillata* was relatively short, this study nevertheless provides the first insights regarding the trophic interactions between these two species in Kiel Fjord based on SI analysis. Previously, based on both field and experimental observations, *C. capillata* has mainly been described as an important predator of *A. aurita* (Bamstedt et al. 1994, Hansson 1997, Titelman et al. 2007), although Hansson concluded that assimilation rate estimates were needed to clearly define the proportion of *A. aurita* in the diet. In contrast, while the short temporal overlap and the absence of significant growth of *C.*

capillata means that this result needs to be treated with caution, our data provide an indication that the role of *A. aurita* in *C. capillata* diet may be lower than previously thought. At the time of first occurrence in Kiel Fjord in September, the δ^{34}S values of *C. capillata* were significantly higher (+ ~8‰) than the values of *A. aurita*. Over the following period of overlap, no temporal approximation in δ^{34}S values – which would be expected under the scenario of *C. capillata* feeding on *A. aurita* and assuming that turnover rates reported by D'Ambra et al. (2014) for *A. aurita* do apply,- occurred. Instead, *C. capillata* δ^{34}S isotope values remained close to pelagic isotopic ratios, which were reflected by the estimated contribution of *A. aurita* to the diet of *C. capillata* of only 15% as indicated by the MixSIR model.

To conclude, this study demonstrates the potential of triple stable isotope datasets to gain novel insights into the feeding ecology and ecological role of jellyfish, which is urgently needed due to the rising concern about worldwide increases of this marine ecosystem component in the course of global change (Gibbons & Richardson 2013). Furthermore, carefully designed experimental designs are required in order to account for potential temporal variation in consumers and their prey to unlock the full potential future of such approaches.

Limitations of the study

The data reported here support the assumption that diet composition of *A. aurita* has changed over time not only from mesozooplankton to microzooplankton food, but also from a more pelagic source to a benthic one. It is important to mention that the MixSIR model results leading to this conclusion were mainly driven by the significant change in δ^{34}S values of *A. aurita*. Results in δ^{13}C and δ^{15}N do not contradict this conclusion, but taken by themselves would have allowed different interpretations as well. In particular, the offset between *A. aurita* and the two assumed dietary source categories (zooplankton and seston) is always larger than >5‰. We assume here that this difference is due to trophic fractionation, which would place *A. aurita* on the upper end of the range reported for other organisms, and larger than the value previously reported by D'Ambra et al (2014). An alternative explanation would be the presence of an additional trophic complexity, e.g., an unidentified dietary source with a higher δ^{15}N value and similar δ^{34}S value compared to seston not included in our mixing model, although the low δ^{34}S values would then still support a more benthic origin of material at the base of the food web in fall (Jaschinski et al. 2008, Mittermayr et al. 2014).

Regarding our conclusion of limited feeding of *C. capillata* on *A. aurita*, it is important to consider that fractionation rates in particular for δ^{34}S, and for jellyfish feeding on other gelatinous prey, have not been reported, i.e., we are assuming that general relationships in isotope ecology will hold true, however this assumption needs validation in the future. Again, δ^{13}C and δ^{15}N values do not contradict this result, but based on C and N alone, a higher importance of *A. aurita* in the prey would have been a possible solution as well.

Finally, as in other isotope ecology studies, it is important to consider that SI fractionation factors may in part depend on the physiological state and the sexual maturity of an organism. However, while the effect of metabolic change on turnover rates has been assessed (Bearhop et al. 2004), little information exists on changes in fractionation. For practical reasons, rates are therefore commonly assumed as stable over time in SI feeding ecology studies (e.g., Michener and Kaufman 2007). *A. aurita* developed gonads in mid-June and its sexual reproduction started in late July (unpublished data) which likely explain the observed slight C:N decrease of *A. aurita* during this time (Milisenda et al. 2014). It is still unclear to which extent the reproductive stage of jellyfish might influence SI fractionation factors, but as no obvious pattern coincided with the timing of reproduction here, we considered the effects as limited.

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Table 1: Temporal biometric data (mean±SD) collected for *A. aurita* and *C. capillata* from June to October 2011

Table 2: Isotope values (mean±SD) of potential food sources (after Mittermayr *et al.* 2014) and jellyfish from June to October 2011.

Fig. 1: Study area in the Western Baltic Sea and the Kiel Fjord with sampling stations Witlingskuhle (circle) and Falkenstein station of Mittermayr *et al* 2014 (plus sign).

Fig. 2: δ^{13}C, δ^{15}N, δ^{34}S of *A. aurita* (diamond), zooplankton (square) and seston (circle) over the course of 5 months in the Kiel Fjord (Jun- Oct 2011). X-axis represents Julian days.

Fig. 3: Stable isotope values of δ^{13}C vs. δ^{15}N (panel A) and δ^{34}S vs. δ^{15}N (panel B) for *A. aurita*. Numbers indicate the date of sampling.The symbols were connected in the temporal order of the data points, thus providing a time trajectory of change in isotope values.

Fig. 4: δ^{13}C, δ^{15}N, δ^{34}S of *C. capillata* (filled diamond) and *Aurelia aurita* (open diamond) over the course of two months in the Kiel Fjord (Sep- Oct 2011).

Fig. 5: Graphical outcome of MixSIR models indicating percentage of mesozooplankton (grey bar) and microplankton (black bar) to the diet of *A. aurita* (panel A) and *C. capillata* (panel B) from June to October 2011 in a biweekly time span.

Supplementary files:

S. 1: : δ^{13}C correction for lipids using two methods of D'Ambra et al. 2014 (diamonds) and Post et al. (triangles) compared to our raw data of this study (cross signs).

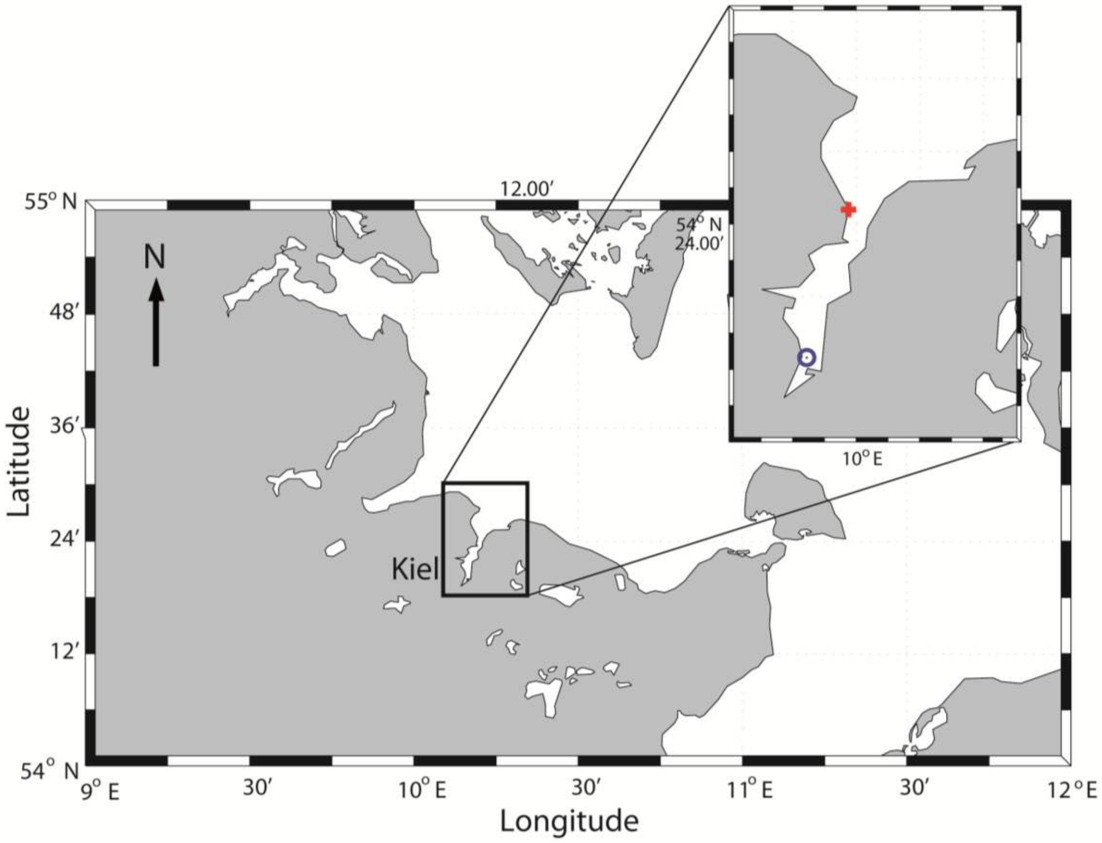
Table 1.

Month (2011)	**Species**	**Sample Size (n)**	**Wet mass (g) (±SD)**	**Length (cm) (±SD)**	**C (µg/4mgDW) (±SD)**	**N (µg/4mgDW) (±SD)**	**C:N Molar ratio**
June	*A. aurita*	9	204.2 (92.1)	16.5 (3.6)	26.1 (9.1)	16.5 (3.6)	5.4 (0.9)
July	*A. aurita*	15	530.9 (355.3)	22.8 (6.9)	25.0 (18.7)	6.5 (5.2)	4.7 (0.9)
August	*A. aurita*	20	367.5 (256.3)	19.8 (5.3)	72.7 (42.6)	18.9 (10.9)	4.5 (0.1)
September	*A. aurita*	10	161.2 (95.0)	17.0 (3.2)	28.7 (13.4)	7.2 (3.6)	4.7 (0.67)
September	C. capillata	11	157.6 (97.5)	12.4 (3)	73.0 (27.4)	19.4 (6.6)	3.7 (0.4)
October	C. capillata	10	232 (161)	13.6 (4)	69.9 (25.3)	19.3 (7)	3.6 (0.2)

Table 2

	June_1	June_2	July_1	July_2	August_1	August_2	September_1	September_2	October_1	October_2
Seston										
δ^{15}N	6.3 ± 1.3	5.1 ± 1.2	4.4 ± 1.4	4.0 ± 1.0	2.3 ± 0.2	5.1 ± 1.0	6.0 ± 0.9	5.0 ± 1.0		
δ^{13}C	-26.0 ± 1.4	-25.0 ± 1.7	-21.6 ± 3.5	-21.1 ± 1.4	-20.5 ± 1.4	-21.0 ± 1.2	-19.1 ± 2.2	-18.7 ± 1.1		
δ^{34}S	12.0 ± 3.2	9.9 ± 3.0	11.9 ± 1.2	11.9 ± 2.9	12.4 ± 0.2	13.2 ± 1.2	14.0 ± 2.9	12.4 ± 1.5		
Zooplankton										
δ^{15}N	6.6 ± 2.0	7.7 ± 1.2	7.1 ± 1.1	6.1 ± 1.1	7.4 ± 0.6	7.3 ± 1.6	6.9 ± 1.9	6.5 ± 1.3		
δ^{13}C	-25.2 ± 2.6	-24.6 ± 2.3	-25.1 ± 0.5	-23.1 ± 1.2	-21.8 ± 0.2	-22.1 ± 1.4	-23.0 ± 1.6	-21.8 ± 3.1		
δ^{34}S	21.0 ± 1.0	18.2 ± 2.0	18.2 ± 1.1	18.6 ± 0.4	18.6 ± 1.2	18.9 ± 1.2	18.9± 1.4	19.1 ± 2.4		
Aurelia										
δ^{15}N	15.1 ± 2.2	14.4 ± 2.6	11.0 ± 2.3	11.5 ± 2.2	11.2 ± 0.7	11.9 ± 0.5	11.3 ± 0.8	11.8 ± 0.7		
δ^{13}C	-22.0 ± 1.5	-22.4 ± 1.9	-20.7 ± 1.7	-22.8 ± 0.5	-21.8 ± 0.3	-21.4 ± 0.5	-20.9 ± 0.5	-20.6 ± 0.3		
δ^{34}S	17.7 ± 1.8	17.0 ± 2.0	19.9 ± 1.5	16.4 ± 0.6	13.9 ± 1.7	11.7 ± 0.3	10.7 ± 0.2	9.8 ± 0.7		
Cyanea										
δ^{15}N							15.9 ± 3.0	12.4 ± 0.5	12.5 ± 0.5	15.3 ± 3.2
δ^{13}C							-22.0 ± 0.6	-20.9 ± 0.2	-20.9 ± 0.5	-20.6 ± 0.4
δ^{34}S							17.8 ± 0.3	18.4 ± 0.9	17.7 ± 0.8	18.4 ± 0.4

Fig. 1



55° N
48′
36′
24′
12′
54° N
9° E
30′
10° E
30′
11° E
30′
12° E
Latitude
Longitude
N
Kiel
12.00′
54° N
24.00′
10° E

Fig. 2

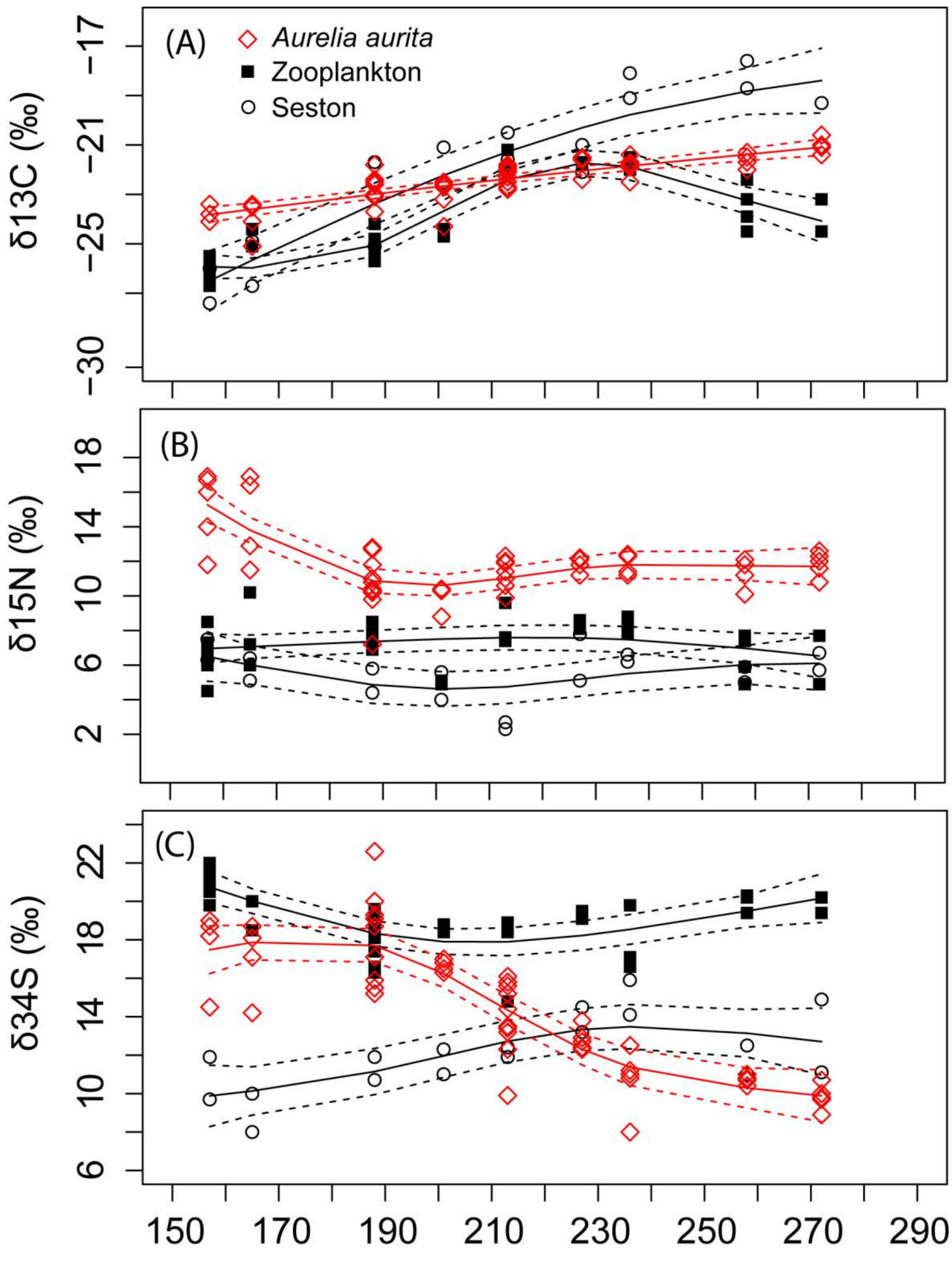

(A)
◇ *Aurelia aurita*
■ Zooplankton
○ Seston
δ13C (‰)
−17
−21
−25
−30
(B)
δ15N (‰)
18
14
10
6
2
(C)
δ34S (‰)
22
18
14
10
6
150
170
190
210
230
250
270
290

Fig. 3

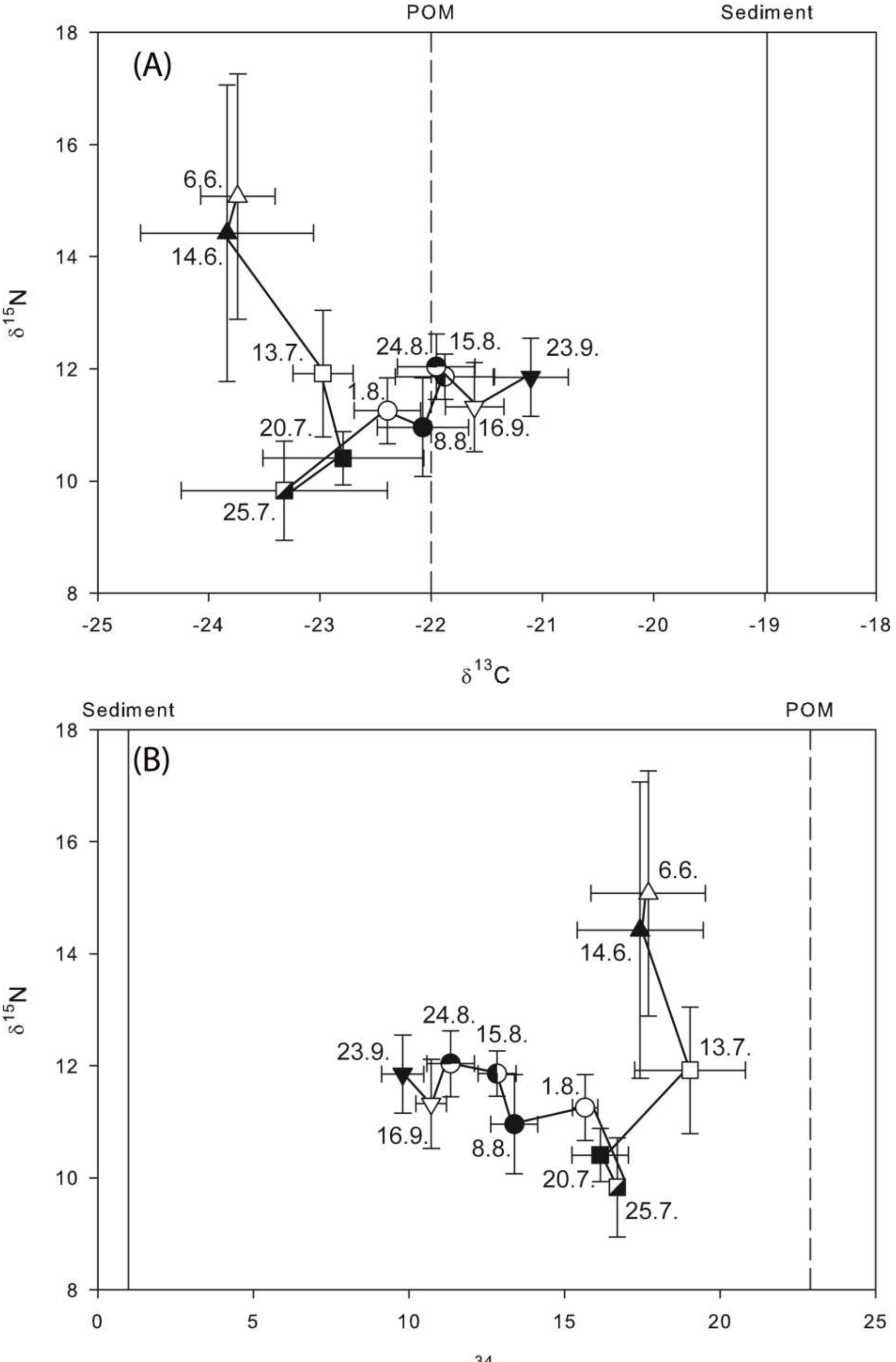

POM
Sediment
(A)
18
16
14
12
10
8
-25
-24
-23
-22
-21
-20
-19
-18
$\delta^{15}N$
$\delta^{13}C$
6.6.
14.6.
13.7.
20.7.
25.7.
1.8.
8.8.
15.8.
24.8.
16.9.
23.9.
Sediment
POM
(B)
18
16
14
12
10
8
0
5
10
15
20
25
$\delta^{15}N$
$\delta^{34}S$
6.6.
14.6.
13.7.
1.8.
20.7.
25.7.
24.8.
15.8.
23.9.
16.9.
8.8.

Fig. 4

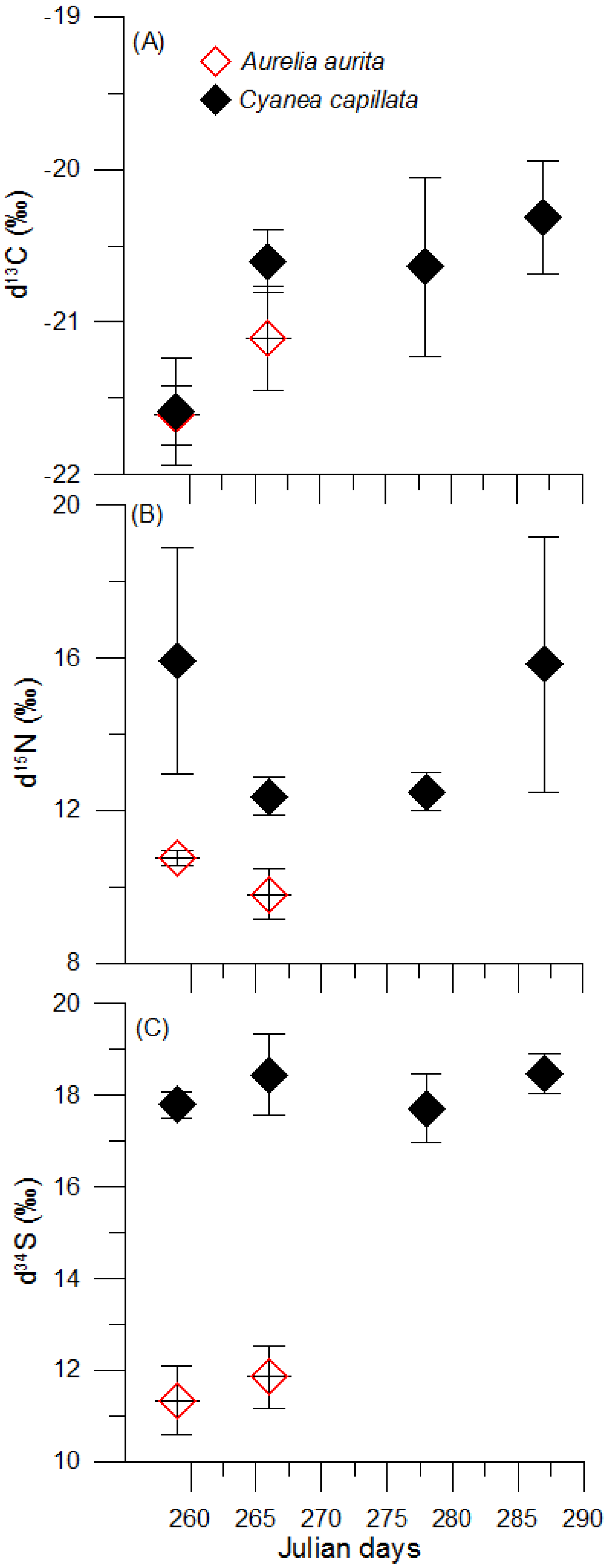
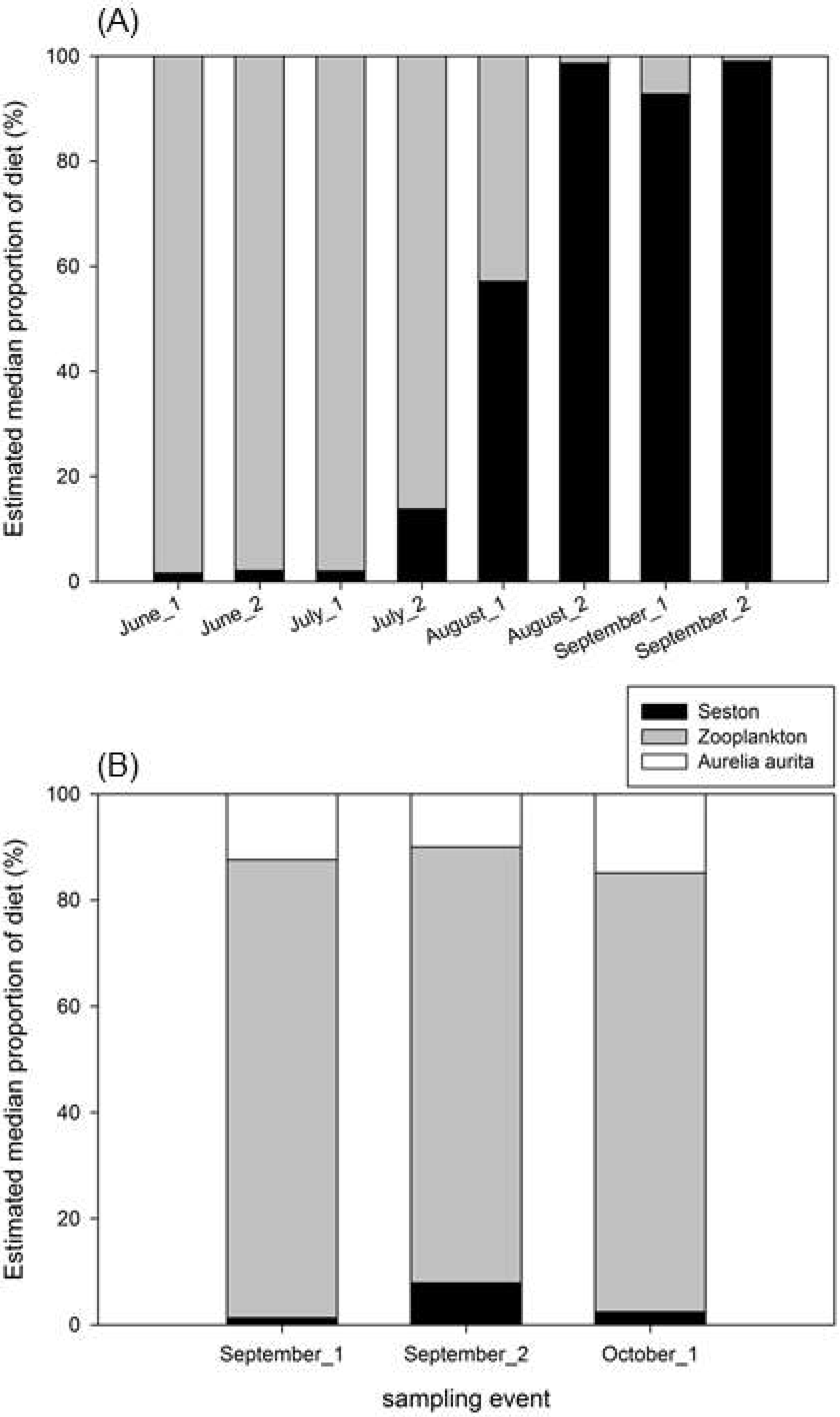

(A)
◇ Aurelia aurita
◆ Cyanea capillata
d13C (‰)
-19
-20
-21
-22
(B)
d15N (‰)
20
16
12
8
(C)
d34S (‰)
20
18
16
14
12
10
260
265
270
275
280
285
290
Julian days

Fig. 5


(A)
Estimated median proportion of diet (%)
0
20
40
60
80
100
June_1
June_2
July_1
July_2
August_1
August_2
September_1
September_2
Seston
Zooplankton
Aurelia aurita
(B)
Estimated median proportion of diet (%)
0
20
40
60
80
100
September_1
September_2
October_1
sampling event

APPENDIX XII

Multispieces model runs and trophic control

Extensive multispecies and ecosystem research has been done in the Baltic in the past about 30 years. ICES has together with several institutes around the Baltic for decades invested substantially in the research on multispecies interactions, ecosystem functioning and integrated assessment. Currently, there exist several multispecies and ecosystem models for the Baltic Sea (for an overview cf. ICES 2012a). SMS results were scrutinised in more detail as they were considered for use in management advice (ICES 2012b, STECF 2012).

There are some concerns regarding the possibility to model multispecies aspects predictably for the future. In particular the multi-species aspects depend on predation data from mainly the 1980s and there is an urgent need to update the information base. Also the current regime in terms of productivity and spatial distribution of fish stocks in the Baltic is different from the earlier period when predation data were collected. However, the present problem in age determination of cod hamper the inclusion of new stomach data collected and put into a database already, and the most probable solution is a purely length based model that is currently under development, but is not yet available to BIO-C3.

Management of fisheries for cod can have an impact on fishing opportunities for sprat and herring, and vice versa. Cod are predatory, and their main prey is sprat and, to a lesser extent, herring and also juvenile cod (cannibalism). In addition, herring and sprat sometimes feed on the eggs of cod. Furthermore, growth of herring and sprat has been density-dependent (Fig. 1), and growth of cod has, to some extent, been dependent on herring and sprat biomass.

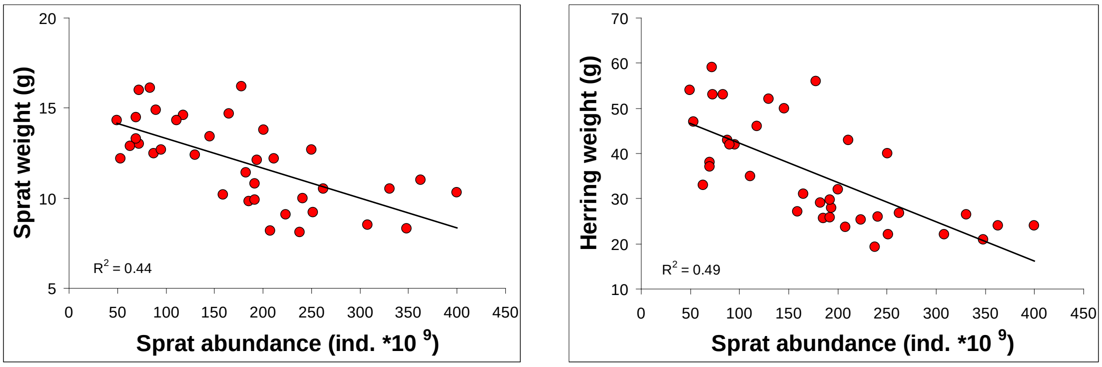


Figure 1. Relation between clupeids mean weights at age 3 and sprat total abundance (data from WGBFAS 2011).

Finally, the relative distributions of predator (cod) and prey (herring and sprat, possibly juvenile cod) have changed substantially during the last years, and for the time being much herring and sprat are outside the predatory reach of cod (Figs. 2 and 3).

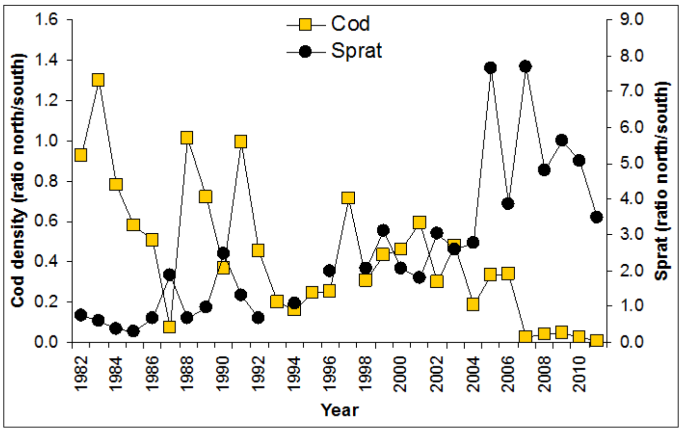


Figure 2. Changes in spatial distribution of Eastern cod and sprat during the past 30 years. This is indicated as density ratio between northern areas (SDs 27-29) and southern areas (SDs 25-26) from acoustic (sprat) and bottom trawl surveys (cod). Modified from Casini et al. (2011).

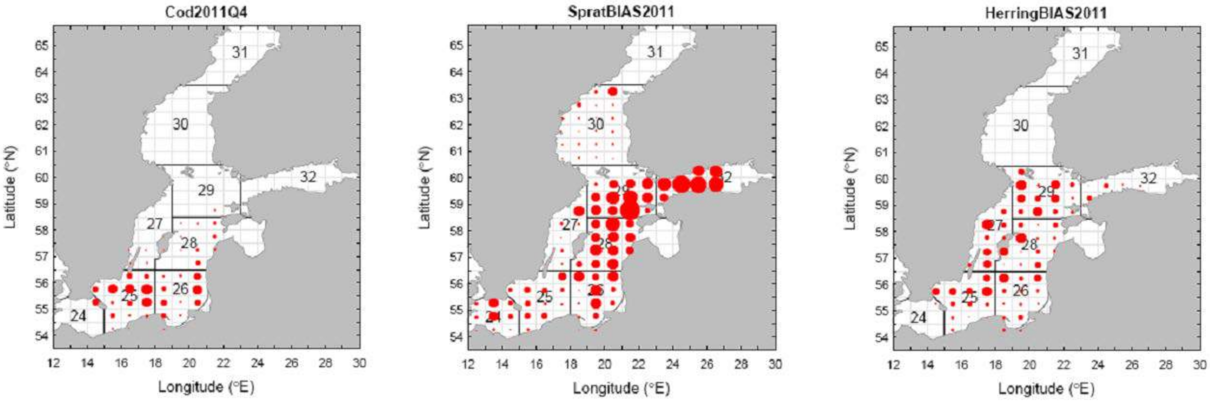


Figure 3. Spatial distribution of Eastern cod, sprat and Central herring in 4th Quarter 2011, from acoustic survey (BIAS, sprat and herring) and bottom trawl survey (BITS, cod).

The combination of an increasing cod stock and low abundance of sprat and herring in SD 25 (in the main distribution area of cod) has resulted in the lowest biomass of clupeids per cod currently available in this area since the 1970s. In line with low biomass of clupeids in the area, the mean weight of older cod (age-groups 4-7) in SD 25 has sharply declined since 2007 (Figure 4).

The mean annual growth rate of grey seal stock in the Baltic has been on average 7.5% annually during the last decade. In 2010, a total of approximately 23 100 grey seals were counted. The increase in stock size was highest in the northern areas and the predation pressure of grey seals on clupeoids has increased accordingly. The diet of grey seal in the Baltic consists of ca. 20 fish species. The most abundant prey items in the Baltic proper are Baltic herring, sprat, and cod, and in the Bothnian Sea and Bothnian Bay Baltic herring, *Coregonus* sp., Baltic salmon, and sea trout. An adult seal consumes on average round 4.5 kg fish per day, of which 55% are clupeoids in the Baltic Main basin and 70% in the Bothnian Sea and Bothnian Bay. According to acoustic estimates, predator– prey distribution patterns, migration patterns, and multispecies analysis (SMS), the predation effect of grey seals on Baltic herring and sprat stocks is still at a very low level. Hence, with present grey seal stock sizes, the impact of seal predation can be ignored in whole Baltic-scale herring and sprat stock management considerations. Locally, however, grey seal–fishery interactions play an important role and should be taken into account in future spatial planning and ecosystem management.

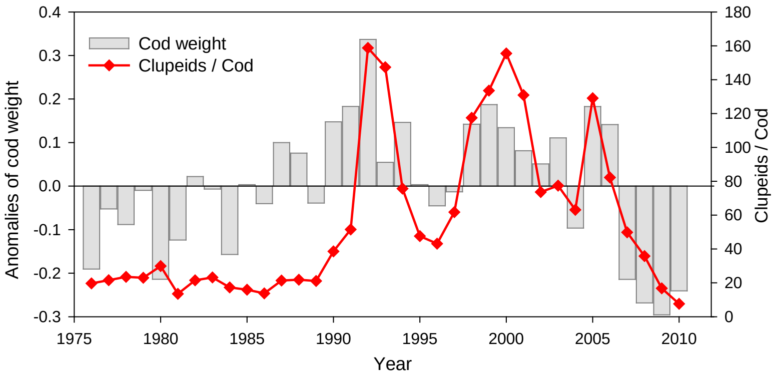


Figure 4. Anomalies in mean weight of cod (average of age-groups 4–7) in SD 25 (bars) compared to changes in the biomass of clupeids (sprat and herring) relative to the number of adult cod (at age 4 and older) in the same area (line).

While the effects of spatial distributions of predator and prey can be assessed in the retrospective runs of the SMS model, for forecast there is a limited knowledge on the processes that lead to changes in spatial distributions. Moreover, when taking clupeid density-dependent growth in consideration, the Fmsy estimated by SMS are very high for both herring and sprat, and the reason for this should be further investigated. For these reasons, both STECF (2012) and ICES (2012b) decided for the so called ‘one-area’ option and also ignored density dependent growth in Management Strategy Evaluations (MSE).

All the multispecies Fmsy values for Eastern Baltic cod, Central Baltic herring and Baltic sprat are higher than the single species values. Particularly for cod and sprat higher Fs give very similar yields on the long term and will give lower SSBs and in some cases risks of stock decline to the “lower biomass” reference points (that is a first suggestion for a lower SSB to avoid impaired recruitment). Model results indicate that although higher Fs on Eastern Baltic cod give little increase in cod yield, a higher cod F gives higher yields from Baltic sprat and Central Baltic herring. As current modelling for Fmsy does not include any structural uncertainty, risks of stock decline and impaired cod recruitment will be higher than those estimated. The presence of year-year constraints in change in cod TAC increases the variability in stock size and the increases are greater in a multi-species system (for detailed results, please cf. STECF 2012; in this report only the main results will be presented).

The present distribution pattern, with a limited distribution range for cod (concentrated in the southern area) and basin wide distribution for herring and sprat (but mainly concentrated in the northern areas, at least in some seasons) (Figs. 2 and 3), implies that an increase in F on cod, not necessarily will result in increasing Baltic wide clupeid stock sizes. Conversely a decrease in F on cod will not necessarily result in a decrease of the Baltic clupeid stock size if it will not be accompanied by a cod expansion to northern areas. However, cod cannibalism will be higher and

limited growth of cod due to food deprivation will become a bigger problem. On the other hand, a reduction of clupeid F in Sub-division 25 will likely improve growth and condition of cod as well as reduce cannibalism. An increase in clupeid F in northern areas (SDs 27-32) will likely not have a negative effect on cod, since this will not affect the stock component distributed in southern areas (SD 25-26). Further, a higher F on clupeids in northern areas would likely reduce density dependence and improve the growth and condition of clupeid stocks (ICES 2012b).
Higher Fmsy proxies for herring and sprat are also obtained when density dependent growth is assumed for the two species, as the stocks compensate by a higher growth at lower stock densities due to either higher fishing mortalities or predation.

Basin scale spatial overlap and its consequences for trophic control

As well as for the whole Baltic Sea (Uzars 1994), also in the Bornholm Basin the number and the weight ratios of herring to sprat in the stomachs of adult cod in March were highly variable (neuenfeldt & Beyer 2006). The variability in number ratio was not related to the predicted variability (Koester *et al.* 2001) in the abundances of herring and sprat (Fig. 5 A).

When accounting for prey biomass instead of prey abundance, it has to be considered that herring and sprat during the late 1980s and early 1990s underwent some decrease in condition due to food limitation (Möllmann *et al.* 2005), selective predation of cod (Jensen & Sparholt 1992), or mixing of sub-stocks with different growth rates (ICES 1997). However, also applying biomass there was no effect on the herring to sprat ratio in cod stomachs identifiable (Fig. 5 B).

As well as abundance or biomass trends do not explain changes in the herring to sprat ratio in cod stomachs, also peaks in the amount of cod in cod stomachs did not follow the relative prey biomasses (**I**).

Therefore, higher order mechanisms in the predator-prey relationship may be responsible. For example, both herring and sprat are schooling pelagics. In spring, herring migrate out of the Bornholm basin in order to spawn in coastal areas (Aro 1989, Klinkhardt 1996). Changes in the timing of this spawning migration might cause variability in the herring abundance in the basin and therefore affect the herring to sprat ratio in cod stomachs.

Due to the vertical gradients in the Baltic, salinity and oxygen thresholds correspond to thresholds depths limiting the habitat volumes of predator and prey. For the pelagic species herring and sprat these habitat volumes can simply be calculated by integrating the volume between upper

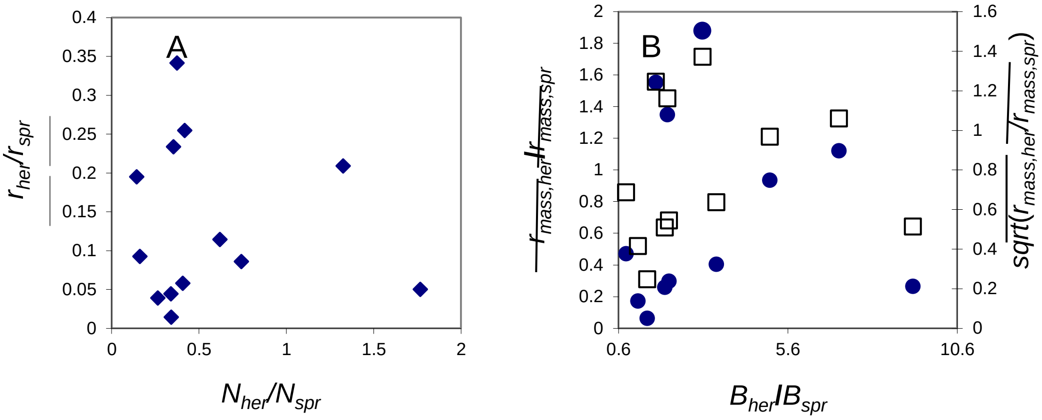


Fig. 5: Abundance ratio of herring to sprat in the stomachs of cod vz. herring age group 1 to sprat age groups 1+ abundance ratio in the sea (A); average herring to average sprat mass ratio in cod stomachs, and square-rooted averages vz, herring to sprat biomass ratio (abundance and biomass data from Koester et al. 2001).

and lower thresholds depths horizontally, using high resolution bathymetry data (MacKenzie *et al.* 2000, Neuenfeldt 2002). When cod are demersal, *i.e.* outside the spawning season, their free vertical range has to be accounted for in the estimation of the cod habitat volume and overlap (Harden Jones & Scholes 1985, Neuenfeldt & Beyer 2006).

The habitat volumes of cod, herring and sprat in the study area vary due to the frequency and intensity of inflows from the North Sea, and they furthermore vary seasonally (Neuenfeldt & Beyer 2003). Under the assumption of homogenous predator and prey distributions inside their habitat volumes, the consequence of these variations is that predator and prey densities vary independent of changes in abundances.

An additional complication arises since the habitat volumes of cod (predator) and the clupeids (prey) often do not overlap completely (Neuenfeldt & Beyer 2003). Such environment-driven overlaps appear to be very common. For example, they have been observed in the field for Atlantic salmon and rainbow smelt in a large-lake ecosystem (Pientka & Parrish 2002), and for Atlantic cod and capelin at Newfoundland (Rose & Leggett 1989). Hydrographic features were correlated to the spatial overlap between larval cod and haddock, and their predators Atlantic herring and Atlantic Mackerel on Georges Bank (Garrison *et al.* 2000). Also the co-occurrence of several fish species on Gorges Bank in commercial trawl catches was correlated to temperature and depth preferences (Murawski & Finn 1988).

With the term PEV_i indicating the potential encounter volume, *i.e.* the water volume where prey i and the predator co-occur (Neuenfeldt 2002**)**, there are two operational ways to formulate predator-prey habitat overlap for integration into food selection and functional response models, both from the predator and from the prey perspective:

$$O_i = \frac{PEV_i}{H_{pred}} \qquad ; \qquad Q_i = \frac{PEV_i}{H_i} \qquad (1)$$

The predator-prey overlap O_i defines the fraction of the predator habitat (H_{pred}), where prey i occurs, whereas the prey-predator overlap Q_i (henceforth termed occupation) defines the fraction of

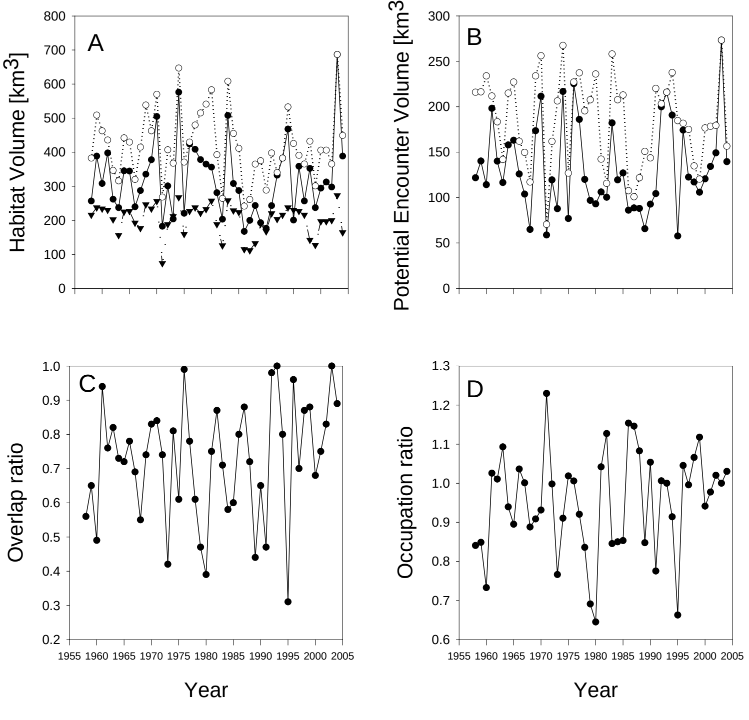


Fig. 6. February-March habitat volumes in the Bornholm Basin for cod (triangles), sprat (circles) and herring (dots) versus time (A). Potential encounter volume for herring (dots) and sprat (circles) (B), and the resulting herring to sprat overlap ratio O_{her}/O_{spr} (C) and occupation ratio Q_{her}/Q_{spr} (D).

the prey habitat (H_i), where prey individuals run the risk of encountering a predator.

In the simplest case, disregarding spatial heterogeneity on scales smaller than the population dispersion scale, and considering the Baltic case with one predator and two prey species, the *per capita* aggregate functional response f_i^* with respect to prey species i (i = 1, 2) is calculated as the weighted average of the individual functional responses f_i in the different strata of the predator habitat with the relative predator abundances n_j / n in the strata as weighting factors. Here, $\rho_{i,j}$ denotes the stratum-specific prey densities, n the total predator abundance, and n_j the predator abundance in stratum j:

$$f_i^* = \sum_{j=0}^{2} \frac{n_j}{n} f_i(\rho_{1,j}, \rho_{2,j}) , \qquad \text{with} \qquad n = \sum_{j=0}^{2} n_j \tag{3}$$

The same concept of using local densities as weights is used in Chesson (2000) and Hassel *et al.* (1991) to determine population dynamics in spatially-varying environments. The new component is that the relative predator abundance n_j / n in each stratum can, in the case of 2 prey species, be represented by environmentally driven habitat overlaps (eq. 1), (Neuenfeldt & Beyer 2006):

$$\frac{n_j}{n} = \begin{cases} 1 - O_1 & ; j = 0 \\ O_1 - O_2 & ; j = 1 \\ O_2 & ; j = 2 \end{cases} \tag{4}$$

The dependence of habitat overlaps on the environmental stratification, which itself depends on climatically driven inflows, partially decouples prey mortality from fluctuations in predator and prey densities. Traditional niche overlap measures (see Krebs 1989 for a review) cannot be used as weighting factors for local predator densities, because predator and prey densities are combined to an ambivalent measure.

The occurrence of specific prey types in predator stomachs from different localities or depths can be used to confirm trawl based potential encounter volumes provided that the evacuation rate of a specific prey is not very high with a simultaneously very low ingestion rate.

When it comes to analysing the importance of population overlap for relative predation rates, the *per capita* aggregate functional response ratio f_2^* / f_1^* is a convenient starting point. Food items other than the two prey species in focus can be neglected, because in the functional response ration the denominator of the multispecies functional response (eq. 4) cancels out.

Stomach content data are useful to approximate f_i^*, if the observed stomach content is quantifiably related to food consumption rates (Pennington 1983). Alternatively to approximating f_i^* directly by ingested prey numbers, f_i^* may be approximated by the mass of the stomach content. However, this approximation demands knowledge of the relationship between the prey-specific stomach mass content and the stomach evacuation rate, in order to derive the food mass consumption rate. This relationship was described in detail by Andersen (2001) who concluded that the so called square root model (Andersen 1998) provided an adequate description of the relationship for cod and other gadoids. Effects of prey characteristics were incorporated into the square root model to produce a generic model of gastric evacuation (Andersen & Beyer 2005a, b). The square root model does not yet account for the effect of hypoxia on the stomach evacuation rate. Under the experimental conditions the stomach evacuation rates of Baltic cod decreased at oxygen saturation below 65 % (Brach 1999). Therefore, the *de facto* food consumption of the cod under hypoxia is likely lower, even if the average stomach content does not differ. Differences in prey–specific evacuation rates might, as long as they are constant, scale the functional response ratio, but will not modify its behaviour at changing predator-prey overlaps.

Using the square root model to estimate food consumption rates in mass units demands individual based data on total mass in the stomach *S*, because $\overline{S^{0.5}}$ is always less than $\bar{S}^{0.5}$ unless all values of *S* are equal (Rao 1965). However, the ICES stomach contents data are generally pooled by predator size. Therefore, only mean values $\bar{S}$ of stomach contents are available. Consumption rates *C* as estimated by $\hat{C} = \bar{r}\,\bar{S}^{0.5}$, where *r* is the evacuation rate constant of the stomach content, are therefore likely to be biased by the frequency distribution of *S*. This bias may be partly abated by multiplying $\bar{S}^{0.5}$ by a correction factor *k* (Andersen 2001). As well as constant differences in the prey specific evacuation rates will scale but not impact the response of the functional response ratio to overlap changes, this is in principal true for the ratio of prey specific k-factors. Unfortunately, k is not necessarily constant and the number of empty stomachs may heavily influence the overall k values. Hence, even if the energy density ratio of herring and sprat is constant, the problem remains that the square-rooted ratio of stomachs including herring and sprat is not constant between years due to changes in occurrence of prey caused by differences in overlap. Therefore, the ratio of the square-rooted averages, if based on the available pooled stomach data from the Baltic, is unlikely to be proportional to the consumption ratio.

Assuming alternatively that the probability to sample a prey specimen in the predator stomach is constant over the whole evacuation period, the average prey specimen will have been in the stomach for half the total evacuation time. The square root model (Andersen 1998) predicts that at this point of time the mass of the prey specimen is a quarter of the mass at ingestion. This assumption is problematic in the presence of mixed meals, because the assumption is violated for at least one prey species when prey-specific evacuation rates differ. However, out of a sample of 389 Atlantic cod from the Bornholm Basin (Neuenfeldt, unpubl. data) only had 2 had both fresh herring and sprat in the stomach. Furthermore, for the same data the probability of having both fresh and further digested food in the stomach was very low with 0.09±0.03 (n=389). Fresh means in this case that prey has been devoured during the last approximately 10 to 12 hours (Johansen *et al.* 2004). Meals of Baltic cod appear from these data to be species-specific and are apparently evacuated in most cases before a new meal is taken.

Applying the stomach data, the hypothesis could be tested that the effects of inflows on the

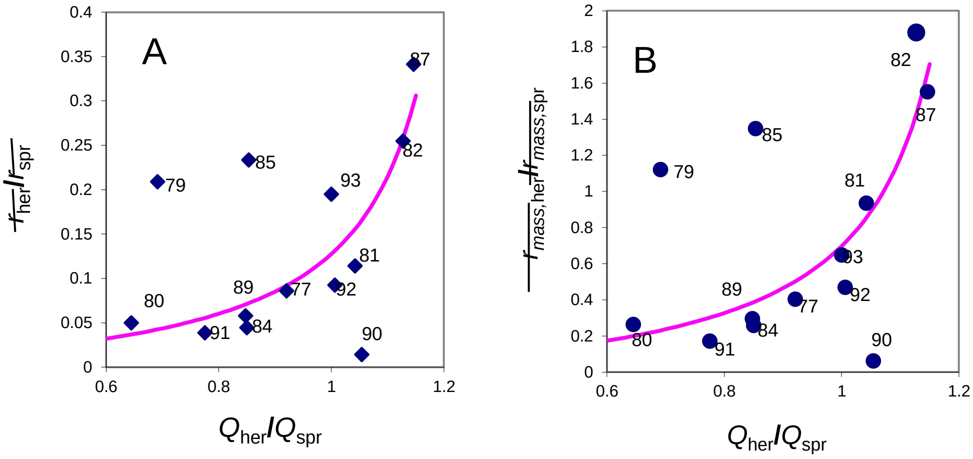


Fig. 7. Average abundance of herring relative to average abundance of sprat (A) and average mass of herring relative to average mass of sprat (B) in cod stomachs (n_{tot} = 2589) versus fraction of the herring habitat occupied by cod divided by fraction of the sprat habitat occupied by cod (Q_{her}/Q_{spr}). The curves represent the model of aggregated functional response ratios with constant coefficients fitted to data. Numbers indicate the sampling year.

aggregate diet are mediated by changes in cod-clupeid overlaps (Fig. 7). The conceptual model developed for this test predicted that the ratio of consumed herring to sprat increases *faster* than proportionally to the ratio of cod-herring to cod-sprat overlaps and indicated furthermore that the functional response of the individual cod followed and active response model (Chesson 1984).

Using ingested biomass instead of ingested numbers is not entirely consistent with an encounter concept focusing on encounters between predator and prey individuals. However, using ingested biomass to identify a probable functional response model exemplified that consumption has to be treated both in terms of ingested numbers and ingested mass when functional response mechanisms are to be identified.

Population consequences of environmentally driven overlaps

In order to clarify the prey population consequences of the overlap dynamics, it is instructive to consider the behaviour of the *per capita* aggregated functional response in situations of extreme predator-prey overlaps. Here, I will demonstrate how overlap dynamics decouple predation mortality from prey and predator abundances, and that this effect varies with the functional response type. The Holling type 2 (Holling 1959) and a simple active response model, (Chesson 1984, Neuenfeldt & Beyer 2006) will be used as examples.

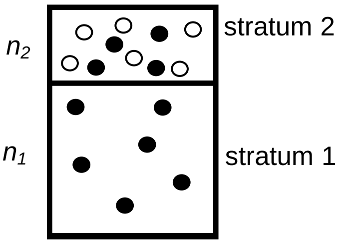


Fig. 8: Schematic representation of the predator habitat (total rectangle). Black dots indicate prey 1, white dots prey 2. Stratum *j* contains n_j predator individuals. In the Baltic case, strata correspond to vertical zones with sprat as prey 1 and herring as prey 2.

The overlap scenario considered is depicted in Fig. 8. Prey population 1 occurs in the total predator population habitat, whereas prey population 2 occurs only in a limited part of the predator habitat (Fig. 8). Habitat volumes of predator and prey are considered constant and predator-prey overlaps are for simplicity considered independent of habitat volumes.

Focus is put upon f_1^* at variable overlap O_2 between the predator and prey population 2, *i.e.* it is investigated what happens to the *per capita* aggregated functional reponse for prey 1, when size changes of the stratum where prey 2 is additionally available for predator individuals.

Multiplying f_1^* with the abundance P of the predator population yields the total amount of prey 1 individuals consumed per unit time. The instantaneous predation mortality rate of prey 1 is given by $dN_1/dt=-(f_1^*)PN_1^{-1}$ (N_1 indicates the abundance of prey 1). Since P is considered constant the term $f_1^*N_1^{-1}$, expressing the relationship between consumed prey individuals per average predator individual and prey abundance, is here sufficient to describe predation mortality dynamics. The derivation of f_1^* for variable O_2 is given in table 1.

Using the Type 2 functional response in this context, different handling time concepts such as relating handling time either to capture only or to capture plus digestion, can be investigated for their implications on predation in different overlap scenarios.

The active response model (henceforth abbreviated ARM) disregards handling time, but considers total consumption constant, *i.e.* independent of prey density. At decreasing prey density, the predator has to intensify searching in order to maintain constant attack rates. Based on the ARM, the effect of compensatory feeding on prey 1 in growing absence of prey 2 (*i.e.* at decreasing O_2) can be investigated.

Table 1: Local individual and per capita aggregated functional responses for prey 1 according to Holling type 2 and active response models.

f_1: individual functional response for prey 1 in stratum 2 (Fig.12);
f_{10}: individual functional response for prey 1 in stratum 1 (1 is the only prey);
f_1*: per capita aggregated functional response for prey 1
f_2* per capita aggregated functional response for prey 2
α_i: prey specific attack rates
h_i: prey specific handling times
f_T: total consumption rate in the ARM
O_i prey specific predator-prey overlaps

	Type 2	ARM (active response model)
f_1	$\alpha_1\rho_1(1+\alpha_1\rho_1 h_1+\alpha_2\rho_2 h_2)^{-1}$	$f_T\alpha_1\rho_1(\alpha_1\rho_1+\alpha_2\rho_2)^{-1}$
f_{10}	$\alpha_1\rho_1(1+\alpha_1\rho_1 h_1)^{-1}$	f_T
f_1^*	$(1-O_2)f_{10}+O_2 f_1$	

Unless explicitly mentioned, $\alpha_1=\alpha_2=0.5$ and $h_1=h_2=1$. Furthermore, f_T was set to 1, and ρ_2 was also set to 1 in the following simulations.

The density ρ_i of prey i is defined as abundance per habitat volume, $\rho_i=N_iH_i^{-1}$. Since H_i is considered constant, the only changes in prey density are caused by changes in prey abundance N_i. Both in the Type 2 and the ARM functional responses, the number of consumed prey individuals per average predator individual and prey abundance decreases at increasing prey abundance even if $O_2=1$, because consumption rates increase slower than prey abundances. However, considering the rate at which the number of consumed prey individuals per average predator individual and prey abundance decreases at increasing N_1, it becomes clear that in the ARM the decrease is more pronounced at $O_2<1$ than at $O_2=1$:

$$\frac{d}{dN_1}\left(\frac{f_1^*}{N_1}\right)=-\frac{1}{N_1^{2}}(1-O_2)f_T-O_2 f_T\frac{\alpha_1^{2}}{\{\alpha_1N_1+(1-\alpha_1)N_2\}^2} \tag{6}$$

If $O_2<1$ in the ARM, then the predators have exclusively prey 1 to obtain a constant amount f_T of consumed fish in a $(1-O_2)$-fraction of their dispersion volume. The number of consumed prey individuals per average predator individual and prey abundance decreases at increasing prey 1 density at a rate that is inversely proportionate to N_1^2 (eq. 6). Therefore, the effect is especially pronounced at $N_1<1$. In consequence, increases of N_1 are amplified in the ARM at $O_2<1$, especially when N_1 is initially small. Here it becomes important to distinguish between the traditional functional response experiments with simultaneous encounters and the sequential encounters in the field. Especially, if N_1 is measured in number of schools per unit volume then a situation where the individual predator has less than 1 encounter per unit time is realistic. Also the density of prey individuals can well be less than 1.

Also in the type 2 functional response model number of consumed prey individuals per average predator individual and prey abundance decreases faster at increasing N_1 if $O_2<1$, however, not at such a high rate as in the ARM:

$$\frac{d}{dN_1}\left(\frac{f_1^*}{N_1}\right)=(1-O_2)\alpha_1\frac{-\alpha_1 h_1}{(1+\alpha_1 N_1 h_1)^2}+O_2\alpha_1\frac{-\alpha_1 h_1}{(1+\alpha_1 N_1 h_1+(1-\alpha_1)N_2 h_2)^2} \qquad (7)$$

Yet, a greater decrease rate of the number of consumed prey individuals per average predator individual and prey abundance at low but increasing N_1 can be observed, if h_1 in the type 2 model is high (eq. 7), for example if the predator individual would not search for new prey during stomach evacuation which, however, contradicts the current concept that searching is resumed already during stomach evacuation (Breck 1993).

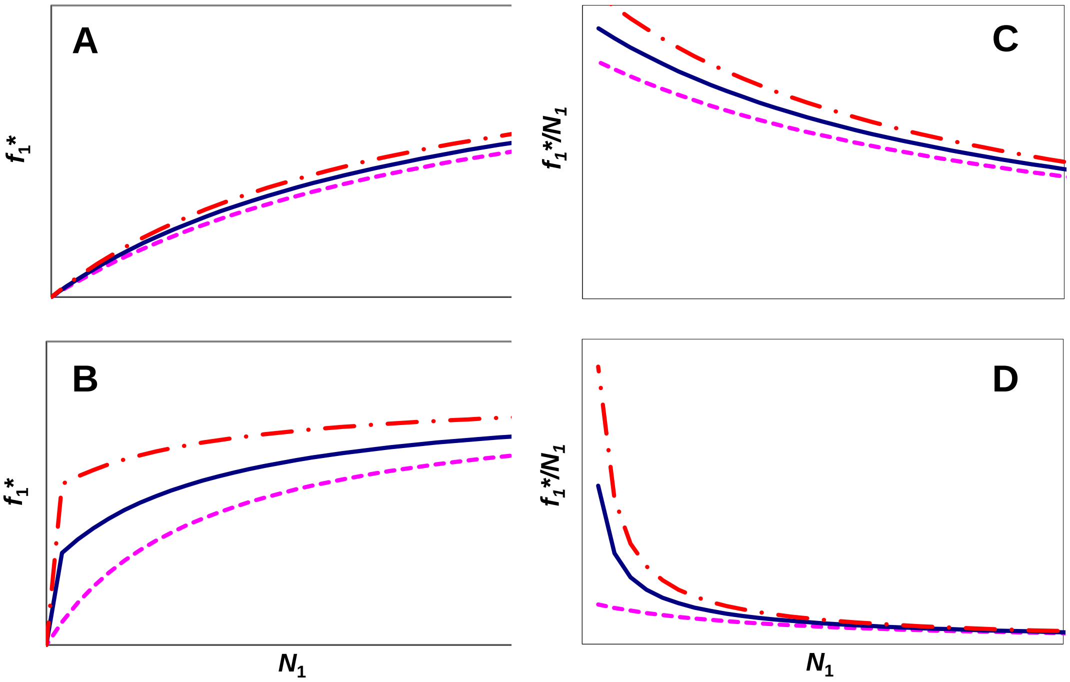


Fig. 9: Aggregated per capita functional response f_1* type 2 (A) and ARM (B) and number of consumed prey individuals per average predator individual and prey abundance f_1*/N_1 for Type 2 (C) and ARM (D). The dashed lines show the functional response and predation rate at O_1=O_2=1, i.e. at complete mixing of predator and prey populations. The solid lines, in contrast, reflect functional response and predation at O_1=1, and O_2=0.7, i.e. in a situation where 30 % of the predator habitat contain prey 1 only. The dash-point lines reflect O_1=1 and O_2=0.4.

In order to allow for a direct comparison with the reference graphs in many textbooks, I plotted a type 2 functional response at constant predator and prey abundances, but differing predator-prey overlaps (Fig. 9). The aim with these panels is to illustrate that overlap influences the number of consumed prey individuals per average predator individual and prey abundance (Fig. 9 C), thereby decoupling predation mortality from predator and prey abundances. These effects are more pronounced in the ARM (Fig. 9 B and D).

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