Impacts of trophodynamics and climate-induced habitat changes on zooplankton distribution and behavior: An optical sampling approach

Dissertation

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Klas Ove Möller
aus Hamburg

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Nothing in the world is purely spatial or purely temporal; everything is process

JM Blaut 1961

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1 Summary

Planktonic organisms serve as the productive base of aquatic ecosystems, play a major role in oceanic food webs and have an important impact on the ocean's carbon cycle. Therefore, it is necessary to understand the processes which control the spatial and temporal distribution as well as the abundance of these organisms. However, resolving this distribution is particularly challenging since aquatic ecosystems are characterized by an immense variability in biotic and abiotic components and traditional methods are limited in their sampling resolution. The present thesis was designed to overcome this spatial and temporal limitation of traditional sampling gears by using an optical approach to investigate distribution patterns and behavioral responses of plankton organisms on small scales. The Video Plankton Recorder (VPR) a digital underwater camera system towed by a research vessel was employed to investigate trophodynamic interactions and potential impacts of climate-induced habitat changes on the distribution and behavior of our model species *Pseudocalanus acuspes*, a key zooplankton species in the Baltic Sea.

The VPR was applied during several cruises in the Central Baltic Sea, specifically in the Bornholm Basin. This non-invasive sampling method allowed the quantification of marine snow aggregates and revealed a pronounced thin layer that was associated with the strong density gradient of the permanent halocline, characteristic for deep Baltic basins. Both features are novel results for the Baltic Sea and generally rarely observed in marine ecosystems. Furthermore, high concentrations of copepods, mainly *P. acuspes*, were observed being directly attached to marine snow aggregates within this layer and demonstrated signs of typical feeding behavior. This indicated that feeding on marine snow in the halocline might be crucial for the survival of copepods that are confined to the deep layers of the basins. No primary production occurs below the halocline and euryhaline copepod species such as *P. acuspes* which need higher salinities in deeper waters experience comparable conditions as zooplankton residing in the mesopelagic zone of the open ocean, where nutrition must be obtained to a large degree via feeding on sinking aggregates.

Observations of individual copepods with the VPR and hydroacoustic estimates of predatory fish biomass were combined to identify behavioral responses and predator-prey interactions. Due to the limited taxonomic resolution of the majority of VPR images, only ovigerous females were used in the analysis since the conspicious external egg sac allowed discrimination from other developmental stages and copepod species and, hence, species-specific behavioral observations of a certain life-stage could be performed. This results revealed a „classical" diurnal migration pattern (DVM) for *P. acuspes*, never observed before. Previous studies used conventional net sampling and due to the coarser vertical resolution were not able to resolve this behavior due to the small vertical migration amplitude which is limited by low salinities above the halocline and low oxygen values below the oxycline. With continuous sampling over the entire day-night cycle this study presents first *in-situ* observations of an immediate response of individual copepods in relation to their predator as well as first *in-situ* evidence of strong individual variation in a marine copepod species´ DVM. Most of the *P. acuspes* females showed a general trend of downward migration during day, but some individuals responded to the presence of predators with an upward movement towards the surface. The two different behavioral modes of *P. acuspes* in the Baltic Sea indicate that different predator avoidance strategies exist within a population. The risk of predation during daytime forced large parts of the *P. acuspes* adult

female population out of their optimal habitat and, hence, this behavior is a trade-off between maximising energy input and minimizing predation risk.

Climate-induced changes in the Baltic Sea during the last decades were reflected by a reduction in the frequency of major inflow events leading to a deepening of the halocline and hypoxic or anoxic conditions of the deep water. Comparing pre- and post-inflow situations elucidated the negative impact of climate-induced habitat changes on this key species during so-called stagnation periods without inflow. Improved food and habitat conditions led to a deeper distribution of individuals. The increased suitable habitat resulted in stronger spatial separation between predatory fish and *P. acuspes* and, hence, to reduced predation pressure. The latter resulted in the observed higher abundances and absence of DVM behavior. Furthermore, uniformly distributed marine snow aggregates in the water column provided improved food conditions under enhanced environmental conditions in the deep water. The deeper distribution of the copepods after the inflow reflected the crucial importance of high salinity levels in combination with high oxygen values for this key species. These results demonstrate that bottom-up (food and hydrography) and top-down (predation pressure) mechanisms in a complex interplay contribute to lower abundances during stagnation periods through both direct- and indirect mechanisms. Furthermore, applying a new developed method of *in-situ* egg production from VPR-derived images revealed that reduced abundances of *P. acuspes* during stagnation periods without inflow events are also caused by negative effects on survival of early life-stages. This results show the strong impact that climate-induced changes in the physical habitat can have on key marine ecosystem species, important for overall ecosystem dynamics.

The present study emphasizes the potential of optical methods to resolve plankton distribution patterns and behavior on appropriate scales which will gain further insight into plankton ecology since the key to prediction and understanding lies in the elucidation of mechanisms underlying the observed patterns (Levin 1992).

2 Zusammenfassung

Planktonorganismen sind die Basis aller aquatischen Ökosysteme, spielen eine fundamentale Rolle in den Nahrungsnetzen der Ozeane und haben außerdem einen beträchtlichen Einfluss auf den Kohlenstoff-Kreislauf in den Weltmeeren. Demzufolge ist es unerlässlich die Prozesse zu verstehen, welche den räumlichen und zeitlichen Verteilungsmustern, sowie der Abundanz dieser Organismen zu Grunde liegen. Die Auflösung dieser Verteilungsmuster ist jedoch eine besondere Herausforderung, da aquatische Ökosysteme durch eine immense Variabilität in deren biotischen und abiotischen Bestandteilen gekennzeichnet sind, und herkömmliche Beprobungs-Methoden nicht die nötige Auflösung für diese Muster besitzen. In dieser Arbeit wurde ein optisches Verfahren eingesetzt um kleinskalige Verteilungsmuster und Verhaltensweisen von Planktonorganismen zu untersuchen und somit die Einschränkungen in der räumlichen und zeitlichen Auflösung von herkömmlichen Beprobungs-Methoden zu überwinden. Der Video Plankton Rekorder (VPR), ein digitales Unterwasser Kamera-System, das von einem Forschungsschiff geschleppt wird, wurde eingesetzt um die Auswirkungen trophodynamischer Interaktionen sowie klimatisch bedingter Habitatsveränderungen auf die kleinskaligen Verteilungsmuster und das Verhalten von *Pseudocalanus acuspes*, einer Schlüsselart des Zooplanktons in der Ostsee, zu untersuchen.

Der VPR wurde während mehrerer Forschungsreisen in die zentrale Ostsee und vor allem in das Bornholm Becken eingesetzt. Die nicht-invasive optische Beprobungs-Methode ermöglichte die Quantifizierung von sogenannten „Marine snow“ Aggregaten, welche sich in dem für die tiefen Ostsee-Becken charakteristischen und ausgeprägten Dichtegradienten, der permanenten Halokline, konzentrierten. Diese konzentrierte Schicht von Marine snow Aggregaten wurde erstmals für die Ostsee beschrieben und auch in anderen marinen Ökosystemen zuvor selten untersucht. Hohe Dichten von Copepoden, vorwiegend *P. acuspes*, wurden innerhalb dieser Schicht beobachtet und wiesen ein für die Nahrungsaufnahme typisches Verhalten auf. Diese Beobachtung impliziert, dass die in der Halokline konzentrierten Marine snow Aggregate eine überlebenswichtige Nahrungsquelle für Copepoden Arten darstellen, deren Lebensraum auf die tiefen Becken beschränkt ist. Unterhalb der Halokline tritt keine Primärproduktion auf und euryhaline Copepoden Arten wie *P. acuspes*, welche an die hohen Salzgehalte im Tiefenwasser gebunden sind, haben daher vergleichbare Bedingungen wie Planktonorganismen in den mesopelagischen Zonen des Ozeans, in denen Nahrung zum Großteil aus herabsinkenden Aggregaten besteht.

Aufnahmen individueller Copepoden mit dem VPR und hydroakustische Daten der Fischverteilung wurden gemeinsam betrachtet um Räuber-Beute Interaktionen und Reaktionen im Verhalten der Copepoden zu entschlüsseln. Aufgrund der geringen taxonomischen Auflösung der meisten VPR Bilder wurden nur reproduzierende, adulte *P. acuspes* Weibchen in der Analyse berücksichtigt, da diese aufgrund des auffälligen Eisacks von anderen Arten und Entwicklungsstadien unterschieden werden konnten und somit eine Identifikation von artenspezifischen und stadienspezifischen Verhaltensweisen ermöglichten. Eine „klassische“ vertikale Tageswanderung (DVM) wurde für *P. acuspes* beobachtet. Dieses Verhalten konnte in vorherigen Studien aufgrund der geringen Wanderungsamplitude, welche von geringen Salzgehalten oberhalb der Halokline und niedrigen Sauerstoffwerten unterhalb der Oxykline beschränkt wird, mit der groben Auflösung von herkömmlichen Beprobungsweisen noch nicht detektiert werden. Die kontinuierliche Probennahme mit dem VPR über den gesamten Tag-Nacht Verlauf ermöglichte zudem die ersten direkten *in-situ* Beobachtungen einer unmittelbaren

Verhaltensreaktion von einzelnen Copepoden gegenüber Räubern, und ausserdem den ersten *in-situ* Nachweis von ausgeprägten individuellen Abweichungen im DVM Verhalten einer marinen Copepoden Art. Während die meisten adulten *P. acuspes* Weibchen ein generelles Wanderungsverhalten in tiefere Wasserschichten zeigten, reagierten einige Individuen mit einem entgegengesetzten Verhalten und einer Wanderung in Richtung der Oberfläche. Die zwei unterschiedlichen Verhaltensweisen deuten darauf hin, dass unterschiedliche Räuber-Vermeidungsstrategien innerhalb einer Population existieren. Generell bewirkt das Risiko von Prädation während des Tages, dass sich die meisten reproduzierenden *P. acuspes* Weibchen aus Ihrem optimalen Habitat entfernen, und das Verhalten stellt somit einen Ausgleich zwischen der Maximierung der Nahrungsaufnahme und der Minimierung des Prädationsrisikos dar.

Während der letzten Jahrzehnte haben sich klimatisch bedingte Veränderungen in der Ostsee besonders durch eine geringere Häufigkeit von sogenannten Einstrom-Ereignissen bemerkbar gemacht, so daß es zu einem Absinken der Halokline und zu hypoxischen bis anoxischen Bedingungen im Tiefenwasser kam. Der Vergleich von Prä- und Post-Einstrom Verhältnissen zeigte deutliche negative Auswirkungen auf das Habitat dieser Schlüsselart zu Stagnationszeiten ohne Einstrom. Verbesserte Nahrungs- und Habitatsbedingungen nach dem Einstrom resultierten in einer tieferen Verteilung von Individuen. Die Vergrößerung des geeigneten Habitates hat vermutlich zu einer stärkeren räumliche Trennung zwischen Fischen und *P. acuspes* und damit zu einem geringeren Prädationsdruck geführt. Letzterer wird als Ursache für den beobachteten Anstieg der Abundanz und dem Ausbleiben des Wanderungsverhaltens angenommen. Weiterhin hat eine gleichmäßige Verteilung von Marine snow Aggregaten in der Wassersäule zu besseren Nahrungsbedingungen unter ebenfalls verbesserten hydrographischen Bedingungen im Tiefenwasser geführt. Die tiefere Verteilung von Copepoden nach dem Einstrom verdeutlicht die kritische Bedeutung von hohen Salinitäten in Kombination mit hohen Sauerstoffgehalten für diese Schlüsselart. Diese Ergebnisse zeigen, dass sogenannte „bottom-up“ (Nahrung und Hydrographie) und „top-down“ (Prädationsdruck) Mechanismen in einem komplexen Wechselspiel stehen, so daß es in Stagnationszeiten zu niedrigeren Abundanzen kommt. Die Ergebnisse einer neu entwickelten *in-situ* Methode zur Bestimmung der Eiproduktionsrate zeigte ausserdem, dass die niedrigen *P. acuspes* Abundanzen während der Stagnationszeiten ebenfalls durch negative Effekte auf das Überleben von jüngeren Lebensstadien bedingt sind. Diese Ergebnisse verdeutlichen welchen starken Einfluss klimabedingte Veränderungen auf das Habitat von Schlüsselarten und damit auf die Struktur mariner Ökosystemen haben kann.

Diese Arbeit betont die neuen Möglichkeiten von optischen Methoden zum Erfassen von kleinskaligen Plankton-Verteilungsmustern und -Verhaltensweisen. Diese Methoden haben die Möglichkeit weiteren Einblick in den Bereich der Planktonökologie zu geben, da der Schlüssel zur Voraussage und dem Verständnis in der Aufklärung der Mechanismen liegt, welche diese beobachteteten Muster bedingen (Levin 1992).

3 Outline of Publications

The following overview outlines the three publications which are included in this thesis and partly already published (manuscript 1) or written as manuscripts with multiple authorship. This list serves as clarification of each authors contribution to the respective manuscript.

Manuscript 1

Marine snow, zooplankton and thin layers: indications of a trophic link from small-scale sampling with the Video Plankton Recorder

Klas O. Möller, Michael St. John, Axel Temming, Jens Floeter, Anne F. Sell, Jens-Peter Herrmann, Christian Möllmann

KOM did all data analyses, graphical presentations and text writing under close cooperation with CM and MStJ. AT provided helpful comments, conducted the VPR sampling together with AFS, JF and JPH in 2002 and provided the data.

The manuscript is published in the peer reviewed journal Marine Ecology Progress Series (MEPS 468:57-69 (2012)).

Manuscript 2

Plasticity in response of a key marine ecosystem player to predation risk

Klas O. Möller, Michael St.John, Axel Temming, Rabea Diekmann, Janna K. Peters, Jens Floeter, Anne F. Sell, Jens-Peter Herrmann, Dominik R. Gloe, Jörn O. Schmidt, Hans H. Hinrichsen, Christian Möllmann

KOM did all data analysis, graphical presentations and text writing under close cooperation with CM and MStJ. AT provided valuable comments. RD contributed to the statistical analyses. JKP provided helpful comments on the analysis. DRG provided acoustic data. AT, JF, AFS and JPH conducted the VPR sampling in 2002 and provided the data. KOM, JKP, JOS and HHH conducted the VPR sampling in 2009.

The manuscript will be shortly submitted to PNAS, a peer reviewed journal.

Manuscript 3

Effects of climate induced habitat changes on a key zooplankton species

Klas O. Möller, Jörn O. Schmidt, Michael St.John, Axel Temming, Rabea Diekmann, Janna K. Peters, Jens Floeter, Anne F. Sell, Jens-Peter Herrmann and Christian Möllmann

KOM did all the data analyses, graphical presentations and text writing under close cooperation with CM and MStJ. JOS provided an earlier version of the manuscript, developed the *in-situ* egg production method and did the egg production analysis. RD contributed to the statistical analyses. AT and JKP provided valuable comments. AT, JF, AFS and JPH conducted the VPR sampling in 2002 and 2003. KOM, JOS and JKP conducted the VPR sampling in 2009.

The manuscript will be shortly submitted to PloS ONE, a peer reviewed journal

4 General Introduction

Optical sampling

Planktonic organisms serve as the productive base of aquatic ecosystems, play a major role in oceanic food webs and have an important impact on the ocean's carbon cycle. Therefore, it is necessary to understand the processes, which control the spatial and temporal distribution as well as the abundance of these organisms. Two main factors influencing these distribution patterns are plankton motility and behavior as well as the physical and chemical settings of the environment. The spatial heterogeneity of plankton which can occur even in a homogeneously mixed water column (Folt & Burns 1999), is a well-studied phenomenon and can lead to concentrations that rise far above levels that would be expected in a random distribution (Mackas et al. 1985). Hence, it is of great relevance for the biological productivity, trophic interactions and foodweb dynamics in marine ecosystems (Mackas et al. 1985, Pinel-Alloul 1995). However, resolving this distribution is particularly challenging, since aquatic ecosystems are charcterized by immense variability in biotic and abiotic components across a continuum of time and space scales from microns to thousands of kilometers (Haury et al. 1978). Historically, a large effort has been devoted to developing various zooplankton-sampling devices (Wiebe & Benfield 2003). These traditional sampling-techniques include water-bottle samplers, pumping systems and nets which can present crucial taxonomic information on species and life-stages. However, these methods present discontinuous single geographic point samples and are, thereby, limited in their spatial and temporal resolution. Additionally, manual taxonomic analysis of samples by taxonomic experts is time consuming, costly and can be biased (Culverhouse et al. 2003). To overcome the spatial limitation, acoustical sampler (Holliday et al. 1989) and non-imaging optical samplers like the Optical Plankton Counter (Herman 1988) have been developed. These instruments provide high-resolution data on biomass and particle size, but not on the taxonomic composition of plankton. In the last two decades, advances in imaging technology have led to the development of new optical imaging devices (e.g. Video Plankton Recorder (VPR), Davis et al. 1992; Underwater Video Profiler (UVP), Gorsky et al. 2003; FlowCam, Sieracki et al. 1998; Shadow Image Particle Profiling Recorder (SIPPER), Samson et al. 2001, see Benfield et al. 2007). The Video Plankton Recorder (Seascan, Fig.4-1) is an optical underwater instrument, i.e. a digital underwater camera system towed by a research vessel.

Fig. 4-1. Image of the Video Plankton Recorder II real time system (VPR) used throughout this study during deployment in the Baltic Sea.

This sampling gear allows high-resolution observation of plankton taxa from micro- (< 1 m) to small-scale (1 – 10 m) over long distances and time periods (e.g. Davis et al. 1992, Ashjian et al. 2005, Davis & McGillicuddy 2006). Another advantage of the VPR is that non-invasive optical sampling obtains data on gelatinous zooplankton (e.g. hydromedusae and appendicularians), colonial phytoplankton (e.g. chain forming diatoms) and other fragile particulate matter and aggregations (e.g. marine snow and aggregated organisms) in their natural orientation and environment (Benfield et al. 2000) without damaging them or destroying the associations (e.g. Jacobsen & Norrbin 2009, Möller et al. 2012). Due to the inability of traditional sampling methods to sample certain organisms or particles quantitativly, the reality of the planktonic world was long time the reality of net designs (Reeve 1988). This traditional sample-reality is also influenced by extrusion of zooplankton from nets, clogging of net mesh and avoidance of the sample gear (Wiebe & Benfield 2003). Furthermore, having the plankton sample in a digital format allows accelarating the classification procedure. Plankton-imaging-system development has been strongly influenced by the desire to reduce sample processing time (Benfield et al. 2007) and advances in image processing (Tang et al. 1998) as well as pattern recognition (Hu & Davis 2005, 2006) of plankton have made it possible to automatically quantify coarse taxonomic composition (and species in some cases) of plankton at sea in real time (Davis et al. 2004, Ashjian et al. 2005). The VPR has been used in numerous studies during recent years, e.g. to develop micro-scale prey fields for larval fish to include in foraging model studies (Lough & Broughton 2006) to resolve how copepod swimming behavior contributes to the formation of fine-scale layers (Gallager et al. 2004) and especially to describe zooplankton distributions in relation to hydrographic structures (e.g. Ashjian et al. 2001, Davis et al. 2004). These studies provided information that is not possible to retrieve with conventional collecting equipment, and, hence showed the potential of optical sampling techniques to give new perspectives and insights on the distribution, composition and interactions in the plankton community as well as on whole ecosystem dynamics. In the present study, we applied an optical sampling approach by using the VPR to obtain data on both the vertical small-scale distribution as well as on the behavior of zooplankton in the Baltic Sea, i.e. the Bornholm Basin. Here we present distribution patterns on scales never before observed in this region, which might have the potential to elucidate new trophic interactions and behavior of copepods.

Baltic Sea

The semi-enclosed Baltic Sea (Fig. 4-2) is the largest brackish water area of the world (Fonselius 1970). The bottom topography is characterized by a cascade of several deep basins, one of them the Bornholm Basin, separated by submarine sills (Matthäus 1995). The hydrographic conditions in the Bornholm Basin are characterized by a pronounced thermocline, established during summer between 20 and 30 m depth while a strong halocline in 50 to 60 m depth separates the water column throughout the whole year (Matthäus & Franck 1992). This permanent halocline hinders vertical mixing and, hence, ventilation of the deep water depends on inflow of high-density saline and oxygenated water by lateral advection from the North Sea. A major effect of climate change on the Baltic Sea during the last decades is a reduction in the frequency of significant inflow events (Hanninen et al. 2000, Feistel et al. 2008; Fig. 4-3). If not ventilated by an inflow, the salinity of the deep water decreases due to the Baltic Seas positive net freshwater balance and biological activity reduces the oxygen content in the deep water to

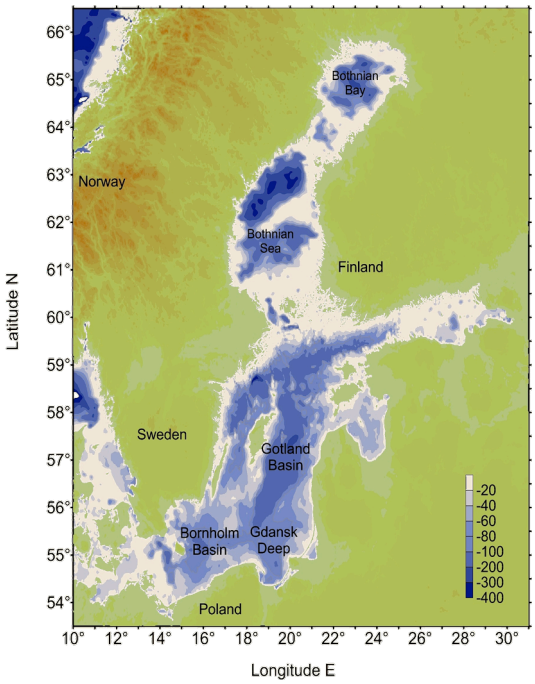

Fig. 4-2. Map of the Baltic Sea indicating the bathymetry and deep basins. The legend represents water depth (m).

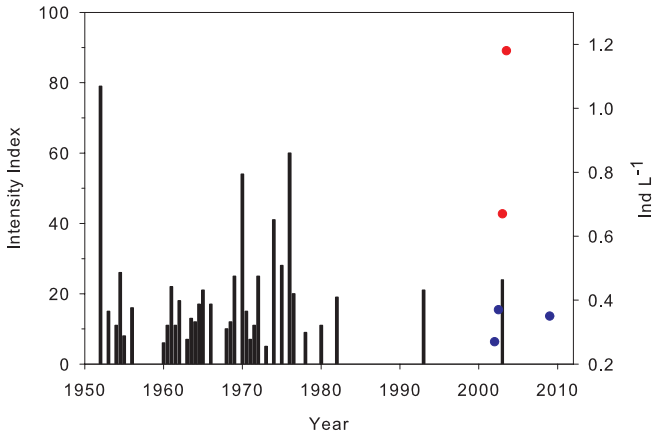

Fig. 4-3. Inflow events with their relative strength (after Matthäus & Schinke 1994); the inflow in 2003 was added by the author; dots indicate the average water column abundance (Ind L^{-1}) of reproductive *P. acuspes* females during stagnation periods (blue, April & May 2002 and May 2009) and directly after an inflow event (red, April & May 2003).

levels detrimental for local plant and animal populations. Baltic inflows are thought to be regulated by atmospheric forcing and while observed regularly until the early 1980s, recently their occurrence is reduced to once per decade (Matthäus & Franck 1992, Schinke & Matthäus 1998, Feistel et al. 2008). The last significant inflows have been recorded in 1993 and 2003 only (Jakobsen 1995, Feistel et al. 2003, Mälkki & Perttila 2012) and, consequently, anoxic bottom waters are now widespread and common in the deep Baltic Sea basins (Kabel et al. 2012). The reduced frequency of major Baltic inflows has been related to changes in the North Atlantic Oscillation (NAO), the dominant mode of climate variability in this region (Mälkki & Perttila 2012).

The lacking Baltic inflows contributed to the major reorganization of the food web during the late 1980s/early 1990s (Alheit et al. 2005, Möllmann et al. 2009). Changes were most pronounced in the fish community that was further amplified by overfishing, resulting in a shift from a previous cod (*Gadus Morhua*) to a planktivorous sprat (*Sprattus sprattus*) dominated system (Casini et al. 2008, Möllmann et al. 2009). However, changes on the zooplankton level were important in this so-called ecosystem regime shift (Möllmann et al. 2008). The dominant zooplankton species in this region are the euryhaline calanoid copepods *Temora longicornis*, species of the genus *Acartia* (i.e. *A. bifilosa, A. longicornis, A. clausi*) and *Pseudocalanus acuspes* (Schulz et al. 2012). The distribution and interactions of the different compartments of the biotic environment are strongly influenced by the vertical structure of the water column leading to an enhanced vertical habitat differentiation. Hence, the interactions between the different trophic levels and compartments depend strongly on the temporal horizontal and vertical overlap. As several zooplankton species live at the edge of their physiological tolerance level in the Baltic Sea (Hernroth & Ackefors 1979), climate-induced bottom-up effects on species' habitat can have major consequences.

The pronounced stratification in the Baltic Sea, i.e. the Bornholm Basin as well as the rather simple structure in terms of species diversity and trophic structure makes it an ideal study area for small-scale sampling with the VPR. We conducted several cruise campaigns during spring bloom conditions between 2002 and 2009 covering stagnation periods and inflow conditions to resolve the complex interplay of physical forcing, trophic bottom-up and top-down processes as well as zooplankton behavior in relation to it.

Pseudocalanus acuspes

Pseudocalanus are common throughout coastal waters from temperate to polar ecosystems, where they are thought to play an important role in the trophic ecology of these systems due to their high rate of production (e.g. Corkett & McLaren 1978, Hopcroft & Kosobokova 2009). *Pseudocalanus acuspes* is a key species in the food web dynamics of the central Baltic Sea (Renz & Hirche 2006) serving as a major food item for the commercially important planktivorous fish species herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) as well as for larval cod (*Gadus morhua*) (Möllmann et al. 2003, 2004, 2008). This species is of marine origin considered to live at marginal physiological conditions in the Baltic Sea, where it has a high affinity to more saline and cold waters, especially the adult females (Renz & Hirche 2006). Oceanographic and hydrological factors are proposed to be the principal control on the variations in the abundance of copepod species of marine origin in the Baltic Sea (Dippner et al. 2008). Consequently, as a result of reduced salinity and increased temperatures by low frequent inflow events (Fonselius & Valderama 2003), the dominance in the zooplankton changed from *P. acuspes* to the euryhaline *Acartia spp.* (Möllmann et al 2003). The population size of *P. acuspes* declined during the last decades, a trend correlated to the salinity level (Möllmann et al. 2003). The process behind the correlation between alterations in *P. acuspes* population size and salinity remained, however, unclear. Additionally, the shift on the fish trophic level from the piscivorous cod to planktivorous sprat increased predation pressure (Möllmann & Köster 2002, Casini et al. 2008, Möllmann et al. 2008). Hence, the decline of the *P. acuspes* population in the Baltic Sea is potentially a combination of climate-induced bottom-up effects and top-down control. Ovigerous adult females use the halocline and the saline deep water as their reproductive habitat (Renz & Hirche 2006) with a peak in the reproduction during April and May (Renz et al. 2007). The halocline is also a feeding hot-spot in and on aggregated marine snow particles (Möller et al. 2012). A key feature of its life-cycle is an ontogenetic vertical migration, i.e. its resident depth increases with individual age represented by developmental stage (Renz & Hirche 2006).

In the present study we put a major focus on the distribution and behavior of ovigerous *P. acuspes* females due to their key role in the Baltic Sea food web structure and since their distinct shape due to the attached egg sac allows discrimination from other copepods from VPR derived images (see Fig. 6-1).

General Objectives:

The aims of this thesis are to investigate the impact of trophodynamic interactions and climate-induced habitat changes on the distribution patterns and behavior of zooplankton in the Baltic sea, i.e. Bornholm Basin. We apply an optical sampling approach by using the video plankton recorder to (i) observe the vertical small-scale distribution of copepods in relation to a marine snow thin layer and assess potential trophic interactions (**manuscript I**), to (ii) identify behavioral responses and individual plasticity of copepods in relation to predators (**manuscript II**), and to (iii) resolve potential effects of climate-induced habitat changes on the abundance, vertical distribution and population dynamics, i.e. egg production, additionally using a novel *in-situ* approach to estimate individual egg production from VPR derived images (**manuscript III**).

Manuscript I - Marine snow, zooplankton and thin layers: indications of a trophic link from small-scale sampling with the Video Plankton Recorder

Marine aggregates of biogenic origin, known as marine snow, are considered to play a major role in the ocean's particle flux (Alldredge & Silver 1988, Graham et al. 2000, Stemmann & Boss 2012) due to their high abundance and rapid sinking rates (Fowler & Knauer 1986, Alldredge & Silver 1988). However, marine snow can also be a food resource for zooplankton, thereby, contributing to the production of higher trophic levels (Dilling et al. 1998, Kiørboe 2011a,b). The link between marine snow and planktonic organisms is presently still underappreciated due to the limitations of traditional sampling methods to quantitatively sample these aggregates. Laboratory feeding studies and gut content analyses have provided evidence that marine snow is a potential food source for zooplankton (Dilling et al. 1998), even if dispersed phytoplankton cells are available (Dilling & Brzezinski 2004). This observation might be especially important since larger crustacean zooplankton would be able to utilize nano- and microzooplankton that are colonizing marine snow aggregates, but are usually too small to be captured (Kiørboe 2001). However, little is known from *in-situ* studies if aggregates are commonly grazed by zooplankton in the field (Kiørboe 2000, Jackson & Checkley 2011).

Typical planktonic features on small vertical scales are thin layers driven by physical and biological processes (McManus et al. 2003, Durham & Stocker 2012). Often these thin layers are found in association with pycnoclines where the density gradient causes the accumulation of particles and plankton organisms (MacIntyre et al. 1995). Biological mechanisms, which bring organisms to thin layers include diel vertical migration, predator avoidance, aggregation in food patches and mate search (e.g. Folt & Burns 1999, Woodson & MacManus 2007). Hence, these layers may be regions of enhanced biological productivity and interactions (Sullivan et al. 2010). Conceivably, thin layers may have an extensive impact on marine ecosystem dynamics and the magnitude of their importance is just now beginning to be quantified (Alldredge et al. 2002, Durham & Stocker 2012, Lyons & Dobbs 2012).

Here we employed a Video Plankton Recorder (VPR) to quantify fragile marine snow aggregates and zooplankton and their relative small-scale vertical distribution. Using this non-invasive optical sampling technique, we recorded a pronounced thin layer of marine snow and copepods associated with a density gradient, an association with a feature rarely observed. We provideindirect evidence of copepods feeding on marine snow aggregates due to a strong spatial

overlap within the thin layer, which is supported by images of copepods being attached to aggregates.

Manuscript II - Plasticity in response of a key marine ecosystem player to predation risk

Predators in ecosystems not only have direct effects on their prey biomass, but can also induce important indirect effects such as trophic cascades or influence diversity, production and nutrient cycling (Greig & McIntosh 2006, Trussell et al. 2006, Schmitz et al. 2008, Schmitz et al. 2010, Strong & Frank 2010). Among these indirect effects of predation are non-consumptive effects on behavior of prey organisms (Preisser et al. 2009). Generally, herbivores and other intermediate trophic level species need to balance the trade-off between maximizing energy or nutrient intake and minimizing predation risk (Werner & Peacor 2003, Preisser et al. 2005). Predator avoidance behavior may force prey species into unfavourable environments in terms of food availability or physical habitat variables such as temperature, salinity and oxygen (Sainmont et al. 2012).

An important adaptive behavior in aquatic ecosystems is diurnal vertical migration (DVM) that has been demonstrated for diverse organisms from protists up to fish (Bollens & Frost 1989, Kaartvedt et al. 2007, Cohen & Forward 2009, Ringelberg 2010). DVM has major ecological consequences at the individual, population, community and ecosystem level (Ramirez-Llodra et al. 2010, Bollens et al. 2012, Sainmont et al. 2012. A number of causes are discussed to induce DVM including light conditions, food availability and temperature (Dupont et al. 2009, Ringelberg 2010) or trade-offs between those (Loose & Dawidowicz 1994). For zooplankton such as copepods, there is general consensus that predator avoidance is a major driver for DVM (Lampert 1989, Bollens & Frost 1989, Bollens & Frost 1991, Hays 2003, Bollens et al. 2011). However, direct observations of the relative behavior of predator and prey in natural enviroments are still rare.

Observing variability in individual plankton behavior in vast natural environments such as the ocean is especially difficult since net sampling typically provides only population mean differences over larger spatial scales. Hence, aggregated group-level data may blur and misrepresent individual behavior, a phenomenon called the „ecological fallacy“ (Wakefield & Shaddick 2006, Clark et al. 2011). However, experimental studies in behavioral ecology have demonstrated strong variation in behavior within single populations which may be due to individual personality and plasticity to environmental cues (Biro & Dingemanse 2009, Dingemanse et al. 2009). Stage-specific variation in predator avoidance has been shown for zooplankton populations in relation to feeding history, size, reproductive status, and pigmentation (Baumgartner et al. 2011, Holliland et al. 2012, Lönnstedt et al. 2012). A wide range of behavioral diversity and plasticity has been observed both between-species as well as within-species (Cohen & Forward 2009, Kaartvedt et al. 2011). Especially trophic interactions in natural environments occur at the individual rather than at the population level (Kiørboe 2008) and investigating predator-prey interactions at the individual level seems therefore essential for a better understanding of zooplankton dynamics.

We applied a modern optical underwater sampling gear, the Video Plankton Recorder (VPR) that allows in contrast to traditional net sampling the investigations of individual, diurnal adaptive behavior of zooplankton in response to predators in the natural oceanic environment. Combining observations of copepod individuals with the VPR and hydroacoustic estimates of predatory fish biomass we here show (i) a DVM of Baltic *P. acuspes* in response to its main

predator, (ii) first *in-situ* observations of a direct short-term reaction of the prey to the diurnal arrival of the predator and (iii) first *in-situ* evidence of strong individual variation in this adaptive behavior with potentially strong effects on individual performance and ecosystem functioning.

Manuscript III: Effects of climate induced habitat changes on a key zooplankton species

Knowledge on how climate change is affecting marine ecosystems has lagged behind that of terrestrial ecosystems, partly due to the size and complexity of the ocean, but also due to the difficulty of taking measurements in marine environments (Hoegh-Guldberg & Bruno 2010). Nevertheless, impacts of climate change on the ocean have become increasingly apparent during recent decades including changes in temperature, circulation, stratification, nutrient, input, oxygen content, and ocean acidification (e.g. Brierley & Kingsford 2009, Doney et al. 2012). Changes in the abiotic environment can have wide-ranging biological effects among them population-level shifts due to physiological intolerance to new environments (Bernhardt & Leslie 2012, MacKenzie et al. 2012).

In the important trophic level of marine zooplankton, changes in key environmental factors can influence the distribution of individual species and assemblages, the timing of important life-cycle events, and abundance and community structure (Richardson 2008, Beaugrand & Kirby 2010, Loeb et al. 2009). Hence, zooplankton is a suitable indicator for the effect of climate change on marine ecosystems and long-term changes in marine zooplankton are well studied (e.g. Planque & Taylor 1998, Möllmann et al. 2000, Beaugrand 2003, Mackas & Beaugrand 2010). Only recently climate induced changes on their physical habitat suitability have been investigated with a focus on potential changes in geographic distribution (Helaouët & Beaugrand 2009, 2011). However, changes in the vertical distribution of water mass characteristics and their importance for zooplankton distribution and migration are not well studied. This is especially important in relation to long-term declines in dissolved oxygen that reduce the habitat for oxygen-sensitive species (Doney et al. 2012).

A major effect of climate change on the Baltic Sea, the largest brackish water system of the world ocean, is a reduction in the frequency of major inflow events (Hanninen et al. 2000, Feistel et al. 2008). Here we investigated the effect of climate-induced habitat changes on *P. acuspes*. The non-invasive sampling with the VPR additionally allowed the observation of reproducing females, identified by attached egg sacs, but usually lost during the net sampling process. To assess the effect of changes in the physical habitat, we compared the small-scale distribution of our target species during non-inflow (i.e. stagnation) and inflow periods. Our study shows an immense increase in the availability of suitable vertical habitat after the inflow event along with increased copepod abundance and their wider vertical distribution(see Fig. 4-3). Applying a new method to estimate *in-situ* egg production rates from VPR derived images revealed no effect of hydrographic changes on individual egg production, pointing towards an effect of low salinity and oxygen on offspring survival. Our observations exemplify the strong impact that climate change can have on the physical habitat of key marine ecosystem species, important for overall ecosystem dynamics.

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5 Marine snow, zooplankton and thin layers: indications of a trophic link from small-scale sampling with the Video Plankton Recorder

Klas O. Möller[1,*], Michael St. John[2,1], Axel Temming[1], Jens Floeter[1], Anne F. Sell[3], Jens-Peter Herrmann[1], Christian Möllmann[1]

[1]Institute for Hydrobiology and Fisheries Science, Center for Earth System Research and Sustainability (CEN), KlimaCampus, University of Hamburg, Grosse Elbstrasse 133, 22767 Hamburg, Germany

[2]National Institute of Aquatic Resources at the Technical University of Denmark, Charlottenlund Castle, 2920 Charlottenlund, Denmark

[3]Johann Heinrich von Thünen-Institut, Institute of Sea Fisheries, Palmaille 9, 22767 Hamburg, Germany

Abstract

Marine aggregates of biogenic origin, known as marine snow, are considered to play a major role in the ocean's particle flux and may represent a concentrated food source for zooplankton. However, observing the marine snow–zooplankton interaction in the field is difficult since conventional net sampling does not collect marine snow quantitatively and cannot resolve so-called thin layers in which this interaction occurs. Hence, field evidence for the importance of the marine snow–zooplankton link is scarce. Here we employed a Video Plankton Recorder (VPR) to quantify small-scale (meters) vertical distribution patterns of fragile marine snow aggregates and zooplankton in the Baltic Sea during late spring 2002. By using this non-invasive optical sampling technique we recorded a peak in copepod abundance (ca. 18 ind l–1) associated with a pronounced thin layer (50 to 55 m) of marine snow (maximum abundance of 28 particles l–1), a feature rarely resolved. We provide indirect evidence of copepods feeding on marine snow by computing a spatial overlap index that indicated a strong positively correlated distribution pattern within the thin layer. Furthermore we recorded images of copepods attached to aggregates and demonstrating feeding behavior, which also suggests a trophic interaction. Our observations highlight the potential significance of marine snow in marine ecosystems and its potential as a food resource for various trophic levels, from bacteria up to fish.

KEY WORDS: Baltic Sea · Marine snow · Small-scale distribution · Thin layer · Trophic interactions · Video Plankton Recorder · Zooplankton

Introduction

Marine aggregates of biogenic origin, known as marine snow, are considered to play a major role in the ocean's particle flux (Alldredge & Silver 1988, Graham et al. 2000, Stemmann & Boss 2012) due to their high abundance and rapid sinking rates (Fowler & Knauer 1986, Alldredge & Silver 1988). This sinking of marine snow is a major mechanism of particulate carbon transport from the productive surface waters to the seafloor. Hence, marine snow represents an important contribution to carbon flux and sequestration. However, marine snow can also be a food resource for zooplankton, thereby contributing to the production of higher trophic levels (Dilling et al. 1998, Kiørboe 2011a,b). The link between marine snow and planktonic organisms is presently underappreciated due to their patchy distribution, which can occur even in a homogeneously mixed water column (Folt & Burns 1999). The patchy distribution of plankton is a well-studied phenomenon and of great relevance for biological productivity, trophic interactions and food web dynamics in marine ecosystems (Mackas et al. 1985, Pinel-Alloul 1995). However, conventional net sampling cannot quantitatively sample marine snow and thus is unable to define small-scale distribution patterns. Recent advances in optical sampling methods, however, have allowed observations of plankton patches from microscale (<1 m) to small scale (1 to 10 m) (e.g. Davis et al. 1992, Ashjian et al. 2001, Jacobsen & Norrbin 2009).

Typical planktonic features on small vertical scales are thin layers, which are driven by physical and biological processes (McManus et al. 2003, Durham & Stocker 2012). Physical processes identified to contribute to thin layers include water column stratification, vertical shear, and shearing by internal waves (Franks 1995, McManus et al. 2005). Often these thin layers are found in association with pycnoclines where the density gradient causes the accumulation of particles and plankton organisms (MacIntyre et al. 1995). Biological mechanisms bringing organisms to thin layers include diel vertical migration, predator avoidance, aggregation in food patches and mate search (e.g. Folt & Burns 1999, Woodson & MacManus 2007). Daly & Smith (1993) suggested that physical forces dominate the formation of large-scale plankton patches while biotic processes become more important at smaller spatial scales. These abiotic and biotic processes lead to high concentrations of bacteria, phytoplankton, zooplankton and/or marine snow (Alldredge et al. 2002, McManus et al. 2003, 2008) that can exceed the concentration of the surrounding environment by orders of magnitude. Hence, these layers may be regions of enhanced biological productivity and interactions (Sullivan et al. 2010a) and have been detected in a variety of marine systems including estuaries (Bochdansky & Bollens 2009), coastal shelves (McManus et al. 2005) and the open ocean (Cowles et al. 1998). Conceivably thin layers may have an extensive impact on marine ecosystem dynamics, and the magnitude of their importance is just now beginning to be quantified (Alldredge et al. 2002, Durham & Stocker 2012, Lyons & Dobbs 2012).

Laboratory feeding studies and gut content analyses have provided evidence that marine snow is a potential food source for zooplankton (Dilling et al. 1998), even if dispersed phytoplankton cells are available (Dilling & Brzezinski 2004). This observation might be especially important since larger crustacean zooplankton would be able to utilize nano- and microzooplankton that are colonizing marine snow aggregates and usually too small to be captured (Kiørboe 2001). However, little is known from in situ studies if aggregates are commonly grazed by zooplankton in the field (Kiørboe 2000, Jackson & Checkley 2011).

Here we employed a Video Plankton Recorder (VPR) to quantify fragile marine snow aggregates and zooplankton and their relative small-scale vertical distribution. Using this non-invasive optical sampling technique we recorded a pronounced thin layer of marine snow and copepods associated with a density gradient, an association in a feature rarely observed. We provide indirect evidence of copepods feeding on marine snow aggregates due to a strong spatial overlap within the thin layer, which is supported by images of copepods being attached to aggregates.

Materials and Methods

Study area

High-resolution images were obtained using the VPR during a spring bloom cruise in April 2002 on RV 'Alkor' in the Bornholm Basin in the central Baltic Sea (Fig. 5-1). The Baltic Sea is the largest brackish water area in the world. During summer a pronounced thermocline is established in the Bornholm Basin between 20 and 30 m depth while a strong halocline in 50 to 60 m depth separates the water column throughout the whole year (Matthäus & Franck 1992). This strong physical stratification makes the central Baltic Sea an ideal area for investigations on thin layers.

VPR

The VPR (Seascan) is a modern optical underwater instrument, i.e. a digital underwater camera system towed by a research vessel. The VPR employed was equipped with a high-resolution digital camera (Pulnix TM-1040) that records 25 image frames s^{-1}. We used a camera setting with a field of view of 0.7 × 0.7 cm, a focal depth of 3.00 cm and a calibrated image volume of 1.45 ml. The camera was set to the largest magnification (f-zoom) due to the small particle and plankton sizes in the sampling area. This magnification was deemed suitable for imaging small-sized adult calanoid copepod species like *Acartia* spp., *Temora longicornis* and *Pseudocalanus acuspes*, known to dominate the mesozooplankton in the Baltic Sea (Möllmann et al. 2000). Illumination for the camera was provided by a strobe (Seascan, 20 W Hamamatsu xenon bulb) with a pulse duration of 1 µs that was synchronized with the camera shutter. Additionally, the VPR was equipped with hydrographic and environmental sensors to measure temperature and salinity (CTD) (Falmouth Scientific) as well as fluorescence (Seapoint, model SCF).

The VPR was mounted on an equipment rack with a v-fin depressor and towed continuously from near bottom to near surface in an undulating way, to obtain data from the whole water column. In order to exclude the influence of turbulence in the ship's wake and to maintain a safe distance from the bottom, the sampled layer was limited to below ~7 m from the surface and above ~8 m from the bottom. We towed the VPR between 23:00 and 11:00 h covering the night/day transition. The gear was towed at 1.5 m s^{-1} (4 knots) and covered a distance of 115 km in total along a star-shaped transect (Fig. 5-1) with an hourly mean sampling volume of 130.4 l (1565 l in total).

Analysis and classification of images

Recorded images and sensor data were sent in real time to an onboard unit via a fibre optic cable. Plankton and other particle images were extracted from each image frame as regions of interest (ROIs) using the Autodeck image analysis software (Seascan) and saved to the computer hard drive as TIFF files. Each ROI was tagged using a time stamp to allow merging with the hydrographic parameters that were written to a separate logfile.

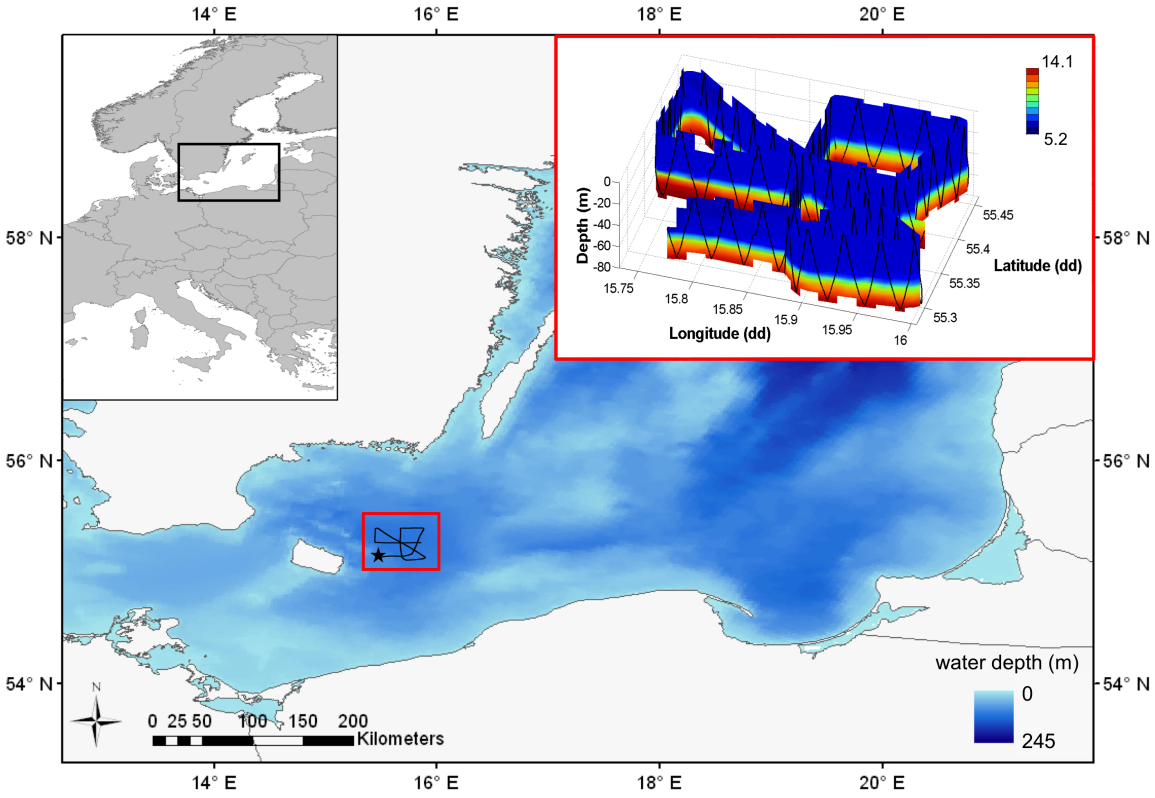

Fig. 5-1. Map of the Baltic Sea with the study area in the Bornholm Basin marked by a red square; the black star indicates the net sampling location. The upper left panel gives an overview of the geographic location of the sampling area and the upper right panel shows the density profile (σt color-coded) along the Video Plankton Recorder (VPR) tow track; black line indicates the VPR tow-yos.

Image classification

All images were classified automatically following a method by Hu & Davis (2006), an approach that includes an automatic correction step. This automatic correction step involves the computation of a confusion matrix from a set of training images in order to determine the detection of false positive rates. These rates are used to correct abundances estimated from the automatic classification results for each category. Aside from the manual sorting required to generate the initial training set of images, this dual-classification method is fully automatic and does not require subsequent manual correction of automatically sorted images. However, all images were also revised manually to avoid major misclassifications. Visual Plankton (Woods Hole Oceanographic Institution), an image processing and visualization application written in MATLAB (Mathworks), was used throughout the process of automatic classification. Once the

ROIs were generated a representative and randomly picked subset of a sufficient number of Training ROIs (trROIs) (approximately 200, see Hu & Davis 2006) was manually sorted into separate taxa or categories that were later used by the automatic classifier. The following step involved feature extraction algorithms that are used in image processing to detect, isolate and measure portions or shapes (features) of a digitised image. These features from the training ROIs were then used to create 2 classifiers in parallel. The first one was built from shape-based features and is called 'learning vector quantization neural network' (LVQ-NN). The second classifier was a 'support vector machine' (SVM) based on texture-based features of the same training samples. Detailed information about the operating modes of both is given elsewhere (Tang et al. 1998, Hu & Davis 2006).

Data handling

Only those taxa and categories of the training samples that had a relatively large number of images and yielded high automatic classification accuracies were selected for automatic classification. Some portions of the extracted images were not in focus and hence not within the imaged volume. Those images were sorted out and put into the category 'blurry'. All copepod species were combined into the general 'copepod' category, as the present classification method cannot distinguish between all genera or species. Rare plankton taxa and those with low automatic classification accuracies were sorted into the 'other' category. This group was composed of larval fish, crustacean larvae, polychaetes, fish eggs, appendicularians, echinoderm larvae and gelatinous zooplankton (e.g. hydromedusae and ctenophores). Marine snow aggregates formed a category varying in shape and size and yielded a high classification accuracy due to their amorphous shapes and distinct texture. Representative example images of marine snow and copepods are given in Fig. 5-2.

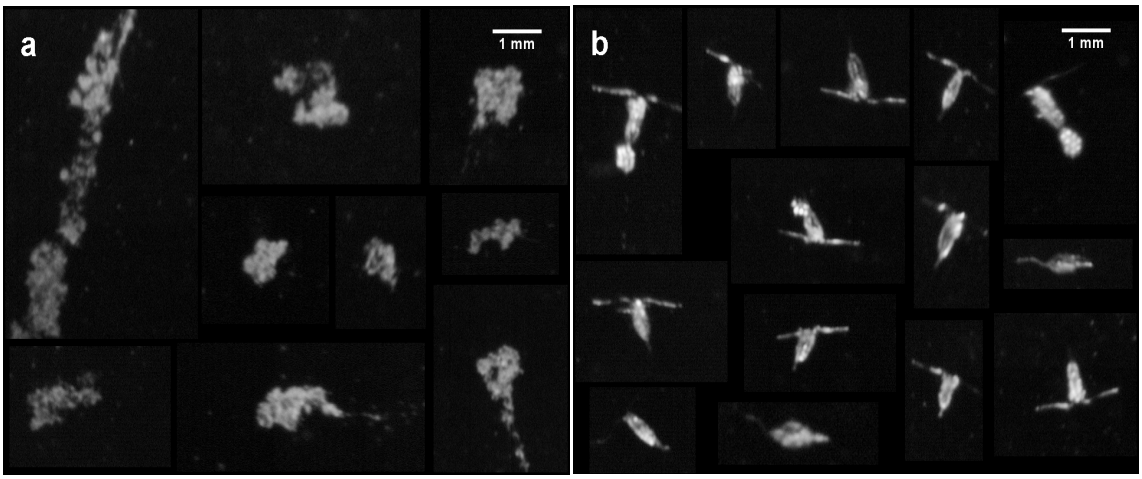

Fig. 5-2. Examples of VPR-derived images: Regions of interest (ROIs) of (a) marine snow and (b) copepods; the copepod category comprises all species (e.g. *Pseudocalanus acuspes* females with egg sacs and *Acartia spp.*).

Additional net sampling

Depth-resolved plankton net samples were collected in close proximity to the VPR transect (Fig. 5-1). We used a Hydrobios Multinet (150 µm mesh size) for comparison and ground-truthing of the VPR data and to obtain taxonomic information on the species level for copepods. Samples were taken between the surface and close to the bottom (~70 m) in stacked 10 m intervals and were immediately fixed in a borax-buffered formaldehyde-seawater solution (4% final formaldehyde concentration) for later analysis. Due to a lack of direct overlap between taxonomic categories (because of different taxonomic identification levels and sensitivity to fragile organisms) of net samples and VPR data, a quantitative comparison was only possible for the VPR-derived copepod category. Net samples were sorted manually to copepod species and stage (nauplii, copepodite stages C1 to C5 and adult males or females). Copepodites and adult copepods of all species were grouped together for comparison. Nauplii data were not used for the analysis.

Spatial overlap index

To assess the vertical overlap between copepods and marine snow aggregates in the water column we employed the spatial overlap index (*O*) described by Williamson & Stoeckel (1990):

$$O = \frac{\sum_{z=1}^{m} (N_z \times n_z) m}{\sum_{z=1}^{m} (N_z) \times \sum_{z=1}^{m} (n_z)}$$

where z represents the depth strata, m is number of depth points sampled, N_z is the density of copepods at a given depth and n_z is the density of marine snow aggregates at a given depth. An overlap index of <1 indicates spatial separation between the copepod and marine snow, an index = 1 indicates a homogeneous distribution, while values of >1 indicate an aggregation of copepods and marine snow in certain strata of the water column.

Overlap indices of cumulative abundances for copepods and marine snow were determined for every 2 h time step along the VPR tow track. There is no statistical test to evaluate the deviation of observed overlap from random expectation. Hence, in order to assess and test for variations in the bi-hourly spatial overlap along the VPR tow track, we computed the overlap index for each 10 min time step, corresponding to one complete up and down cast.

Results

Hydrography

As background information for our investigations on the small-scale distribution of marine snow and mesozooplankton we recorded the hydrographic environment along the VPR transect. We found the vertical distribution of temperature, salinity and density to represent a typical spring situation in the central Baltic Sea (Fig. 5-3), which included a surface mixed layer with a mean temperature of 4.5°C that was observed down to 50 m. No thermocline was recorded in the upper layer, but slightly elevated temperatures near the surface indicated the seasonal warming of surface waters. However, a thermocline separated the upper water layer from the winter deep water where temperatures increased to 9°C. The deep thermocline matched exactly with the permanent halocline, where salinity increased with depth from 8 to a maximum of 18. Together the thermocline and the halocline represented a strong density gradient, i.e. a pycnocline.

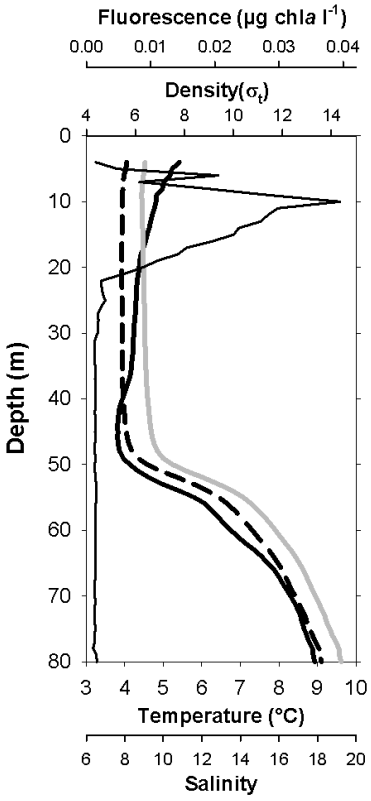

Fig. 5-3. Environmental data: Vertical profiles of temperature (°C, bold black line), salinity (black dashed line), density (σ_t, grey line), and relative fluorescence (µg chl *a* l^{-1}, thin black line), averaged over the whole VPR tow track for each 1 m depth interval.

Phytoplankton

We recorded the chlorophyll *a* (chl *a*) concentration as an index of the phytoplankton biomass by performing fluorescence measurements (Fig. 5-3). Due to our sampling scheme VPR measurements were not performed close to the surface. However, we observed phytoplankton primarily in the uppermost sampling stratum closest to the surface and not deeper than 20 m. Mean values of relative chl *a* were generally low (max. 0.04 µg l^{-1}) and peak abundances occurred to be patchy along the transect (max. 0.4 µg l^{-1}).

Marine snow

Marine snow aggregates were the second most abundant category recorded by the VPR next to copepods. The particle size spectra ranged from 0.2 to 7 mm in diameter with the highest abundances in the size class of ~0.5 mm. Fine-scale sampling with the VPR revealed a dense thin layer of marine snow aggregates at the pycnocline between 50 and 55 m depth (Fig. 5-4a). This thin marine snow layer was observed to occur over the whole transect with peak abundances of marine snow aggregates of 28 particles l^{-1}. Background aggregate concentrations in the water column varied between 5 and 8 particles l^{-1} with a mean (±SD) abundance of 6.4 ± 0.5 particles l^{-1}. Less dense patches of marine snow not related to the pycnocline were observed at some locations in the upper mixed layer. Visual examination of our marine snow images identified the phytoplankton origin of most aggregates, with many of the larger aggregates having the characteristic rod shape of diatoms. Additionally, appendicularians might have contributed to the marine snow production, since they produce houses that, once discarded, look like diatom flocks. Smaller and more abundant aggregates had a mucoid matrix including senescent diatom cells, detrital material and, due to the high abundance of copepods, probably also copepod exoskeletons as well as fecal pellets.

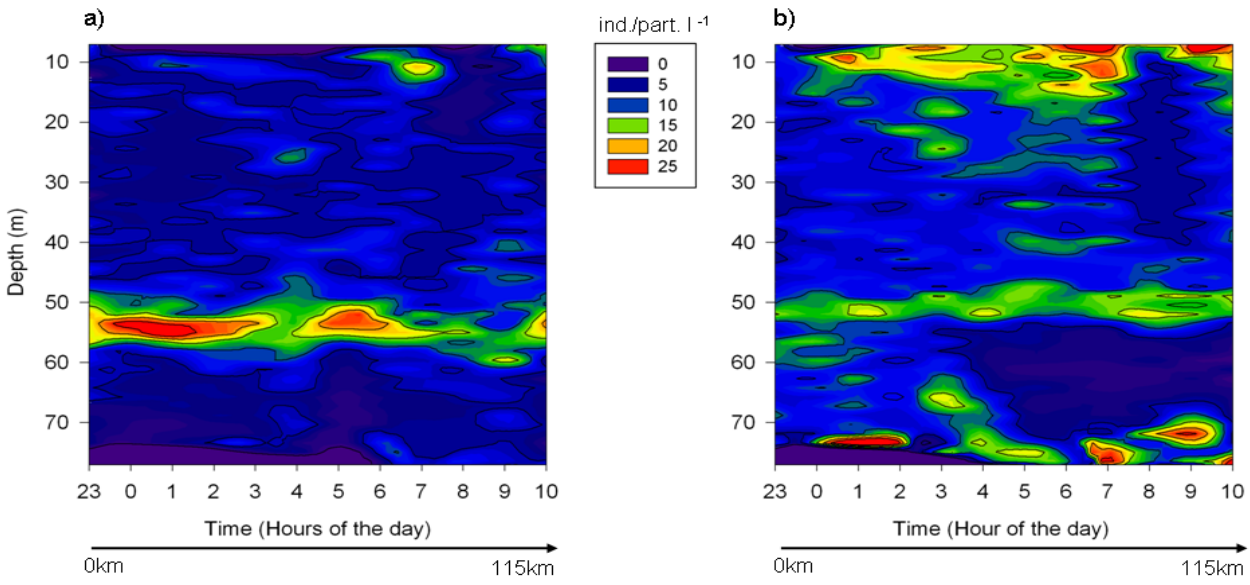

Fig. 5-4. Contour plots showing the average hourly depth distribution and abundance of (a) marine snow (particles l^{-1}) and (b) copepods (ind. l^{-1}) recorded with the Video Plankton Recorder (VPR); the black arrow indicates the cumulative distance travelled by the ship along the tow track.

We assume the absence of a fluorescence signal within the thin layer observed in our study to be due to the decay of phytoplankton cells. This could also explain the presence of an amorphous and flocculent film visible on all images. Furthermore, we observed no major changes in marine snow abundance or particle size that could be attributed to diel influences during night and day transition.

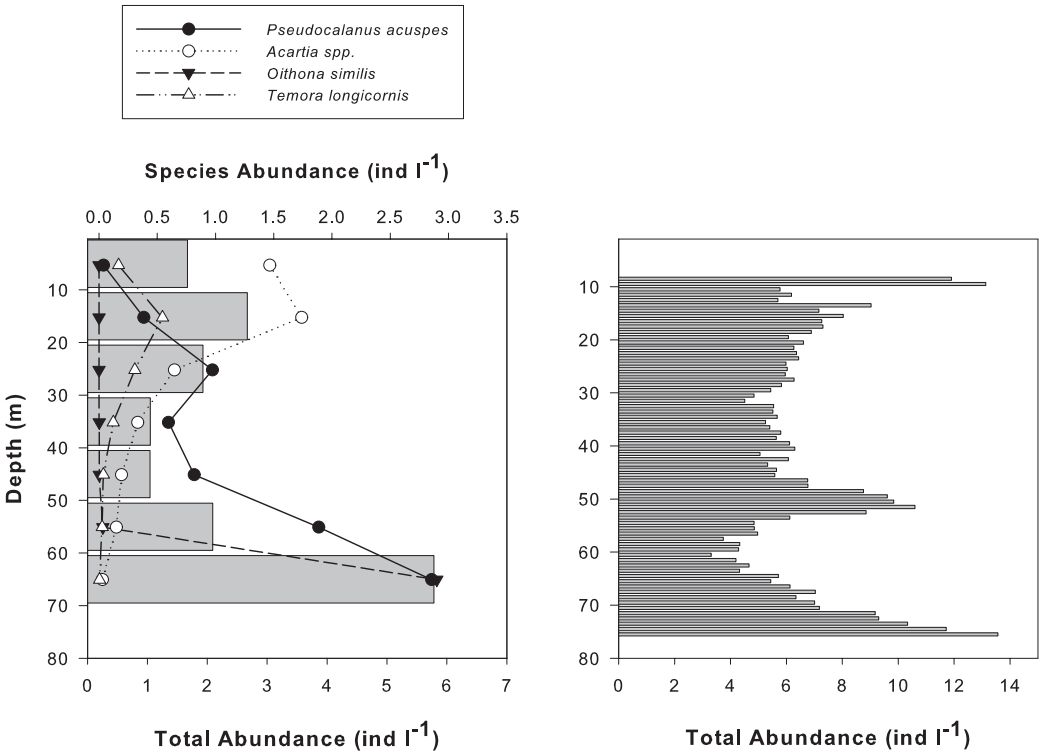

Fig. 5-5. Vertical copepod distribution patterns: (a) Total copepod abundance (ind. l^{-1}) combining all species in one category in 10 m depth bins (from 0 – 70 m), and species specific depth distribution of copepods (ind. l^{-1}) (including adults and copepodite stages) sampled with the Multinet; (b) Copepod abundance (ind. l^{-1}) sampled with the Video Plankton Recorder (VPR) averaged over the whole tow track in 1m depth bins (from 8 – 76 m depths).

Mesozooplankton

Copepods were the dominant group comprising 60% of all mesozooplankton organisms classified. Additionally we recorded approximately 30% appendicularians and 10% gelatinous organisms (comprising small hydromedusa and ctenophores) in the identified mesozooplankton category. We observed a vertical maximum in abundance of copepods in the upper surface layer (28.2 ind l^{-1}) and close to the bottom (23.4 ind. l^{-1}), and a distinct peak within the pycnocline (18.2 ind. l^{-1}) (Fig. 5-4b). Background copepod concentrations between those peak abundances in the water column varied between 5 and 9 ind. l^{-1} with a mean abundance of 7.9 ± 1.0 ind. l^{-1}. The abundance peak in the pycnocline was stable during most of the transect, with high copepod abundances overlapping with the marine snow thin layer. Dense patches of copepods were also found close to the bottom as well as near the surface. In comparison with the deep water

aggregations these patches were more heterogenous in distribution and less pronounced, while the surface aggregations tended to have a more widespread vertical distribution and were more patchy horizontally. There was no indication of diel changes in the vertical copepod distribution.

We used conventional net sampling and manual sorting to evaluate the copepod species composition not resolved by the VPR (Fig. 5-5a). The copepod community was dominated by *Acartia* spp., *Temora longicornis*, *Pseudocalanus acuspes* and *Oithona similis*. Copepods displayed species-specific vertical distributions, which allowed a mapping of species to the different vertical peaks in the copepod category sampled by the VPR. *Acartia* spp. and *T. longicornis* dominated the upper layer <30 m depths, while *P. acuspes* and *O. similis* were found in the pycnocline and in aggregations in the deep water above the bottom.

A comparison of the 2 sampling techniques revealed strong differences in abundance values recorded and in the ability to resolve vertical distribution patterns (Fig. 5-5). For this purpose copepod abundance values sampled with the VPR along the tow track were averaged in 1 m depth bins. All copepod species sampled with the Multinet were grouped into one abundance category to match the VPR sampling, but, due to the nature of the sampling gear, in 10 m depth bins only. Mean abundances sampled with the VPR were generally higher (max. 13.5 ind. l^{-1}) than with the Multinet (max 5.8 ind. l^{-1}). As expected the vertical distribution pattern showed clear differences as well. The fine-scale resolution of the VPR profile allowed discrimination of the copepod layer at 50 to 55 m, while this feature could not be observed with the coarse vertical resolution of the Multinet sampling.

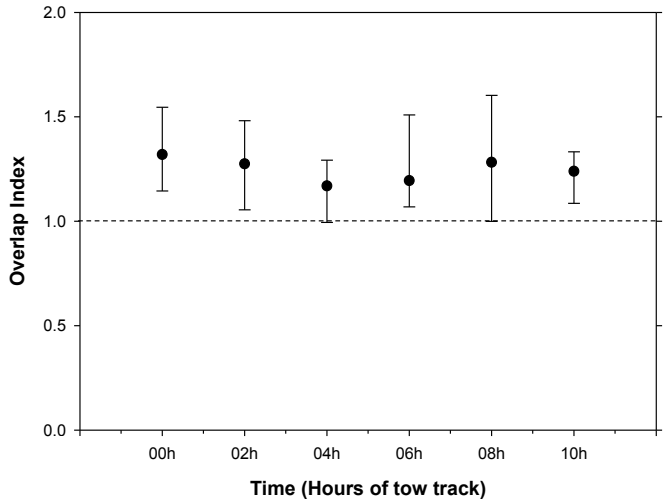

Fig. 5-6. Spatial overlap of marine snow and copepods: Black dots represent bi-hourly estimates of the Williamson spatial overlap index during the sampling period; error bars show minimum and maximum variations of the overlap index for each 20 min. time step; overlap indices <1 indicate spatial segregation between the copepod and marine snow; overlap indices = 1 indicate that copepods and/or marine snow are uniformly distributed in the water column; overlap indices >1 (black dotted line) indicate aggregation of copepods and marine snow in certain strata of the water column.

Indications for marine snow–mesozooplankton interaction

As an indication of the feeding interaction between copepods and marine snow, we calculated an index of their vertical overlap. Overlap indices for marine snow and copepods ranged from 1.04 to 1.51 along the whole tow track, indicating a permanent positively correlated distribution pattern (Fig. 5-6). Peak concentrations of marine snow aggregates and high abundances of copepods coincided within a vertical narrow band along the pycnocline. Because there was no indication of diel changes in the abundance and distribution of copepods and marine snow, a stable overlap was observed. Only minor variations were observed in the overlap index for single up and down casts of the VPR between index values of 0.99 and 1.6.

A portion (approx. 5%) of the marine snow aggregate images obtained with the VPR showed copepods directly attached to the aggregates, suggesting an active feeding behavior. However, this estimate may be low due to occlusion of copepods by aggregates and the ROI extraction method. Furthermore, many of those images that were in focus and of high quality allowed species identification. Most of these images showed *Pseudocalanus acuspes* with its antennae in feeding position (Broughton & Lough 2006) suggesting active feeding by copepods on marine snow (Fig. 5-7). Feeding behavior of *Temora longicornis* could be identified on a few images as well.

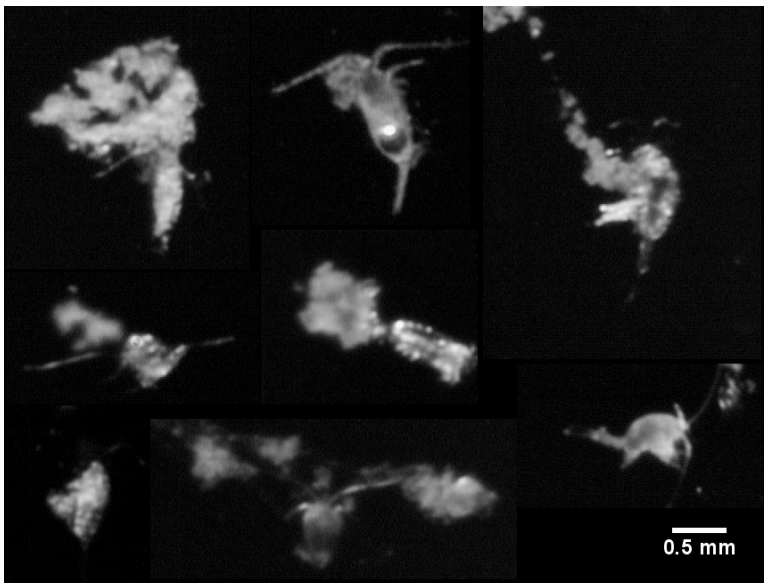

Fig. 5-7. Example of VPR-derived images of copepods attached to marine snow indicating a trophic interaction.

Discussion

Marine snow occurrence

In this study we quantified the abundance of fragile marine snow aggregates as well as their sizes. We evaluated the results through a comprehensive literature review on studies quantifying marine snow aggregates (Tab. 5-1). Our review revealed a large range of particle abundance and sizes. Generally, several factors influence the quantification of marine snow metrics in the field and can explain the variability between studies. For example, variability in particle concentrations can be the result of the sampling location or sampling technique. Many previous studies are based on quantitative observations by divers, which are often limited to particles readily visible (≥3 mm) and restricted to the upper surface layer (Lampitt et al. 1993b). Technical advances within the last 2 decades have led to an increased number of data sets that have been assessed by optical sampling methods and generally tend to result in decreasing aggregate sizes. Furthermore, size estimates based on *in situ* measurements with camera systems always depend on the respective field of view, which may explain differences between particular studies. Our estimates of particle abundance are in the upper range of studies using the VPR, which all displayed similar particle size ranges. Similar values were also recorded using Underwater Video Profilers. Based on the literature review, we consider our marine snow abundance estimates as reliable.

Mesozooplankton abundance and distribution

A comparison of copepod abundances revealed estimates derived by the VPR to be generally more than 2-fold higher than those obtained with the Multinet. Similar results have been reported by Benfield et al. (1996) and Broughton & Lough (2006) when comparing zooplankton abundances obtained with a VPR and a MOCNESS (Multiple Opening/Closing Net and Environmental Sampling System) sampler (). Differences between the 2 gear types are probably due to different sampling efficiencies for earlier copepodite stages as well as smaller copepod species such as *Oithona similis*, which are typically underrepresented in net samples due to net extrusion and/or avoidance (Gallienne&Robins 2001, Broughton & Lough 2006). The high spatial and temporal resolution of sampling with the VPR relative to the Multinet was critical for resolving vertical thin layers. Clearly our net sampling was not able to resolve the pronounced small-scale abundance peak in the halocline, which was only visible in the VPR data. However, the strength of the Multinet data is in the provision of abundance information on the species level. Manual analysis of Multinet samples allowed a clear attribution of the dominant copepod species to the layers of high copepod abundance observed by the VPR. Similar to earlier studies (Hansen et al. 2006), we found Acartia spp. and Temora longicornis inhabiting the upper water column. Both species have a preference for high water temperatures and low salinities (Holste & Peck 2006, Holste et al. 2009), and hence may exploit the chl *a* maximum in the upper water column. Conversely, *Pseudocalanus acuspes* and *Oithona similis* are marine species with preferences for high salinities and low temperatures (Hansen et al. 2004, Renz & Hirche 2006), hence dominate the zooplankton abundance in the deep and saline marine snow layer. Due to its ontogenetic vertical distribution, the vertical distribution of *P. acuspes* showed a minor

Sampling Technique	Sampling location	Abundances (particle l^{-1})	Particle size spectra (Ø mm)	Reference
Video Plankton Recorder	Baltic Sea	≤ 28 ($\bar{x}$ 6.4)	0.2 - 7 ($\bar{x}$ 0.5)	Möller et al. (this study)
	Beaufort Sea, Arctic	≤ 80	0.5 - 6 ($\bar{x}$ 1.66±0.69 mm)	Ashjian et al. (2005)
	Georges Bank	≤ 3.8	nd	Gallager et al. (2004)
	Georges Bank	≤ 5	$\bar{x}$ 1.8 - 2.1	Ashjian et al. (2001)
	Georges Bank	≤ 7	up to a few milimeters	Norrbin et al. (1996)
Video Plankton Recorder & Camera system	Central North Pacific	≤ 13	0.5 - 3	Pilskaln et al. (2005)
Underwater Video Profiler	Northwest Mediterranean Sea	0.14 - 1108	0.2 - 0.55	Stemmann et al. (2008)
	Equatorial Pacific	$\bar{x}$ 0.62 - 9.95 between stations	nd	Gorsky et al. (2003)
	Northwest Mediterranean Sea	6 - 30	$\bar{x}$ 0.17 - 0.40	Stemmann et al. (2000)
Camera system	Equatorial Atlantic, Deep sea	< 6	> 0.5	Bochdansky et al. (2010)
	Cape Blanc, Upwelling	≤100 (max. 30 - 100 between stations)	0.15 - >1 ($\bar{x}$ 0.37 - 0.53)	Iversen et al. (2010)
	East Sound Fjord	25 - 250	$\bar{x}$ 2.9 - 5	Alldredge et al. (2002)
	Santa Barbara Channel, California	max. 10 - 80	nd	Graham et al. (2000)
	off Central California	6 - 110	0.5 to 10	MacIntyre et al. (1995)
	Northeast Atlantic	≤ 12	0.6 - 9.9	Lampitt et al. (1993a)
	Northeast Atlantic	≤ 300	0.6 - 9.8	Lampitt et al. (1993b)
Scuba divers with camera	Cape Lookout Bight	2.63 to 371 ($\bar{x}$ 125)	0.2 to several mm (few >2)	Shanks (2002)
	off Charleston, Atlantic	14 - 63	1 - 2 (length)	Shanks and Walters (1997)
	Parks Bay, Pacific	3 - 11	> 1 - 6 (length)	Shanks and Walters (1997)
	West Sound, Pacific	0.1 - 2	90 - 171 (length)	Shanks and Walters (1997)
	West Sound, Pacific	0.07 to 1.23	$\bar{x}$3.2 to 16	Shanks and Walters (1996)
Scuba divers	Santa Barbara Channel	nd	0.5 - 14.7	Goldthwait et al. (2004)
	Gulf of Mexico	0.007 to 0.05	3000 - 4000	Green and Dagg (1997)
	Northern Adriatic Sea	nd	<200 (length)	Bochdansky and Herndl (1992)
	Northern Adriatic Sea	nd	aggregates ≤5 (clouds up to 3 m)	Kaltenböck and Herndl (1992)

Table 5-1: Literature review of studies quantifying marine snow occurrence using different sampling techniques. VPR: Video Plankton Recorder; UVP: Underwater Video Profiler; nd: no data

abundance peak at ~25 m depth, which can be attributed to younger copepodite stages (Renz & Hirche 2006).

Thin layers of marine snow and mesozooplankton

The formation of thin layers of passive particles is controlled by physical and biological factors (Sullivan et al. 2010b). For example marine snow particles have been identified to form thin layers by settling on strong density gradients due to reduced sinking rates caused by changes in the density of the medium (Alldredge et al. 2002). Processes leading to aggregations of motile planktonic organisms in thin layers are much more complex. They include active swimming, sexual reproduction, predator avoidance, food availability and responses to chemical or physical cues (Dekshenieks et al. 2001, McManus et al. 2003, 2005, Gallager et al. 2004, Malkiel et al. 2006, Benoit-Bird et al. 2010, Holliday et al. 2010).

Although the mechanisms described above have substantial consequences for the ecological importance of thin layers, they have to a large extent remained untested in the field (Benoit-Bird et al. 2009). Generally, the existence of thin vertical layers has been rarely investigated, which is due to the low sampling efficiency of traditional net sampling for marine snow and the generally low ability of this method to resolve small-scale distribution patterns (Widder et al. 1999, Alldredge et al. 2002, Jacobsen & Norrbin 2009). Studies using optical and acoustical instruments have only recently started to quantitatively describe thin layers of marine snow at gradients (Alldredge et al. 2002, McManus et al. 2003). In the present study, we describe an intense thin layer of marine snow aggregates associated with a strong vertical density gradient in the permanent halocline typical for a deep Baltic basin, using results from small-scale sampling with a VPR. In contrast to earlier studies (Cheriton et al. 2007, Sevadjian et al. 2010), we found this layer of marine snow aggregates persisting throughout our whole sampling track, most likely due to the stability of the halocline.

Furthermore, we observed a thin layer of copepods directly attached to marine snow aggregates that showed signs of typical feeding behavior. Hence, we suggest that copepods actively respond to dense marine snow aggregations with the goal to use them as a food source. A number of laboratory experiments have shown that copepods are able to detect and exploit food patches in thin layers (Tiselius 1992). However, field observations of zooplankton behavior in relation to thin layers of marine snow, such as in the present study, are still rare. Previous field studies mainly investigated zooplankton thin layers acoustically (e.g. McManus et al. 2003, Benoit-Bird et al. 2009, Holliday et al. 2010). However, these studies were not able to investigate the species composition in these layers, which is an important issue as behavioral responses are often species-specific. Alldredge et al. (2002), using a camera system, observed high densities of planktonic organisms above and below a thin layer of marine snow. They suggested that most macrozooplankton taxa avoid the thin layer because of very high marine snow abundances that potentially lead to clogging of their delicate feeding appendages. In agreement with our observations Malkiel et al. (2006) reported elevated abundances of calanoid copepods with maximum concentrations of marine snow. However, they interpreted this pattern as predator avoidance behavior, since high concentrations of large detritus particles may provide shelter from visual predators (Malkiel et al. 2006). Based on our high-resolution imaging study using the VPR, we suggest that zooplanktonic copepods aggregate in thin layers as a response to a concentrated food source of marine snow.

Trophic interactions between marine snow and zooplankton

The key finding of this study is a strong association of copepods showing signs of typical feeding behavior with a thin layer of marine snow. Our results support earlier laboratory and field studies that provide direct evidence of feeding interactions between marine snow and euphausiids (Dilling et al. 1998, Dilling & Brzezinski 2004), ostracods and amphipods (Lampitt et al. 1993b), as well as copepods (Steinberg 1995, Koski et al. 2007, Chen et al. 2010, Wilson & Steinberg 2010). A few studies provided, as we have here, indirect evidence of feeding interactions through close spatial associations between zooplankton and marine snow aggregates (Shanks & Edmondson 1990, Bochdansky & Herndl 1992, Green & Dagg 1997, Shanks & del Carmen 1997, Malkiel et al. 2006). Despite these indications of a trophic interaction, there still remains the question whether marine snow is a viable food source for zooplankton. There is still little information on the trophodynamic importance of marine snow in marine ecosystems since these aggregates are difficult to quantify both in the lab and the field. In our study, marine snow particles consisted primarily of mucoid detritus derived from phytoplankton cells, which usually represents a viable food source for copepods (Dilling et al. 1998). In the Baltic Sea, feeding on marine snow in the halocline may even be crucial for the survival of copepod populations. In or below the halocline of the deep Baltic basins no primary production occurs, and copepod species such as *Pseudocalanus acuspes* and *Oithona similis*, which need the elevated salinities in deeper waters (Hansen et al. 2004, 2006, Renz & Hirche 2006), experience comparable conditions as zooplankton residing in the mesopelagic zone of the open ocean. At this depth nutrition must be obtained to a large degree via feeding on sinking aggregates (e.g. Steinberg 1995). Lipid biomarker studies support this hypothesis and show that Baltic *P. acuspes* is an opportunistic feeder, feeding mainly on sinking algae, detritus and microzooplankton (Peters et al. 2006). Furthermore, these investigations suggest that ciliates, which generally show elevated concentrations on marine snow aggregates (Silver et al. 1984, Lombard et al. 2010), have a high relevance in the food spectrum of *P. acuspes* (Peters et al. 2006).

Our observations support the hypothesis that marine snow aggregates are an important food source for mesozooplankton, especially in stratified regions such as the central Baltic Sea. Furthermore, marine snow may have an important function in the ecosystem as an important additional food source for benthic suspension feeders (Newell et al. 2005) and possibly larval and juvenile fish (Larson & Shanks 1996, Green & Dagg 1997). Our observations highlight the significance of marine snow in marine ecosystems and its potential as a food source for various trophic levels, from bacteria up to fish.

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6 Plasticity in response of a key marine ecosystem player to predation risk

Klas O. Möller[1,*] , Michael St.John[2,1], Axel Temming[1] , Rabea Diekmann[1], Janna K. Peters[1], Jens Floeter[1] , Anne F. Sell[3] , Jens-Peter Herrmann[1] , Dominik R. Gloe[1], Jörn O. Schmidt[4], Hans H. Hinrichsen[5], Christian Möllmann[1]

[1]Institute for Hydrobiology and Fisheries Science, Center for Earth System Research and Sustainability (CEN), KlimaCampus, University of Hamburg, Germany

[2]National Institute of Aquatic Resources at the Technical University of Denmark, Denmark

[3]Johann Heinrich von Thünen-Institut, Institute of Sea Fisheries, Germany

[4]Sustainable Fishery, Department of Economics, University of Kiel, Germany

[5]Helmholtz Centre for Ocean Research Kiel, GEOMAR, Germany

Abstract

Predators in ecosystems not only have direct impact on biomass but also indirect, non-consumptive effects on the behavior their prey organisms. A characteristic response of some zooplankton species in aquatic ecosystems is predator avoidance by diurnal vertical migration (DVM), a behavior which is well studied on the population level. A wide range of behavioral diversity and plasticity has been observed both between- as well as within-species and hence investigating predator-prey interactions at the individual level seems therefore essential for a better understanding of zooplankton dynamics. However, *in-situ* observations of zooplankton on the individual level is due to the complexity of vast aquatic ecosystems particularly challenging. Here we applied a modern optical underwater sampling gear, the Video Plankton Recorder (VPR), that allows in contrast to traditional net sampling the investigations of individual, diurnal adaptive behavior of zooplankton in response to predators in the natural oceanic environment. We studied the zooplanktonic copepod *Pseudocalanus acuspes* that is key to ecosystem functioning of the pelagic foodweb of the Central Baltic Sea. Combing observations of zooplankton individuals with the VPR and hydroacoustic estimates of predatory fish biomass we here show (i) a DVM of Baltic *P. acuspes* in response to its main predator, (ii) first *in-situ* observations of a direct short-term reaction of the prey to the diurnal arrival of the predator and (iii) first *in-situ* evidence of strong individual variation in this adaptive behavior with potentially strong effects on individual performance and ecosystem functioning.

KEY WORDS: Diurnal vertical migration · Individual behavior · Predator avoidance · Baltic Sea · Trophic interactions · Video Plankton Recorder · Zooplankton · *Pseudocalanus*

Introduction

Predators in ecosystems not only have direct effects on their prey biomass, but can also induce important indirect effects such as trophic cascades or influences on diversity, production and nutrient cycling (Greig & McIntosh 2006, Trussell et al. 2006, Schmitz et al. 2008, Schmitz et al. 2010, Strong & Frank 2010). Among these indirect effects of predation are non-consumptive effects on behavior of prey organisms (Preisser et al. 2009). Generally, herbivores and other intermediate trophic level species need to balance the trade-off between maximizing energy or nutrient intake and minimizing predation risk (Werner & Peacor 2003, Preisser et al. 2005). Such an adaptive foraging behavior hence can have important implications for ecosystem functioning, e.g. by affecting transfer efficiencies between trophic levels. Furthermore, predator avoidance behavior may force prey species into unfavourable environments in terms of food availability or physical habitat variables such as temperature, salinity and oxygen (Sainmont et al. 2012). Hence, from an evolutionary perspective species in danger of predation need to respond adaptively to balance fitness gains from foraging with fitness losses from predation (Mangel & Clark 1988, Lima & Dill 1990, Lima 1998).

An important adaptive behavior in aquatic ecosystems is diurnal vertical migration (DVM) that has been demonstrated for diverse organisms from protists up to fish (Bollens & Frost 1989, Kaartvedt et al. 2007, Cohen & Forward 2009, Ringelberg 2010). DVM has major ecological consequences at the individual, population, community and ecosystem level (Ramirez-Llodra et al. 2010, Bollens et al. 2012, Sainmont et al. 2012). Furthermore, it plays an important role in global biogeochemical cycles by modifying and transporting inorganic and organic material throughout the water column (Robinson et al. 2010). DVM of planktonic organisms in lakes and oceans represents one of the most widespread and massive migration of animals on Earth (Williamson 2011).

A number of causes are discussed to induce DVM including light conditions, food availability and temperature (Dupont et al. 2009, Ringelberg 2010) or trade-offs between those (Loose & Dawidowicz 1994). For zooplankton such as copepods, the most wide-spread animal group in the world ocean, there is general consensus that predator avoidance is a major driver for DVM (Lampert 1989, Bollens & Frost 1989, Bollens & Frost 1991, Hays 2003, Bollens et al. 2011). The most common pattern of zooplankton DVM involves an avoidance of shallower depth during daylight and hence visual predators such as planktivorous fish (e.g. Zaret & Suffern 1976, Frost 1988, Buskey et al. 2011).

The role of predation in DVM and, hence adaptive foraging behavior of an ecologically important group such as marine copepods is unequivocal (Sainmont et al. 2012), but direct observations of the relative behavior of predator and prey in natural enviroments are still rare. Traditional net sampling methods often fail to resolve the spatial and temporal scales necessary for observing DVM in plankton and parallel predator biomass measures are frequently lacking. Observing variability in individual plankton behavior in vast natural environments such as the ocean is especially difficult since net sampling typically provides only population mean differences over larger spatial scales. Hence, aggregated group-level data may blur and misrepresent individual behavior, a phenomenon called the „ecological fallacy“ (Wakefield & Shaddick 2005, Clark et al. 2011).

Experimental studies in behavioral ecology have demonstrated strong variation in behavior within single populations which may be due to individual personality and plasticity to

environmental cues (Biro & Dingemanse 2009, Dingemanse et al. 2009). Stage-specific variation in predator avoidance has been shown for zooplankton populations in relation to feeding history, size, reproductive status and pigmentation (Baumgartner et al. 2011, Holliland et al. 2012, Lönnstedt et al. 2012). A wide range of behavioral diversity and plasticity has been observed both between-species as well as within-species (Cohen & Forward 2009, Kaartvedt et al. 2011). Especially trophic interactions in natural environments occur at the individual rather than at the population level (Kiørboe 2008). Therefore, investigating predator-prey interactions at the individual level seems essential for a better understanding of zooplankton dynamics. This is important since predator hunting strategy and, consequently, prey escape strategy can be viewed as key functional traits that partly control the top-down interactions in ecosystems (Legreneur et al. 2012).

We applied a modern optical underwater sampling gear, the Video Plankton Recorder (VPR), that allows in contrast to traditional net sampling the investigations of individual, diurnal adaptive behavior of zooplankton in response to predators in the natural oceanic environment. We studied the zooplanktonic *Pseudocalanus acuspes,* a key species for the ecosystem functioning of the pelagic foodweb of the Central Baltic Sea (Möllmann et al. 2009). The population of *P. acuspes* has recently suffered from climate induced changes in the physical environment (Möllmann et al. 2003). Furthermore, overfishing the local top-predator cod (*Gadus morhua*) caused a strong predation pressure on *P. acuspes* by planktivorous small pelagic fish (Casini et al. 2008, Möllmann et al. 2008).

Combining observations of zooplankton individuals with the VPR and hydroacoustic estimates of predatory fish biomass, we here show (i) a DVM of Baltic *P. acuspes* in response to its main predator, (ii) first *in-situ* observations of a direct short-term reaction of the prey to the diurnal arrival of the predator and (iii) first *in-situ* evidence of strong individual variation in this adaptive behavior with potentially strong effects on individual performance and ecosystem functioning.

Materials and Methods

The model species

We studied DVM of the calanoid copepod *Pseudocalanus acuspes* that is an important component in the Baltic ecosystem (Möllmann et al. 2009; Fig. 6-1) as are other species of the genus *Pseudocalanus* spp. in most areas of the world ocean (McLaren & Corkett 1978, Hopcroft & Kosobokova 2009). A key feature of its life-cycle is an ontogenetic vertical migration, i.e., its resident depth increases with individual age represented by developmental stage (Renz & Hirche 2006). Later larval, i.e. copepodite stages and adult *P. acuspes* reside in deep waters specifically in the layer of highest salinity, the so-called halocline (Renz & Hirche 2006, Hansen et al. 2006). This habitat provides them with concentrated food through marine snow aggregates (Möller et al. 2012) and a salinity level that allows successful reproduction (Renz & Hirche 2006). However, the downside of this habitat is a high predation risk since populations of small pelagic and planktivorous fish feed here during daytime (Köster & Schnack 1994). Hence, our model species within its special habitat is ideal for studying adaptive behavior because *P. acuspes* individuals need to deal with the trade-off between maximizing energy or nutrient intake and minimizing predation risk.

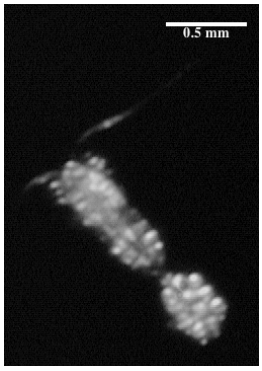

Fig. 6-1. Example image of a *Pseudocalanus acuspes* female with egg sac.

Data acquisition with the Video Plankton Recorder

We obtained high resolution images of plankton using the Video Plankton Recorder (VPR) during field campaigns in April 2002 and May 2009 aboard the research vessel „Alkor" in the Bornholm Basin of the Central Baltic Sea (Fig. 6-2). The VPR (Seascan) is a modern underwater camera system towed by a research vessel. Our VPR was equipped with a high-resolution digital camera (Pulnix TM-1040) which records 25 image frames s^{-1}. We used a camera setting with a field of view of 0.7 x 0.7 cm and a calibrated image volume of 1.45 ml in 2002 and 1.10 ml in 2009. The camera was set to the largest magnification (f-zoom) due to the small particle and plankton size in the Baltic Sea. Illumination for the camera is provided by a strobe (Seascan / 20 W Hamamatsu Xenon bulb) with a pulse duration of 1 µs, which was synchronized with the camera shutter. Additionally, the VPR was equipped with hydrographic and environmental sensors for temperature and salinity (CTD) (Falmouth Scientific Inc.) as well as fluorescence (Seapoint Inc., model SCF). Additionally, oxygen data were obtained using a CTD-probe equipped with an oxygen sensor measuring vertical profiles after each VPR haul.

The VPR was mounted on an equipment rack with a v-fin depressor and undulated continuously from near bottom to near surface, hence, surveying the whole water column. In order to exclude the influence of turbulence in the ship's wake and to maintain a safe distance from the bottom, sampling was only conducted between app. 5 m below the surface and 8 m above the bottom, but varied between the VPR surveys (see Fig. 6-3). The VPR was towed between 11pm and 11am covering the full night/day transition from April 25th and 26th in 2002. In 2009 four separate VPR hauls were conducted between May 17th (4pm) and 18th (7pm) covering day-, nighttime and the day/night transition. The gear was towed with a speed of 1 – 1.5 m s^{-1}, covering a distance of 115 km and a total sampling volume of 1565 l in 2002 and a distance of 91.2 km and a total sampling volume of 1242 l in 2009. Recorded images and sensor data were sent in real time to an onboard unit via a fiber optic cable. Plankton and other particle images were extracted from each image frame as regions of interest (ROIs) using the Autodeck image analysis software (Seascan) and saved to the computer hard drive as TIFF files. Each ROI was tagged using a timestamp to allow merging with the hydrographic parameters that were written to a separate logfile. Most ROIs did not allow discrimination of copepods to the species level. Hence, we only used ovigerous *P. acuspes* females in our analysis since these are best identified due to their distinct shape caused by attached egg sacs (Fig. 6-1). In the following we refer to ovigerous females only as *P. acuspes* females.

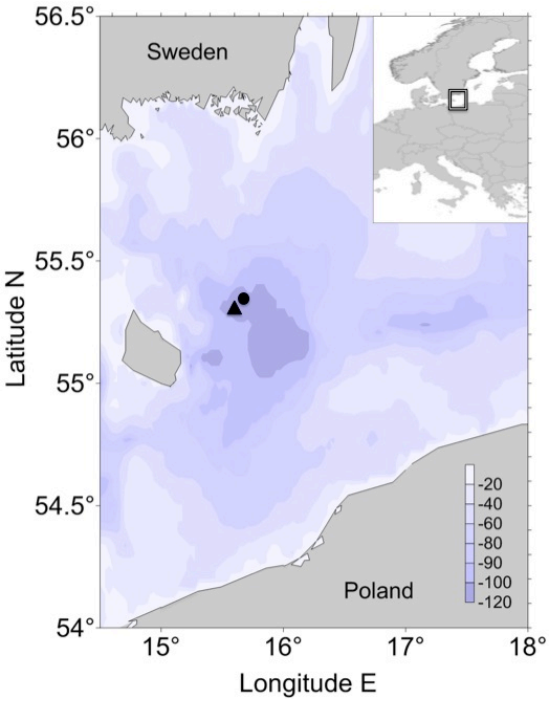

Fig. 6-2. Map of the Central Baltic Sea with the study area in the Bornholm Basin; the black dot indicates the area of the Video Plankton Recorder (VPR) sampling location in 2002 and the black triangle the VPR and Multi-Net sampling location in 2009. The upper right panel gives an overview of the geographic location of the sampling area.

Hydroacoustic fish biomass recording

We measured the biomass and vertical distribution of the planktivorous fish population using hydroacoustic techniques during the cruise in 2009. The research vessel was equipped with a Simrad echosounder EK60 using a hull-mounted 38-kHz split-beam transducer. Hydroacoustic measurements were performed according to standard procedures for Baltic Sea acoustic surveys (ICES 2001). Calibration settings of the echosounder and data processing were conducted using the Sonardata ECHOVIEW 4.9 software (Sonardata ECHOVIEW 2010) applying a volume backscattering coefficient threshold of S_V –60 dB. Hydroacoustic recording and VPR sampling cannot be performed parallel due to different ship speeds required. Hence hydroacoustic recording was conducted the day following the VPR sampling which we assume does not introduce a major bias since the vertical pelagic fish distribution appears to be quite stable during spring in the study area (Stepputtis 2011).

Numerical analyses

We were interested in diurnal differences in the depth distribution of *P. acuspes* females. Thus, all VPR data were seperated into day- (4 am to 22 pm) and nighttime (22 pm to 3 am) values. Sampling times at dusk or dawn when copepods presumably migrate vertically were excluded from the analysis. Abundances of *P. acuspes* females were averaged for each hour of sampling and 1m depth intervals. The centre of the copepod distribution was calculated as the median depth and the corresponding upper and lower quartiles as variability measures. A modified two-sample Kolmogorov-Smirnov test was applied to test the null hypothesis of equal day and night depth distributions (Solow et al. 2000).

A modified test statistic *W* was applied

$$W = \frac{max\left(\left|P_{jk} - P_{jk}\right|\right)}{\left(\sum_{k=j}^{k}\left(p_{jk} - p_{jk}\right)^2\right)^{\frac{1}{2}}}$$

with P_{jk} being the sample cumulative distribution function of depth for the sample *j* and p_{jk} the relative abundance of sample *j* in depth bin *k*. The significance of *W* was assessed by a randomization scheme underlying Fisher's exact test of independence in a contingency table. During randomization, both samples were pooled and divided at random into two samples of sizes n_1 and n_2. Row sums and column sums need to be the same as from the original samples. Eventually, W was calculated for 100000 permutations of the dataset and the significance level was estimated by the proportion of simulated W-values larger than the observed values. All calculations were performed within the statistical environment of R, version 2.14.2 (R Development Core Team 2012).

As an index of vertical location of planktivorous fish, hourly weighted mean depths (WMD) (Bollens & Frost 1989) were computed as:

$$\mathrm{WMD} = \left(\frac{(\sum n_i d_i)}{\sum n_i}\right)$$

where n_i is the fish density expressed as nautical area scattering coefficient (NASC) of the population in depth stratum i with midpoint depth d_i. The same index was computed for all female *P. acuspes* individuals, grouped together from both sampling periods to obtain the mean diurnal distribution during the day. Since *P. acuspes* individuals showed variable vertical distribution patterns during daytime, the mean of the hourly nighttime WMD was used as a threshold to compute average lower and upper WMD for each daytime hour.

We further investigated within-population variability of DVM. We tested for departures from unimodality in the migratory behavior of the female *P. acuspes* population in response to their predators. We used Hartigans´ dip test (Hartigan & Hartigan 1985) implemented in the dip function in the R diptest library. The test is very conservative and the distribution of the test statistic is based on asymptotic and empirical samples relative to a uniform distribution (Bestelmeyer et al. 2011). The power of the test (for $\alpha = 0.05$) is 80% when sample size = 50. Since our sample sizes were much larger we used *p*-values ≤ 0.01 to indicate statistically significant evidence for departure from unimodality.

Results

Physical environmental habitat

As background information for our investigations on DVM of *Pseudocalanus acuspes* females, we recorded their physical habitat simultaneously to the VPR sampling (Fig. 6-3a,d). In upper water layers, the seasonal thermocline was visible in 2009, but not in 2002, which was due to the earlier cruise date in 2009. In deeper water layers, we clearly observed the permanent halocline at c. 50 to 60 m depths, representing the typical habitat of *P. acuspes* females. Oxygen and salinity depth profiles were almost identical in both years showing a decline in oxygen levels below the halocline to just above oxygen deficiency. Below the permanent halocline temperature increases, which is characteristic for the Central Baltic Sea.

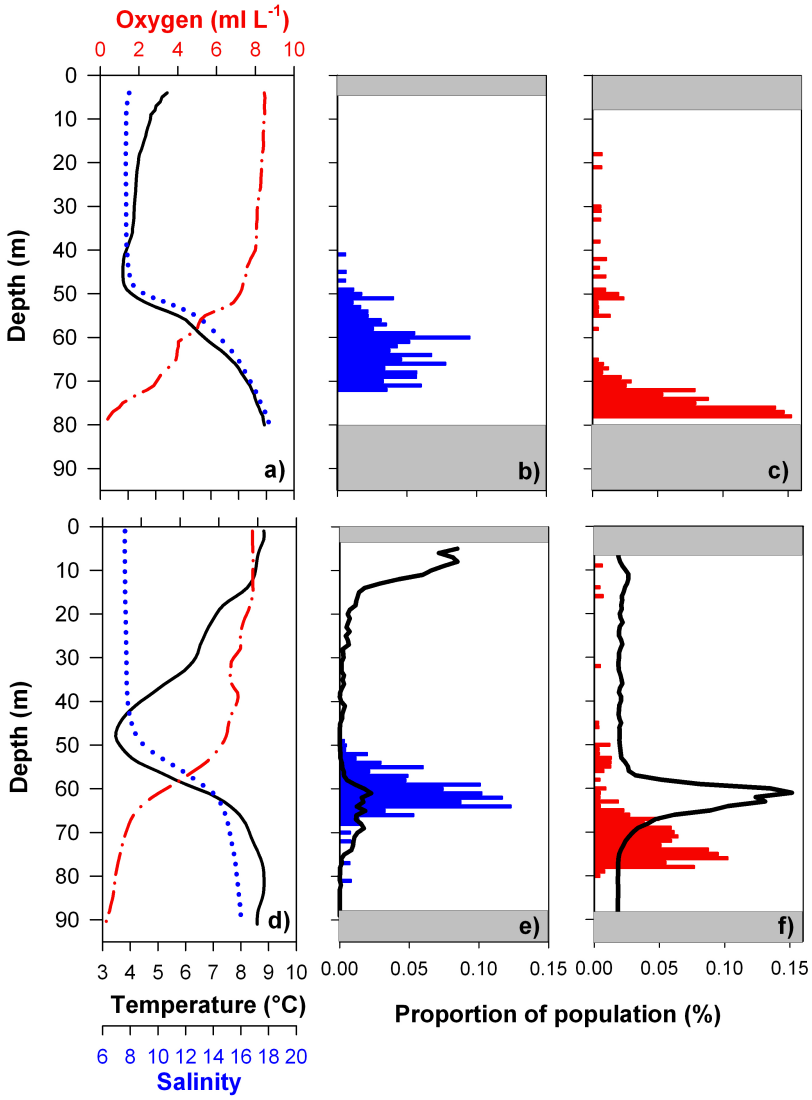

Fig. 6-3. Environmental data and vertical day and night distribution patterns of ovigerous *Pseudocalanus acuspes* in 2002 (upper row) and 2009 (lower row): (a) & (d) Vertical profiles of temperature (°C, black line), salinity (blue line) and oxygen (mg ml^{-1}, red dot-dashed line) averaged for each VPR tow track in 1 m depth intervals; (b,c) & (e,f) Vertical average nighttime (b & e, blue bars) and daytime (c & f, red bars) proportional distribution (%) of ovigerous *P. acuspes* in 1 m depth bins sampled with the Video Plankton Recorder (VPR). Black line indicates the vertical distribution of fish (proportional Nautical area scattering coefficient); Grey areas indicate the average sampling depth representing the ranges to surface and bottom without sampling;

Average DVM patterns of prey and predator

The average water column abundance of *P. acuspes* females integrated over all 1 m sampling bins was similar in both years with 0.3 Ind. L^{-1} during night and a slightly lower abundance of 0.23 Ind. L^{-1} during day. Overall 403 and 388 individual *P. acuspes* females were observed with the VPR in 2002 and 2009, respectively. We recorded maximum abundance (average depth interval concentrations) of 2.14 (day) and 1.52 Ind. L^{-1} (night) in 2002, and slighty lower values in 2009 with 1.45 (day) 2.82 Ind. L^{-1} (night).

We found evidence of a „classical" average DVM for *P. acuspes* females during both sampling campaigns (Fig. 6-3 b, c,& e, f) because of significantly deeper mean distributions during daytime (K-S test modified for patchy distributions; $p < 0.001$ for both years). Weighted median depth of the copepods was 75 and 63 m in 2002, and 72 and 61 m in May 2009 for day and night, respectively. Variability of the mean vertical distribution is generally low with deviations from the median (weighted upper and lower quartiles) of 5 and 7 (day) and 9 and 5 m (night) for 2002 and 2009, respectively. During daytime, the spread of the distribution was increasing and hence, individuals were observed between the surface and 50 m depth. We found the deeper daytime distribution of *P. acuspes* females related to the presence of the predator population. Planktivorous fish themselves performed DVM being close to the surface during night (average WMD night = 23.8 m) and in and below the halocline during daytime (average WMD day = 58.5 m) (Fig. 6-3 e & f).

Within population variability in P. acuspes female DVM

In a second step we made use of the special ability of the VPR to identify individual *P. acuspes* and investigated if the DVM was performed unidirectionally by all members of the population or if there exists within-population variability. We, therefore, combined observations from both sampling campaigns into one diurnal dataset since average vertical day- and nighttime profiles were not significantly different between 2002 and 2009 (modified K-S test, $p_{day} < 0.05$; $p_{night} < 0.01$. Moreover, the significance of the difference between years was lower than day-night differences (2002: $p < 0.0001$; 2009: $p < 0.001$). Following all 912 sampled female *P. acuspes* along the time-axis reveals a striking immediate response of the zooplankton population to the DVM of their predators (Fig. 6-4). During night, nearly all copepods dwelled uniformely in or around the halocline between 50 and 70 m. In response to the downward migration of the fish predators at sunrise, most individual zooplankter migrated into deeper water depths and aggregated in 70 – 80 m (max. average WMD = 71.7 m) where they remained during the day. Unexpectedly, a smaller part of the population avoided the predator population by moving in the opposite direction towards shallower depths.

Hence, our observations demonstrate substantial within population variability in DVM of *P. acuspes*. The results of the visual inspection is supported by a daytime vertical distribution that is significantly different from unimodality (Hartigans´ dip test: $p< 0.01$). Rather, we observed a bimodal vertical distribution with the „shallower fraction" of the copepod population displaying more variability than the „deeper fraction". Eventually, at sunset, the predator population returns towards the surface and female *P. acuspes* return to concentrate unimodally in the center of their usual halocline habitat.

Potential consequences of prey avoidance behavior

The results of our study revealed an adaptive behavior of our model species with a fast response to the presence of its predator population that let them leave their preferred habitat during daytime. Hence we assumed the nighttime distribution to represent optimal conditions for *P. acuspes* females in terms of hydrographic and food conditions while leaving these can lead to reduced growth, reproductive success and survival. As an indication of potential negative effects of the DVM, we calculated the proportion of the population that is migrating out theoptimal conditions as a consequence of predator presence (Fig. 6-5).

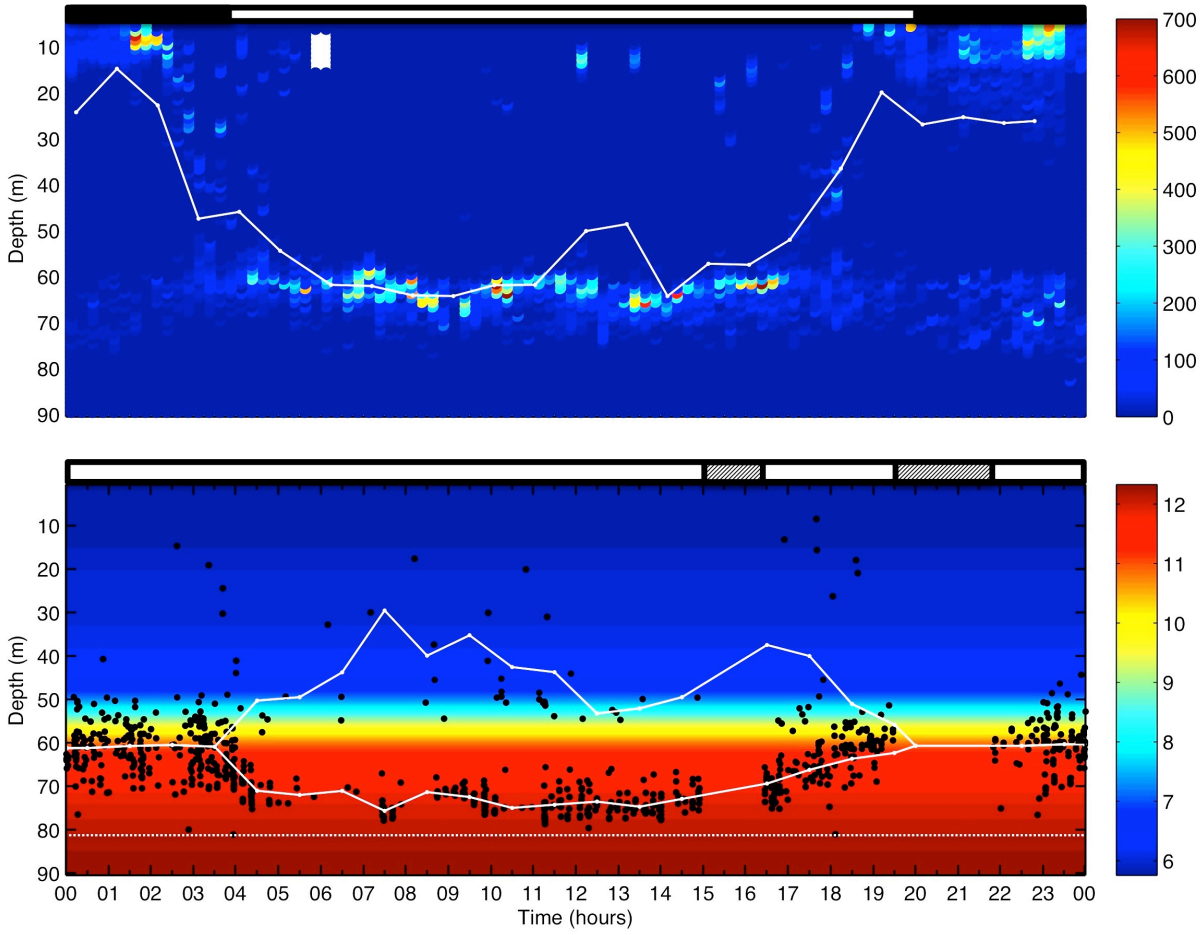

Fig. 6-4. Vertical diurnal distribution of fish and ovigerous *Pseudocalanus acuspes*: (upper panel) Diel echogram showing the vertical distribution of fish densities and expressed by the nautical area scattering coefficient (NASC). White line indicates hourly weighted mean depths of vertical fish distribution in NASC. Bar on top of the panel represents day- (white) and nighttime (black). (lower panel) Vertical distribution of all individual ovigerous *P. acuspes* (black dots) sampled with the Video Plankton Recorder (VPR) in 2002 and 2009 in relation to an averaged schematic density profile (σ_T) of 2009. White lines indicate hourly weighted mean depths for all individuals (night), individuals showing escape behavior during day (upper line) and individuals performing vertical migration during day (lower line). Bar on top of panel indicates VPR sampling (white) and data gaps (black shaded). White dashed line indicates the maximum depth where individuals occurred.

Based on our observations, we determined the distribution range between 55 and 65 m (WMD during night equaled 60 m) as the undisturbed situation. On average, we observed 13 % of the population to dwell shallower compared to the undisturbed depth range during daytime, hence, facing lower than optimal salinity conditions (< 13). A large proportion of 79 % was observed deeper than the optimal depth range, hence, facing suboptimally low oxygen conditions. The individually experienced oxygen minimum of *P. acuspes* corresponded to 0.8 ml L^{-1}.

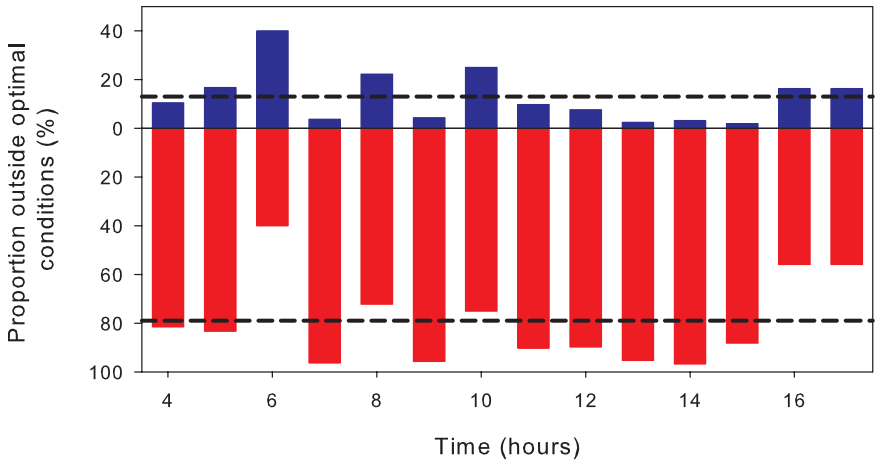

Fig. 6-5. Proportion of individual *P. acuspes* outside the preferred habitat. Blue bars indicate proportion (%) of individuals above (adverse salinity), red bars below (adverse oxygen) the preferred habitat during daytime (4am – 6pm); black dotted line indicates the mean daytime proportion (%) of individuals above and below the preferred habitat.

Discussion

In this study, we combined observations of zooplankton individuals with a VPR and hydroacoustic estimates of predatory fish biomass and were able to show a DVM of Baltic *P. acuspes* in response to its main predator. We observed a classical DVM of zooplankton that has been shown for many species in conventional studies before (e.g. Zaret & Suffern 1976, Frost 1988, Bollens et al. 1992). However, for the first time, enabled by using a modern optical underwater observation technique, we showed *in-situ* an immediate reaction of a zooplankton population to the behavior of the predator. Predation avoidance is the most likely reason for DVM of *P. acuspes* females in the Baltic Sea since light conditions do not change significantly over the depth range of the migration in the deep basin. Furthermore, our observations strongly suggest that the copepods leave the food-rich marine snow layer in the halocline directly in response to the predators (Möller et al. 2012). Although we are confident in our results, the ultimate evidence that predation induces the DVM would be a control situation with no predators present during daytime and consequently no occurance of DVM. We unfortunately did

not encounter such a situation yet. Furthermore, the trigger for the migration still remains unclear and waits for experimental verification. A candidate trigger is mechanoreception that is widely used by copepods to detect and react to hydrodynamic disturbances created by approaching predators (Bollens et al. 1994, Buskey et al. 2011). Furthermore, the chemoreception of kairomones released by fish predators is another commonly discussed trigger (Cohen & Forward 2009).

Baltic *P. acuspes* is a marine species with preferences for high salinities and low temperatures and hence we observed egg-carrying females in the deep and saline bottom water confirming earlier studies (Hansen et al. 2006, Renz & Hirche 2006). Most previous studies have been using conventional net sampling and due to the coarser vertical resolution were not able to show DVM for this key species. However, despite its ability to identify individual planktonic animals on small spatial and temporal scales, species identification still remains problematic based on VPR-derived images. We focused our study on ovigerous adult females of *P. acuspes* that carry their eggs until hatching in a conspicious external egg sac (Fig. 6-1). This special life-history feature allowed a discrimination from other developmental stages and copepod species, usually impossible with VPR-derived pictures. This procedure might have introduced a bias in the perception of the DVM of the whole adult population since it ignores non-reproducing females and male *P. acuspes* (Bollens & Frost 1989, Holliland et al. 2012). We, however, recorded the part of the population that is mainly important for producing the subsequent generation. Net sampling on the other side would have resulted in a loss of the egg sacs and, hence, a discrimination between egg-carrying and not reproducing females would have been impossible.

An important result of our study is the first *in-situ* evidence of strong individual variation in a marine copepod species´ DVM. Most of the *P. acuspes* females showed a general trend of a downward migration during day, but some individuals responded to the presence of predators with an upward movement towards the surface. Previous studies have shown stage-specific differences in behavior due to size or reproductive status (e.g. Bollens & Frost 1991, Holliland et al. 2012), but we show that behavioral responses additionally can also vary between individuals of one developmental stage. The two different behavioral modes of *P. acuspes* in the Baltic indicate that different predator avoidance strategies exist in a population. The regular DVM of most of the individuals might represent an evolutionary developed behavior imprinted in the population. The upward movements of single individuals are most likely more spontaneous responsive escape behavior. Recent research has shown that the decision to migrate might depend on individual traits, such as size, sex or conditional state (Olsson et al. 2006, Brodersen et al. 2008, Hansson & Hylander 2009). The direction of the response in our case is likely to depend on the position of the copepods in the water column relative to their predators.

In our opinion, our VPR observations deliver strong evidence for individual variability in behavior of Baltic *P. acuspes* females. However, the ultimate evidence for individual-based behavior would be to track many animals over time which is impossible with zooplankton *in-situ*. Laboratory experiments would be necessary allowing individual tracking with camera systems, but the natural setting observed in our study will be difficult to reproduce. We were able to observe individual variability due to the ability of the VPR to conduct a continuous sampling over a long period of time and on small spatial scales at a stationary sampling site that serves as an comparable method for tracking individuals, although not following it. Furthermore, we observed the behavioral pattern repeatedly during two different cruises making an observation based just on chance unlikely.

Our results indicate that the risk of predation during daytime forces large parts of the *P. acuspes* adult female population out of their optimal habitat. The latter, which they inhabite during night, is characterized by suitable salinity and oxygen conditions (Renz & Hirche 2006) as well as accumulations of marine snow where they find food (Bochdansky et al. 2010, Möller et al. 2012). Hence, during daytime most of the population is forced into low oxygen zones, which can function as a refuge against predation (Larsson & Lampert 2011), but might have detrimental effects on the survival of the females and their offspring (Marcus et al. 2004). Conversely, the part of the population that is avoids predation by migrating to the surface encounters potentially too low salinity conditions which have negative consequences for their physiological performance (Gaudy et al. 2000, Renz & Hirche 2006). The animals may, however, account for the period of sub-optimal conditions during day by making use of their saline, oxygenated, and food-rich habitat undisturbed by predators during night.

In summary, combining observations of zooplankton individuals with the VPR and hydroacoustic estimates of predatory fish biomass, we here show (i) a DVM of Baltic *P. acuspes* in response to its main predator, (ii) first *in-situ* observations of a direct short-term reaction of the prey to the diurnal arrival of the predator and (iii) first *in-situ* evidence of strong individual variation in this adaptive behavior. Furthermore, we show that this adaptive behavior to predation has the cost of accepting unfavourable conditions in terms of physical habitat parameters and feeding conditions. Thus, the increase in planktivorous predator populations through trophic cascading (Casini et al. 2008, Möllmann et al. 2008) may have caused direct consumptive effects on the population of *P. acuspes*, but as shown here indirect non-consumptive effects as well (Schmitz et al. 2008).

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7 Effects of climate induced habitat changes on a key zooplankton species

Klas O. Möller[1,*], Jörn O. Schmidt[4], Michael St.John[2,1], Axel Temming[1], Rabea Diekmann[1], Janna K. Peters[1], Jens Floeter[1], Anne F. Sell[3], Jens-Peter Herrmann[1] and Christian Möllmann[1]

[1] Institute for Hydrobiology and Fisheries Science, Center for Earth System Research and Sustainability (CEN), KlimaCampus, University of Hamburg, Germany

[2]National Institute of Aquatic Resources at the Technical University of Denmark, Denmark

[3]Johann Heinrich von Thünen-Institut, Institute of Sea Fisheries, Germany

[4]Sustainable Fishery, Department of Economics, University of Kiel, Germany

[5]Helmholtz Centre for Ocean Research Kiel, GEOMAR, Germany

Abstract

Impacts of climate change on marine ecosystems have become increasingly apparent during the last decades. In the Baltic Sea, deep water salinity and oxygen levels declined over the last years due to climate driven changes in the frequency of inflowing North Sea water. In consequence, it is necessary to study how these alterations can affect the habitat and population dynamics of organisms. Here we used a Video Plankton Recorder (VPR), a modern optical underwater technology to investigate the effect of climate-induced habitat changes on the copepod *Pseudocalanus acuspes*, a key species in the Baltic Sea. Non-invasive sampling allowed the observation of reproducing females, identified by attached egg sacs, usually lost during traditional net sampling. We compared the small-scale distribution of our target species during non-inflow (i.e. stagnation) and inflow periods. Our study shows an immense increase in the availability of suitable vertical habitat after the inflow event along with increased copepod abundance and their deeper and wider vertical distribution due to improved oxygen and salinity conditions. Applying a new optical approach to estimate *in-situ* egg production rates from VPR derived images revealed no changes on individual egg production, pointing towards an effect of low salinity and oxygen on offspring survival. Our observations exemplify the strong impact that climate change can have on the physical habitat of key marine ecosystem species, important for overall ecosystem dynamics.

KEY WORDS: Baltic Sea · *Pseudocalanus* · Small-scale distribution · Climate change · Habitat · Video Plankton Recorder · Hypoxia · Egg production · Inflow event

Introduction

Knowledge on how climate change is affecting marine ecosystems has lagged behind that of terrestrial ecosystems, partly due to the size and complexity of the ocean, but also due to the difficulty of taking measurements in marine environments (Hoegh-Guldberg & Bruno 2010). Nevertheless, impacts of climate change on the ocean have become increasingly apparent during recent decades including changes in temperature, circulation, stratification, nutrient, input, oxygen content, and ocean acidification (e.g. Brierley & Kingsford 2009, Doney et al. 2012). Changes in the abiotic environment can have wide-ranging biological effects among them population-level shifts due to physiological intolerance to new environments (Bernhardt & Leslie 2012, MacKenzie et al. 2012). Resulting changes in community structure and species interactions may eventually change overall ecosystem functioning and alter the provision of services to depending people and societies (Doney et al. 2012).

In the important trophic level of marine zooplankton, changes in key environmental factors can influence the distribution of individual species and assemblages, the timing of important life-cycle events, abundance and community structure (Richardson 2008, Loeb et al. 2009, Beaugrand & Kirby 2010,). Hence, zooplankton is a suitable indicator for the effect of climate change on marine ecosystems and long-term changes in marine zooplankton are well studied (e.g. Planque & Taylor 1998, Möllmann et al. 2000, Beaugrand 2003, Mackas & Beaugrand 2010). Only recently, climate induced changes on their physical habitat suitability have been investigated, with a focus on potential changes in geographic distribution (Helaouët & Beaugrand 2009, 2011). However, changes in the vertical distribution of water mass characteristics and their importance for zooplankton distribution and migration are not well studied. This is especially important in relation to long-term declines of dissolved oxygen that reduce the habitat for oxygen-sensitive species (Doney et al. 2012).

A major effect of climate change on the Baltic Sea, the largest brackish water system of the world, is a reduction in the frequency of major inflow events (Hanninen et al. 2000, Feistel et al. 2008). These are lateral transports of oxygen rich North Sea waters into the deep Baltic basins, where vertical mixing of deep waters is hindered by a permanent halocline. If not ventilated by an inflow, biological activity reduces the oxygen content in the deep water to levels detrimental for local plant and animal populations. Major Baltic inflows are thought to be regulated by atmospheric forcing and while observed regularly until the early 1980s, recently their occurence is reduced to once per decade (Matthäus & Franck 1992, Schinke & Matthäus 1998, Feistel et al. 2008). The last major inflows have been recorded in only 1993 and 2003 (Jakobsen 1995, Feistel et al. 2003, Mälkki & Perttila 2012) and consequently anoxic bottom waters are now widespread and common in the deep Baltic Sea basins (Kabel et al. 2012). The reduced frequency of major Baltic inflows has been related to changes in the North Atlantic Oscillation (NAO), the dominant mode of climate variability in this region (Mälkki & Perttila 2012).

Lacking Baltic inflows contributed to the major reorganization of the food web during the late 1980s/early 1990s (Alheit et al. 2005, Möllmann et al. 2009). Changes were most pronounced in the fish community since amplified by overfishing, but zooplankton changes were important in this so-called ecosystem regime shift (Möllmann et al. 2008). A key zooplankton species in the Baltic ecosystem is the calanoid copepod *Pseudocalanus acuspes,* a major food item for commercially important herring (*Clupea harengus*) and sprat (*Sprattus sprattus*) as well as for larval cod (*Gadus morhua*) (Möllmann et al. 2003, 2004, 2008). *P. acuspes* is of marine origin and

later larval stages and egg-carrying adults use the halocline and the saline deep water as their reproductive habitat (Renz & Hirche 2006), where they also feed on marine snow (Möller et al. 2012). Lacking inflows reduced salinity in the deep water (Fonselius & Valderama 2003) a trend correlated with the declining population size of *P. acuspes* (Möllmann et al. 2000, 2003), additionally suffering from high predation pressure (Möllmann & Köster 2002, Casini et al. 2008, Möllmann et al.2008). The process behind the salinity effect is, however, still unclear. Renz & Hirche (2006) suggested, e.g. a direct physiological effect of low salinity on growth and reproduction. The apparent detrimental effect of low salinity on *P. acuspes* may, however, be rather due to co-occurring low oxygen levels. Low salinity may force reproducing adult females to move into anoxic deeper waters potentially affecting egg production or survival of adults, the attached eggs or hatched nauplii stages.

Investigating the effects of climate-induced changes on the deep water habitat of *P. acuspes* and, hence, their population dynamics requires high frequent observations of the animals on small spatial scales in relation to the environmental conditions (Möller et al. 2012). Conventional net sampling, resolving the water column only in depth intervals of a few meters do not provide the needed sampling resolution in time and space. Here we used a Video Plankton Recorder (VPR), a modern underwater imaging technology to investigate the effect of climate-induced habitat changes on *P. acuspes*. Additionally, the non-invasive sampling with the VPR additionally allowed the observation of reproducing females identified by attached egg sacs, but lost during the net sampling process. To investigate the effect of changes in the physical habitat we compared the small-scale distribution of our target species during non-inflow (i.e. stagnation) and inflow periods. Our study shows an immense increase in the availability of suitable vertical habitat after the inflow event along with increased copepod abundance and their wider vertical distribution. Applying a new method to estimate *in-situ* egg production rates from VPR derived images, revealed no effect of hydrographic changes on individual egg production, pointing towards an effect of low salinity and oxygen on offspring survival. Our observations exemplify the strong impact climate change can have on the physical habitat of key marine ecosystem species important for overall ecosystem dynamics.

Materials and Methods

Video Plankton Recorder (VPR) observations

The Video Plankton Recorder (Seascan) is a modern optical underwater instrument, i.e. a digital underwater camera system towed by a research vessel. The VPR was deployed on four cruises in the Bornholm Basin in the Central Baltic Sea to obtain high resolution images of plankton and particles. The surveys covered the period before (April & May 2002) and after (April & May 2003) the last significant inflow event (Fig. 7-1). Additionally, another VPR survey was conducted in the Bornholm Basin in May 2009 to obtain data of the environmental restucturing during the persisting stagnation period (Fig. S7-1). The VPR was mounted below a v-fin depressor and towed with a ships speed of 3 to 5 knots continuously from near bottom to near surface in an undulating way, to obtain data of the whole water column. The sampling layer was limited to approximately 4 m below the surface and 8 m above the bottom to exclude influence of turbulences in the ships wake as well as maintain a safe distance to the bottom. Only VPR transects with a depth > 60 m, the depth of the halocline before the inflow event, were used in the analysis to exclude any sampling bias in the vertical distribution.

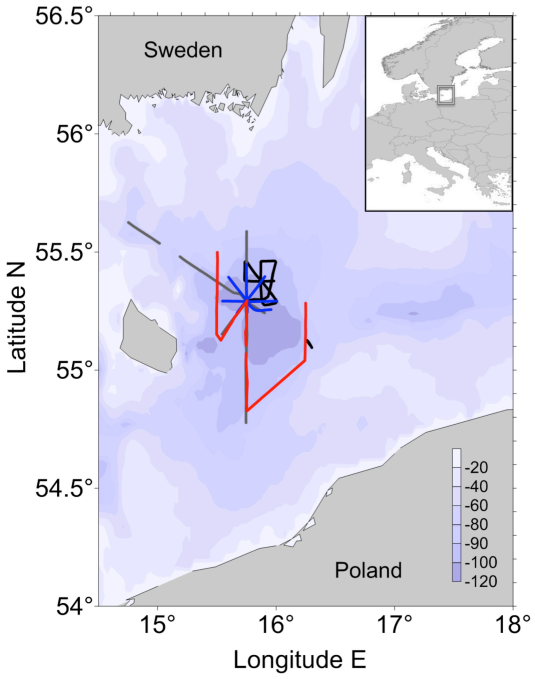

Fig. 7-1. Map of the Central Baltic Sea with the study area in the Bornholm Basin; colored lines indicate Video Plankton Recorder (VPR) surveys in 2002 (April: black line, May: red line) and 2003 (April: grey line, May: blue line). The upper right panel gives an overview of the geographic location of the sampling area. The legend indicates water depth (m).

VPR technical settings

The VPR was equipped with a high-resolution digital camera (Pulnix TM-1040) monitoring the imaged water volume with a rate of 25 image frames s^{-1}. The sampling volume changed due to different calibration settings between the cruises. We used a camera setting with a field of view of 0.8 x 0.8 cm and a calibrated image volume of 1.45 ml in April 2002 and 0.85 ml in May 2002 resulting in a total sampling volume of 1438 l and 1012 l per cruise, respectively. A smaller sampling volume of 0.65 ml was used during both cruises in 2003 resulting in a total sampling volume of 989 l in April and 576 l in May. A calibrated image volume of 1.01 ml was used in May 2009 resulting in an overall sampling volume of 1242 l. The camera was set to the largest magnification (f-zoom) due to the small particle and plankton size in the sampling area. This magnification was deemed suitable for imaging small sized adult copepod species like *Pseudocalanus acuspes*. Illumination for the camera was provided by a strobe (Seascan / 20 W Hamamatsu Xenon bulb) with pulse duration of 1 µs, which was synchronized with the camera shutter. Additionally, the VPR was equipped with hydrographic and environmental sensors for temperature and salinity (CTD) (Falmouth Scientific Inc.) as well as fluorescence (Seapoint Inc., model SCF). Oxygen data and hydrographic information of the 2003 surveys were obtained from vertical profiles with a stand-alone CTD equipped with an oxygen sensor in between the VPR hauls. All hydrographic measurements were averaged in 1 m depth bins over the respective survey period.

Data processing and classification

Recorded images and sensor data were sent in real time to an onboard unit via a fiber optic cable. Plankton and other particle images were extracted from each image frame as regions of interest (ROIs) using the Autodeck image analysis software (Seascan), then saved on the computer hard drive as TIFF files. Each ROI was tagged using a timestamp to allow merging with the hydrographic parameters written to a separate logfile.

Since most ROIs did not allow discrimination of copepods to species level, only ovigerous *P. acuspes* females (further on called *P. acuspes* females only) were used in the analysis. Taxonomic identification was performed manually as the present automatic classification method yielded only low accuracies for distinguishing copepods with and without egg sacs. However, reproductive *P. acuspes* females could be clearly identified manually due to their distinct shape (Fig. 7-2). In total 2444 individuals have been imaged during all VPR sampling surveys.

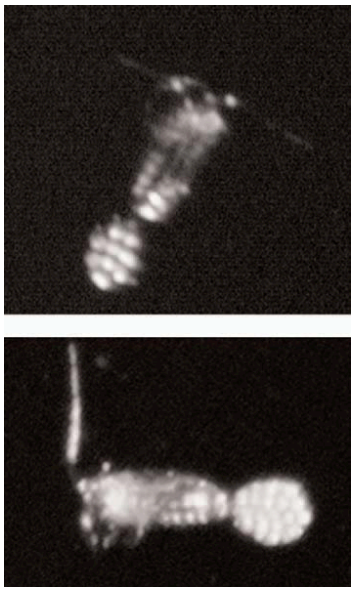

Fig. 7-2. VPR-images of female *Pseudocalanus acuspes* carrying egg sacs; upper image from 2002 survey; lower image from 2003 survey.

Afterwards, to obtain information about the abundance and distribution of marine snow aggregates, a potential food source for *P. acuspes* (Möller et al. 2012), we applied an automatic classification method with an automatic correction step. Here we followed an approach by Hu & Davis (2006). A randomly picked subset of a sufficient number of Training ROIs (trROIs) (approximately 200) was manually sorted into categories that were later used by the automatic classifier. Aside from the manual sorting required to generate the initial training set of images, this dual-classification method is fully automatic and does not require subsequent manual correction of automatically sorted images. Some portions of the extracted images were not in focus and hence not within the imaged volume**.** Those images were sorted out and put into the category "blurry". Marine snow aggregates formed another classifier category and yielded a high classification accuracy due to their distinct shape and texture. All other plankton and particles were combined into an "other" category. For more information on the automatic classification method and representative example images see Möller et al. (2012). Post-processing of the data was done with specially developed Matlab routines.

Data analysis

As an index for the vertical distribution of ovigerous *P. acuspes*, weighted mean depths (WMD) (Bollens & Frost 1989) were computed as:

$$\mathrm{WMD} = \left(\frac{(\sum n_i d_i)}{\sum n_i}\right)$$

where n_i is the mean abundance (Ind L^{-1}) of *P. acuspes* females with egg sac averaged over the whole survey time in depth stratum i (1 m depth interval) with midpoint depth d_i. WMD values were compared with a weighted t-test. Significance levels of pairwise comparisons were adjusted with the Dunn-Šidák method (Ury 1976).

Since *P. acuspes* females show different vertical distribution patterns during day- and nighttime (Möller et al. - Manuscript 2), we split the dataset by time (daytime = 04h to 20h & nighttime = 20h to 04h) and calculated the average abundance per 1 m depth bin for both. Thereafter, we calculated mean abundance of day- and nighttime in order to avoid a DVM dependent bias in the distribution patterns and exclude different day and night sampling lengths.

We used VPR derived high-resolution images of *P. acuspes* females to calculate an index of *in-situ* egg production for pre- and post-inflow situations, representing different reproductive conditions. The egg sac and six eggs within each egg sac of each individual recorded dorso-ventrally or laterally (each sampling period n = 20) were measured using the software ImageJ (Rasband 1997-2012). The volume (V) of the egg sacs was computed using the formula for ellipsoid spheres:

$$V = \frac{4}{3}\pi abb'$$

with *a*, *b* and *b'* being the length, width and depth of the measured object (Fig. 7-3). Width and depth were considered equal as only one dimension could be measured. The diameter of the eggs was measured and the volume was calculated using the equation for spheres (*b* = *b'*). Using the arithmetic mean of the six measured eggs and assuming a random loose packing of spheres (Weitz 2004), the number of eggs per sac was calculated from the measured clutch size (V). With the results, a daily individual egg production was calculated using the temperature dependent development time for *P. acuspes* eggs taken from McLaren et al. (1989):

$$\mathrm{D} = 1949(\mathrm{T}+12.59)^{-2.05}$$

where D is generation time in days and T the ambient temperature in °C.

To investigate the effect of the inflow on the mortality of *P. acuspes* eggs and nauplii, we finally computed an index of survival, i.e. the ratio of nauplii to females. Therefore, abundance numbers of *P. acuspes* females and nauplii were taken from Renz & Hirche (2006). Finally, marine snow concentrations were computed as average pre-inflow (April and May 2002) and post-inflow (April and May 2003) abundances in 1m depth bins.

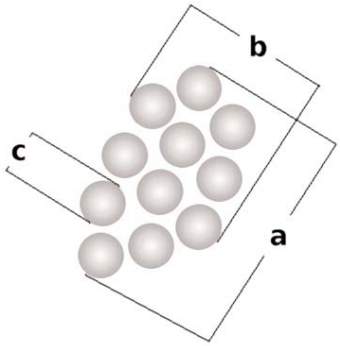

Fig. 7-3. Scheme of measurement on an egg clutch of *Pseudocalanus acuspes*; a – length; b – width; c – egg diameter.

Results

Individual vertical distribution

We recorded high-resolution images of individual *P. acuspes* females before and after the last significant Baltic inflow event and observed strong differences in their vertical distribution (Fig. 7-4). During pre-inflow conditions most individuals were found between 50 and 70 m, with a WMD of 65.3 m. Hence, most of the population was distributed around the pycnocline at c. 60 m depth. After the inflow in early 2003, the WMD of *P. acuspes* females was observed at 72 m, significantly deeper than before the inflow (t-test, $p < 0.001$). In addition the vertical distribution range inhabited by the copepods more than doubled after the inflow and most individuals were found between 40 and 84 m. While the depth of the pycnocline was observed to be shallower after the inflow (at c. 45 m), *P. acuspes* females were now observed mainly in deeper water, which has increased in density. Water mass characteristics and vertical distribution of individual copepods in 2009, after an extended stagnation period without inflows resembled the shallower and more concentrated pre-inflow conditions once more(Fig. S7-2).

Water mass characteristics and abundance

Hydrographic conditions changed markedly before and after the inflow in early 2003 (Fig. 7-5a&b). Temperature-salinity-oxygen relationships show two distinct water bodies before the inflow event. Cold (3.8 – 6 °C) and low saline (7 – 10) water in the upper water column was associated with maximum oxygen values (7 – 8.5 ml L^{-1}) close to the surface. This shallow water mass was separated from the high saline (12 – 17.5), warm (6 – 8.9 °C) and less oxygenated (0.3 – 4 ml L^{-1}) deep water by the permanent halocline. Between April and May oxygen contents in the deep water decreased, while warmer surface waters in May compared to April reflect the beginning of the seasonal stratification. With the inflow temperature decreased severely in the intermediate and deep layers. The saline water intruded by the inflow resulted in a shallower halocline (60 m in 2002, 45 m in 2003). Additionally, maximum salinity values in the deep water increased from 17.5 to 19.4 and oxygen content increased below the halocline from oxygen deficiency to in minimum 5 and 3.2 ml L^{-1} in April and May, respectively. Vertical profiles for temperature, salinity and oxygen are given in Fig. S7-3 & S7-4 in the supporting information.

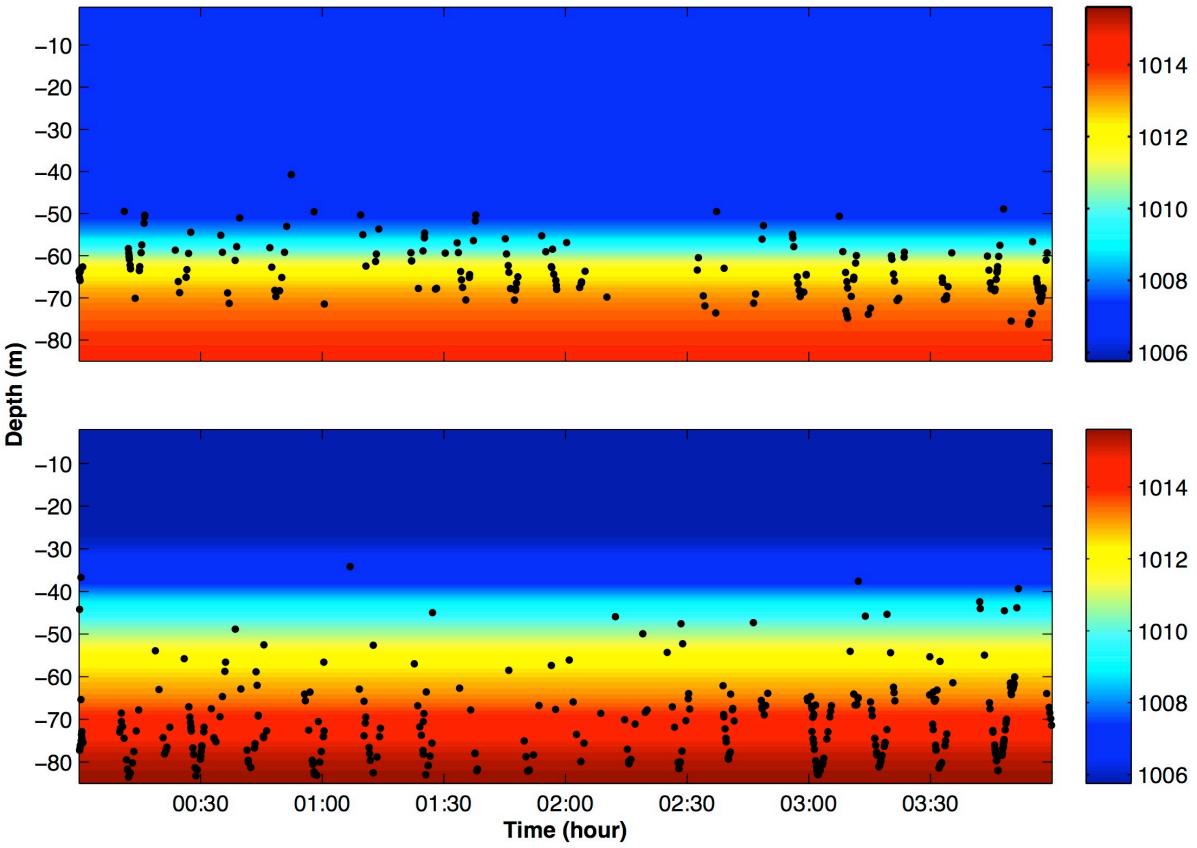

Fig. 7-4. Nighttime distribution of reproductive *P. acuspes*: Vertical distribution of all individuals (black dots) sampled with the VPR before (upper panel; April & May 2002) and after (lower panel; April & May 2003) the inflow event between 00h and 04h in relation to an averaged schematic density profile (σ_t).

We further examined the relationship between the vertical distribution of *P. acuspes* females and oxygen and salinity levels (Fig. 7-5c,d). Before the inflow the depth of peak abundance (3.7 Ind. L^{-1} in April and 4.8 Ind. L^{-1}) was 78 m in April and 70 m in May. After the inflow increased peak abundances (4.57 Ind. L^{-1} in April and 5.17 Ind. L^{-1} in May) were observed considerably deeper, i.e. in 88 and 85 m in April and May, respectively. The deeper distribution of the copepods is obviously related to improved salinity and oxygen conditions after the inflow. Also, overall average water column abundance increased between 2002 (0.3 Ind. L^{-1} in April to 0.34 Ind. L^{-1} in May) and 2003 (0.58 Ind. L^{-1} in April and 1.6 Ind. L^{-1} in May). Again, water mass characteristics and abundances of *P. acuspes* females returned to pre-inflow conditions in 2009 (Fig. S7-5).

Average vertical distribution

We investigated iwhether the hydrographic changes in the copepod's habitat due to the inflow influenced the vertical distribution and diurnal migration behavior (Fig.7-6). During both night and day *P. acuspes* females were found to have a significantly deeper distribution after the inflow (t-test for all WMDs, $p < 0.028$, see Tab. S7-1 in the supporting information) due to increased salinity and oxygen levels. Nighttime WMD increased in April from 61 to 71 m and in May from 64 to 70 m.

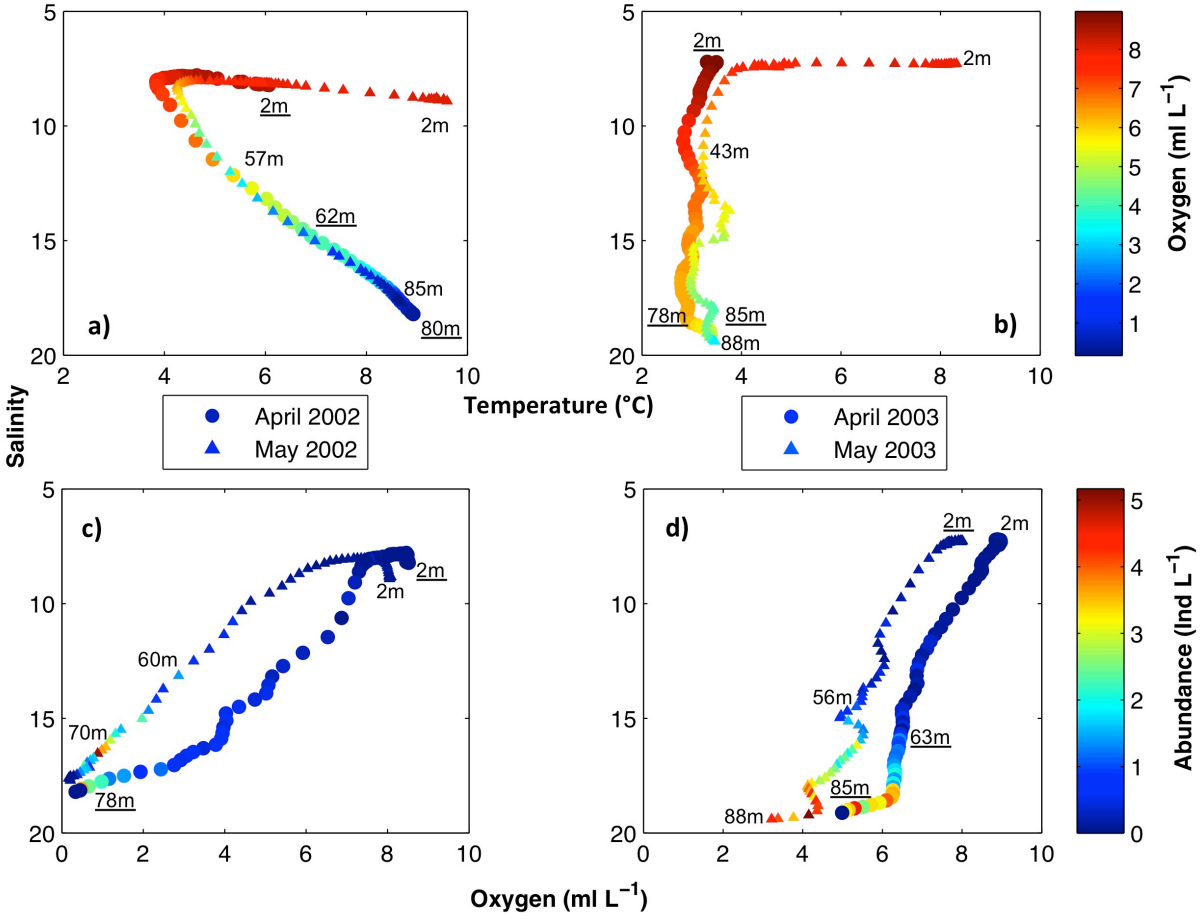

Fig. 7-5. Watermass characteristics and related abundances of *P. acuspes*: Temperature-salinity-diagram indicating the oxygen (ml L^{-1}; color-coded) distribution along the water column (a) before and (b) after the inflow event. Oxygen-salinity-diagram indicating average abundances of reproductive *P. acuspes* (Ind L^{-1}; color-coded) (c) before and (d) after the inflow event. Numbers indicate water depth (m); underlined: April and without line: May.

The same pattern was visible in the daytime WMD increasing in April from 70 to 74 m and in May from 66 to 76 m. Diurnal comparisons of WMD differences revealed a significant DVM pattern (t-test, p = 0.0000) in April before the inflow. Most individuals were sampled within the halocline during night, while peak abundances occurred during daytime directly at the oxycline, limited by an oxygen value of 0.8 ml L^{-1} from below. A similar pattern was not observed in May before the inflow. However, high copepod abundances at the oxycline during daytime again corresponded to oxygen values of 0.8 ml L^{-1} in 70 m depth. The diurnal comparison of WMD revealed no pronounced DVM behavior after the inflow. Day- and nighttime vertical distribution in May was significantly different (t-test, p = 0.0007) but, with a more uniform distribution. Generally, the distribution in 2003 was vertically more spread with highest abundances in the high saline but now oxygenated deep layer. Again, the vertical distribution pattern of reproducing *P. acuspes* (Fig. S7-6) during the stagnation period in 2009 matched with the pre-inflow distribution pattern.

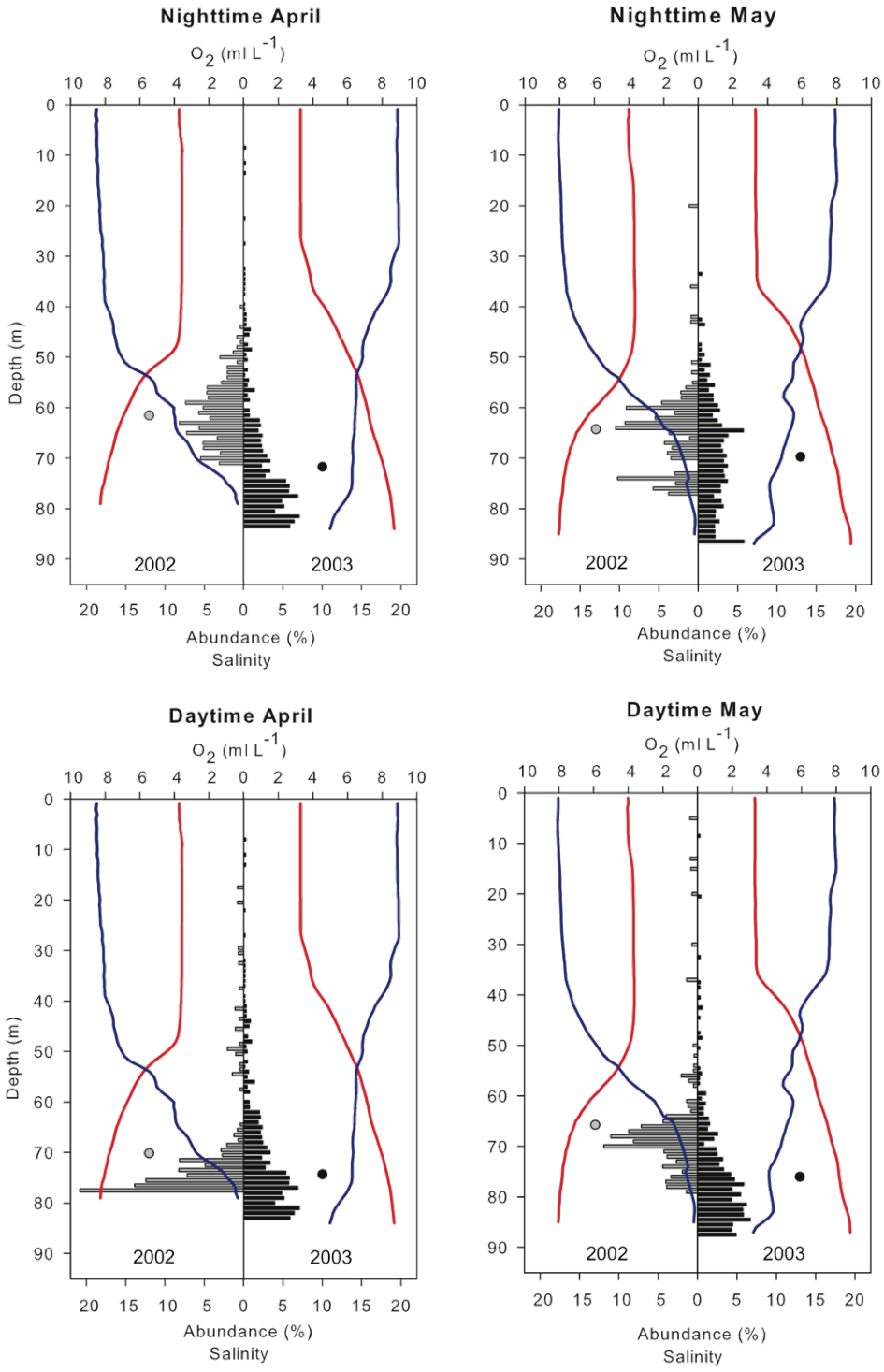

Fig. 7-6. Average vertical night- and daytime distribution (%; 1 m depth bins) of reproductive *P. acuspes* before (2002: grey) and after (2003: black) the inflow event in relation to key environmental factors; Red line indicates salinity- and blue line oxygen (ml L^{-1}) profiles. Filled circles indicate the weighted mean depths.

Egg production and survival

To evaluate the effect of the hydrographic conditions in the habitat of *P. acuspes* females on the reproduction of the animals, we estimated the mean clutch size and egg production using VPR-derived pictures. We compared individual egg production rates (EPR) between April 2002 and May 2003 to minimize the temperature effect of the colder inflow water. Clutch size, i.e. average number of eggs in an egg sac, was slightly higher after the inflow in May 2003 (n=22) as compared to pre-inflow conditions in April 2002 (n=19). However, individual egg production (IEP) was slightly higher in 2002 (4.3 female^{-1} day^{-1}) before compared to after the inflow (3.2 female^{-1} day^{-1}). This difference was due to a higher temperature in April 2002 (7°C) compared to May 2003 (3°C), which resulted in a shorter egg development time of 4.4 days in 2002 compared to 7 days in 2003. As an index for the survival of eggs and early nauplii, we calculated the ratio of nauplii to female abundance which increased strongly from 27.6 (2002) to 53.1 (2003) indicating higher survival.

Discussion

Here we investigated the effect of changes in the physical habitat of *P. acuspes* females by comparing its vertical small-scale distribution during non-inflow (i.e. stagnation) and inflow periods. Our study shows an immense increase in the availability of suitable vertical habitat after the inflow event along with increased copepod abundance and a deeper and wider vertical distribution. During stagnation periods *P. acuspes* females were mainly distributed in a narrow band around the halocline confirming earlier observations by Hansen et al. (2006) as well as Renz & Hirche (2006). After the inflow, a significant vertical shift occurred and individuals were able to stay deeper and occupy a broader depth range, i.e. the extend of their suitable habitat strongly increased.

The deeper distribution of the copepods after the inflow points towards the crucial importance of high salinity levels for the reproduction of this species of marine origin (Corkett & McLaren 1978). Suboptimal salinity conditions are known to increase respiration rates in copepods (Farmer & Reeve 1978, Gaudy et al. 2000), which results in a need of supplementary energy for osmoregulation (Kinne 1964). This is even more critical for reproducing *P. acuspes* since ovigerous females have elevated respiration rates due to additional energy costs of carrying eggs and egg respiration (Svetlichny et al. 2012). Our results show that *P. acuspes* females maximize ambient salinity by descending as deep as possible into the deep Baltic basins. Hence, Renz & Hirche (2006) suggested, e.g. a direct physiological effect of low salinity on growth and reproduction. The apparent detrimental effect of low salinity on *P. acuspes* may, however, be rather due to co- occurring oxygen levels. Shallowing of the halocline after the inflow causes the vertical expansion of the habitat and, hence, salinity appears to be the limiting factor for the upper boundary in the copepods distribution while low oxygen values are potentially more critical and limit the vertical extent from below. This mechanism is displayed by the narrow distribution during the stagnation period, where the trade-off between high salinity and low oxygen resulted in abundance peaks close to the oxycline at 0.8 ml L^{-1}. High aggregations of organisms at the interface to hypoxic waters are consistent with previous studies (Zhang et al. 2009, Craig 2012) where copepods were reduced or absent in hypoxic bottom waters with less

than 1 ml L^{-1} (Qureshi & Rabalais 2001). Oxygen minimum zones (OMZ) can on the other hand also have positive effects by providing refuge from predators (Gilly et al. 2012), depending on the trade-off between predation risk and increased demographic costs of living in hypoxic waters (Larsson & Lampert 2011). However, this might not be the case for Baltic *P. acuspes* females displaying strong avoidance of hypoxic water layers by aggregating at the oxycline during this vulnerable life cycle-stage.

We observed lower abundances of *P. acuspes* females during stagnation periods and a strong increase after the inflow in combination with improved habitat conditions. Applying a new method to estimate *in-situ* egg production rates from VPR derived images revealed no effect of hydrographic changes on individual egg production. Rather low salinity and oxygen may affect offspring mortality as shown by increased survival rates after the inflow. Recent investigations on other copepod species showed that suboptimal oxygen conditions can reduce egg production and survival (Stalder & Marcus 1997, Sedlacek & Marcus 2005, Richmond et al. 2006).

We estimated clutch size and daily egg production rates using pictures derived by the VPR. Our average estimates of clutch size (19 and 22 eggs f^{-1} in May 2002 and April 2003, respectively) were on the upper limit of those recorded by conventional methods, i.e. maximum values of 2 - 25 eggs f^{-1} between March 2002 and May 2003 reported by Renz et al. (2007). The number of eggs per female may be overestimated in our method because the egg sac does not represent a perfect sphere, hence slightly biasing the results. Consequently, our estimates of individual egg production (4.3 eggs f^{-1} day^{-1} in 2002 and 3.2 eggs f^{-1} day^{-1} in 2003, respectively) are higher than when observed by conventional techniques, yielding in maximum 3.6 eggs f^{-1} day^{-1} in April 2002 and 2.1 eggs f^{-1} day^{-1} in April 2003 (Renz et al. 2007). Our results showed no significant change in individual egg production before and after the inflow and are consistent with a previous study by Renz et al. (2007) who estimated EPR of *P. acuspes* with a traditional method. A possible explanation for our results might be the lower food concentration, i.e. marine snow abundance, which has been shown to be the limiting factor for egg production of *P. acuspes,* rather than lower temperatures after the inflow (Isla et al. 2008, see marine snow results in the supporting information and Fig. S7-7).

Beside the bias in the clutch size estimation, an additional factor causing the overestimation in egg production by our method may have been introduced by the temperature dependent development time for the eggs. These are derived in fully marine systems (McLaren et al. 1989) and may not be valid for the special conditions in the brackish Baltic Sea (Renz et al. 2007). However, measurements from field samples as well as from laboratory-reared females provide a calibration option, which should improve our results in the future. Our method offers a new opportunity to obtain information on population dynamics parameters from optical sampling gears.

Our results suggest that egg production can be excluded as the mechanism leading to the increased nauplii abundance in 2003. Consequently, increased survival of eggs and nauplii may be the reason for enhanced recruitment as shown by the increase in the ratio of nauplii to females. Increasing offspring mortality may have contributed to the long-term decline of *P. acuspes* (Möllmann et al. 2000, 2003). Low oxygen concentrations rather than low salinities are likely to be the reason for the lower egg and nauplii survival during stagnation periods. This has also been demonstrated in earlier studies for other calanoid copepods (Stalder & Marcus 1997, Marcus et al. 2004, Richmond et al. 2006).

Furthermore, low oxygen concentrations might not only directly affect the abundance of early life stages, but also adults. The risk of the strategy applied by *P. acuspes* to migrate into the high saline layer of the deep basins during stagnation periods implies the danger to encounter suboptimal oxygen conditions (Hansen et al. 2006, Renz & Hirche 2006). Our observations with lower abundances of reproductive females during stagnation periods are consistent with other regions where copepods generally decreased when hypoxia developed (Kimmel et al. 2009, Zhang et al. 2009). Direct impact through mortality has been observed both in benthic organisms (Rabalais et al. 2002) as well as in the pelagic, including zooplankton (Roman et al. 1993, Stalder & Marcus 1997). However, due to the strong avoidance behavior of hypoxic water layers by reproducing females the negative effect of low oxygen levels on the abundance of adult *P. acuspes* might rather be an indirect than a direct effect. Hypoxic zones alter habitat use and may increase bioenergetic costs of marine organisms, potentially resulting in decreased growth rates and condition (Eby & Crowder 2012). Additionally, the compressed habitat and hence also limited DVM amplitude during stagnation periods might reduce the spatial distance between predators and prey.

Generally, predation by planktivorous fish is an important agent of mortality for *P. acuspes* in the Baltic Sea (Möllmann & Köster 1999, 2002). Our results indicate that adverse hydrographic conditions during stagnation periods force the female copepods into a narrow band in the halocline where Baltic clupeids herring and sprat feed during daytime (Möllmann et al. 2004). Increased fish predation would likely have a more severe impact on ovigerous *P. acuspes*, since egg sacs increase copepod susceptibility to visual predators (Devrekker et al. 2008). We observed in consistence with a previous study (Möller et al. – Manuscript 2) that reproducing females are counteracting increased predatory pressure especially during stagnation periods through vertical migration into deeper layers. Since non-reproductive females without egg sac are suggested to differ in their behavior (Bollens & Frost 1991, Holliland et al. 2012), habitat compression may have caused higher predation rates particularly on non-reproductive adult females. This explanation is supported by the higher abundance of females observed after the inflow (Renz & Hirche 2006). Copepods are the primary diet of secondary consumers in marine environments and top-down control of their population is possible, particularly in the Baltic Sea (Möllmann et al. 2008). Conclusively, top-down and bottom-up effects in combination are likely to contribute to lower abundances during stagnation periods.

Vertical migration in stratified environments is often a trade-off between higher predation-risk and less favorable food conditions in deeper waters. A consequence of this trade-off is that predator avoidance mechanisms should only be activated when predation risk is above a certain threshold (Loose & Dawidowicz 1994). Our results show a pronounced DVM behavior only during stagnation periods under compressed habitat conditions. Hence, the increased suitable habitat followed by a vertical downward shift in distribution of individuals potentially reduced predation pressure due to increasing spatial separation between predators and *P. acuspes*. Additionally, high concentrations of marine snow aggregating at the halocline, a potential feeding hot-spot for *P. acuspes* (Möller et al. 2012), were only observed during stagnation periods. Particles were distributed uniformly throughout the water column after the inflow and, hence, also available under improved conditions in the deep water. Consequently, the climate-induced habitat changes after the inflow enabled *P. acuspes* to move into the preferred deep water layer under improved food conditions and reduced predation pressure, hence making DVM unneccessary.

An additional source for higher abundances of *P. acuspes* after the inflow may have been advective transport of individuals with the inflowing water masses. Occasionally, the congener *P. elongatus* has been observed in the Baltic Sea after inflow events (Holmborn et al. 2010) and, hence, might have due to its similar morphology contributed to the increased number of ovigerous individuals. This has been recently genetically confirmed by Grabbert et al. (2010), but only in low numbers and therefore, does not explain the strong increase. Furthermore, nauplii residing in the upper layers, which are not influenced by the inflow have increased to a larger degree (Renz et al. 2007), deeming advection as the major process of population variability as unlikely.

In conclusion, our study shows the strong impact that climate-induced changes in the physical habitat can have on key marine ecosystem species, important for overall ecosystem dynamics. Our results provide indications that the population reduction of Baltic *P. acuspes* with salinity (Möllmann et al. 2000, 2003, 2008) is mainly caused by low oxygen conditions during stagnation periods. We show that inflow events improve the conditions for this copepod, while periods of stagnation result in habitat compression and abundance decreases. Unfortunately, anthropogenic climate change is projected to result in extended periods of stagnation in the Baltic Sea, a trend that has been already observed during the last decades (Meier et al. 2012, Neumann et al. 2012), hence, leading to further decrease in *P. acuspes* abundance (Otto 2012). Sustaining decline of this key species has the potential to propagate up the food web and affect the economically important fish trophic level and, hence, shows the impact of climate induced habitat changes (Möllmann et al. 2008). Additionally, we developed and applied a new method for *in-situ* egg production estimations, which offers a considerable opportunity to obtain additional information from optical gears, that are currently becoming more widespread in aquatic ecosystem research.

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Supporting information

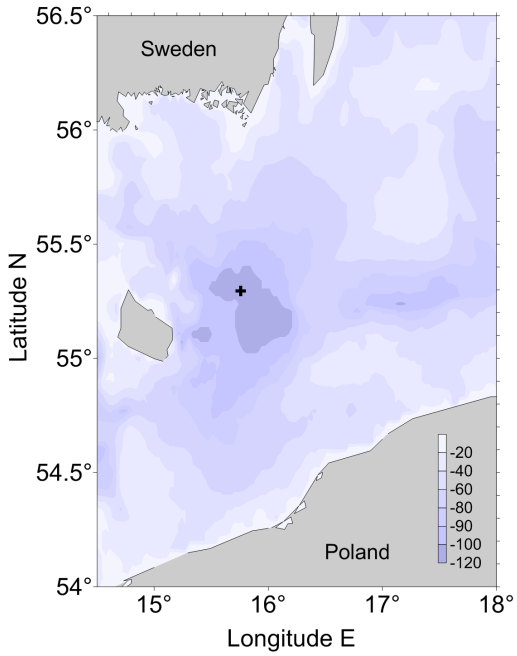

Fig. S7-1. Map of the Central Baltic Sea with the study area in the Bornholm Basin; Black cross indicates the location of Video Plankton Recorder (VPR) surveys in May 2009. The legend indicates water depth (m).

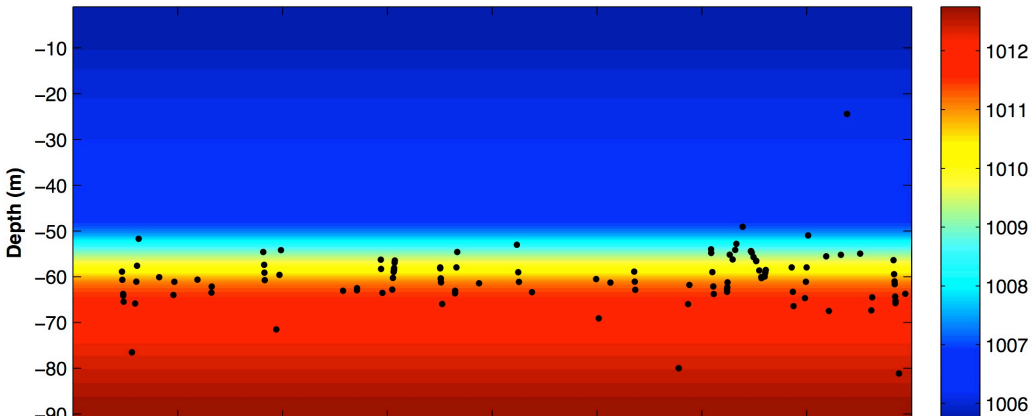

Fig. S7-2. Nighttime distribution of reproductive *P. acuspes*: Vertical distribution of all individuals (black dots) sampled with the VPR during the May 2009 survey period between 00h and 04h in relation to an averaged schematic density profile (σ_t).

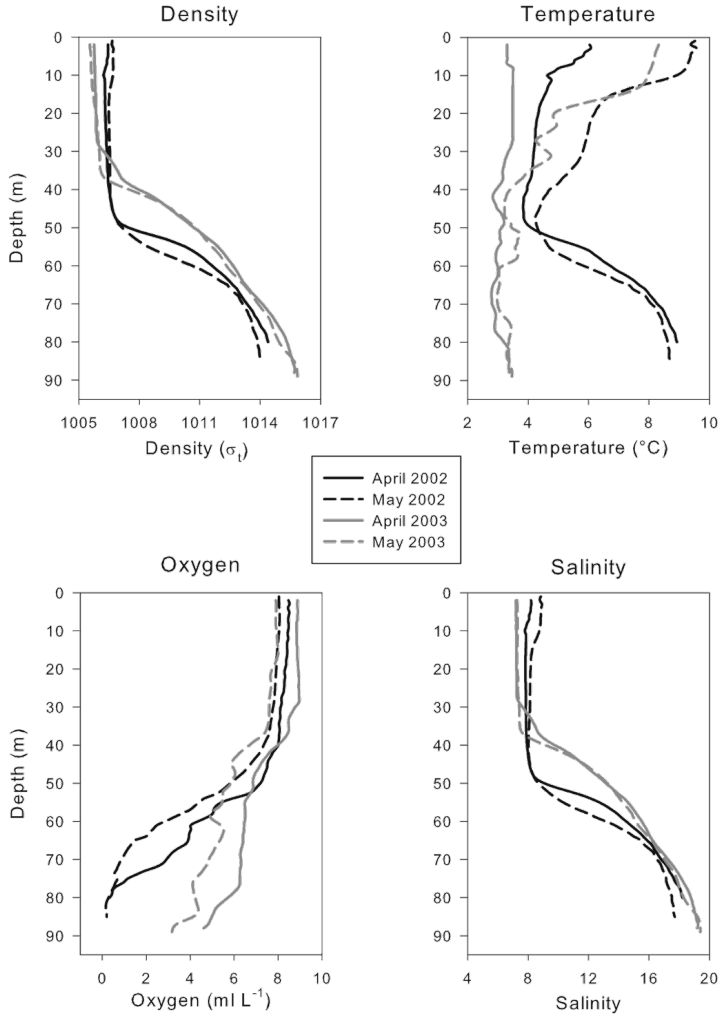

Fig. S7-3. Average hydrographic water column profiles of density (σ_t), temperature (°C), oxygen (ml L^{-1}) and salinity before (black lines: 2002) and after (grey lines: 2003) an inflow event.

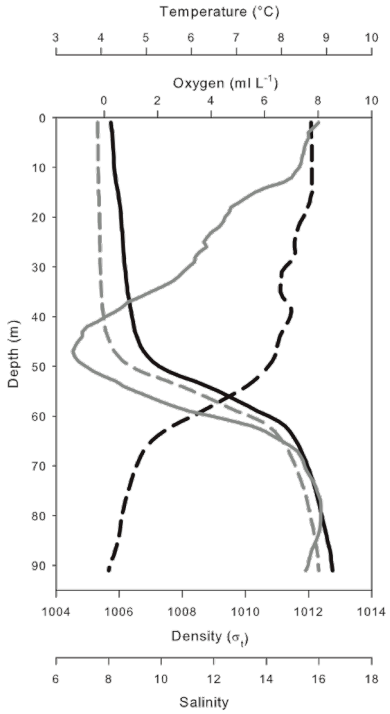

Fig. S7-4. Average hydrographic water column profiles of density (σ_t; black line), temperature (°C ; grey line), oxygen (ml L^{-1} ; black dashed line) and salinity (grey dashed line) during VPR sampling in May 2009.

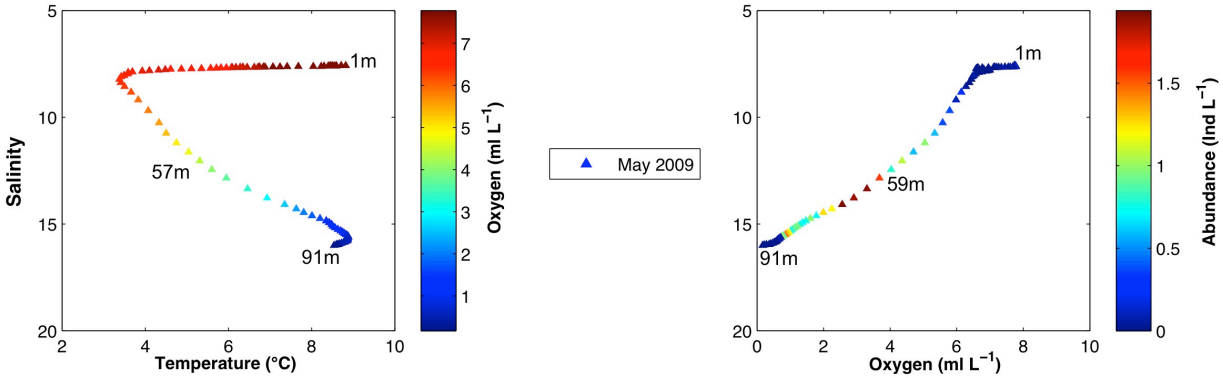

Fig. S7-5. Watermass characteristics and related abundances of *P. acuspes*: (a) Temperature-salinity-diagram indicating the oxygen (ml L^{-1}; color-coded) distribution along the water during VPR sampling in May 2009. (b) Oxygen-salinity-diagram indicating average abundances of reproductive *P. acuspes* (Ind L^{-1}; color-coded) during VPR sampling in May 2009. Numbers indicate water depth (m).

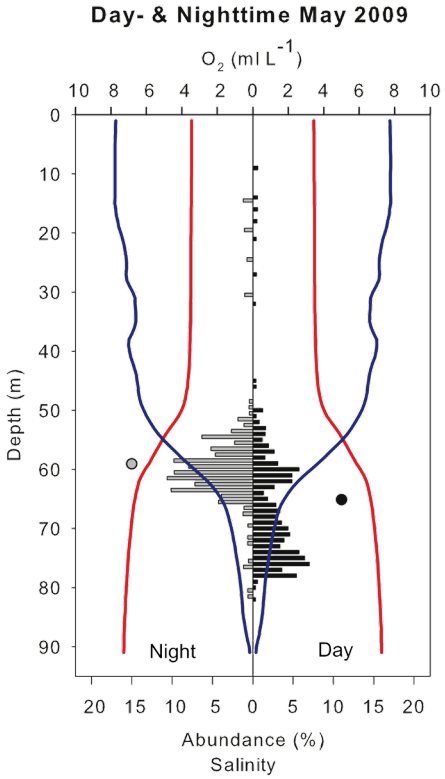

Fig. S7-6. Average vertical night- and daytime distribution (%; 1 m depth bins) of reproductive *P. acuspes* during VPR sampling in May 2009 in relation to key environmental factors; Red line indicates salinity- and blue line oxygen (ml L^{-1}) profiles. Filled circles indicate the weighted mean depths.

Marine snow distribution and abundance

We observed strong alterations in the abundance and vertical distribution of marine snow aggregates between stagnation and inflow conditions (Fig. S7-7). The average water column abundance decreased considerably from 6.3 particles L^{-1} during pre- inflow conditions to 2.2 particles L^{-1} afterwards. Peak concentrations of 17.4 particles L^{-1} were observed within the halocline in 2002 while aggregates were evenly distributed with slightly elevated concentrations close to surface and bottom in 2003. Marine snow abundance drastically increased (11.2 particles L^{-1}) again with peak concentrations at the halocline in May 2009.

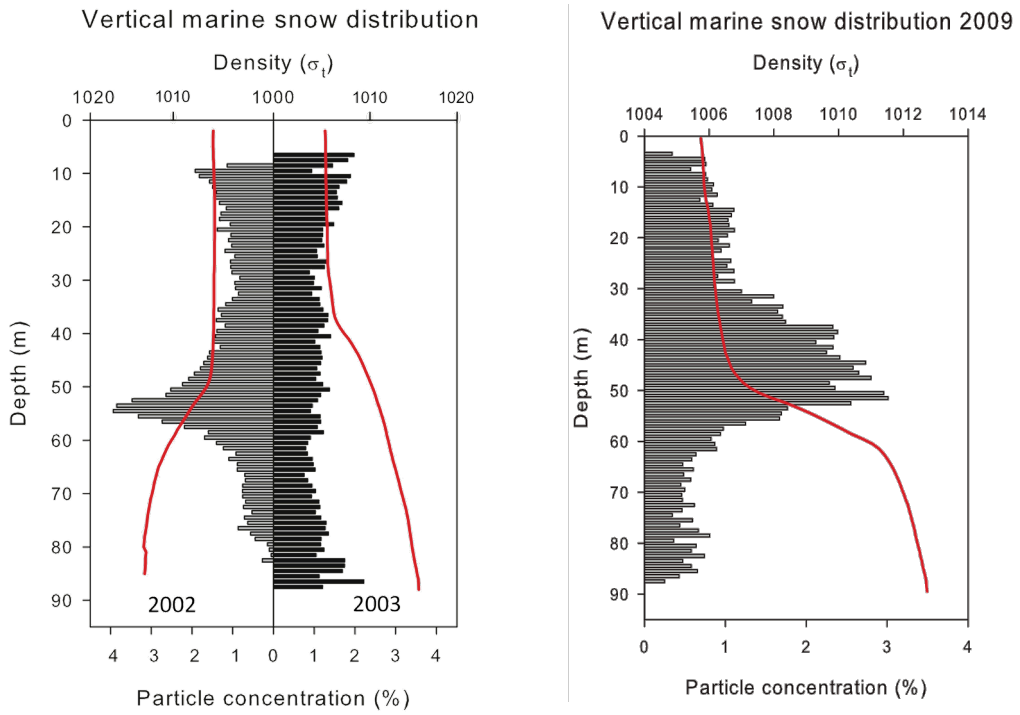

Fig. S7-7. Average vertical distribution (%; 1 m depth bins) of marine snow aggregates: (left panel) before (2002: grey) and after (2003: black) the inflow event in relation to the averaged density profile (σt); (right panel) vertical distribution in May 2009.

Comparison between years			
Month	Daytime	WMD-difference (m)	*p*-value
April	d	4.18	*0.0278*
April	n	10.14	*0.0000*
May	d	10.28	*0.0000*
May	n	5.48	*0.0014*
Day-night comparison			
Month	Year	WMD-difference (m)	*p*-value
April	2002	8.59	*0.0000*
May	2002	1.52	0.3677
April	2003	2.64	0.1798
May	2003	6.31	*0.0007*

Tab. S7-1. Interannual and diurnal comparison of weighted mean depths (WMD) of average vertical reproductive *P. acuspes* distribution. *p*-values are corrected values from pairwise t-tests.

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8 General Discussion

Key findings

Here we applied an optical sampling approach to investigate the impact of trophodynamic interactions and climate-induced habitat changes on distribution patterns and behavior of *Pseudocalanus acuspes*, a key zooplankton species in the Baltic Sea. We displayed high concentrations of copepods within a pronounced thin layer of marine snow and presented evidence of a strong trophic link. We identified behavioral responses of copepods in terms of a diurnal vertical migration in relation to predator occurence within this layer and presented first *in-situ* observations of a direct short-term reaction of the prey to the diurnal arrival of the predator as well as first *in-situ* evidence of strong individual variation and plasticity in a marine copepod. Furthermore, we assessed climate-induced effects on the habitat structure of this copepod and identified strong effects on abundance and distribution patterns as well as potential feedback implications on the copepods behavior. Finally, we developed and applied a novel *in-situ* method for egg production estimates, which offers an opportunity to obtain additional information from optical sampling gears in the future. All these results present novel findings for the Baltic Sea ecosystem and, furthermore, have been so far only rarely observed in other marine systems. In the following our results and their potential implications for ecosystem dynamics are discussed and put into context. The key findings of the three manuscripts are set into perspective using a conceptual diagram (Fig. 1).

Marine snow as a food source

First, we identified an intense thin layer of marine snow aggregates associated with the strong vertical density gradient in the permanent halocline typical for a deep Baltic basin using results from optical small-scale sampling with the VPR (**manuscript 1**). In contrast to earlier studies in other regions (Cheriton et al. 2007, Sevadjian et al. 2010), we found that this layer of marine snow aggregates persisted throughout our whole sampling track, most likely due to the stability of the halocline during stagnation conditions (**manuscript 3**). Furthermore, we observed a thin layer of copepods directly attached to marine snow aggregates that showed signs of typical feeding behavior. Hence, we can conclude that copepods actively respond to dense marine snow aggregations with the goal to use them as a food source. A number of laboratory experiments have shown that copepods are able to detect and exploit food patches in thin layers (e.g. Tiselius 1992). However, field observations of zooplankton behavior in relation to thin layers of marine snow, as in this study, are still rare. Previous field studies mainly investigated zooplankton thin layers acoustically (e.g. McManus et al. 2003, Benoit-Bird et al. 2009, Holliday et al. 2010). However, these studies were not able to investigate the species composition in these layers, which is an important issue as behavioral responses are often species-specific, and as shown in this thesis can even vary among individuals within one species (**manuscript 2**). In the Baltic Sea, feeding on marine snow in the halocline may be crucial for the survival of copepod populations. In or below the halocline of the deep Baltic basins no primary production occurs and copepod species such as *P. acuspes*, which need the elevated salinities in deeper waters (**manuscript 3**) experience comparable conditions as zooplankton residing in the mesopelagic zone of the open ocean where nutrition must be obtained to a large degree via feeding on sinking aggregates (e.g. Steinberg 1995). Hence, our observations highlight the significance and possible bottom-up

control of marine snow in the Baltic Sea, but its potential as a food source for various trophic levels from bacteria up to fish might be crucial in other marine ecosystems as well. Thin layers have been detected in a variety of marine systems including estuaries (Bochdansky & Bollens 2009), coastal shelves (McManus et al. 2005) and the open ocean (Cowles et al. 1998). Conceivably, thin layers may have an extensive impact on marine ecosystem dynamics and the magnitude of their importance is just now beginning to be quantified due to the availabilty of new sampling techniques (Alldredge et al. 2002, Durham & Stocker 2012, Lyons & Dobbs 2012).

Predator-prey interactions

After identifying the marine snow layer as an important feeding hot-spot for *P. acuspes* (**manuscript 1**)**,** we combined observations of individual copepods with the VPR and hydroacoustic estimates of predatory fish biomass to identify potential behavioral responses to its main predator (**manuscript 2**). We observed a classical DVM of zooplankton that has been shown for many species by conventional studies before (e.g. Zaret & Suffern 1976, Frost 1988, Bollens et al. 1992), but never before for *P. acuspes* in the Baltic Sea. Most previous studies used conventional net sampling and due to the coarser vertical resolution were not able to show a DVM for this key species due to their small vertical amplitude caused by habitat compression during stagnation periods (**manuscript 3**). However, enabled by the modern optical underwater observation technique, we showed *in-situ* an immediate reaction of a zooplankton population to the behavior of the predator for the first time. Malkiel et al. (2006) reported in agreement with our observations elevated abundances of calanoid copepods with maximum concentrations of marine snow (**manuscript 1**). However, they interpreted this pattern as predator avoidance behavior, since high concentrations of large detritus particles may provide shelter from visual predators (Malkiel et al. 2006). Here, predation avoidance is the most likely reason for DVM of *P. acuspes* females in the Baltic Sea, since main predators like sprat feed likewise in the halocline (**manuscript 2**). Furthermore, our observations strongly suggest that copepods leave the food-rich marine snow layer in the halocline directly in response to the predators (Möller et al. 2012) and return to it after the predators migrate back to the surface over night. Hence, our results support the cascading migration theory by Bollens et al. (2011), since migrations occuring on the fish trophic level affect migration patterns on the zooplankton trophic level below. In the special case of *P. acuspes* in the Baltic Sea both predator and prey perform a „classical" DVM pattern migrating to deeper layers during day and shallower layers during night, while zooplankton often perform reverse DVM in presence of migrating predators, due to preferred conditions at the surface, i.e. food and temperature (e.g. Ohman et al. 1983). While the need for elevated salinities and the downward movement of *P. acuspes* is linked to critical oxygen values during stagnation periods (**manuscript 3**) it can also be an advantage, as this reduces potential encounters with predators, possible for reverse migrating species during transition periods. Our results suggest that predator avoidance is the major cause for DVM of Baltic *P. acuspes* rather than environmental cues due to its short-term reaction in the presence of fish. However, DVM is a well studied predator avoidance behavior of zooplankton in all aquatic systems. As shown here, it can occur on small spatial scales and hence, is potentially undersampled with the coarse sampling resolution of traditional techniques.

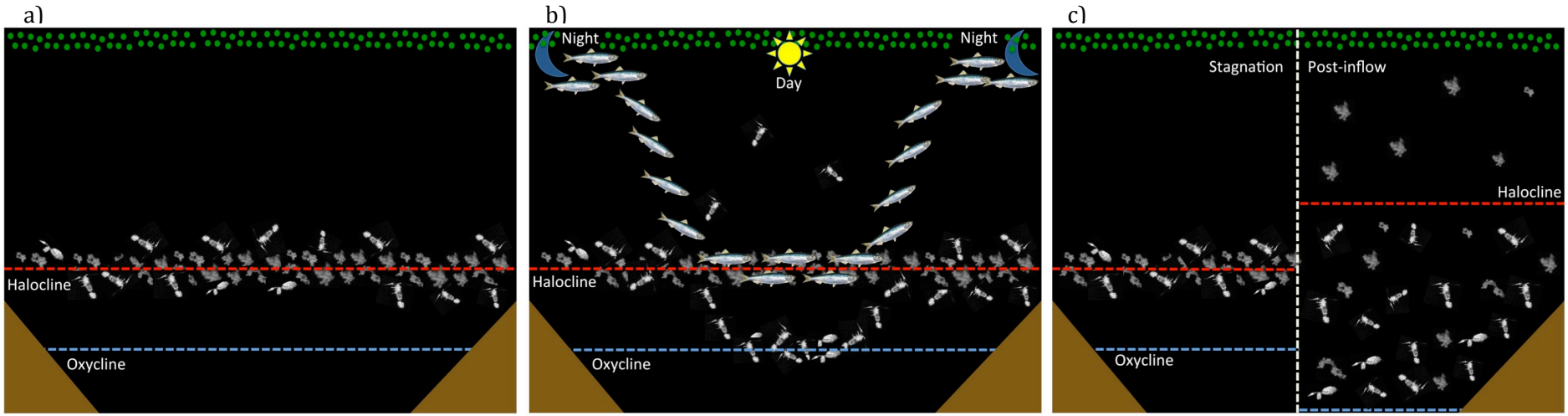

Fig. 8-1. Conceptual diagram of the Bornholm Basin (central Baltic Sea): Overview of key findings within this thesis; (a, left panel) aggregation and trophic interaction of copepods within a thin layer of marine snow (manuscript 1); (b, center panel) predator-prey behavior (DVM) and individual plasticity (manuscript 2); (c, right panel) impact of climate-induced habitat changes on distribution and behavior (manuscript 3).

Individual plasticity

Another important result of our study is the first *in-situ* evidence of strong individual variation in a marine copepod species´ DVM (**manuscript 2**). Most of the *P. acuspes* females showed a general trend of downward migration during day, but some individuals responded to the presence of predators with an upward movement towards the surface. Previous studies have shown stage-specific differences in behavior due to size or reproductive status (e.g. Bollens & Frost 1991, Holliland et al. 2012), but we show that behavioral responses can also vary between individuals of one developmental stage as well. The two different behavioral modes of *P. acuspes* in the Baltic indicate that different predator avoidance strategies exist in a population. The regular DVM of most of the individuals might represent an evolutionary developed behavior imprinted in the population. The upward movements of single individuals are most likely more a spontaneous responsive escape behavior. Recent research has shown that the decision to migrate might depend on individual traits, such as size, sex or conditional state (Olsson et al. 2006, Brodersen et al. 2008, Hansson & Hylander 2009). The direction of the response in our case is likely to depend on the position of the copepods in the water column relative to their predators. Our results indicate that the risk of predation during daytime forces large parts of the *P. acuspes* adult female population out of their optimal habitat (**manuscript 3**). The latter, which they inhabite during night, is characterized by suitable salinity and oxygen conditions as well as accumulations of marine snow where they find food (**manuscript 1**). The animals may however account for the period of sub-optimal conditions during day by making use of their saline, oxygenated, and food-rich habitat undisturbed by predators during night. Our results add new complexity to the understanding of population DVM in copepods in particular, but also on plankton migration and behavior in general. Our results demonstrate that a focus on individual personality and scales relevant to individuals, contributes information beyond what can be resolved from targeting the mean population in typical integrated net samplings and, hence, contributes to bridging the gap between field ecology on one hand and theory and experiments on the other (Kaartvedt et al. 2011)

Impact of habitat changes

Finally, after identifying the importance of marine snow aggregations in the halocline as a food source (**manuscript 1**) and resolving individual behavior and vertical migration as a trade-off between maximizing energy input and minimizing predation risk (**manuscript 2**), we investigated the effects of changes in the physical habitat of *P. acuspes* females by comparing its vertical small-scale distribution during non-inflow (i.e. stagnation) and inflow periods (**manuscript 3**). Additionally, we split the dataset into night- and daytime sampling periods to exclude bias due to the different diurnal distribution patterns observed as well as to assess potential changes in DVM behavior (**manuscript 2**).

During stagnation periods, *P. acuspes* females were mainly distributed in a narrow band around the halocline during nighttime and aggregated at the oxycline during daytime, confirming our earlier observations (**manuscript 1,2**) and, hence, DVM behavior. After the inflow, a significant vertical shift occurred and individuals were able to stay deeper and occupy a broader depth range, i.e. the extend of their suitable habitat strongly increased in combination with increasing abundances (**manuscript 3**), while a pronuounced DVM behavior was now absent. Vertical migration in stratified environments is often a trade-off between higher predation-risk and less favorable food conditions in deeper waters. A consequence of this trade-off is that predator

avoidance mechanisms should only be activated when predation risk is above a certain threshold (Loose & Dawidowicz 1994). Our results show a pronounced DVM behavior only during stagnation periods under compressed habitat conditions. Hence, the increased suitable habitat followed by a vertical downward shift in distribution of individuals potentially reduced predation pressure due to increasing spatial separation between predators and *P. acuspes*. Additionally, high concentrations of marine snow aggregating at the halocline, a potential feeding hot-spot for *P. acuspes* (**manuscript 2**), were only observed during stagnation periods. Particles were distributed uniformly throughout the water column after the inflow and, hence, also available under improved conditions in the deep water. Consequently, the climate-induced habitat changes after the inflow enabled *P. acuspes* to move into the preferred deep water layer under improved food conditions and reduced predation pressure, hence making DVM unnecessary. The deeper distribution of the copepods after the inflow reflects the crucial importance of high salinity levels in combination with high oxygen values. Shallowing of the halocline after the inflow causes the vertical expansion of the habitat and hence, salinity appears to be the limiting factor for the upper boundary in the copepods distribution while low oxygen values are potentially more critical and limit the vertical extent from below. This mechanism is displayed by the narrow daytime distribution of *P. acuspes* females during the stagnation period, where the trade off between high salinity and low oxygen in combination with increased predation pressure due to daytime feeding of visual predators in the halocline results in aggregations close to the oxycline (**manuscript 2**). The adaptive behavior to predation during stagnation has the cost of accepting unfavourable conditions in terms of physical habitat parameters and feeding conditions (**manuscript 2**), hence, lower abundances of *P. acuspes* females might be a direct result of low oxygen values. Recent investigations on other copepod species showed that suboptimal oxygen conditions can reduce egg production and survival (Stalder & Marcus 1997, Sedlacek & Marcus 2005, Richmond et al. 2006). Additionally, the compressed habitat might result in higher predation rates, especially for non-ovigerous females which are suggested not to migrate (Bollens & Frost 1991, Holliland et al. 2012, **manuscript 2**). Thus, the increase in planktivorous predator populations through trophic cascading (Casini et al. 2008, Möllmann et al. 2008) may have caused direct consumptive effects on the population of *P. acuspes*, but as shown here indirect non-consumptive effects as well (Schmitz et al. 2008). Copepods are the primary diet of secondary consumers in marine environments and top-down control of their population is possible, particularly in the Baltic Sea (Möllmann et al. 2008). Applying a new method to estimate *in-situ* egg production rates from VPR derived images, revealed no effect of hydrographic changes on individual egg production. Rather low salinity and oxygen may affect offspring mortality as shown by increased survival rates after the inflow. A possible explanation for our results might be the lower food concentration, i.e. marine snow abundance, which has been shown to be the limiting factor for egg production of *P. acuspes,* rather than lower temperatures after the inflow (Isla et al. 2008). Our results show that both bottom-up (food and hydrography) and top-down (predation pressure) mechanisms in a complex interplay contribute to lower abundances during stagnation periods. Although inflow events are a distinct feature of the Baltic Sea our results exemplify the strong impact that climate-induced habitat changes can have on key marine ecosystem species, important for overall ecosystem dynamics

Method

We applied an optical sampling approach by using the VPR to investigate the impact of trophodynamic interactions and climate-induced habitat changes on the distribution patterns and behavior of zooplankton on scales never observed before in the Baltic Sea. Non-invasive small-scale sampling allowed us to quantify fragile marine snow aggregates within a pronounced thin layer, a feature rarely observed and not resolvable with traditional sampling methods (**manuscript 1**). We provide indirect evidence of copepods feeding on marine snow aggregates due to a strong spatial overlap within the thin layer, which is supported by images of copepods being attached to aggregates. Although VPR derived images did not allow discrimination of copepepods on species level, we successfully used traditional net sampling to overcome this limitation of our optical method. Due to the pronounced stratification in the Baltic Sea, which structures the water column into vertical microhabitats, we were able to attribute the high copepod concentrations within the thin layer to mostly adult *P. acuspes*. Although it might be more complicated to link supporting taxonomic information from net sampling to VPR scales in more complex and diverse marine ecosystems, recent and ongoing improvement of optical methods is already providing better image quality and, hence, increased taxonomic identification. Additionally, implementation of color information in the automatic classification process will help to improve the classification accuracy.

A major problem when studying behavior and migration of small organisms is that many of the questions addressed for larger animals are not possible to formulate due to constraints on tracking smaller animals. However, to observe species specific predator-prey behavior we focused in our study on ovigerous adult females of *P. acuspes* that carry their eggs until hatching in a conspicious external egg sac (**manuscript 2, 3**). This special life-history feature allowed a discrimination from other developmental stages and copepod species. We identified behavioral responses of ovigerous *P. acuspes* in terms of a diurnal vertical migration in relation to predator occurence within this layer by combining VPR results with hydroacoustics and present first *in-situ* observations of a direct short-term reaction of the prey to the diurnal arrival of the predator (**manuscript 2**). Furthermore, we present first *in-situ* evidence of strong individual variation and plasticity within one life-stage for reproductive *P. acuspes* (**manuscript 2**). This procedure might have introduced a bias in the perception of the DVM of the whole adult population since it ignores non-reproducing females and male *P. acuspes* (Bollens & Frost 1991, Holliland et al. 2012). However, we recorded the part of the population that is mainly important for producing the subsequent generation. Net sampling on the other side would have resulted in a loss of the egg sacs and, hence, a discrimination between egg-carrying and not reproducing females would have been impossible. Previous *in-situ* observations of individual zooplankton organisms were so far only made for larger jellyfish that were tracked with hydroacoustics (Kaartvedt et al. 2011) while smaller *Daphnia* were tracked in the laboratory using nanotechnology (Lard et al. 2010). In our opinion, our VPR-observations deliver strong evidence for individual variability in behavior of Baltic *P. acuspes* females. However, the ultimate evidence for individual-based behavior would be to track many animals over time which is so far impossible with smaller zooplankton *in-situ*. Laboratory experiments would be necessary allowing individual tracking with camera systems, but the natural setting observed in our study will be difficult to reproduce. We were able to observe individual variability due to the ability of the VPR to conduct a continuous sampling over a long period of time and on small spatial scales at a stationary sampling site that serves as an comparable method of tracking individuals, although not following it. Furthermore, high-frequency environmental data recorded in combination with

high-frequency images of *P. acuspes* allowed the exact description of habitat changes as well as changes in the distribution patterns in relation to it (**manuscript 3**). Hence, optical sampling methods offer new opportunities to routinely study zooplankton responses to light, food and predation, opening up advancements within research areas such as diel vertical/horizontal migration, partial migration and other differences in intra- and interspecific movements and migration as well as individual behavior in zooplankton ecology.

Furthermore, we developed a new method to estimate *in-situ* egg production for copepods carrying external egg sacs (**manuscript 3**). The number of eggs per female may be overestimated in our method because the egg sac does not represent a perfect sphere, hence slightly biasing the results. However, measurements from field samples as well as from laboratory-reared females provide a calibration option, which should improve our results in the future. Additionally, this method allows „non-invasive" *in-situ* estimation from VPR derived images, while results from individuals under laboratory conditions can also be biased due to non-natural food conditions or temperature (Saiz et al. 1997). Our method offers a new opportunity to obtain information on population dynamics parameters from optical sampling gears., which recently became more distributed and, as shown in this thesis, will help to gain further insights in plankton ecology.

Conclusion

In conclusion, optical sampling with the VPR provided data on the distribution and behavior of zooplankton in the Baltic Sea never observed before. For the first time, the presence of a prominent thin layer of marine snow was observed at the density gradient of the permanent halocline in the Baltic Sea in combination with high concentrations of copepods (Fig. 8-1a). This thin layer was shown to be a characteristic feature of the Baltic Sea basins during stagnation periods where copepods are feeding on marine snow particles. The importance of the permanent halocline for the trophodynamics and behavior of the model species *Pseudocalanus acuspes* is apparent and literally running through all three manuscript as a red thread (Fig. 8-1a,b,c). While previous studies suggested low salinities to be the major reason for the decline of this key species population with marine origin our results indicate that it is rather the co-occuring low oxygen levels during stagnation periods. Furthermore, our results show that predation is likely to be contributing to the decline and, hence, it is an complex interplay of both, bottom-up and top-down processes on distribution and behavior during periods of stagnation within a compressed habitat between halo- and oxycline. However, predators did not only have direct effects by predation, but also non-consumptive effects on the behavior of *P. acuspes*. Small-scale sampling with the VPR revealed a DVM behavior of reproductive *P. acuspes* females and, furthermore, individual plasticity within this behavior (Fig. 8-1b). Comparison of pre- and post-inflow situations elucidated the negative impact of climate-induced habitat changes on this key species (Fig. 8-1c). Improved food and habitat conditions in combination with decreasing predation pressure due to habitat expansion and a deeper distribution of individuals led to an increase in the abundance and, hence, absence of DVM behavior. Furthermore, the new developed method of *in-situ* egg production revealed that reduced abundances of *P. acuspes* during stagnation periods are also caused by negative effects on survival of early life-stages. Conclusively, climate induced changes in the Baltic Sea ecosystem due to a reduction of inflow events controls the distribution and behavior of this key species and is responsible for the decline of its population during recent decades. This thesis shows the strong impact that climate-induced changes in the physical habitat can have on key marine ecosystem species,

important for overall ecosystem dynamics. Unfortunately, anthropogenic climate change is projected to result in extended periods of stagnation in the Baltic Sea (Meier et al. 2012, Neumann et al. 2012), a trend that has been already observed during the last decades and, hence, leading to further decrease in *P. acuspes* abundance (Otto 2012). Sustaining decline of this key species has the potential to propagate up the food web and affect the economically important fish trophic level.

Future perspectives

Our results show the potential of optical sampling methods to gain more insight in the distribution and behavioral patterns in aquatic ecosystems and, hence, the potential to further increase our understanding of overall ecosystem dynamics. Although this method is still limited in taxonomic resolution recent improvement of image technology and classification software are promising. Small-scale features in the ocean like thin layers or horizontal frontal systems need further exploration and unraveling the role of individual behavior within plankton communities as well. Additionally, calibrating our *in-situ* egg production method with egg sac measurements from laboratory reared individuals will help to obtain fast and reliable egg production estimations from egg sac carrying copepods in the field. Furthermore, the digital image format of the plankton sample contains manifold information which allows to draw conclusions on the feeding conditions of individuals (e.g. arm length of planktonic echinoderm larvae) or behavior (e.g. solitary, raptorial feeding of the heterotrophic dinoflagellate *Noctiluca scintillans* versus aggregation in "feeding webs") to name only a few examples. Hence, there is a lot more information to obtain from optical sampling gears additionally to the powerful ability to map small-scale distribution patterns. However, based on our results it is also important to further investigate the effect of hypoxia on zooplankton physiology, i.e. secondary production, growth and mortality in laboratory experiments. This not only important for the special case of *P. acuspes* in the Baltic Sea since hypoxia is a becoming a wide spread problem in marine systems caused by global warming. Furthermore, the trigger for the observed migration in *P. acuspes* remained still unclear and waits for experimental verification.

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11 Eidesstattliche Versicherung *Declaration on oath*

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe.

I hereby declare, on oath, that I have written the present dissertation by my own and have not used other than the acknowledged resources and aids.

Hamburg, den 06.12.2012