Prey quantity and quality effects on larval Atlantic herring (*Clupea harengus* L.) growth in the western Baltic Sea

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Summary

Recruitment in many marine fish populations is subject to a strong inter-annual variability and larval survival is seen as one of its key drivers. Apart from predation, nutrition constitutes one of the most influential factors on larval survival rates as it determines growth and, hence, influences the duration time that a larva remains in the highly sensitive larval stage. In light of the enduring low recruitment of western Baltic spring spawning herring (WBSSH), the studies conducted in the framework of this thesis aim at providing information about the relation between the nutritional situation and the growth of Atlantic herring larvae in three important nursery areas of the western Baltic Sea (Kiel Canal, Greifswalder Bodden, Kiel Fjord).

First, food quantity and its quality in terms of essential fatty acid (EFA) concentrations were analyzed and related to larval growth rates in the Kiel Canal and the Greifswalder Bodden in 2011. The results indicated that both prey quantity and its quality affect larval growth rates. In the study, highest EFA concentrations in the prey were reflected in highest EFA concentrations in the larvae; the latter of which positively correlated with larval growth. Over the course of the season, both habitats provided beneficial and unfavorable growth conditions for the larvae in, however, opposing temporal order.

Analyses of a six-year time series of the Kiel Canal revealed a consistent seasonal pattern of prey availability and larval herring growth in this watershed. Larval growth correlated positively with the abundance of the dominant copepod species (*Eurytemora affinis*), of which highest abundances occurred throughout May. Moreover, by the end of May/ beginning of June, with increasing water temperatures (≥ 15°C) the copepod abundance and subsequently larval growth rates strongly decreased. In contrast, larval growth linearly increased in the Greifswalder Bodden with increasing temperature. The coinciding decline of copepod abundance and larval growth was recurrently observed, which indicates that herring larvae in the Kiel Canal undergo food-limited growth by the end of each spring season.

Potential effects of protozooplankton (PZP) biomass on growth of spring-hatched herring larvae were investigated in the Kiel Canal in 2014. No effects of PZP biomass on larval herring growth rates were detected. However, the abundance of copepods – which

are known to represent the preferred prey of larval herring – was high during the investigation period. It is therefore possible that PZP is only of minor importance to larval herring, if larger prey items occur at adequate concentrations.

Size-dependent larval growth is frequently observed in marine larval fish. In contrast, larval herring growth and size data in combination with seasonal mean zooplankton abundance during a six-year time series from the Kiel Canal revealed a decreasing size-dependency in larval growth with increasing prey abundance. This outcome suggests that size-dependent larval growth may function as an indicator for suboptimal feeding conditions particularly for small larvae.

Spatial variability in larval growth was investigated in the Greifswalder Bodden in 2010 and a comparison between seasonal mean growth in the Greifswalder Bodden and the Kiel Canal was conducted between 2010 and 2012. With one exception (large larvae in the channel-like Strelasund area of Greifswalder Bodden), temporal variability was higher than spatial variability in larval growth, both within the Greifswalder Bodden and between habitats. This finding was supported by the results of coastal mesozooplankton surveys which suggested that variability in prey was greater temporally as opposed to spatially.

Changes in larval growth and copepod abundance were analyzed in the Kiel Canal (2007-2012), the Kiel Fjord (2007-2012) and the Greifswalder Bodden (2010-2012) with respect to changes in the Baltic Sea Index (BSI), a broadscale, atmospheric climate index. Changes in the winter BSI were unrelated to changes in larval growth rate or copepod abundance. Only a negative trend between the abundance of the copepod *Pseudocalanus* spp. and the winter BSI existed with decreasing abundance at increasing BSI. Moreover, a highly significant correlation between the growth rates of small larvae (< 14 mm) and the mean seasonal copepod abundance from all investigation areas was observed, whereas this was not found for larger larvae (14 mm – 20 mm).

Based on 9 years of data, a significant correlation between WBSSH recruitment and the yearly mean abundance of the copepod *Pseudocalanus* spp. was found. In the 1990´s, WBSSH recruitment was on average twice as high as between 2000 and 2012. In line with that, peak abundances of copepods and cladocerans were twice as high in 1991-1995 and 1999 compared to the period thereafter. Weight-at-age 1 did not change over time and was significantly lower compared to same-age conspecifics from the Kattegat/Skagerrak area, independent of recruitment strength. The findings suggest that

herring recruitment is close to the carrying capacity of the pelagic ecosystem in the western Baltic Sea.

Overall, this thesis provides comprehensive insight into the relationship between food quantity/ food quality and larval Atlantic herring growth. Food quality significantly affected larval growth; however, food quantity appeared to be of paramount importance especially for smaller, less developed larvae. The outcome of the thesis indicates that WBSSH stock recruitment is significantly affected by prey availability, both due to effects on larval growth/survival and density-dependence during the juvenile stage.

Zusammenfassung

Die Rekrutierung von marinen Fischbeständen ist durch starke inter-annuelle Variabilität gekennzeichnet. Die Überlebensrate der Larven spielt dabei als einer der Haupttreiber eine zentrale Rolle. Neben Prädation ist die Ernährungssituation der Larven von übergeordneter Bedeutung: sie beeinflusst das Larvenwachstum und damit die Dauer, die die Larven in der hoch sensiblen Larvalphase verbringen. Angesichts der andauernden niedrigen Rekrutierung des frühjahrslaichenden Heringsbestandes der westlichen Ostsee (engl. *western Baltic spring spawning herring*, WBSSH) wurden die Studien dieser Thesis durchgeführt. Dies geschah mit dem Ziel weitergehende Erkenntnisse über die Beziehung zwischen der Ernährungssituation der Heringslarven und deren Wachstum in drei wichtigen Aufwuchsgebieten der westlichen Ostsee (Nord-Ostsee-Kanal, Greifswalder Bodden, Kieler Förde) zu gewinnen.

In der Frühjahrssaison 2011 wurden Nahrungsquantität und Nahrungsqualität in Form der Konzentration der essentiellen Fettsäuren (engl. *essential fatty acids*, EFA) im Greifswalder Bodden und im Nord-Ostsee-Kanal analysiert und zum Heringslarvenwachstum in Beziehung gesetzt. Es zeigte sich, dass sowohl Nahrungsquantität als auch –qualität das Larvenwachstum beeinflussen. Die höchsten EFA-Konzentrationen in den Larven wurden beobachtet, als auch die EFA-Konzentrationen in der Beute am höchsten waren; die EFA-Konzentrationen in den Larven korrelierten signifikant mit deren Wachstum. Im Laufe der Saison boten beide Habitate sowohl gute als auch schlechte Nahrungsbedingungen, jedoch in diametraler zeitlicher Anordnung.

Die Analysen einer sechsjährigen Zeitserie des Nord-Ostsee-Kanals zeigten ein wiederkehrendes saisonales Muster von Futterverfügbarkeit und Larvenwachstum in diesem Habitat. Im Mai eines jeden Jahres wurden die höchsten Abundanzen von *Eurytemora affinis* beobachtet, und das Larvenwachstum korrelierte positiv mit der Abundanz dieser Copepodenart. Ende Mai/ Anfang Juni bei Wassertemperaturen ≥ 15°C sanken die Copepodenabundanzen stark, und in der Folge auch das Larvenwachstum. Im Gegensatz dazu war eine positiv lineare Beziehung zwischen Larvenwachstum und Wassertemperatur im Greifswalder Bodden zu beobachten. Das zeitgleiche Auftreten von abnehmender Copepodenabundanz und abnehmendem Larvenwachstum wurde

wiederkehrend beobachtet, was zeigt dass Heringslarven im Nord-Ostsee-Kanal nahrungslimitiertem Wachstum am Ende jeder Frühjahrssaison unterliegen.

Potentielle Effekte der Protozooplankton (PZP)-Biomasse auf das Wachstum von Heringslarven während der Frühjahrssaison wurden 2014 im Nord-Ostsee-Kanal untersucht. Es wurden keine Effekte der PZP-Biomasse auf das Larvenwachstum beobachtet. Allerdings war die Copepodenabundanz hoch im Untersuchungsjahr, und die unterschiedlichen Lebensstadien der Copepoden stellen das bevorzugte Futter der Heringslarven dar. Daher ist denkbar, dass PZP eher eine geringe Bedeutung für Heringslarven hat, wenn hohe Abundanzen von anderem Futter verfügbar sind.

Wachstums- und Größendaten von Heringslarven wurden in Verbindung mit mittleren saisonalen Zooplanktonabundanzen einer sechsjährigen Zeitserie im Nord-Ostsee-Kanal untersucht. Dabei wurde eine abnehmende Größenabhängigkeit im Larvenwachstum bei zunehmender Futterabundanz beobachtet. Dieses Ergebnis legt nahe dass größenabhängiges Larvenwachstum als Indikator für suboptimale Futterbedingungen insbesondere für kleine Larven dienen könnte.

Die räumliche Variabilität des Larvenwachstums wurde 2010 im Greifswalder Bodden untersucht. Zudem wurde ein Vergleich zwischen den mittleren saisonalen Larvenwachstumsraten in Greifswalder Bodden und Nord-Ostsee-Kanal zwischen 2010 und 2012 durchgeführt. Von einer Ausnahme abgesehen (große Larven im kanalartigen Strelasund) war die Larvenwachstumsvariabilität generell zeitlich höher als räumlich, sowohl innerhalb des Greifswalder Boddens als auch beim Vergleich Greifswalder Bodden und Nord-Ostsee-Kanal. Dieses Ergebnis wurde unterstützt von Ergebnissen einer Mesozooplankton-Analyse längs der Schleswig-Holsteinischen Ostseeküste, wo ebenfalls die zeitliche Variabilität höher war als die räumliche.

Effekte eines Klimaindices (Baltic Sea Index, BSI) auf das Larvenwachstum und die Copepodenabundanz wurden im Nord-Ostsee-Kanal (2007-2012), der Kieler Förde (2007-2012) und dem Greifswalder Bodden (2010-2012) untersucht. Es wurden keine Effekte des Winter-BSI auf das Larvenwachstum oder die Copepodenabundanz gefunden. Allerdings wurde ein negativer Trend zwischen der Abundanz des Copepoden *Pseudocalanus* spp. und dem Winter-BSI beobachtet (abnehmende Abundanz bei zunehmendem BSI). Außerdem wurde eine hoch signifikante Korrelation zwischen dem Wachstum von kleinen Larven (< 14 mm) und der mittleren saisonalen Copepodenabundanz von allen Untersuchungsgebieten beobachtet, wohingegen dies bei großen Larven (14 mm – 20 mm) nicht der Fall war.

Futtereffekte auf die Bestandsrekrutierung des WBSSH wurden analysiert. Basierend auf einer neunjährigen Datengrundlage wurde eine signifikante Korrelation zwischen der WBSSH-Rekrutierung und der mittleren jährlichen Abundanz des Copepoden *Pseudocalanus* spp. beobachtet. In den 1990er Jahren war die Rekrutierung des WBSSH im Durchschnitt doppelt so hoch wie zwischen 2000 und 2012. In Übereinstimmung damit waren die Peak-Abundanzen von Copepoden und Cladoceren doppelt so hoch 1991-1995 und 1999 im Vergleich zu der Periode danach. Das Gewicht der einjährigen Heringe jedoch änderte sich nicht zwischen 1991 und 2012, war unabhängig von der Rekrutierungsstärke und generell signifikant niedriger im Vergleich zu gleichaltrigen Jungfischen aus dem Kattegat/Skagerrak-Gebiet. Diese Ergebnisse legen nahe dass die Heringsrekrutierung dicht an der Tragfähigkeit des pelagischen Ökosystems der westlichen Ostsee stattfindet.

Zusammengefasst lässt sich sagen, dass diese Thesis umfassende Einblicke in die Beziehung von Nahrungsmenge/ Nahrungsqualität und Heringslarvenwachstum gewährt. Die Nahrungsqualität hat das Larvenwachstum signifikant beeinflusst; jedoch schien die Nahrungsmenge von übergeordneter Bedeutung zu sein, insbesondere für die gering entwickelten kleinen Larven. Die Resultate dieser Thesis weisen darauf hin, dass die Rekrutierung des WBSSH signifikant von der Nahrungsverfügbarkeit beeinflusst wird; die Ergebnisse legen nahe, dass dies sowohl für Wachstum und Überleben der Heringslarven, als auch für Dichteabhängigkeit bei den Juvenilen gilt.

General Introduction

Systematics of clupeid fishes

Clupeomorpha are amongst the phylogenetically oldest fish orders. These fish have inhabited the earth's oceans for over a hundred million years (Klinkhardt, 1996). Today, the family of the herring comprises 179 species within 55 genera. The Atlantic herring *Clupea harengus* is taxonomically classified in the family Clupeidae and the order Clupeoidei (Fig. 1); defining characters for clupeoids are dorsal and ventral body profiles, an order-specific caudal fin skeleton and a sensory canal system, coupled with a swimbladder-ear connection (Whitehead, 1985). Within the genus *Clupea* another two species exist, which are the Pacific herring *Clupea pallasii*, and the Araucanian herring *Clupea bentincki*.

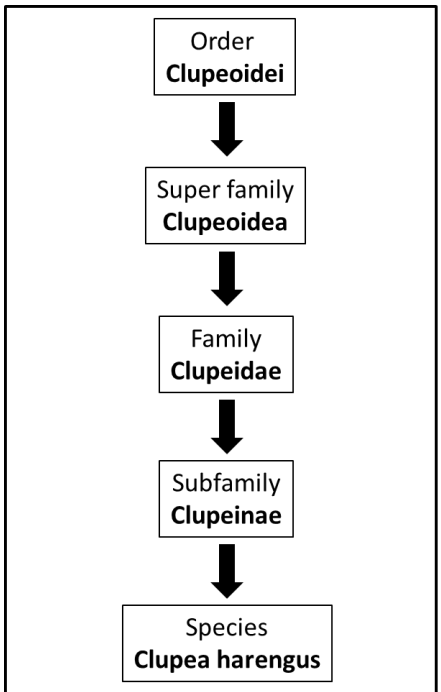

Fig.1: Taxonomic classification of the Atlantic herring *Clupea harengus* L. after Whitehead (1985)

Biology of the herring and importance for humans

Herrings are widely distributed in boreal marine waters, and their distribution area can be restricted to the 6°C and 12°C isotherms in the Northern hemisphere (Klinkhardt, 1996), which may correspond to a geographical range between 35°N and 70°N (Blaxter, 1985). Though principally marine, herring is a highly plastic species that lives and/or spawns in a wide range of salinities of 2 up to 33 (Blaxter, 1985; Haegele and Schweigert, 1985). This high salinity tolerance could be the main reason for the herring's fast colonization of the brackish water habitats of the geologically young Baltic Sea. In the North-Eastern Baltic Sea they manage to constantly live at salinities below 6. However, living in low saline environments leads to growth depression: while the average size of herring is 20 cm to 30 cm, with a maximum length of 40 cm (Blaxter, 1985; Muus and Nielsen, 1999), average total length of herring in low-saline environments such as the Windebyer Noor, a small enclosed water body in northern Germany, and the north-eastern Baltic Sea

is about 15 cm (Neb, 1952; Blaxter, 1985). Given that herring are a preferred prey of various predators and that fishing mortality is high, the maximum values of both, size and age, are rarely realized (Neb, 1952; Klinkhardt, 1996).

Characteristic for many marine ecosystems is a "wasp-waist" food web structure (Bakun, 2006), i.e., only a single or few planktivorous fish species dominate their trophic level while feeding on a highly diverse plankton community. The diversity of predators feeding on the dominant planktivorous fish species is high again. Typically, the dominant planktivorous fish species are clupeoid fish such as menhaden (*Brevoortia* spp./*Ethmidium maculatum*), anchovy (*Engraulis* spp.), sardine (*Sardinella* spp., *Sardinops* spp., *Sardina pilchardus*) and herring. As herring primarily feed on zooplankton (e.g. Last, 1989; Arrhenius and Hansson, 1993; Arrhenius, 1996; Cardinale and Arrhenius, 2000; Dalpadado et al., 2000; Bernreuther, 2007), they serve as a trophic link and ensure a highly efficient energy flow from the plankton to large predators. As the herring´s habitats are located in plankton-productive boreal waters, herring are able to potentially build up very high biomasses (www.ices.dk). Due to these facts, herring are very important to the trophodynamic structure and function of many marine and brackish water habitats by serving as the prey for various top predators such as piscivorous fish, sea birds, and marine mammals. However, not only predatory animals target on herring, but also substantial parts of the world´s fisheries. Atlantic herring is of high economic importance, and amongst the three quantitatively most important fish species in the world, with a yearly catch of about 2 million metric tons (FAO, 2014).

The western Baltic spring spawning herring

In the western Baltic Sea, two herring stocks exist: the spring spawners, and the autumn spawners. While the former seek low saline, shallow waters in spring, the latter lay their eggs in deeper waters (10 m to 20 m) and exposed to currents (Neb, 1952; Weber 1971; Ojaveer 2003). Before World War II, about a third of the herring caught were assigned to the autumn spawners. Compared to the spring spawners, autumn spawners were larger on average, which was attributed to a lower fishing mortality as spawning places were variable over time, and targeted fishing on the autumn spawners was difficult (Neb, 1952).

The western Baltic spring spawning herring (WBSSH) spawns from March to May. The extension of the spawning period depends on temperature development. Shallow and low saline waters near the coast such as enclosed fjords and bays are preferred spawning habitats. In contrast to most other clupeid fishes, herring are substrate spawners. Baltic Sea spring spawning herring stocks preferentially spawn on macroalgae (Aneer, 1989). During the spawning period adults can be found in these habitats for at least two months, though temporally in fluctuating abundances as the herring enter their spawning sites in waves (Brandhorst, 1955).

While the majority of the stock spawns in estuarine, low saline waters in Germany and Denmark, small portions of the stock are known to spawn in the fully marine conditions of the Swedish west coast. Herring spawning events intensively affect the respective coastal spawning sites for the following reasons: Firstly, large shoals of adult herring entering the spawning site represent an easily accessible food source for piscivorous fish (e.g., pike (*Esox lucius*) and pikeperch (*Sander lucioperca*)) and birds (e.g., cormorants (*Phalacrocorax carbo*) and European herring gulls (*Larus argentatus*)). Occasionally, harbor porpoises (*Phocoena phocoena*) and even common bottlenose dolphins (*Tursiops truncatus*; observed in Kiel Fjord in spring 2016) follow the herring into the inner fjords to feed on them (own observations). Secondly, the large amount of sperm released to the water represents an additional nutrient input to the coastal waters. Thirdly, the large amount of eggs functions as a food source for fish species such as three-spined stickleback (*Gasterosteus aculeatus*) or perch (*Perca fluviatilis*) (Kotterba et al., 2014) and sea birds alike (Kils, 1992). Finally, after hatching, the larvae serve as food source for small predatory fish (Hansson et al., 1997; Lappalainen et al., 2001), jellyfish (Möller, 1984) or small seabirds (Kils, 1992).

Soon after metamorphosis, the juveniles start to emigrate out of their nursery areas into the outer coastal areas. They will stay there as long as they are immature, and start feeding migrations at the age of 2 to 3 years (Brandhorst, 1955; Muus and Nielsen, 1999). Right after spawning in the coastal waters of the western Baltic Sea, the adults head northwards to feed in the transition area to the North Sea over the summer (Neb, 1952; Aro, 1989). Kattegat and Skagerrak are the feeding grounds for mature western Baltic spring spawning herring.

In the Øresund/Öresund (Danish and Swedish spelling), the waters between the Danish island Zealand and the Swedish south-west coast, the majority of the stock aggregates over winter (Biester, 1979). However, it is also reported that the herrings overwinter in the deep flumes close to the spawning grounds (Neb, 1952). When temperatures begin to increase at the end of the winter, the herrings migrate towards their spawning grounds in the inner coastal areas (Aro, 1989).

The herring´s spawning grounds and nursery areas at the German Baltic Sea coast are very diverse (Weber, 1971; Klinkhardt, 1996). They comprise of estuaries such as the Schwentine River, the Trave River, or the Warnow River, fjords such as Flensburg Fjord, Schlei Fjord, Eckernförde Fjord or Kiel Fjord, artificial canals such as the Kiel Canal or shallow bays like the Greifswalder Bodden (Fig. 2). Despite their diverse structure, all spawning sites feature the same important basic conditions such as low salinity (but not lower than 4 (Brandhorst, 1955)), the presence of submerged vegetation as spawning substrate, and a low water depth. The latter is beneficial, as small and/or shallow waters quickly warm in spring. Apart from that, the main spawning grounds such as Greifswalder Bodden, Kiel Canal and Schlei Fjord are rich in nutrients, and therefore support the development of large quantities of mesozooplankton, especially calanoid copepods such as *Eurytemora affinis* and *Acartia* spp., which are important for the nutrition of the herring larvae. Additionally, spawning habitats known to be preferentially used by the herring are retention areas (as shown for the Greifswalder Bodden; Bauer et al., 2013), ensuring that the vast majority of the larvae remains in the habitat they hatched in.

The Greifswalder Bodden is considered to be the most important spawning ground and nursery area of the WBSSH stock (Biester, 1989). It is a large enclosed bay with an average depth of 5.6 m and a spatial extension of 514 km^2, the water is characterized by a low salinity (6-8).

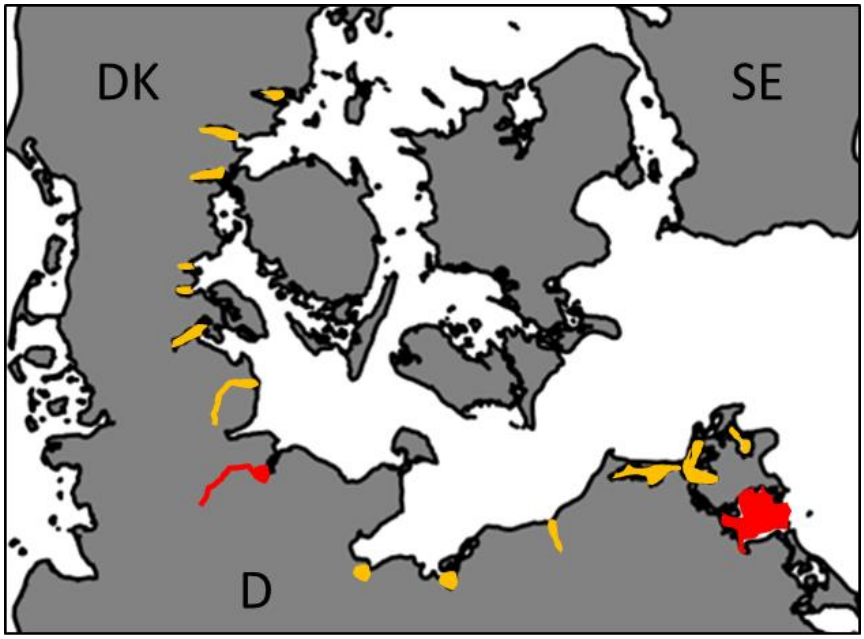

Fig. 2: Spawning areas of western Baltic spring spawning herring along the Danish (DK) and German (D) Baltic Sea mainland coast. Coloring indicates known spawning areas (note: no data available for the Danish islands). Red coloration denotes the investigation areas: Kiel Canal and Kiel Fjord (left); Greifswalder Bodden (right).

Another important nursery area is the Kiel Canal (Weber, 1971), an artificial canal built at the end of the 19th century. Already one year after its opening (1895), it was entered by large herring schools and also herring larvae were already caught (Hinkelmann, 1897). Compared to the Greifswalder Bodden, it is relatively deep with an average depth of 11 m. Hydrological features shared with the Greifswalder Bodden are the low salinity (6-10) and the well-mixed water column, which exists due to the frequent shipping traffic. The investigation area Kiel Canal provides ideal conditions to research basic larval fish ecology, as it is narrow and characterized by only weak currents. Therefore, cohorts can be followed over time and effects of changing prey fields on the larvae´s condition can be studied. Time series are a fundamental tool to investigate population dynamics and are equally important to describe biological processes. In the Greifswalder Bodden, during the spawning and larvae season (March-June) a comprehensive survey has been conducted since 1992 (Rügen herring larvae survey, RHLS). The spatial resolution is 35 stations, and these stations are sampled on a weekly basis. It was shown that the total number of herring larvae reaching a length of 20 mm correlates well with the number of recruits of the stock (N20-index; Oeberst et al.,

2009b). The North Sea-counterpart of the RHLS is the International Herring Larvae Survey (IHLS) which has been conducted since 1967. Here, the abundance of yolk-sac larvae serves as a proxy for spawning stock biomass. In order to investigate questions of larval fish ecology (focus on herring), a time series on a weekly basis is conducted in both Kiel Fjord and Kiel Canal, western Baltic Sea, Germany, since 2005 (GEOMAR ichthyoplankton time series). Unfortunately, stock data of WBSSH only exist for the time after the German Reunion, starting in 1991. In the beginning, a decrease of spawning stock biomass (SSB) correlated with an increase in fishing mortality while recruitment (defined as number of young-of-the-year) was high (Fig. 3). From 2001 on, however, recruitment strongly decreased to half of the 1990´s level. Ever since 2006, recruitment remained on a low level. In North Sea herring, significant effects of climate fluctuations (North Atlantic Oscillation and Atlantic Multidecadal Oscillation) and the winter abundance of the calanoid copepod *Pseudocalanus* spp. on recruitment were observed (Gröger et al., 2010; Alvarez-Fernandez et al., 2015). Similarly, the winter Baltic Sea Index was found to correlate with WBSSH recruitment (Gröger et al., 2014). However, in which way remains unexplained.

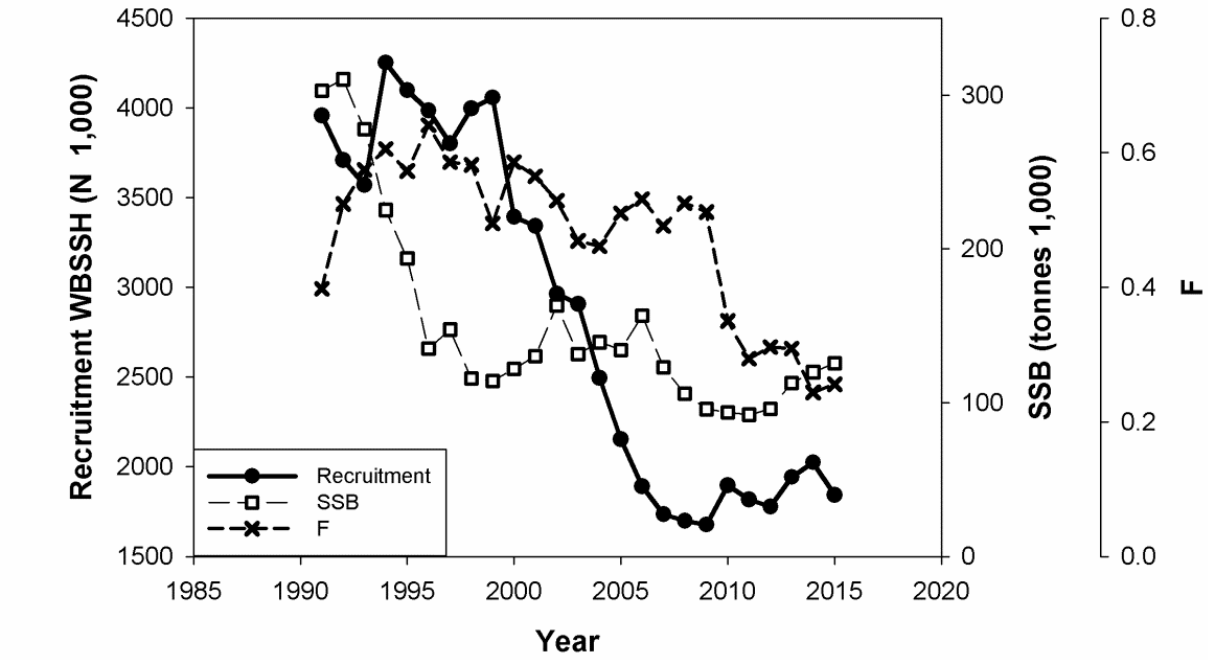

Fig. 3: Temporal development of recruitment, spawning stock biomass (SSB) and fishing mortality (F) of the western Baltic spring spawning herring

Recruitment and larval fish ecology

The high recruitment variability is a phenomenon not only observed in herring, but is a general feature of the population dynamics of many marine fish stocks. Recruitment is a multifactorial process and affected by various factors such as spawning stock biomass (Ottersen and Sundby, 1995; Axenrot and Hansson, 2003; Brander, 2005), stock structure (Marteinsdottir and Thorarinsson, 1998; Vallin and Nissling, 2000), nutrition of the parents (Brooks et al., 1997; Izquierdo et al., 2001; Perez and Fuiman, 2015) and egg mortality (Nissling, 1994; Köster and Möllmann, 2000), predation on larvae and juveniles (Gjøsæter and Bogstad, 1998; Takasuka et al., 2003), and nutrition and growth of the larvae and juveniles (e.g. Houde, 2008 and references therein; Jørgensen et al., 2014; Robert et al., 2014; Alvarez-Fernandez et al., 2015). In several stocks, predation is assumed to be an important agent of mortality (Houde, 2008).

It is important to highlight that a complex array of processes and both intrinsic and extrinsic factors can affect recruitment strength. Depending on stock and year, respective factors may have varying influence, leading to changes with respect to the stages in which recruitment is determined (Houde, 2008). Due to this, it is difficult to reliably predict recruitment. Nevertheless, for some fish stocks the life stage where recruitment is mainly determined was detected. For example, for Baltic Sea cod *Gadus morhua* the recruitment determining life stages were detected between late egg and early larval stage, while the critical period for sprat *Sprattus sprattus* was found to be between the late larval and early juvenile stage (Köster et al., 2003). However, the sensitive, recruitment determining stage might change over time. This was reported for walleye pollock (*Theragra chalcogramma*), where the recruitment determining stage switched from the larval to the juvenile stage, caused by a regime shift (Bailey, 2000).

Until the beginning of the 20th century it was believed that fluctuations observed in the abundance of local fish stocks were caused by changes in the fishes´ migration behavior. The Norwegian fisheries scientist Johan Hjort observed the exceptionally large year class of 1904 Norwegian spring spawning herring, and its dominance within the stock in subsequent years. He hypothesized that fluctuations are primarily caused by changes in larval survival, and linked it to larval nutrition during the transition phase from endogenous feeding by their yolk-sac to exogenous feeding. At this stage of first feeding, he argued, sufficient prey needs to be available to ensure high larval survival (Hjort, 1914, *Critical Period hypothesis*).

Johan Hjort set the basis for ecological research on marine fish larvae by creating a link between this highly sensitive life stage and recruitment variability. However, over the years it became apparent that his hypotheses were too one-dimensional (Houde, 2008). This was true for both, a restriction of larval ecology to feeding ecology and a restriction of recruitment success to larval survival (see above).

Cushing (1974; 1990) extended Hjort´s Critical Period hypothesis. In the *Match-Mismatch hypothesis* he proposed that larval survival is linked to the timing of hatching with regard to zooplankton production, i.e. an overlapping time window. In contrast to Hjort, he did not restrict the important period to the time of first feeding.

Several authors linked prey availability to physical processes. For example, Lasker (1978; 1981) developed the *Stable Ocean hypothesis*, after the observation of high recruitment for North-Pacific anchovy (*Engraulis mordax*) during calm weather conditions. The explanation for this is the stratification of the water column under calm conditions, thereby concentrating both fish larvae and their prey and improving larval feeding conditions.

Cury and Roy (1989) and Rothschild and Osborne (1988) showed that micro-turbulences can strongly enhance encounter rates between larvae and their prey. Here, a possible explanation can be found why reared larvae need exceptionally high prey concentrations for good survival rates. These concentrations were far beyond those normally occurring in nature.

Iles and Sinclair (1982) further set focus on physical processes in their *Larval Retention/Membership-Vagrancy* hypothesis. They claimed that retention of the larvae in suitable conditions is most important, ensured by a good choice of their parents concerning the spawning grounds.

Basically, larval survival can be affected by prey availability in three different ways. First, larvae need to live in an environment where prey availability is sufficient. Prey availability is influenced by several factors, such as its abundance, water turbulence, turbidity, and patchy distribution. This means that larvae depend on a best-fit position in space and time. When larvae do not learn to forage successfully, they will reach the *point-of-no-return* (Blaxter and Ehrlich, 1974). If they do, the respective larvae will die, regardless of possibly improving feeding conditions at a later time point. Second, growth rates of the larvae are of importance. The faster larvae grow, the shorter they are in this highly vulnerable stage. This was taken up by Houde (1987) and Anderson (1988) in the *Stage duration hypothesis*, which is based on the fact that mortality rates generally

decrease with increasing larval size. Third, Jørgensen et al. (2014) pointed out that larval survival might be affected by prey availability even if no changes in larval growth rates can be detected yet. The more hungry larvae are, the more active they are while searching for prey, and the easier they are detected by predators. Generally, it has to be considered that larval foraging requirements increase sevenfold at temperatures between 5°C to 25°C (Peck et al., 2012).

Prey quality – general considerations

Not only prey availability is of importance for larval fish nutrition, but also prey quality. This comprises three different aspects: prey size, species composition, and the biochemical composition of the prey.

There is always an optimum prey size for a fish larva. Small prey might be easier to catch, but the gain of energy is low compared to the effort of catching. Big prey is appealing, but also much more difficult to capture. Focusing on large prey might lead to many unsuccessful feeding strikes until a single one is successful. Hence, the risk of putting more energy into the attempts than gaining from them when being successful is high. Between these two extremes, there lays an optimum prey size where energy loss during foraging is lowest and energy gain is highest after a successful attempt. This optimum prey size changes during larval ontogeny, i.e., small larvae need a high availability of small prey, while larger larvae depend on a sufficient amount of larger prey (Schoener, 1971; Pyke et al., 1977; Munk, 1992). Already in 1924, feeding by larval North Sea herring on flagellates was observed (Hardy, 1924). Hence, it is remarkable that although protozooplankton can be considered as potential prey for small or first feeding larval fish (Montagnes et al., 2010; Mitra et al., 2014 and references therein), it has largely been neglected in field studies on nutritional situations for larval fish. In this respect, the work by Busch (1993) on larval herring was an exception, showing experimentally that small herring larvae provided a natural zooplankton assemblage fed on synchaetes, which are protists. Nevertheless, though potentially important, field work-based knowledge about effects of protozooplankton on the growth rates of spring-hatched herring larvae is lacking at present.

The species composition and life history characteristics of the prey might affect the larvae in four different ways: size of the taxon (see above), the spatial and temporal overlap of the productive period of the prey in accordance with the peak hatch of the larvae (i.e., the Critical Period and Match-Mismatch hypotheses), movement

patterns/predator avoidance of the taxa and the prey´s biochemical composition (see below).

The spatial and temporal overlap between prey peak and peak hatch is crucial, and it was observed that recruitment of North Sea cod decreased as the dominance in the copepod community shifted from *Calanus finmarchicus* to *Calanus helgolandicus*. The distribution of the cold water adapted *C. finmarchicus* shifted northwards as a consequence of global warming. As peak abundance of *C. helgolandicus* occurs about three weeks earlier than that of *C. finmarchicus*, a temporal mismatch between cod larvae and copepods was the consequence, leading to low recruitment (Beaugrand et al., 2003). Similarly, recruitment of cod decreased and recruitment of sprat increased as a shift in the copepod community from *Pseudocalanus acuspes* to *Acartia* spp. took place in the central Baltic Sea at the end of the 1980´s (Alheit et al., 2005; Möllmann et al., 2005). This regime shift was again driven by changes in the abiotic environment: a salinity decrease due to reduced salt water inflows from the North Sea in combination with increasing temperatures.

Apart from issues in the timing, larval fish may focus on dominant prey taxa. However, this selectivity seems to be plastic, in a way that the larvae may switch from one prey item to another one, when the latter becomes dominant. Despite this, most studies investigating prey effects on larval growth did not consider prey selectivity as only total zooplankton abundance was analyzed, without further taxonomical differentiation (Robert et al., 2014, and references therein). The authors assume that one of the reasons for the frequently observed missing correlation between larval growth and prey availability is the lack of taxonomic differentiation. A possible reason for prey selectivity might be the prey´s movement in two ways: firstly, the larvae might be embossed by movement patterns of the dominant prey taxon. Secondly, some taxa might be more successful in escaping certain predators (e.g. herring larvae), thereby indirectly supporting prey selectivity effects.

Food quality effects on larval fish development and growth

The biochemical value of a prey item for a larva is defined by providing energy for respiration (caloric value), molecules to build up the own body tissue (total amount of amino acids and fatty acids), the stoichiometry (P and C:N), and essential components, i.e., components that the larva cannot synthesize for itself, but that are indispensable for

its survival and growth. The latter comprise amino acids, vitamins, minerals, and fatty acids (Holt, 2011).

For marine animals, three fatty acids are essential: docosahexaenoic acid (DHA), eicosapentaeonic acid (EPA), and arachidonic acid (ArA). In the marine environment, these essential fatty acids (EFA) are primarily synthesized by bacteria and the phytoplankton. Additionally, some protist species are able to perform a trophic upgrading, which means that they synthesize highly unsaturated fatty acids (HUFA) while elongating the carbon chain of precursor fatty acids from their prey. Generally, the phytoplankton is the quantitatively most important producer of EFAs (Dalsgaard et al., 2003).

Primarily, the fatty acid composition of the phytoplankton is determined by its taxonomical composition. Apart from that, the phytoplankton´s quality changes during blooms. EFA concentrations are high during the exponential growth phase, when the cells divide frequently. During the stationary phase however, the cells accumulate triglycerids as storage lipids, thereby decreasing the relative concentration of the EFAs (Dalsgaard et al., 2003, and references therein). Accordingly, the EFA concentrations in one and the same copepod species vary in space and time (El-Sabaawi et al., 2009).

As changes in the biochemical composition of the fish larvae´s prey are observed, the question arises in how far these changes affect development and growth of the larvae? Food quality is a big issue in aquaculture, as the concentrations of EFAs in the most common food organisms used for rearing marine fish larvae, the rotifer *Brachionus* spp. and nauplii from the brine shrimp *Artemia* spp., are insufficient to meet the requirements of marine fish larvae (Izquierdo, 1996). Therefore effects of food quality on development, growth and survival of fish larvae are extensively investigated in aquaculture research (Holt, 2011). Negative consequences for larvae fed with low-EFA level food have been observed including, e.g. abnormal swimming behavior, impaired visual capabilities, reduced immune activity, malpigmentation, lowered growth rates and increased mortalities (e.g., Sargent et al., 1994; 1995 & 1999; Ishizaki et al., 2001; Copeman et al., 2002; Cutts et al., 2006). It was experimentally shown that the quality of the primary producers affects the condition of fish larvae (St. John et al., 2001; Malzahn et al., 2007a).

Despite the aforementioned findings, potential issues of food quality have been largely ignored in field studies until recently (but see Malzahn et al. 2007b & 2009). However, it was shown that feeding on low quality prey items can lead to reduced larval herring growth rates in the field (Paulsen et al., 2014). Though habitat-specific

differences in the prey´s quality are potentially important, currently no studies comparing nursery areas in terms of the food quality exist.

Condition indices

To investigate food effects on fish larvae, their nutritional condition needs to be analyzed. Several methods to analyze larval fish condition and growth are established. For instance, cohort analyses observe changes of mean length and/or dry weight of a cohort over time (e.g. Schnack, 1972; Oeberst et al., 2009a). While this approach provides information about mean growth of a cohort, other methods have to be used to analyze the nutritional condition of individual larvae. Otolith analysis offers the opportunity to analyze the age (Pannella, 1971) and the growth history (e.g. Brothers et al., 1976) of a larva. To investigate the current condition status, biochemical growth indices are suitable, such as activities of digestive or metabolic enzymes (e.g. Ueberschär, 1988) , lipid content (e.g. Balbontin et al., 1973) or the RNA:DNA ratio (e.g. Clemmesen, 1994).

The use of RNA:DNA as a proxy for nutrition and growth is based on the principle that the amount of DNA in a cell is relatively constant. Contrary, the RNA concentrations vary, depending on the condition of the larva. While in the beginning the analysis was restricted on bulk samples with many larvae (Buckley, 1979; Buckley, 1984), highly sensitive methods were developed in the meanwhile, allowing the analysis of individual larvae (Clemmesen, 1994). Because the analyses of RNA:DNA from different analytical protocols led to different results in terms of absolute values, results from different labs were not comparable. To overcome this issue, the protocols were intercalibrated (Caldarone et al., 2006). Today, RNA:DNA can be used to calculate larval instantaneous growth rates by using species-specific growth models or, if not available, multi-species growth models (Buckley et al., 2008). RNA:DNA analysis is an established and frequently used method to assess the condition and growth of larval fish (e.g. Clemmesen, 1994; Folkvord et al., 1996; Esteves et al., 2000; Malzahn et al., 2007b; Höök et al., 2008; Huwer et al., 2011; Grote et al., 2012; Illing et al., 2015). At present, no studies comparing larval herring growth and growth conditions in different nursery areas in the western Baltic Sea exist. Similarly, spatial variability of larval herring growth in western Baltic Sea retention areas is not investigated so far. Information on this issue would allow an effort-efficient, empirical-based sampling design for larval growth analyses.

Thesis Outline

In light of the recruitment issue of western Baltic spring spawning herring (WBSSH) and the importance of the Greifswalder Bodden as a nursery area, larval herring growth rates were investigated in relation to the nutritional situation for the larvae. This was done in three important nursery areas for WBSSH, which are the Greifswalder Bodden, the Kiel Fjord and in the Kiel Canal. The latter is particularly suitable for both, between-site comparisons as well as more general studies on larval fish ecology, it provides the opportunity to study larval cohorts over time. Temporally highly resolved data were analyzed, according to the short response time of the analytical method used (RNA:DNA analyses). The thesis touched different novel aspects, such as a qualitative comparison of the prey field between nursery areas, effects of protozooplankton availability on spring-hatched larval herring, and further investigations of known phenomena, for instance food-limited and size-dependent growth. Accordingly, six central questions form the basis of this thesis:

1. Does the Greifswalder Bodden provide qualitatively better food than other nursery areas?
2. Can food-limitation be a recurring issue affecting the growth and potentially survival of herring larvae?
3. Does protozooplankton affect growth rates of spring-hatched herring larvae?
4. Does size-dependent growth in larval herring depend on food availability?
5. Do growth rates of larval herring exhibit significant spatial variability within large, seemingly homogenous nursery areas?
6. Are broad-scale patterns in climate variability such as the Baltic Sea Index correlated with larval fish growth rates?

Chapter 1 *Nutritional situation for larval Atlantic herring (*Clupea harengus *L.) in two nursery areas in the western Baltic Sea.* Though many studies assessed nutritional situations for larval fish in the field using RNA:DNA analysis, only very few compared different habitats and no studies exist that compare the nutritional situation of larval WBSSH. Similarly, the issue of food quality was largely neglected in this respect in the past, especially in comparative approaches investigating the suitability of habitats.

The Greifswalder Bodden is assumed to be the most important spawning ground and nursery area for WBSSH. However, reasons for the large impact of the Greifswalder Bodden on WBSSH recruitment are unknown. Chapter one compares two important larval WBSSH nursery areas, Greifswalder Bodden and Kiel Canal, aiming at a comparison of both food quantity and food quality in terms of the essential fatty acid concentrations of the prey. Biochemically derived larval growth rates (RNA:DNA ratio) served as a response variable to the nutritional situation. In this chapter, the potential importance of food quality on larval fish growth and its interaction with food quantity is highlighted. No basic differences concerning food quality between Greifswalder Bodden and Kiel Canal were detected.

Chapter 2 *Food-limited growth of larval Atlantic herring* Clupea harengus *recurrently observed in a western Baltic Sea watershed.* Apart from its quality, prey also has to be sufficiently available in the numerical sense. Regarding to literature, food-limited growth of larval fish seems to be an exception. In previous studies in the Kiel Canal, however, larval growth was repeatedly found to simultaneously decrease with prey abundance in the Kiel Canal. Hence, chapter five used a time series consisting of 6 consecutive spring seasons in order to investigate in how far these events occur regularly, if they indicate food-limited growth in larval herring and which mechanisms drive these events. Results indicated that food-limitation can be a re-occurring problem in certain habitats, and highlight the paramount importance of certain copepod species for larval nutrition.

Chapter 3 *Preliminary insights into effects of protozooplankton on growth of spring-hatched Atlantic herring (*Clupea harengus *L.) larvae.* Additionally to the biochemical composition of the prey, the prey´s size is another major food quality aspect. The prey´s size is of crucial importance for small larval fish. Protozooplankton (PZP) is small and considered as important prey for first-feeding larvae. Recently, results from experimental work showed that larval herring benefit from microzooplankton occurrence during the yolk-sac phase (Illing, 2016). However, effects of PZP on the condition of larval fish in general and spring-hatched herring larvae in particular were not investigated in field studies so far. In this study, effects of PZP on the nutritional condition of small herring larvae (8 mm to 12 mm) and adult female calanoid copepods (*Eurytemora affinis*) were investigated. Furthermore, chlorophyll *a* concentrations were included as a quantitative measure for phytoplankton biomass. Chapter three follows a comprehensive

approach, as four trophic levels were included into analyses (phytoplankton, protozooplankton, mesozooplankton and ichthyoplankton). This chapter shows that growth of spring-hatched herring larvae is not directly affected by protozooplankton biomass.

Chapter 4 *Size-dependent growth of larval fish is not an issue in a world of plenty.* Size-dependent larval growth is a phenomenon observed in several fish species. A possible reason for this might be better foraging capabilities of large larvae compared to small ones, which is especially advantageous at relatively low prey abundances. At high prey abundances however, the advantage of being large is expected to be smaller. In order to test effects of prey abundance on size-dependent larval herring growth rates, a 6-year time-series from the Kiel Canal was analyzed in chapter six. This chapter demonstrates decreasing size effects in larval herring growth rates with increasing prey abundance.

Chapter 5 *Spatial variability of larval Atlantic herring (*Clupea harengus *L.) growth rates in the western Baltic Sea*. Spawning and nursery areas of marine fish species that reproduce in estuaries and lagoons are distributed along coastlines. The geographic distance between and large spatial extension of the nurseries potentially leads to different growth conditions and, thus, different larval growth rates. In order to test spatial variability of larval Atlantic herring growth rates, five stations in the Greifswalder Bodden were analyzed throughout one spring-season. Seasonal mean larval growth was compared between the Greifswalder Bodden and the Kiel Canal in three consecutive years. Further, yearly mean mesozooplankton data from five coastal sampling stations were available from three consecutive years. Results showed that spatial variability in larval growth rates and mesozooplankton availability is low compared to temporal variability.

Chapter 6 *Effects of the Baltic Sea Index (BSI) on larval herring growth and copepod abundance in the western Baltic Sea.* Effects of climate variability on marine fish stock population dynamics was observed in several species, including Atlantic herring in the North and western Baltic Seas. In case of North Sea cod and the pelagic ecosystem of the central Baltic Sea it was shown that climate variability affects the composition of the plankton, with potential effects for larval nutrition and ultimately recruitment. Though it is generally assumed that larval growth affects recruitment, until now no studies investigating effects of climate variability on biochemically determined larval fish growth exist. In light of the ongoing low recruitment of WBSSH, larval herring growth and prey abundance data from a 6-year time-series from the Kiel Fjord, a 5-year

time-series from the Kiel Canal and a 3-year time series from the Greifswalder Bodden (here prey abundance data were only available from one year) were used to investigate effects of the Baltic Sea Index (BSI), a broad-scale climate indicator, on larval herring growth rates and prey availability. No significant effects of the BSI on larval growth or copepod abundance were observed, though a negative trend for one copepod species (*Pseudocalanus* spp.) existed. Further, a highly significant correlation between growth rates of small herring larvae (< 14 mm) and copepod abundance was observed.

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Chapter 1

Nutritional situation for larval Atlantic herring (*Clupea harengus* L.) in two nursery areas in the western Baltic Sea

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Abstract

The Greifswalder Bodden (GWB) is considered to be the most important spawning and nursery area for the Western Baltic spring-spawning herring. However, the biotic and abiotic reasons for this are still unclear. Consequently, we investigated larval growth conditions in the GWB and in the Kiel Canal (KC), another nursery and spawning area of Baltic herring. We investigated prey quantity and quality (copepod abundance and essential fatty acid (EFA) concentration) as well as biochemically derived growth rates and fatty acid content of larval herring in the spring of 2011. A significant correlation between larval growth and larval EFA concentration could be observed in the GWB. The highest growth rates and EFA concentrations in the larval herring coincided with high food quality. Compensating effects of food quality on food quantity and vice versa could be observed in both the GWB and the KC. Whilst larval growth rates in the KC were high early in the season, highest growth rates in the GWB were achieved late in the season. In conclusion, neither area was superior to the other, indicating similar growth conditions for larval herring within the region.

Keywords: food quality, essential fatty acids, DHA, EPA, growth, prey density

Introduction

Early life stages are crucial for the determination of year class strengths. A hundred years ago, Hjort (1914) already hypothesised that high numbers of suitable prey items during the stage of first feeding are responsible for good recruitment in marine fish stocks. This has been the basis of recruitment research ever since, and his hypothesis was continually refined and supported by modelling, experimental and field workers alike (Anderson, 1988; Buckley and Durbin, 2006; Cury and Roy, 1989; Cushing, 1974; Cushing, 1990; Rosenthal and Hempel, 1970; Sinclair, 1988; Sinclair and Tremblay, 1984). However, it is still not possible to reliably predict recruitment simply on the basis of biotic and abiotic parameters. Hence, other uninvestigated, or only rarely investigated factors seem to play an important role as well.

Although the strong effects of food quality on larval rearing are well-known from aquaculture as well as experimental work, this issue is mostly neglected in field studies. In particular, the effect of essential fatty acid (EFA) supply on the performance of marine fish larvae is well documented in experiments. Total amounts of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) as well as their ratio, along with arachidonic acid (ArA) affect development, growth and survival of marine larval fish (Bell *et al.*,

1995; Copeman and Laurel, 2010; Copeman *et al.*, 2002; Cutts *et al.*, 2006; Furuita *et al.*, 1998; Mourente *et al.*, 1991; Van Anholt *et al.*, 2004). These dietary components are mainly synthesised by the phytoplankton and subsequently transferred to higher trophic levels. Therefore, the basic EFA pattern in marine food webs is determined by the planktonic primary producers. Two of the most important and widespread phytoplankton classes, diatoms and dinoflagellates, differ fundamentally in their EFA ratios. Diatoms are rich in EPA and poor in DHA, while dinoflagellates provide high amounts of DHA and substantially less EPA (Dalsgaard *et al.*, 2003). The relative amount of EFA is also higher in the exponential growth phase of the phytoplankton (e.g. during a spring bloom) when cell division occurs frequently and decreases as it reaches the growth plateau when storage lipids then accumulate (Falk-Petersen *et al.*, 1998; Kattner *et al.*, 1983; Morris, 1981). Within certain ranges, i.e. physiologically possible or tolerable limits, the EFA concentration of the fish larvae´s food is determined by the phytoplankton. Significantly improved growth was observed in larval cod fed with copepod nauplii originating from adult copepods grown on dinoflagellates when compared to a diatom-based diet (St. John *et al.*, 2001). Malzahn *et al*. (2007a) were also able to show nutritional effects that travelled up the food chain to larval fish. Since the total EFA concentration as well as the ratios between the different EFAs can differ strongly between habitats and during the spring season, food quality is expected to vary for larval fish in space and time.

In current ICES stock assessment practice, Ruegen herring spawning in GWB is considered the major component for western Baltic spring-spawning herring. Oeberst *et al.* (2009a) found a strong correlation between the number of 20 mm larvae within the GWB and the number of recruits found in the western Baltic Sea during hydroacoustic surveys in autumn. It is characteristic for spring-spawning herring in the Baltic Sea to seek low saline, shallow coastal, and protected habitats for spawning like the GWB, the KC or the Schlei Fjord (Aneer, 1989; Aneer *et al.*, 1983; Biester, 1989a; Neb, 1952; Weber, 1971). Höök *et al*. (2008) were able to show that nursery conditions for larval herring were better in coastal sheltered areas by judging the quality of the different habitats based on the RNA/DNA ratios of larval herring and the RNA content of *E. affinis*, an important larval herring food source (Schnack, 1972).

In light of the apparent dominance of the GWB as a herring spawning ground the question arises as to what the particular qualitative differences are between this major spawning ground and the many other quantitatively less important spawning grounds, such as the Kiel Canal (KC). In contrast to the natural habitat of the GWB, the KC is an

artificial inland waterway. Despite the obvious differences between GWB and the KC, they have important hydrological features in common; for example high nutrient load, no anoxia due to a well-mixed water column and low salinity. The latter is especially important in order for a spawning ground to be suitable for Baltic herring. The question remains if both areas are similarly suitable as nursery grounds for herring hatchlings.

The principle of using RNA/DNA ratios as an indicator of condition is based on the assumption that the DNA content of a cell is constant, while the RNA content varies with the nutritional condition of the cell The RNA/DNA ratio is a well established biochemical method to determine the condition of fish larvae (Caldarone *et al.*, 2003; Clemmesen, 1994; Grote *et al.*, 2012; Malzahn *et al.*, 2007b; Meyer *et al.*, 2012). Standardising the RNA/DNA ratios (Caldarone et al., 2006), and using a multispecies fish larvae growth model allows for the calculation of instantaneous growth rates for a comparative approach (Buckley et al., 2008). Increase in EFAs, especially in DHA, in the diet of laboratory reared cod larvae was reflected in an increase in larval growth using this method (St. John et al., 2001).

On the basis of our observations that larval growth is affected by food quality in experimental work and that the GWB potentially provides more recruits than other spawning areas of the western Baltic spring spawning herring we defined the following two hypotheses: i) Food quality as determined by concentrations of EFAs significantly affects larval growth *in situ* and ii) The GWB provides better nutritional conditions for larval growth than the KC. To test this, we analysed and compared food quantity and quality in terms of DHA and EPA and investigated larval growth, based on larval RNA/DNA ratios, as well as DHA and EPA concentration of larval herring simultaneously from the GWB and KC.

Material and Methods

Sampling

Herring larvae (*Clupea harengus* L.) were sampled along with mesozooplankton and abiotic parameters to compare the growth conditions of larval herring in different spawning sites. One sampling site was located in the KC at a station 15 km inland from the open Baltic Sea (ICES subdivision 22; Fig.1, A), whereas the other sampling site was located in the GWB (ICES subdivision 24; Fig.1, B). All samples were collected between April and June of 2011 during the seasonal occurrence of larval herring.

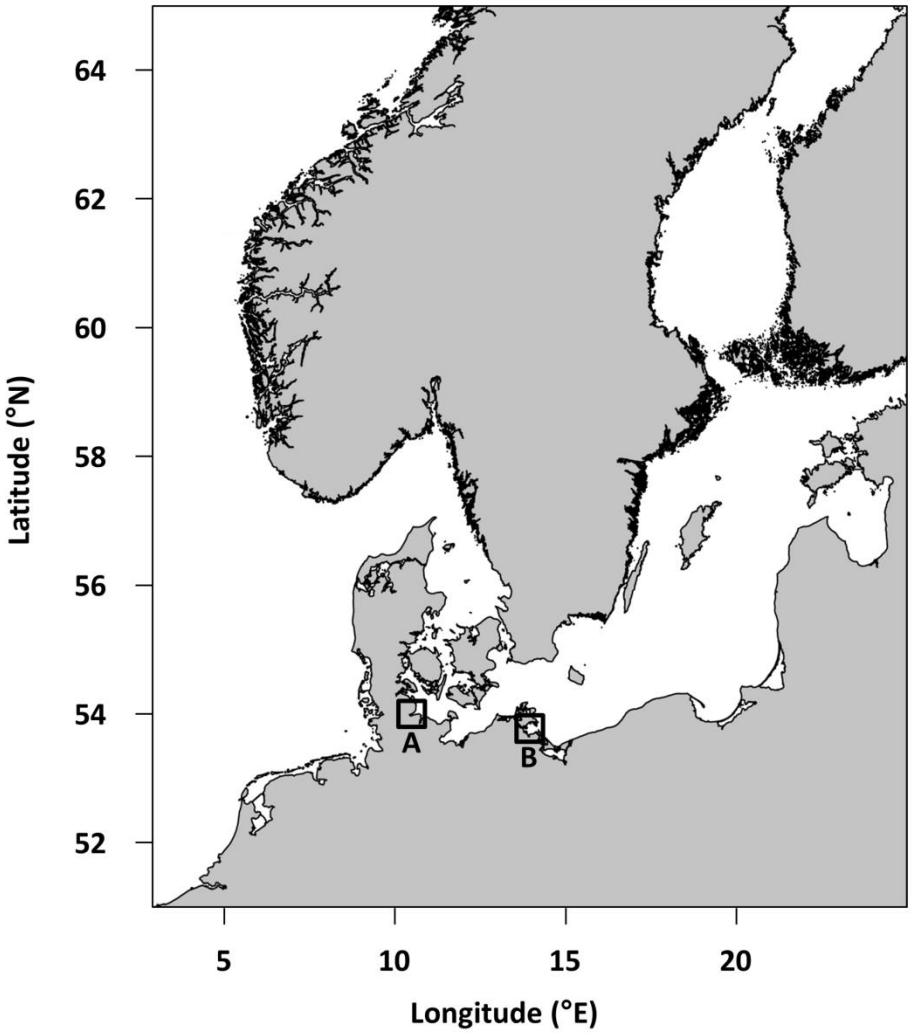

Fig. 1: Sampling sites for larval herring and copepods in the western Baltic Sea during the spring season. A = Kiel Canal, B = Greifswalder Bodden

Herring larvae were sampled with a bongo net (60 cm diameter, 335 µm and 500 µm mesh size respectively) that was heaved in an oblique haul. All larvae were frozen on board within 30 minutes of the haul. Prior to further analysis, larval standard length was measured to the lower 0.1 mm and freeze-dried for 24 hours using a freeze drier (CHRIST ALPHA 1-4 LSC). Afterwards the larvae were weighed to the nearest 0.1 µg (SARTORIUS microbalance SC2). The prey field was sampled with a WP2-net (200 µm mesh size) that was hauled once vertically from 3 m above the bottom to the surface in the KC, and from 1 m above the ground in the GWB.

Mesozooplankton abundance

The mesozooplankton samples, conserved with 4 % formaldehyde, were separated in a plankton divider (Kott, 1953) up to the point where at least 100 individuals of the most abundant copepod species were available in the section that was counted. All copepods were determined to the species level where possible although in the case of *Acartia* spec. some remained classified at the genus level.

Fatty acids

Fatty acids (FA) were measured as fatty acid methyl esters (FAMEs) by means of gas chromatography slightly modified after Malzahn et al. (2007). Lipids were extracted with dichlormethane/methanol (vol. 2:1) for a minimum of 72 hours at -80°C. After the extraction, larvae were removed and stored in a desiccator to vaporise the adhering dichlormethane/methanol. Copepod samples were treated similarly, but with an additional 30 min. of ultrasound bath after the 72 h extraction at -80°C.

RNA/DNA analysis

For better comparability of the RNA/DNA ratio and the essential fatty acid concentration of the larvae, RNA/DNA ratio and fatty acids were measured in the same larvae individuals. This was possible by first defatting the herring larvae and then homogenising the defatted carcass for the RNA/DNA analysis according to Clemmesen (1993) and Belchier et al. (2004). For both analyses complete larvae were used. Therefore the ratio determined is a whole larva respond neglecting the fact that different tissue types respond differently to changes in food availability (Olivar *et al.*, 2009). Some modifications were necessary because of the increased elasticity of the larvae due to the missing lipids. The cells of the defatted larvae were homogenised in three steps: 1)

freeze-dried larvae were placed in a cell mill for 15 minutes together with different sized glass beads (diameter 2.0 mm and 0.17–0.34 mm) 2) supersonic treatment in Tris-SDS buffer (Tris 0.05 mol*l^{-1}, NaCl 0.01 mol*l^{-1}, ethylenediaminetetracetic acid (EDTA) 0.01 mol*l^{-1}, sodium dodecyl sulfate (SDS) 0.01 %) 3) larvae together with buffer and glass beads were placed in the cell mill for 15 minutes. Then, the homogenate was centrifuged at 3829 g (6,800 rpm) at 0°C for 8 min (Sigma Laboratories Centrifuge 3-18k). A combined fluorometric measurement of RNA and DNA in the homogenate in a microtiter fluorescence reader (Labsystems, Fluoroscan Ascent) followed. Next, RNase was added to the samples to digest the RNA (30 minutes at 37°C) and the remaining DNA was measured. The difference of the sum of total nucleic acids and the remaining DNA was assigned to be RNA. By using the calibration curve fitted to the standard measurements (23s r RNA Boerhinger) the amount of RNA was calculated. The RNA calibration was repeated every measurement day. The DNA concentrations were calculated using the relationship between RNA and DNA described by Le Pecq and Paoletti (1966) with a slope ratio of 2.2. for DNA to RNA.

Growth calculation

Larval instantaneous growth rates were calculated according to Buckley et al. (2008). The best-fit multi species growth model that was chosen for further calculation was:

$$G_i = 0.0145 * sRD + 0.0044 * (sRD * T) - 0.078$$

where G_i is the instantaneous growth rate, sRD is the standardised RNA/DNA ratio (Caldarone et al., 2006) and T the temperature at the given date. Results have to be interpreted in the way that a value of 0 would mean no growth at all and a value of 1 would be a doubling of the weight of the larva per day.

Estimation of larval herring production in both nursery areas

To gain a rough estimation of the larval herring production of the KC and the GWB to relate the productivity of both systems, available larval abundance data (n * m^{-3}) from the whole season were used to calculate the median of larval herring abundance. For the GWB (area: 512 km^2), abundance data of 36 stations were available and used to get the best approximation possible. Analyses of larval growth as well as chl *a* data from 4 stations in the GWB have shown limited spatial variability (Paulsen *et al.* in prep.),

indicating comparable conditions within the system. Due to logistic constraints only a single sampling station was analysed in the KC with the assumption that this is representative for the relatively small nursery area (area: 6 km^2).The median of larval abundance was multiplied by the volume of water of the spawning sites. The value of the GWB was then divided by the KC´s value to relate both areas. Since the sampling sites from which abundance data were used are spread over the whole area of the GWB, the whole water volume of the GWB was used for calculation. However, in the KC only approximately 40 km of the total area is used for spawning and this was accounted for in the calculation.

Silica and chlorophyll a concentrations

Silica as well as chlorophyll a concentrations were analysed according to Grasshoff et al. (1999).

Statistics

Statistical analyses were performed using the statistic software package STATISTICA (version 6). The data were checked for normal distribution and homogeneity of variances using the Shapiro-Wilk- and the Levene-test. When variances were heterogeneous, data were transformed by extracting the cube and fourth root, respectively. To check between sampling days, a one-way ANOVA was conducted and a TukeyHSD test was used for post hoc comparison. Linear regressions were performed to test for effects of larval EFA on larval growth. To test for differences between the two habitats, the season was split into two time windows, where drastic changes in prey availability and larval growth were observed. Larval growth rates, larval and copepod EFA concentration as well as copepod abundance within each time window and region were pooled. Afterwards the different parameters were tested with a t-test between the two regions.

Results

Greifswalder Bodden (GWB)

The mesozooplankton assemblage of the GWB consisted mainly of copepods. *Acartia* spp. was the dominant genus until 25 May, contributing 60 % to 80% to the copepodid community. The strong increase in prey abundance on 1 June was driven by increasing *Eurytemora* abundance. This species contributed 70% of the copepodids on that day. Thereafter, the contribution of *Eurytemora* decreased strongly and *Acartia* became dominant again on 15 June (97% of all copepodids). In the GWB, instantaneous growth rates of larval herring followed the copepodid abundance (Fig. 2i). An exception was the time window between 1 and 15 June, when growth remained constantly on a high level despite an approximate halving in prey abundance. Prey DHA concentration increased significantly between 4 May and 18 May (ANOVA, TukeyHSD, $p<0.05$), which was reflected in the significantly increasing DHA concentration of the larvae (ANOVA, TukeyHSD, $p<0.05$, Fig. 2ii). Growth rates increased significantly on 1 June (ANOVA, TukeyHSD, $p<0.01$) when prey abundance increased six fold. However, not only growth rates increased, but also average larval standard length (Tab. 1). This affects larval growth rates, since during adequate growth conditions larger larvae generally have higher growth rates than smaller ones (Clemmesen, 1994). While copepodid abundance decreased by 50 % between 1 and 15 June, DHA concentration of the copepods showed an increasing trend and larval growth remained constant. Variance in growth and DHA concentration of the herring larvae was low when nutritional conditions were bad between 4 and 25 May. Although growth conditions in terms of prey abundance and copepod DHA concentration were similar between 18 May and 15 June, larval growth rates were significantly higher on 15 June. However, temperatures were 7°C higher on 8 June than on 11 May (Fig. 8). The EPA concentration of the copepods increased similar to the DHA concentration between 27 April and 18 May. As a result, EPA concentrations increased in the larvae as well (Fig. 2iii).

Tab.1: Average length per date in the Greifswalder Bodden

Date	27.4.	4.5.	11.5.	18.5.	25.5.	1.6.	8.6.	15.6.
Length	9.5±2.0	9.8±1.8	10.8±2.4	12.4±2.5	12.9±3.4	15.3±2.3	14.0±2.8	13.1±1.9

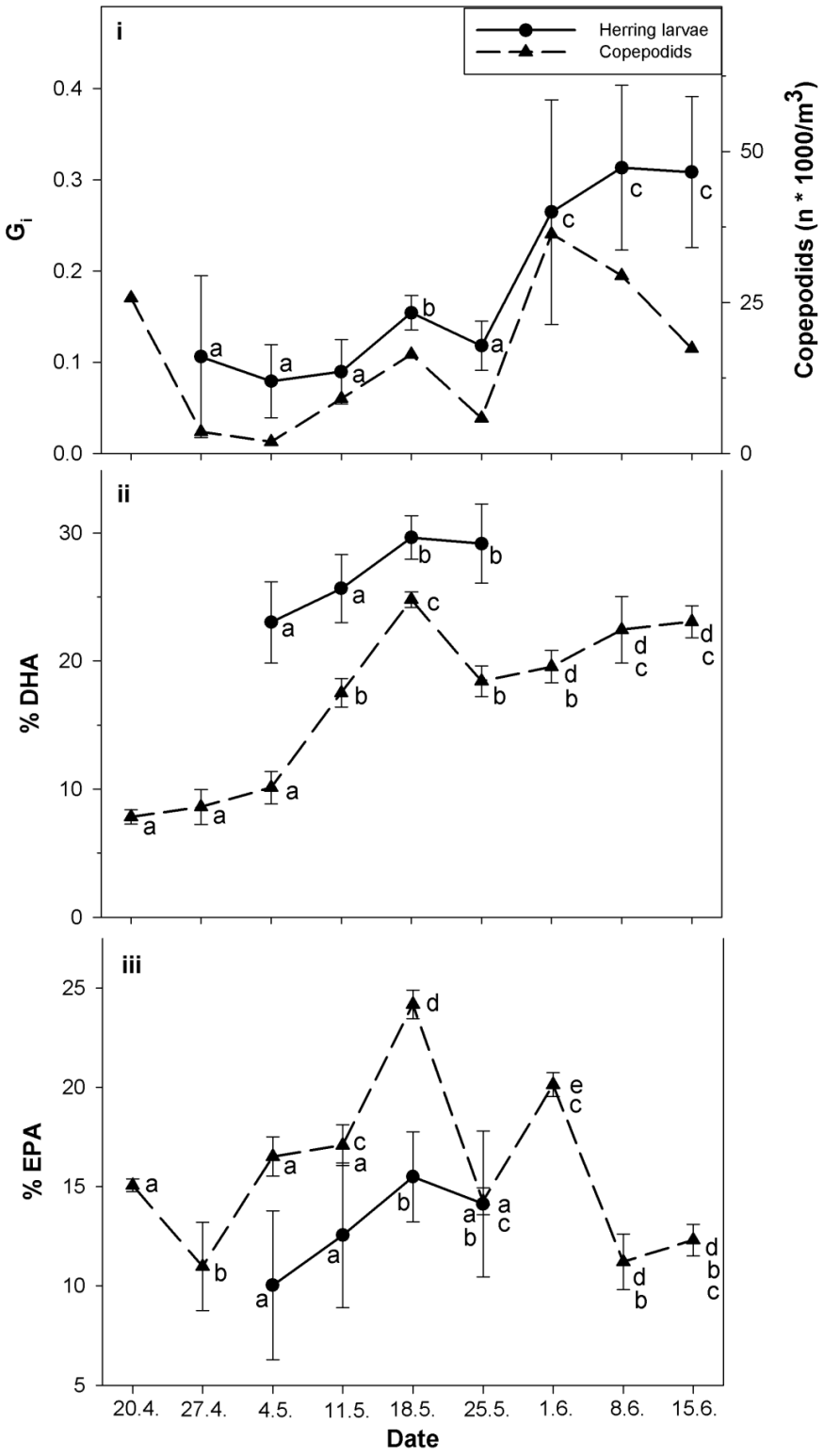

Fig. 2: Greifswalder Bodden. Error bars indicate standard deviations. i) Instantaneous growth rate (G_i) of larval herring and copepodid abundance per cubic meter over time ii) DHA concentration of larval herring and copepods over time iii) EPA concentration in

larval herring and in copepods over time. Different letters beside the data point denote significant differences.

Kiel Canal (KC)

Eurytemora dominated the copepodite assemblage during late April and throughout May (90 % to 100 % of all copepodites sampled) in the KC. From late May on, *Acartia* dominated (70 % to 80 % of all copepodids). Similar to the GWB, growth rates of larval herring followed prey abundance in the KC (Fig. 3i). However, growth rates remained constant, even when prey abundance increased eight fold on 13 May. This occurred when DHA concentration of the copepods decreased significantly (ANOVA, TukeyHSD, $p<0.05$) and DHA of the larvae showed an increasing trend (Fig. 3ii). Since larvae were larger on 13 May compared to 3 May (Tab. 2), a faster growth due to the increase in size would be expected. When food quantity abruptly became limited on 31 May, growth rates of the larvae decreased significantly. While DHA concentration in the larvae increased during the whole season (Fig.3ii), EPA remained constant after an initial increase (Fig. 3iii).

Tab.2: Average length per date in the Kiel Canal

Date	3.5.	13.5.	17.5.	24.5.	31.5.	7.6.
Length	9.0±1.1	11.1±1.4	10.1±2.0	12.5±2.2	13.1±1.5	18.6±1.2

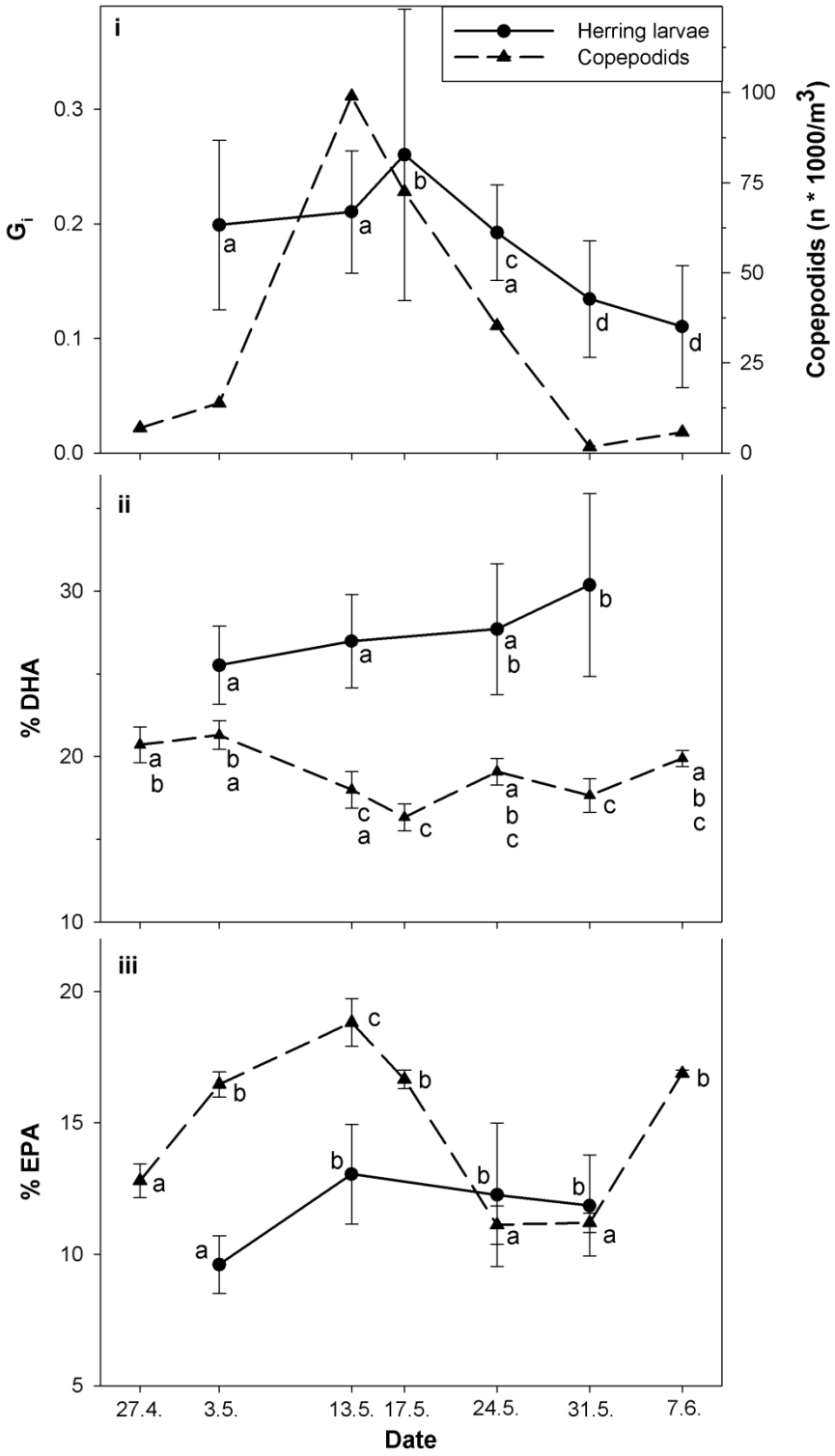

Fig. 3: Kiel Canal. Error bars indicate standard deviations. i) Instantaneous growth rate (G_i) of larval herring and copepodid abundance per cubic meter over time ii) DHA concentration of larval herring and copepods over time iii) EPA content in larval herring and in copepods over time. Different letters beside the data point denote significant differences.

Comparison of both areas

In the GWB, larval growth was significantly correlated with the DHA and EPA concentration in the larvae ($p<0.01$, Fig. 4 and Fig. 5). Highest growth rates were achieved at highest DHA and EPA concentrations in the larvae. When DHA and EPA concentrations in the copepods were highest on 18 May, larvae had the highest DHA and EPA concentrations and grew at the highest rates. In contrast to this, no significant correlation between DHA and larval growth was detected in the KC (Fig. 6), though one between larval growth and EPA was found (Fig. 7).

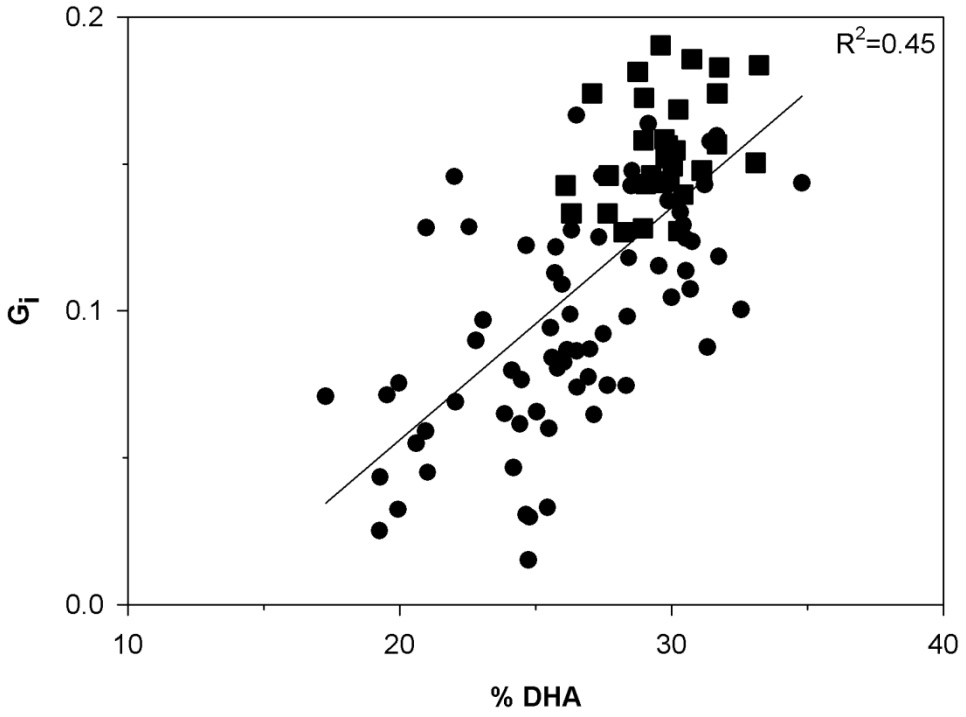

Fig. 4: *Greifswalder Bodden* Significant correlation ($p < 0.01$) between instantaneous growth rates (G_i) of larval herring and their DHA concentration. Dots indicate all available data, squares show data from 18 May, when DHA concentration in copepods was highest.

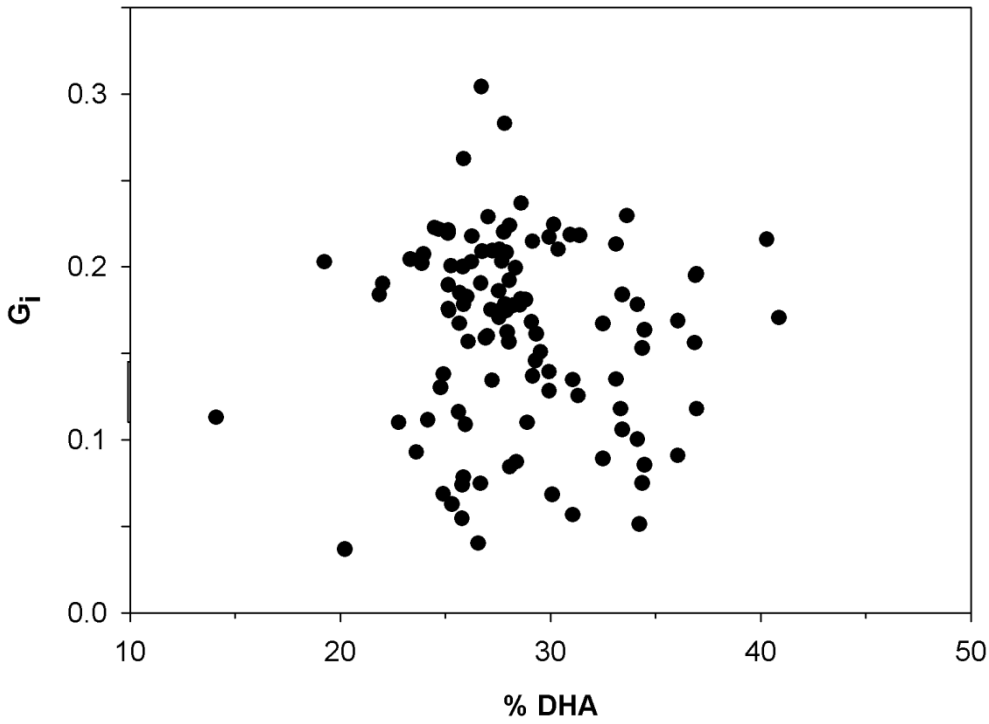

Fig. 5: *Kiel Canal* Correlation between instantaneous growth rates (G_i) of larval herring and their DHA content. The correlation is not significant.

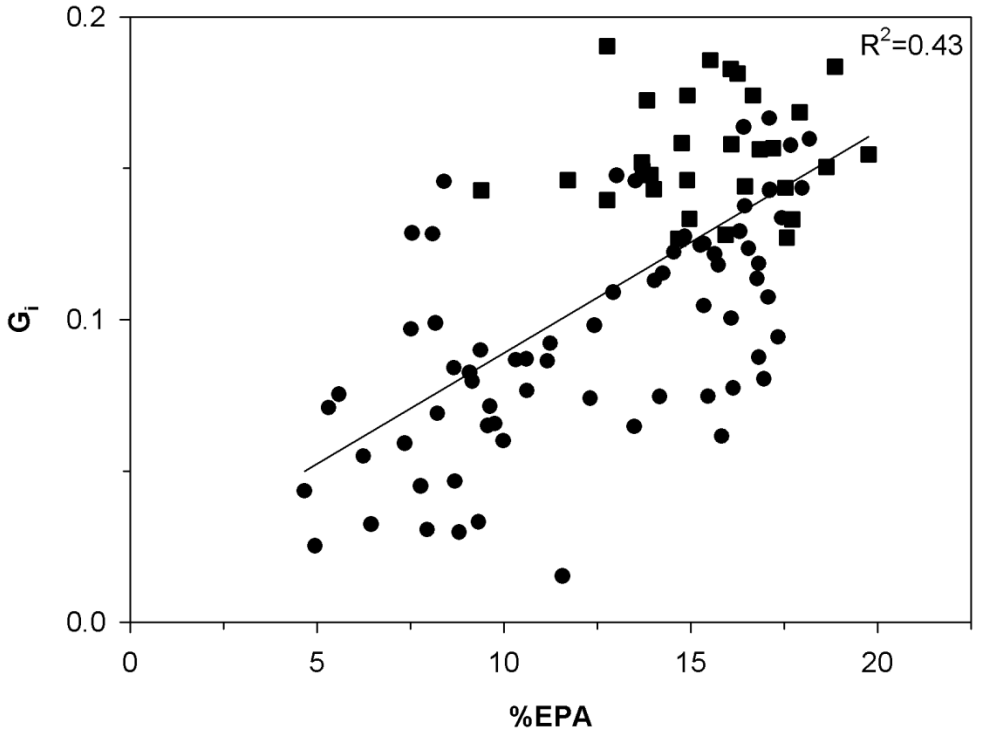

Fig. 6: *Greifswalder Bodden* Significant correlation ($p<0.01$) between instantaneous growth rates (G_i) of larval herring and their EPA concentration.

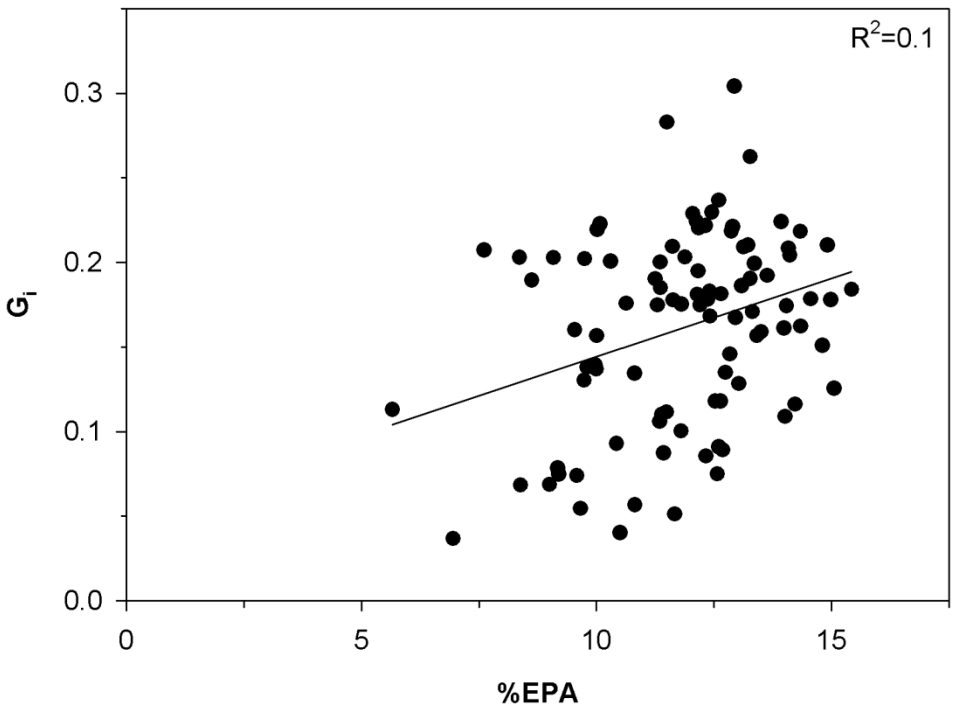

Fig. 7: *Kiel Canal* Significant correlation ($p<0.01$) between instantaneous growth rates (G_i) of larval herring and their EPA concentration.

Over the whole season, chlorophyll *a* values were higher in the GWB when compared to the KC (Fig. 8 and Fig. 9). Silica values were very low (< 1 µmol * l^{-1}) in the GWB until 11 May, when silica values started to recover (Fig. 8). In contrast, silica values were above 21 µmol * l^{-1} in the KC throughout the whole season (Fig. 9).

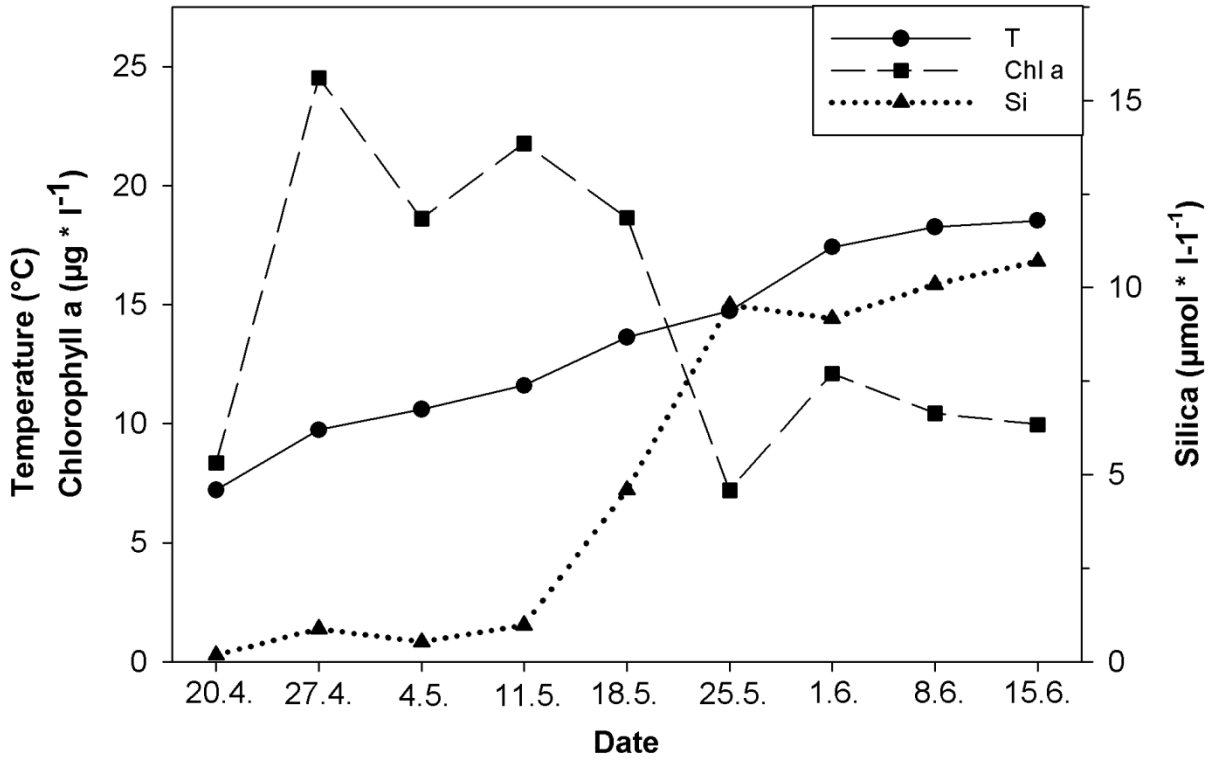

Fig. 8: *Greifswalder Bodden* Temperature, Silica and chlorophyll a over time

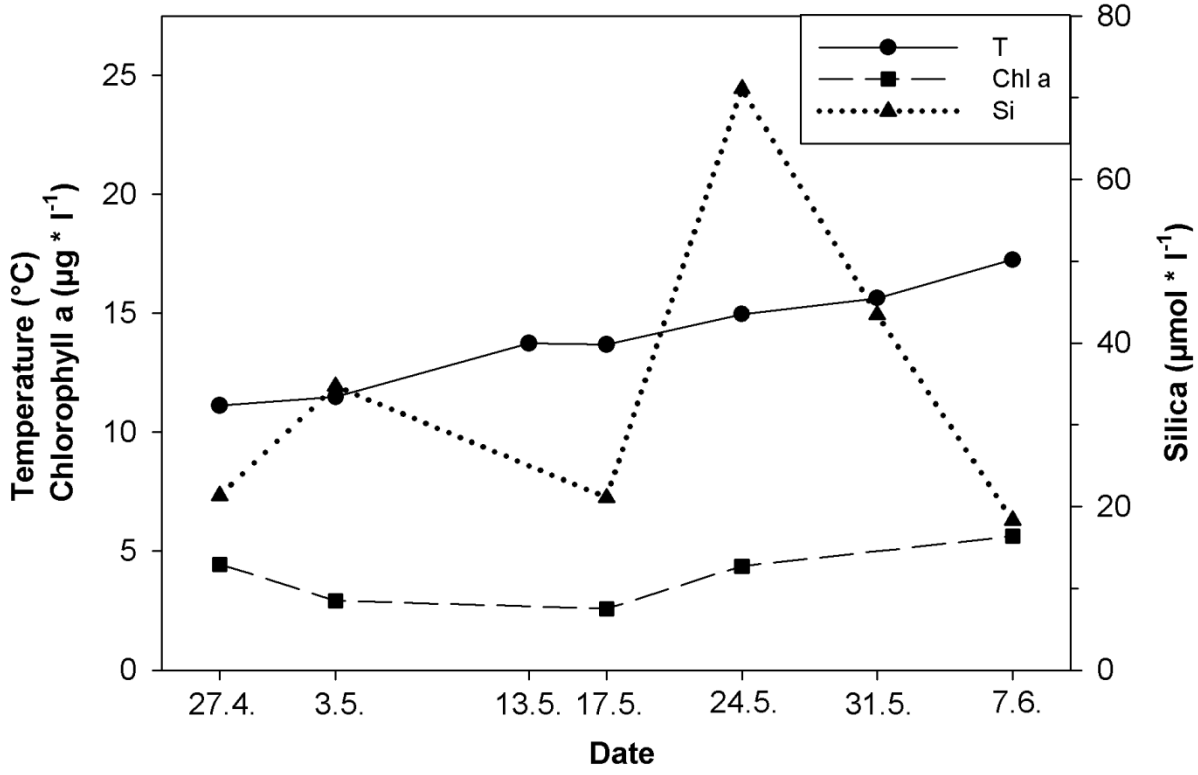

Fig. 9: *Kiel Canal* Temperature, Silica and chlorophyll a over time

As aforementioned, to better compare both habitats the season was divided into two time windows according to drastic changes in prey availability and larval growth. The first time window reached from 27 April to 25 May, while the second one comprised of 2 or 3 samplings from 31 May to 15 June, depending on the habitat. In contrast to significantly different prey abundances in both time windows ($p < 0.05$, Tab. 3), prey DHA concentration was only significantly different in the second time window ($p < 0.05$, GWB > KC, Tab. 3). In the first time window, larvae grew significantly faster in the KC, whereas growth rates were significantly higher in the GWB in the second time window (Tab. 4). This was true for all ontogenetic stages. Larval fatty acid data were only available for the first time window. Contrary to larval growth rates, DHA and EPA concentrations were only significantly different in certain ontogenetic stages (Tab. 1). Yolk-sac larvae (< 9 mm) had significantly higher DHA and EPA concentrations in the KC than larvae from the GWB. At first feeding, no differences could be detected, while EPA was significantly higher in pre-flexion larvae (11-14 mm) of the GWB. In post-flexion larvae EPA as well as DHA was significantly higher in the GWB compared to the KC irrespective of the bad growth conditions in terms of prey abundance and prey EFA concentration during that time window.

The calculation of the rough estimate of total larval production of the different spawning sites revealed a 24-fold higher production of larval herring in the GWB compared to the KC.

Tab. 3: Results from t-tests between prey quantity and quality of Greifswalder Bodden (GWB) and Kiel Canal (KC).

	27 April - 25 May	31 May - 15 June
Prey abundance	p<0.05; KC>GWB	p<0.05; GWB>KC
% DHA	p>0.05	p<0.05, GWB>KC
% EPA	p>0.05	p>0.05

Tab.1: Results from t-Tests of larval herring originating from Greifswalder Bodden (GWB) and Kiel Canal (KC). The length classes reflect different ontogenetic stages of the larvae: yolk-sac (< 9 mm), first feeding (9 - 11mm), pre-flexion (11 – 14 mm), and post flexion (> 14 mm). The season is divided into two time windows, according to drastic changes in prey availability and larval growth in both habitats. G_i I and DHA and EPA are results from time window one, reaching from 27 April to 25 May, while G_i II shows results from time window two, 31 May to 15 June. Larval fatty acid data to test exist only from time window one, these results are shown.

	< 9 mm	9 - 11 mm	11 - 14 mm	> 14 mm
G_i I	$p < 0.001$; KC > GWB	$p < 0.001$; KC > GWB	$p < 0.001$; KC > GWB	$p > 0.05$; KC > GWB
G_i II			$p < 0.001$; GWB > KC	$p < 0.001$; GWB > KC
% DHA	$p < 0.05$; KC > GWB	$p > 0.05$	$p > 0.05$	$p < 0.01$; GWB > KC
% EPA	$p<0.05$; KC>GWB	$p>0.05$	$p<0.01$; GWB>KC	$p<0.01$; GWB>KC

Discussion

Though growth of fish larvae is dominantly affected by food availability and temperature, other factors such as salinity, oxygen and the larvae's interactions with other organisms are known to be influencing factors (Clemmesen, 1994; Johnes, 2002). Furthermore, it is known from experimental work that food quality can also be an important factor (Copeman *et al.*, 2002; Malzahn and Boersma, 2009). The present study shows that high food quality is able to compensate for low food quantity and vice versa for larval fish in the field. This can lead to constant growth rates in larval herring even when both parameters develop in opposing directions. Examples for these effects can be found on 15 June in the GWB and on 13 May in the KC. However, the increase in copepod DHA was relatively modest compared to the decrease in prey abundance on 15 June in the GWB, which leads to the assumption that other uninvestigated factors, for instance essential amino acids, vitamins, sterols (Cahu *et al.*, 2003), or abiotic factors, might have played a role in addition to the increase in DHA. A possible explanation for this compensatory

effect is that the demand for essential components is assumed to be constant, depending on the developmental stage of the larva. When food quality decreases, the larva needs to capture more prey to accommodate this demand, which is an energy consuming process. In addition, the EFA are needed as construction material and precursors to build up neural tissue along with cell membranes and hormones (Sargent et al., 1999). Therefore growth is limited when essential component supply is limited.

Even though it is possible to compensate for nutritional value and food quantity to a certain extent, this does not necessarily happen. During the early season in the GWB very low food quantity was coupled with extremely low food quality, and this led to very low growth rates. Variances in larval growth data were very small during this period, apart from the first date when yolk sac larvae appeared who had not begun to externally feed. Growth of aquatic larval organisms is at least partly genetically determined (Meyer and Manahan, 2010), and slow growing individuals may have an advantage when growth conditions are poor. Fast growing individuals starve during bad feeding conditions leading to small variances in larval growth. In contrast, variance of larval growth is high when growth conditions are moderate or good (Houde, 1987; Voss *et al.*, 2006). In this case relatively slow and fast growing individuals occur simultaneously, even though the fast growing individuals may have an ecological advantage.

Growth of larval herring in the GWB increased significantly and remained on a constant high level from 1 June onwards. Here, different aspects might have played a role: Firstly, food quantity increased strongly on 1 June, which enabled the larvae to take up more prey with a constant quality. Secondly, temperature increased more than 3°C between 25 May and 1 June. This enabled the larvae to take full advantage of the improved nutritional situation. Similarly, a temperature increase of 7°C is a possible reason for higher growth rates despite very similar food quality and quantity conditions between 18 May and 15 June.

We could show in the GWB that larval growth increases with increasing DHA and EPA concentration in the larvae. The highest DHA and EPA concentrations of the copepods were reflected in the highest DHA and EPA concentrations of the larvae and both these concentrations were likely two important factors that led to the highest larval growth rates. Conversely, only larval EPA showed an effect on larval growth in the KC, whilst larval DHA did not affect larval growth in our correlation analysis. The reason for the pronounced effects of larval EFAs in the GWB and EPA in the KC is that the development of prey EFA was consistent whilst prey quantity remained constant over the

course of several weeks consequently leading to an accumulation or dilution of EFA in the larvae. In the KC, no correlation between larval DHA and their growth rates existed. Here, high food quality was offset by low food quantity, and vice versa, and this might have been a reason for the very similar DHA values found in the larvae over a four week period. Nevertheless prey DHA probably affected larval growth in the KC, for example on 13 May, even though the strong decrease in larval DHA did not lead to decreasing DHA concentrations in the larvae themselves due to high prey quantities. St. John and Lund (1996) showed that it takes 13 days until larval cod FAs were in equilibrium with the prey. However, this is just the complete effect on the FA in the larvae themselves, and not related to larval growth. The larvae are not able to take up EFA selectively, but depend on the composition delivered by the food. FAs in the larvae change successively, starting at the point when food with a different FA composition is taken up. As a result the larvae are able to grow faster when more essential components are delivered, leading to an up-regulating of their RNA content. Contrary to their FA metabolism, larval growth is actively regulated since they are able to build up and catabolise RNA actively. Though we suppose that this is an ongoing process, we expect a detectable time delay between the DHA concentration of the prey and larval growth of about 3-4 days at the latest, according to experimental data regarding the time delay in herring larvae facing different prey quantities (Clemmesen, 1994). However during short time frames, significant effects in larval DHA are expected only when prey quality changes strongly. Even when the effect of a changing food quality or quantity on larval DHA is not significant yet, the process is nonetheless ongoing. This could explain the constancy of larval growth and EFA during adequate growth conditions in the KC, because contrary developments of EFA in the prey and prey abundance were occurring. Additionally, other parameters not investigated in this study influence larval growth, for instance essential amino acids and sterols. In both areas, *Acartia* spp. as well as *Eurytemora* were by far the most dominant copepod genera. Interestingly, in both habitats the highest abundances were reached by *Eurytemora*. Schnack (1972) as well as Donner (2006) and Busch (1996) showed that both genera in all developmental stages (nauplii, copepodids & adults) are the preferred prey items of larval herring in the estuarine habitats Schlei Fjord, KC and GWB. The different concentrations of especially DHA in the prey between both areas are likely due to differences in the taxonomic composition of the primary producers. In the KC, *Chlorophyceae* dominate the spring bloom while diatoms are negligible (A. Stuhr, pers. comm.). This is supported by high silica values over the season, because silica is an

essential nutrient for diatoms and therefore is decreasing in the water column during a diatom bloom. *Chlorophyceae* are known to be DHA poor (Dalsgaard *et al.*, 2003). However, substantial amounts of DHA and EPA could be detected in the copepods of the KC. A possible explanation for this might be trophic upgrading by protists, as shown by Klein Breteler et al. (1999). The underlying process is the elongation of HUFA precursors which leads to the synthesis of EPA and DHA. In contrast to the *Chlorophyceae* dominated KC, diatoms are the major phytoplankton taxon during the spring bloom in the GWB until the depletion of nutrients, and flagellates start to take over (Edler, 2008). Diatoms are a DHA poor taxa, while dinoflagellates are rich in this EFA (Dalsgaard *et al.*, 2003). The extremely low silica values in the beginning of our sampling period indicate a constant uptake of silicate by diatoms. From the middle of May onwards, silica values started to recover strongly and remain on a high level afterwards which might indicate the succession from diatoms to dinoflagellates. The increasing DHA values of the copepods also support this assumption.

Interestingly, yolk-sac larvae from the KC grew significantly faster and had a significantly higher DHA as well as EPA concentration than in the GWB. Therefore, maternal effects in terms of increased levels of EFAs in the eggs seem to influence not only hatching rate (Navas *et al.*, 1997; Pickova *et al.*, 1997), but also larval growth, at least in the early phase when larvae do not feed yet. A possible explanation for the different EFA concentrations and growth rates in the yolk-sac larvae is that the females from both areas fed on different quality food. Izquierdo et al. (2001) concluded in their review that the amount of DHA in the eggs partly depends on the diet of the females.

Mesozooplankton samples in the KC were taken only on one station. But since the same succession of copepods has been observed in the KC since 2005, a single haul could be argued to be representative for observing prey field development in this area. In the GWB similar patterns in larval growth rates were observed between 2010 and 2012 (data not shown), which indirectly shows that similar succession patterns of the prey field occur in the GWB inter-annually. The prey field was sampled over the whole water column, and it was not distinguished between prey quantity and prey availability. Copepods tend to accumulate in patches, e.g. in fine layers close to the thermocline. However, both areas, GWB and KC, are well mixed due to very shallow waters in the GWB (5.6 m in depth on average) or heavy shipping traffic in case of the KC. Though it is not expected that patchiness does not occur in these areas, it might be reduced due to the strong mixing. Since larval growth data from both areas follow quite well the observed prey field

development, we are confident that our method is reliable to describe prey field development in our investigation areas. According to Schnack (1972), prey biomass of 14 mm estuarine western Baltic herring larvae consisted of 90 % copepodids and adults, and only 10 % of nauplii. At least at the first feeding-stage it is expected that the larvae feed on nauplii and protists. However, because most of the analyzed larvae were beyond that stage, it is assumed that samples taken with 200 µm mesh size sufficiently describe prey field conditions for estuarine larval herring.

In the KC prey abundance was significantly higher and larval growth rates were significantly higher compared to the GWB up until 25 May. As the season proceeded however, in the GWB the larvae experienced favourable growth conditions with significantly higher prey abundance and DHA concentration of the prey that led to significantly higher growth rates than in the KC. In contrast, the KC growth conditions worsened late in the season due to a strong decline in prey abundance. Interestingly, at least in recent years this pattern of larval growth conditions seems to be typical for both nursery areas. The time series of the KC (Catriona Clemmesen, GEOMAR) shows the same trend in prey field succession since 2005 (Donner, 2006; Paulsen, 2010; Peschutter, 2008), indicating that recruits from the KC originate mainly from early in the season. In contrast, data from the GWB time series (Institute of Baltic Sea Fisheries) revealed that the late season provided the majority of recruits in recent years (Polte *et al.*, 2013). At this time, growth rates of the larvae were also highest. Since predators selectively prey upon slow growing larvae (Takasuka *et al.*, 2003), larval growth data in the present study support the recruitment data mentioned above. Our results are also consistent with calculations by Houde (1987) where even relatively small changes in growth rates can lead to strongly increased mortality in larval Atlantic herring.

Since no strong differences in larval growth conditions of both areas could be detected, there have to be other reasons for the potential importance of the GWB as a nursery area (Oeberst *et al.*, 2009b). The rough estimate of total production of both GWB and KC showed that the GWB produced 24 times as many larvae as the KC did. Even though our sampling site in the KC is at the edge of the main spawning area and the true production is probably higher than the calculated one, results indicate that a possible reason for the importance of the GWB as a nursery area is simply a size effect. Since the volume of water used for spawning of the GWB is 41 times larger than the volume of the KC this is tenable. This would also mean that whilst the KC may provide more recruits per cubic meter of water available, in absolute numbers the GWB is superior to the KC.

We are well aware that this is a very rough estimate and not a precise calculation, but it does provide one possible reason for the GWB´s importance.

In conclusion, we found support for our first hypothesis that food quality significantly affects larval fish growth *in situ.* Food quality was able to compensate food quantity effects, and vice versa. However, our second hypothesis that the GWB generally provides better nutritional conditions for larval herring than other nursery areas in this region do, was rejected. Therefore we propose that other factors, like habitat size, might be the reason for the great importance of the GWB as a nursery area for the western Baltic spring-spawning herring.

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Chapter 2

Food-limited growth of larval Atlantic herring *Clupea harengus* recurrently observed in a coastal nursery area

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Abstract

Food-limited growth of larval fish, defined as growth rates lower than observed in other habitats or from laboratory experiments at a given temperature, is rarely reported in field studies. This would imply that either larval fishes are living in an environment characterized by plenty of food, that nutritional condition selective mortality (i.e., eliminating the weak) is very strong, or this impression is caused by misinterpretation of data concerning e.g. poor taxonomical resolution of potential prey items, i.e. total potential prey abundance is high, but positively selected food is actually scarce. We analyzed RNA:DNA derived growth rates of herring larvae (*Clupea harengus* L.) and taxonomically differentiated prey field data of six consecutive spring seasons from the Kiel Canal, an artificial waterway in northern Germany in order to test if food-limited growth in larval fish can occur regularly in coastal habitats. In all years analyzed, larval growth rates decreased simultaneously with prey abundance at the end of each larval season. Furthermore, larval growth rates were observed to be lower than mean growth rates observed in another herring larvae nursery area at temperatures above 15°C. Asymptotic relationships between prey abundance and larval growth rates were observed, further supporting the hypothesis of food-limitation. As larval growth was best explained by the abundance of the numerically dominant calanoid copepod *Eurytemora affinis*, the paramount importance of the dominant prey item is highlighted. We conclude that food limitation can be a severe and re-occurring issue for larval fish in coastal habitats, and that certain prey items play a crucial role in determining larval growth rates, and therefore potentially recruitment.

Key words: RNA:DNA, *Eurytemora affinis*, time series analysis, western Baltic Sea, Kiel Canal

Introduction

Over a century ago Johan Hjort (1914) hypothesized that the nutrition of first feeding larvae is an important recruitment-driving factor. Since then, generations of fishery scientists worked on this issue, but it took decades until they started "emerging from Hjort´s shadow" (Houde 2008), interpreting the issue of larval nutrition in a broader context. This includes biotic as well as abiotic factors influencing the nutritional situation for fish larvae, and focusing not only on the time of first feeding (e.g., Anderson 1988; Cury & Roy 1989; Meekan & Fortier 1996; Buckley & Durbin 2006).

Nevertheless, the issue of food limitation in the field has rarely been examined in larval growth analyses, and remains a matter of debate. Numerous field studies reported that only small proportions of marine fish larvae starve (e.g., Clemmesen et al. 1997; Chicharo et al. 1998; Diaz et al. 2011; Yandi & Altinok 2015). Consequently, it was recently hypothesized that intermediate or low prey abundance indirectly affect larval recruitment by enforcing more active foraging and, thus, higher risk of predation by easier detection by predators (Jørgensen et al. 2014) rather than starvation itself. Accordingly, starvation of larvae would rather be exceptional at very low levels of prey abundance. For example, studies on clupeoid fish larvae showed that lowest threshold values for early survival are ~4-5 copepod nauplii L^{-1}, and that larval growth was increasingly positively affected until upper threshold concentrations of ~50 copepod nauplii L^{-1}(Peck et al. 2013, and references therein). These concentrations are strongly affected by prevailing temperatures, as fish metabolism is temperature dependent (Peck et al. 2013). Relatively few studies found evidence that growth rates of marine fish larvae are occasionally food limited (Grønkjær et al. 1997; Buckley et al. 2004; Voss et al. 2006; Huwer et al. 2011). Such observations, however, seem to be exceptions, since food limitation only occurred in certain situations, e.g., in a single year, or in certain water depths, but not on a regular basis.

All the above cited studies were conducted off-shore, providing relatively stable environmental conditions in terms of salinity and showing relatively slow temperature changes. In contrast, conditions in coastal habitats such as lagoons and estuaries are highly variable, including changing salinities and subsequent changes in prey composition (e.g., Greenwald & Hurlbert 1993; Schallenberg et al. 2003; Jeppesen et al. 2007; Brucet et al. 2009; Telesh & Khlebovich 2010). Small waterbodies and low water depths are typical for such areas, leading to strong short-term changes in temperature during spring time (e.g., Fey 2001; Newton & Mudge 2003), potentially with qualitative and quantitative implications for prey availability (Ambler 1985).

In previous studies we observed food limited growth rates of larval herring in the Kiel Canal (Paulsen et al. 2014a; Paulsen et al. 2014b), an artificial waterway in northern Germany. In this follow-up study we analyzed a 6-years-time series including RNA:DNA derived growth rates of 1177 larval herring and taxonomically differentiated prey field data in order to test the hypothesis that food-limited growth of larval fish can occur regularly in coastal habitats.

Material and Methods

Sampling

Time series analyses of biochemically derived larval herring growth rates along with prey field and temperature data of six consecutive spring seasons (2007 to 2012) in the Kiel Canal at a station 13 km inland to the open Baltic Sea (54°20'45 N, 9°57'02 E, Fig. 1A) were conducted. The Kiel Canal has an average width of about 100 m, and is characterized by a mean depth of about 11 m. The water column is constantly mixed due to the heavy shipping traffic (highest frequency of container ship use in the world), and the salinity is low, ranging from 6 to 10. The Kiel Canal is a suitable habitat to examine prey effects on larval fish, due to its very confined space and weak currents. Larval fish and zooplankton were sampled on a weekly basis. This sampling frequency was chosen according to the response time of the biochemical larval growth indicator used (RNA:DNA), which is several days to a week (Clemmesen 1994). Vertical profiles of salinity and temperature were taken (CTD 48 M, Sea & Sun Technology GmbH, Trappenkamp, Germany). To analyze the prey field, a vertical haul was carried out with a WP2-net (200 µm mesh size; the issue of losing the nauplii by using this mesh size is referred to in the discussion) and fixed with 4 % borax buffered formalin solution. Larval herring were sampled by using a bongo net with 500 µm mesh size which was retrieved in an oblique haul with a speed of 2.5 knots. Two hauls were carried out: the first one was preserved with 4% borax buffered formalin solution and used for the determination of larval abundance. The second one was taken to sample larvae for RNA:DNA analysis. This sample was cooled with crushed ice immediately, and all larvae were sorted out within 30 minutes after the haul. The larvae were frozen on board at -20°C, and stored after the return to the institute (about 2 to 3 hours after the haul) at -80°C. To define and detect food-limitation, larval growth data from Kiel Canal were compared to larval growth data from another important habitat for western Baltic Sea spring spawning herring, the Greifswalder Bodden (Bodden is the German term for an enclosed, shallow and low saline bay at the Baltic Sea coast; Fig.1A; for further information on this site, please see Paulsen et al. 2014b) with samples collected in the spring seasons 2010 to 2012. The sampling procedure was the same as in the Kiel Canal, apart from the fact that larvae were frozen in liquid nitrogen on board.

Larval standard lengths were measured to the lower 0.1 mm. Prior to RNA:DNA analysis, the larvae were freeze dried for 24 h (Christ Alpha 1-4 LSC freeze dryer) and then weighed to the nearest 0.1 µg (Sartorius microbalance SC2).

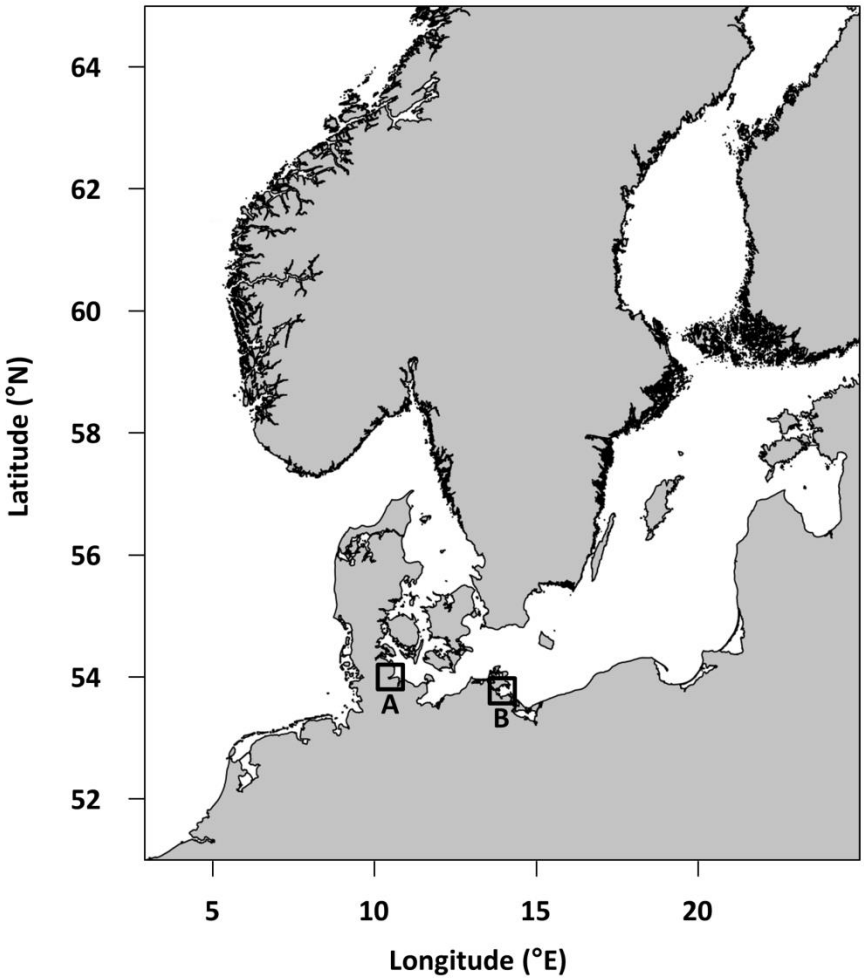

Fig. 1: Location of the sampling sites in northern Germany: A Kiel Canal and B Greifswalder Bodden

RNA:DNA analysis and instantaneous growth rates (G_i)

RNA and DNA concentrations of whole individual larvae were measured. A detailed description of the analysis is given by Clemmesen (1988; 1994). Larvae caught in 2011 however, were defatted before analyzing RNA:DNA and analyzed according to Malzahn et al. (2007a; 2007b) to analyze the effects of essential fatty acids on larval growth rates (Paulsen et al. 2014b). Comparison with the classical approach by Clemmesen (1988; 1994) showed no significant differences between RNA:DNA of

defatted and non-defatted larvae. We analyzed a minimum of 10 larvae and a maximum of 50 larvae, but on average 20-30 larvae per sampling day. Two size classes were used for further analyses: Length classes smaller and larger than 14 mm were chosen. At 14 mm the dorsal fin is clearly protruded (own observations; Doyle 1977). This fin differentiation is assumed as an indicator for an ethological change, due to enhanced swimming and therefore hunting capabilities.

The RNA:DNA ratios were converted to standardized RNA:DNA ratios (sRD; Caldarone et al. 2006) and used to calculate dry weight related instantaneous growth rates (G_i) of the larvae (Buckley et al. 2008) based on the following relationship:

$$G_i = 0.0145 * sRD + 0.0044 * (sRD * T) - 0.078$$

With G_i being the instantaneous growth rate, sRD the intercalibrated RNA:DNA ratio and T the temperature determined at the sampling time. Interpretation of G_i-values has to be done in the way that a value of 0 means no growth at all and a value of 1 would be a doubling of the weight of the larva per day. Larval growth data were used in different ways for regression analyses with prey abundance: 1) as individual larval growth data without any size differentiation, 2) mean per sampling day (including all larvae analysed at a given date) and 3) seasonal mean growth rates. The different approaches were chosen in order to 1) display individual variability, 2) visualize sampling date variability, and 3) test for inter-annual variability, which would be completely masked by taking individual data due to the high range of variability of both larval growth data and prey abundance throughout the course of the seasons.

Mesozooplankton analysis

Mesozooplankton samples were divided with a Kott plankton splitter (1953) up to the point, where at least 100 individuals (including copepodite and adult stages, excluding nauplii) of the most abundant copepod species were found in a single subsample. If less than 100 individuals were counted, additional subsamples were analyzed until at least 100 individuals were reached. All taxa were recorded and distinguished to the lowest possible taxonomic level using the taxonomic guide by Sars (1895).

Further analyses were restricted to calanoid copepods and cirriped nauplii, as these organisms were shown being the major prey items for larval herring in the Kiel Canal (Donner 2006).

Defining food-limitation

In order to define and detect food-limitation, negative effects of increasing temperatures on larval growth needed to be excluded. In order to do so, larval herring growth data (means per sampling date) from the Kiel Canal were compared to growth data from the Greifswalder Bodden, where growth rates further increased at temperatures above 15°C, indicating that the optimal temperature was not reached yet. Trend lines were fitted to larval growth vs. temperature data from the Greifswalder Bodden (2010 to 2012) and the Kiel Canal (2007 to 2012), respectively, including 95% confidence intervals (CI). When the upper 95% CI of larval herring growth data from the Kiel Canal was below the lower 95% CI of larval growth data from the Greifswalder Bodden, these larvae were assigned to be food-limited, as their growth rates were lower than possible.

Statistics

The statistic package Statistica (version 6) was used to perform statistical analyses. For all statistics, the significance level was set to $\alpha = 0.05$. By using the Shapiro-Wilk- and the Levene-Test, data were analyzed for normal distribution and homogeneity of variances, respectively. To test for differences in larval growth rates during each spring season, one-way analyses of variance (ANOVA) were conducted and Tukey honestly significant difference (HSD) tests were used for *post hoc* comparison. Growth data used for this approach were not further differentiated with respect to size classes, but used to generally test for significant declines in larval growth rates towards the end of the season in the Kiel Canal. Mann-Whitney-U-tests were used to test for significant differences between larvae smaller (range: 4.8 mm to 13.9 mm) and larger than 14 mm (range: 14 mm to 20 mm) at given dates. In order to test for prey effects on larval growth, individual larval growth data and mean growth per sampling date were correlated to prey abundance observed at that sampling date. Additionally, seasonal mean larval growth data and seasonal mean prey abundance were correlated. As the 2009-season was temporally not fully covered, that year was excluded from the seasonal mean approach. As temperature is included as a term in the multi-species growth model used (Buckley et al. 2008), larval G_i could potentially rather reflect temperature changes than changes in the larvae´s nutritional condition. To test in how far larval G_i was determined by the nutritional condition of the larvae, a regression analysis between G_i and sRD was performed. Further, generalized additive models (GAMs) were performed in order to test for explained variability of sRD for both *Eurytemora* abundance and temperature in

combination. This was only possible for larvae <14 mm, as *Eurytemora* abundance and temperature were significantly negative correlated for the data set of larvae >14 mm. This was based on the fact that larger larvae only occurred from the middle of the season onwards. At that time point, *Eurytemora* abundances were always at their highest levels, and decreased thereafter.

Results

The zooplankton community in the Kiel Canal was dominated by *Eurytemora affinis*, *Acartia tonsa* and cirriped nauplii (Tab. 1). *Eurytemora affinis* was the numerically dominant species among the copepods found in the Kiel Canal (Fig. 2, Tab. 1). On average *Eurytemora* made up 79 % of the total copepod abundance across all years investigated. Total copepod abundance (all species named in Tab. 1, including *Eurytemora*) and *Eurytemora* abundance alone correlated significantly throughout the investigated seasons (analyzed time frame and sampling frequency same as for herring larvae growth analyses; $y = 0.9568x - 2859.9$, $R^2 = 0.97$, $p < 0.001$, $N = 43$).

Tab. 1: Seasonal mean zooplankton abundance (N m^{-3}), consisting of calanoid copepods and cirriped nauplii, in the Kiel Canal in the years 2007 to 2012. The time frame used for calculation corresponds to larval growth data available. Numbers after ± indicate standard deviations.

	Eurytemora affinis	***Acartia tonsa***	***Pseudocalanus elongatus***	***Temora longicornis***	***Centropages hamatus***	**Cirriped nauplii**
2007	8,144 ± 10,314	1,287 ± 2,655	167 ± 130	32 ± 47	48 ± 85	3,127 ± 3,773
2008	466 ± 454	535 ± 712	58 ± 35	5 ± 7	8 ± 7	4,046 ± 5,620
2009	27,618 ± 26,485	5,398 ± 5,551	3,605 ± 5,617	0 ± 0	0 ± 0	9,650 ± 12,403
2010	11,644 ± 10,445	2,378 ± 1,029	22 ± 36	5 ± 16	0 ± 0	3,663 ± 6,520
2011	44,445 ± 49,774	3,004 ± 2,329	563 ± 777	0 ± 0	0 ± 0	9,843 ± 9,373
2012	17,823 ± 19,147	458 ± 500	226 ± 308	5 ± 16	0 ± 0	2,248 ± 3,042

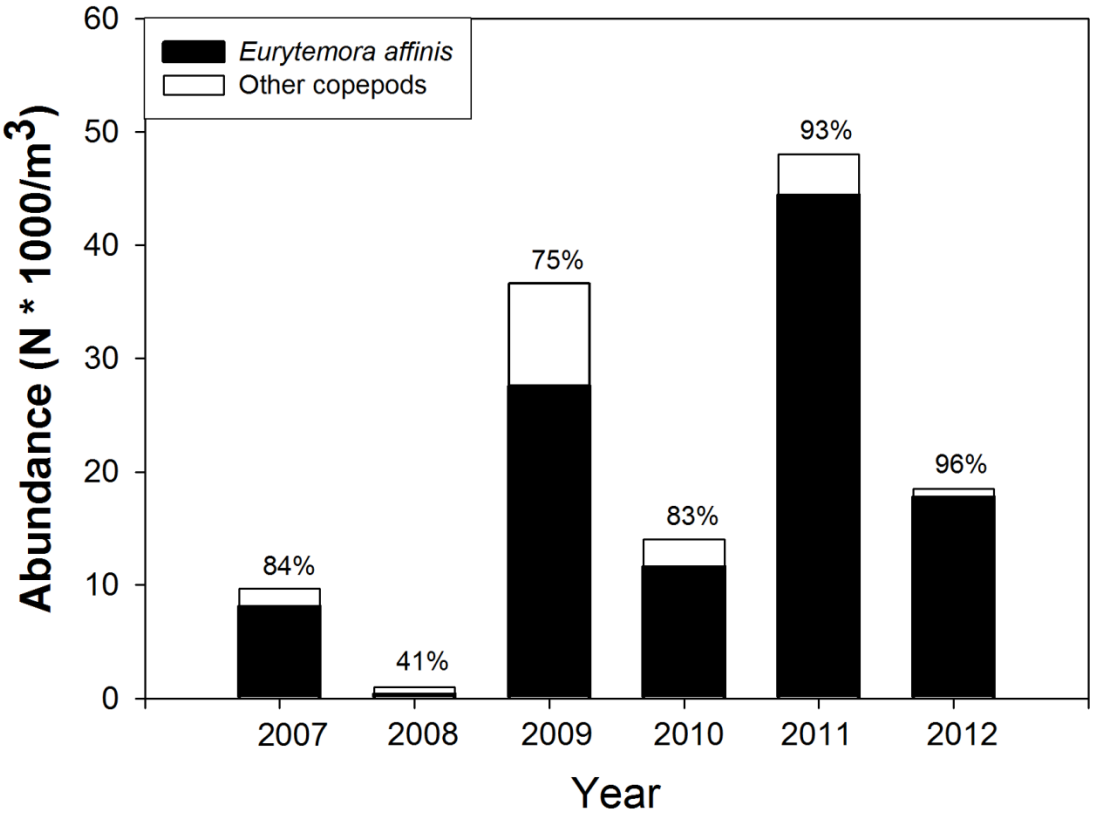

Fig. 2: Inter-annual variability of the copepod abundance in the Kiel Canal and the contribution of *Eurytemora affinis*. *Other copepods* comprise calanoid copepods of the genera *Acartia*, *Pseudocalanus*, *Temora* and *Centropages*. Seasonal means are displayed.

Results from regression analyses were significant between larval herring seasonal mean G_i and seasonal mean *Eurytemora* abundance (R^2 = 0.9, $p < 0.05$, N = 5) and insignificant for the other taxa ($p > 0.05$; *Acartia tonsa* R^2 = 0.34, *Pseudocalanus elongatus* R^2 = 0.71, *Temora longicornis* R^2 = 0.39, *Centropages hamatus* R^2 = 0.38, cirriped nauplii R^2 = 0.56). Therefore, further analyses of prey effects on larval growth rates concentrated on *Eurytemora* (all stages from CI to CVI).

Generally, significantly positive relationships between larval instantaneous growth rates and *Eurytemora* abundance were observed (Fig. 3 a-c). Individual larval growth rates showed a high variability at given *Eurytemora* abundances (Fig. 3 a, $p < 0.01$, N = 1177), mirrored in a low explained variability of 19 %. Explained variability of larval growth rates increased to 30 % using weekly means of larval growth rates (Fig. 3 b, $p < 0.01$, N = 43), while 90 % of larval growth variability was explained using seasonal means (Fig. 3 c, $p < 0.05$, N = 5).

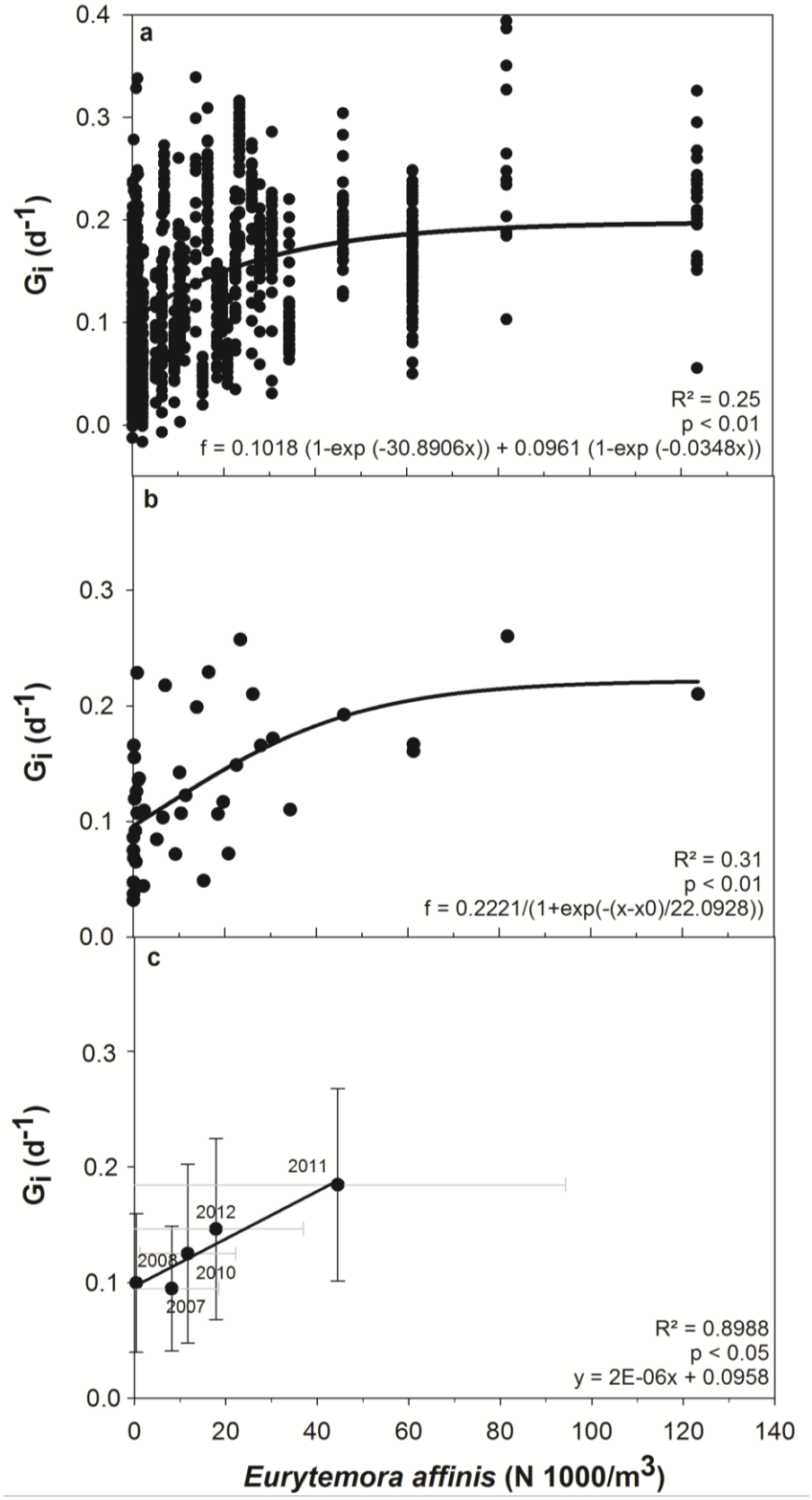

Fig. 3: Relationships between seasonal mean larval herring growth rates (including large and small larvae) versus the dominant copepod species in the Kiel Canal, *Eurytemora affinis*. **a)** Individual larval instantaneous growth rates (G_i (d^{-1}); N = 1177)) versus the abundance of *Eurytemora affinis*; data from the years 2007 to 2012. **b)** Weekly mean larval growth rates vs. the *Eurytemora* abundance observed at the given sampling date (N = 44); data from the years 2007 to 2012. **c)** Seasonal mean larval growth data versus seasonal mean *Eurytemora* abundance (N = 5). Error bars indicate standard deviations. Here, the season 2009 is not included, as this season was not fully covered by the sampling.

Over the years, a recurring seasonal pattern was observed in the Kiel Canal: during the early season, *Eurytemora* abundances increased or were on a high level and larval growth rates increased simultaneously (Fig. 4). Larvae larger than 14 mm grew significantly faster than smaller larvae in 2/3 of the cases (Mann-Whitney-U-test, $p < 0.05$, $N = 13$), but in 1/3 of the cases ($N = 6$) growth rates of small and large larvae were not significantly different (Fig. 4). 83.3 % of the insignificant cases ($N = 5$) were observed at food limited situations. Temporal trends did not differ between large and small larvae.

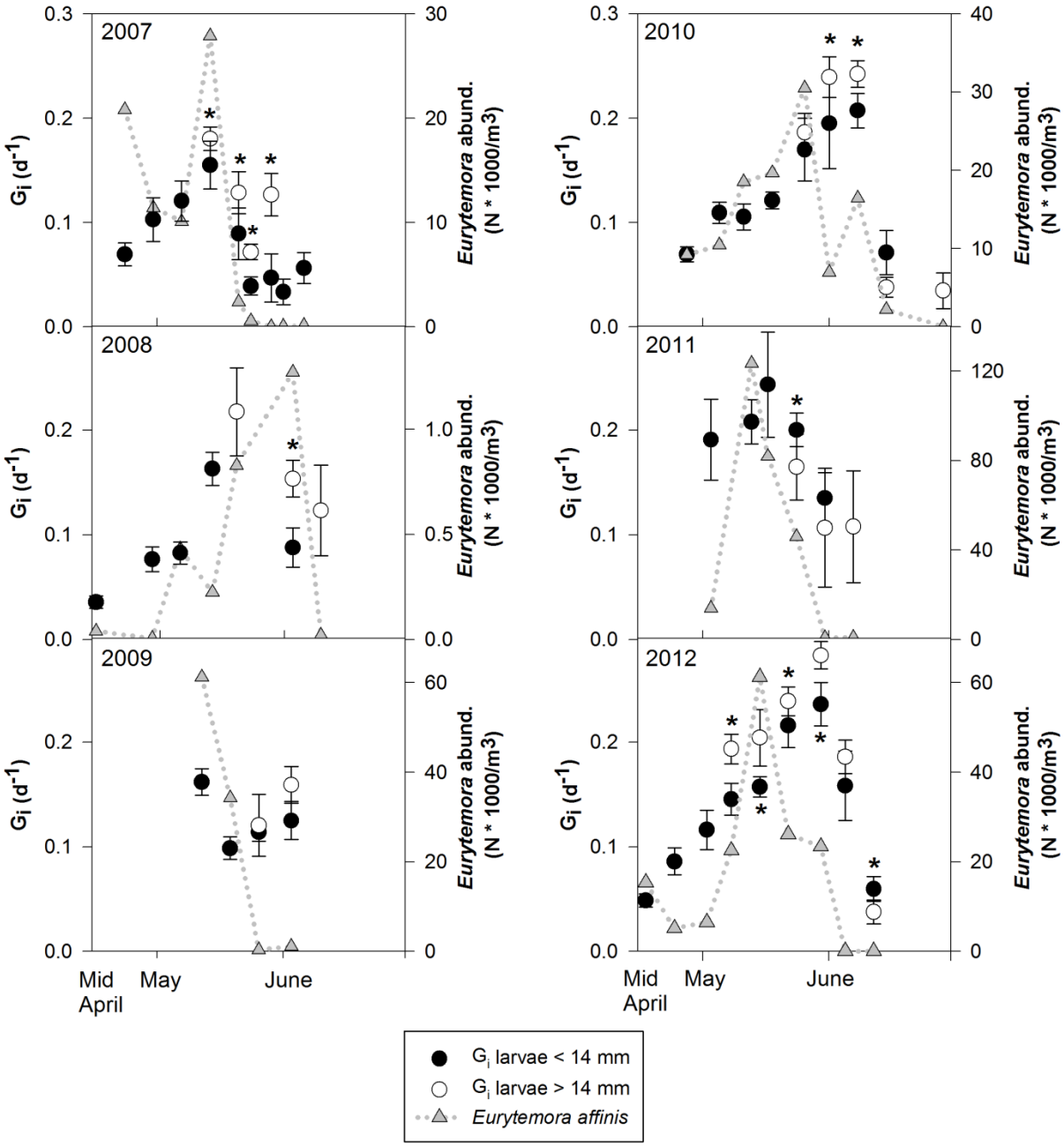

Fig. 4: Seasonal course of larval growth rates and prey availability from 2007 to 2012. Growth rates of herring larvae smaller and larger than 14 mm were differentiated. Circles indicate the median of larval growth rates, error bars show 95% confidence intervals. Stars denote significant differences of growth rates of small and large larvae. Note the different scales of *Eurytemora* abundances.

In the late season at temperatures above 15°C *Eurytemora* abundances decreased and remained at very low levels (Fig. 5), and larval growth rates decreased significantly (ANOVA, $p < 0.05$). Up to 15°C larval growth rates in the Kiel Canal were similar to the mean larval growth rates observed in the Greifswalder Bodden in the seasons 2010 to 2012 (Fig. 6). Thereafter, the upper 95 % confidence interval of the Kiel Canal-larval growth data was constantly below the lower 95 % confidence interval of the Greifswalder Bodden-larval growth data.

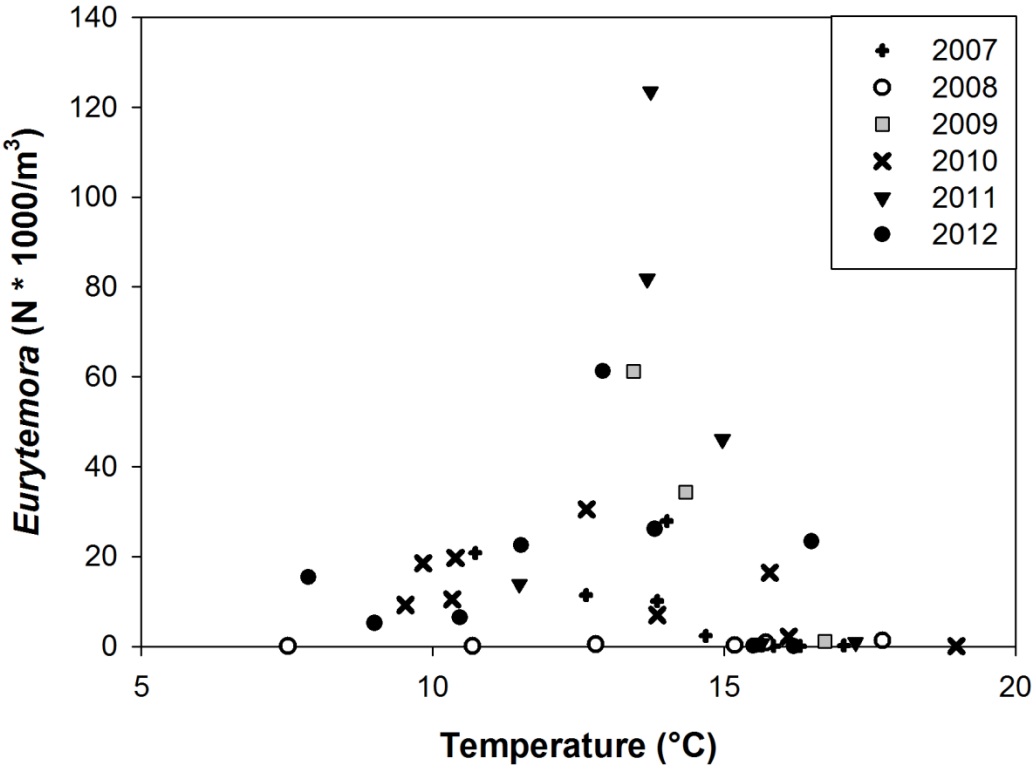

Fig. 5: Relationship between *Eurytemora* abundance and temperature (N = 43). Different symbols indicate different years.

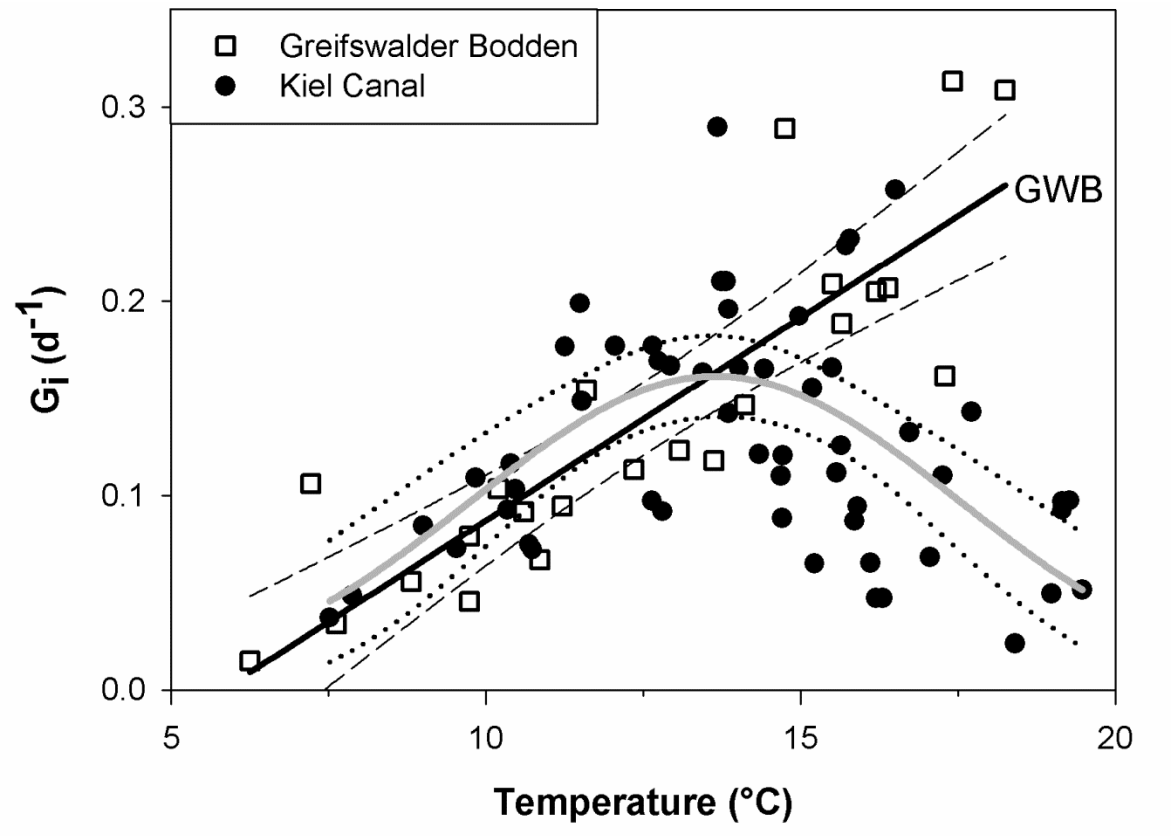

Fig. 6: Definition of food-limitation in the present study. Instantaneous larval growth rates (G_i) vs. temperature in the Kiel Canal. Means of sampling dates are displayed (N = 43). The grey trend line allocates to Kiel Canal data and dotted lines indicate 95 % interval. The black regression line allocates to Greifswalder Bodden larval growth data in the seasons 2010 to 2012 (N = 23), the dashed lines, are indicating 95 % confidence intervals (CI). Larval growth rates were defined to be food-limited, when the upper 95 % CI from Kiel Canal was constantly below the lower 95% confidence interval of larval growth rates from the Greifswalder Bodden, as their growth performance was lower than possible.

82 % of larval G_i variability (all size groups) was explained by the standardized RNA:DNA ratio (sRD), indicating major effects of larval nutritional condition on larval G_i (Fig. 7, $p < 0.001$, N = 1177). Further statistical analyses on the effects of prey availability and temperature on sRD variability could only be performed on larvae <14mm due to an autocorrelation between prey and temperature for the period when large larvae occurred in the Kiel Canal. Results from generalized additive model (GAM) analyses showed that sRD for larvae <14 mm was explained to 44.4% by *Eurytemora* abundance and temperature. However, using exclusively *Eurytemora* as independent variable, explained variability of sRD was 40.9% (Fig. 8a), while temperature alone explained 24.1% of sRD variability (Fig. 8b).

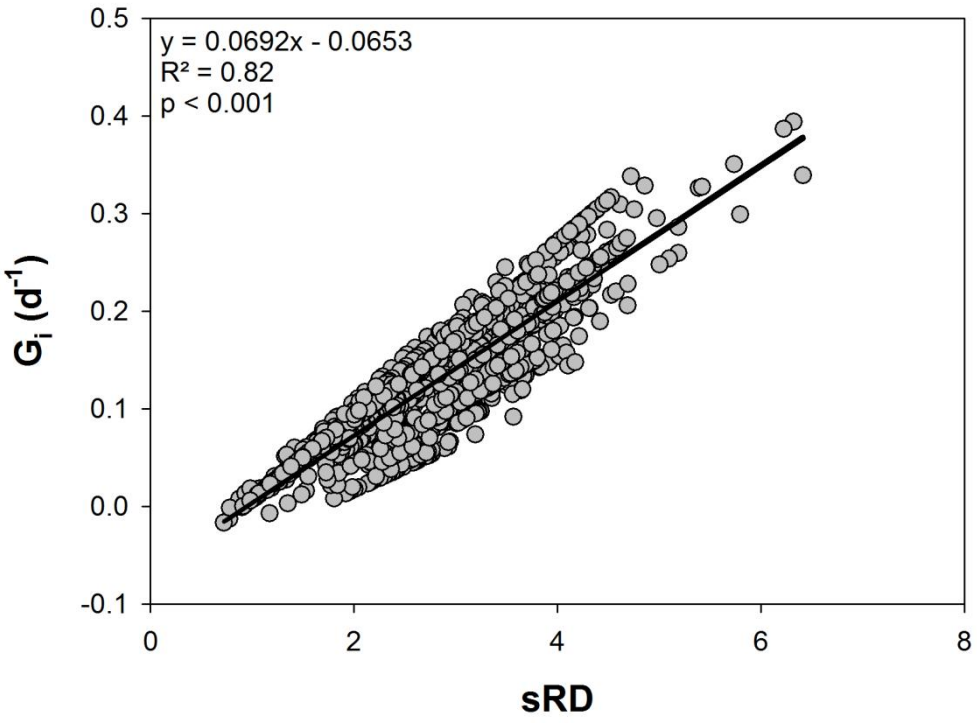

Fig. 7: Regression analysis of larval instantaneous growth rate G_i (d^{-1}) versus standardized RNA:DNA ratio (sRD; N = 1177).

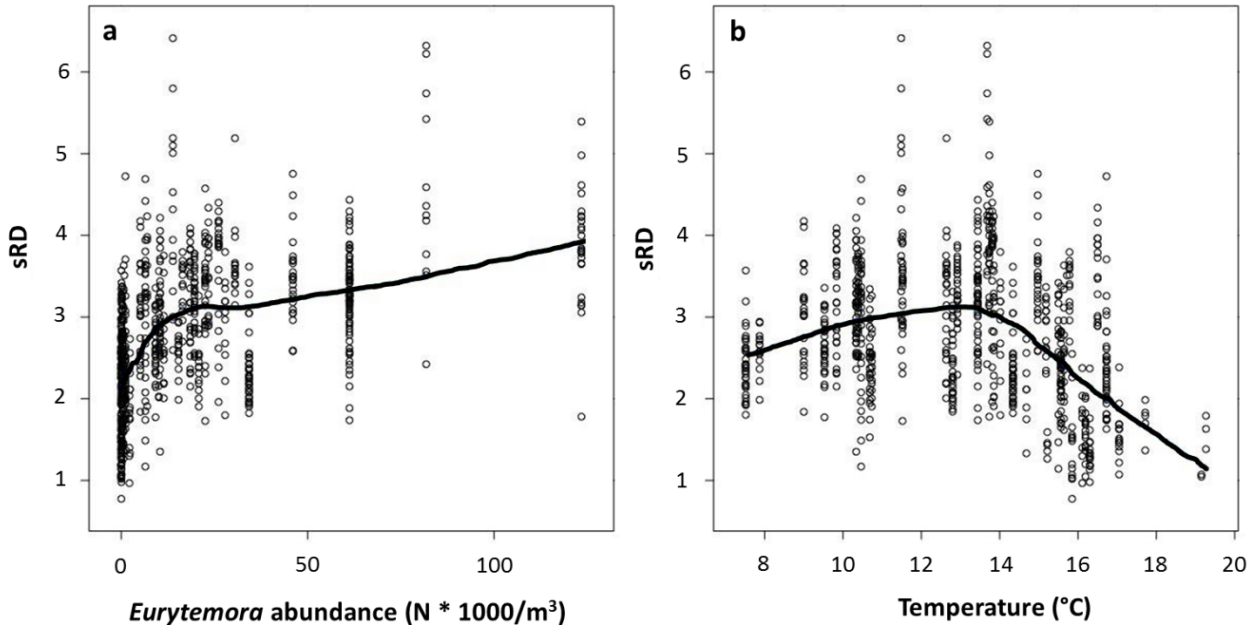

Fig. 8: General additive model (GAM) analyses of larvae <14 mm (N = 820) of **a**) standardized RNA:DNA ratio (sRD) versus *Eurytemora* abundance (explained variability: 40.9 %) and b) sRD versus temperature (explained variability: 24.1 %). GAMs could only be performed for larvae <14 mm, as an autocorrelation between prey and temperature existed for the period when large larvae occurred in the Kiel Canal.

Abiotic conditions (salinity and temperature) showed considerable differences between the years, especially in terms of salinity, which ranged between 5.5 and 11.5 (Fig. 9a). While salinity was extraordinary high in 2011, it was lowest in 2007. In contrast to the relatively high salinity variability, temperature development was linearly throughout the years, ranging from to 7.5°C to 19.4°C (Fig. 9b). In 2010, temperatures were lower on average compared to the other seasons. Neither *Eurytemora* abundance, nor larval G_i correlated significantly with temperature or salinity.

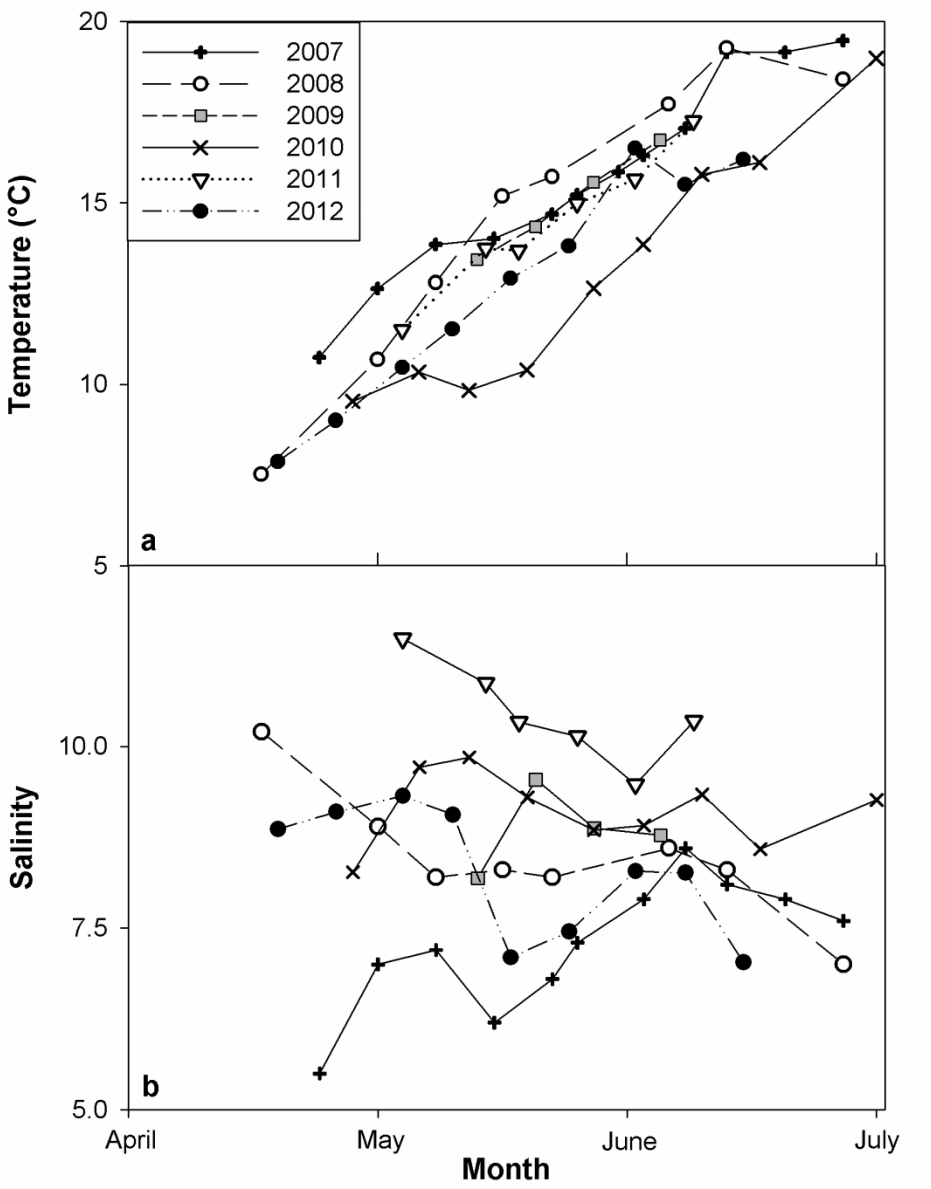

Fig. 9: Seasonal course of **a**) temperature, and **b**) salinity during the years of investigation in the Kiel Canal. Different symbols indicate different years.

Discussion

Based on contradicting results of studies dealing with prey availability and larval fish growth, it was recently hypothesized that fluctuating prey availability affects survival rates not primarily via larval growth rates, but rather indirectly by changes in larval behavior and therefore increasing predation rates (Jørgensen et al. 2014). Nevertheless, evidence shows that stage duration and therefore growth rates are of importance for recruitment (Houde 2008, and references therein). We demonstrate that larval growth rates can be regularly food-limited in coastal habitats, which is, to the best of our knowledge, reported for the first time. Variability of mean larval herring growth rates was best explained when the analysis was restricted to the dominant copepod, *Eurytemora affinis*.

In the present study, food-limited growth was defined as lower growth compared to growth rates of herring larvae observed in a different habitat (Greifswalder Bodden) at the same temperature, which indicates a suboptimal growth performance. Similar approaches have been used by other authors before. For example, growth rates of central Baltic Sea cod larvae were correlated with temperature and compared to growth rates from Georges Bank and mesocosm experiments (Huwer et al. 2011). Similarly, body weight of field-caught radiated shannies *Ulvaria subbifurcata* were correlated with prey biomass and compared to larval weight from experimental work (Bochdansky et al. 2008). Additionally to the comparison of larval growth data from the Kiel Canal with those from the Greifswalder Bodden, the asymptotic relationship between larval growth and *Eurytemora* abundance serves as an indicator for food-limitation in itself, as larvae grew less at low prey abundances. Indirect temperature effects (increasing copepod abundance with increasing temperature) can be excluded, as *Eurytemora* abundance and temperature were not correlated.

Food-limited growth has rarely been observed in off-shore regions (Grønkjær et al. 1997; Buckley et al. 2004; Voss et al. 2006; Huwer et al. 2011). As regularly occurring food-limitation was observed in a coastal habitat in this study, the question arises, which basic differences between coastal and off-shore habitats do exist that potentially drive food-limitation? As food-limitation was observed each year, a regularly occurring mechanism limiting copepod abundance is the most likely explanation. As most coastal habitats such as lagoons, estuaries and canals are characterized by a relatively small water body, they sensitively react to climate and weather events such as rainy seasons and

strong short-term temperature changes which typically occur in boreal regions during spring time. In the study at hand, temperatures above 15°C negatively affected copepod abundance. Even though salinity might not be drastically changed by rain or river-run off, relatively small changes could produce effects on prey availability. For example, small long term-changes in salinity correlated with decreasing copepod biomass caused by an altered species composition (decreasing abundance of large species) in the northern Baltic Sea (Vuorinen et al. 1998), leading to food-limitation for adult herring (Flinkman et al. 1998). However, *Eurytemora* abundance was not negatively but rather positively affected by this development (Vuorinen et al. 1998). In the Kiel Canal, no significant correlation between salinity and the abundance of the brackish water copepod *Eurytemora* was observed at salinities between 5.5 and 11.5. Nevertheless, highest *Eurytemora* abundances were observed when salinity was highest in 2011. Experimental work showed that egg production does not significantly differ at salinities between 5 and 15 (Devreker et al. 2009).

Though larval herring appear to be selective feeders, they seem to be rather plastic concerning the choice of their preferred prey item, with a preference for the dominant species (for references, see below). This might explain the significant correlation between larval growth rates and *Eurytemora*, but not other copepod species in the present study. It was previously shown that larval herring feed selectively on *Pseudocalanus* and *Oithona* (Checkley 1982), while *Acartia* was negatively selected. Contrary, Busch (1993) and Hesse (2010) found herring larvae selectively feeding on *Acartia* spp.. Results by Buckley and Durbin (2006) showed highest correlation coefficients between prey abundance and growth rates of larval cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*), when analyses were restricted to their preferred prey, *Pseudocalanus* spp.. Similarly, abundances of the copepod *Sinocalanus sinensis* were observed to significantly affect growth rates of larval Japanese temperate bass (Lateolabrax japonicus; Islam et al. 2006), while this was not observed for other co-occurring copepod species. Peterson & Ausubel (1984) found larval Atlantic mackerel (*Scomber scombrus*) selectively feeding on certain copepod species as well.

As abundances of adult herring remained quite constant in the northern Baltic Sea after changing copepod species composition caused by changing salinities (Flinkman et al. 1998), the early life stages of herring were apparently not strongly affected by these changing conditions. However, the situation might be different for other fish species or in different habitats. It was shown that the North Atlantic oscillation has the potential to

cause regime shifts, including changing dominance of copepod species and leading to declining or low level cod (*Gadus morhua*) stocks (Beaugrand et al. 2003; Alheit et al. 2005) in the North and Baltic Seas. Further, droughts were observed to cause hypersalinity in estuaries in the tropics and subtropics with severe ecological consequences (Savenije & Pagès 1992), or increasing salinities were observed in coastal lakes caused by sea-level rise (Schallenberg et al. 2003). Effects of changing abiotic conditions on prey composition and therefore larval fish nutrition and survival and ultimately recruitment are expected to be case-dependent. Here, particularly the suitability of new dominant copepod species as prey organisms is important. Factors determining this suitability are primarily temporal occurrence and quality aspects (e.g., size, energy content and biochemical composition (especially essential fatty acid concentrations, but also C:N)).

Using a theoretical framework with larval fish behavior included (Jørgensen et al. 2014), it was shown that low prey abundances lead to a more active larval behavior, and therefore better detection by predators and consequently increased predation rates. In that model, behavioral adjustment concerning food availability was included by vertical migration (Fiksen & Jørgensen 2011). Due to the shallowness of the canal, the possibility for vertical migration is restricted. Consequently, the opportunity of behavioral adjustment is reduced for fish larvae in shallow nursery areas such as the Kiel Canal, but also lagoons or estuaries serving as nurseries (e.g., Fey 2001; Barletta-Bergan et al. 2002; Franco et al. 2006; Polte et al. 2013), and such areas might be exceptions of the validity of the model.

Generally, larval growth is used as an indirect indicator for mortality rates (Houde 2008) assuming that a better nutritional situation for the larvae is reflected in better larval growth and lower larval mortality rates, and vice versa (Houde 1987; Anderson 1988). Indeed, results of otolith analyses of the survivors in the Kiel Canal showed that the vast majority of the survivors hatched early in the season 2011, when growth conditions were good (Hesse 2012). This supports the findings of the present study, were non-food-limited situations in the beginning of the season were observed, defined by increasing larval growth rates. Only very few survivors originated from the mid-season or shortly before food-limitation occurred. Overall, the survivors were larger during the season compared to the mean size of the larvae (Hesse 2012), supporting the Stage-Duration hypothesis and "bigger is better" (Houde 1987; Anderson 1988).

Potentially, food-limitation can have strong negative effects on recruitment (e.g., Houde 2008; Jørgensen et al. 2014). Consequently, the question arises in how far recruitment might be affected by the food-limitation observed each season in the present study? In the Kiel Canal, food-limitation occurred only temporally during the season, and not consistently throughout the whole season. During the non-food limited period, which includes the particularly sensitive phase of first feeding of the peak-hatch cohort, copepod availability was high. Therefore, food-limitation most likely primarily affects survival of late hatched larvae. This means, that the time point of food limitation is of crucial importance for larval survival.

Though significant differences in growth rates between larvae smaller and larger than 14 mm existed in the majority of the cases tested, observed trends did not differ between size classes. Results from experimental work indicated that large herring larvae grow faster than small ones (positive linear relationship; Clemmesen 1994). In our study, in one third of the cases no significant differences between small and larger larvae were found, mostly during the food-limited periods. A possible reason might be that larger larvae have a higher energy demand than smaller ones, which is difficult to meet during food-limitation. This is especially true for the late season, where temperatures were relatively high. Instantaneous larval growth was found to increase approximately 0.01 per °C increase in temperature (Houde 1989), and increasing temperatures enhance larval metabolism and therefore energy demand (Houde 1989), which is critical under food limitation. Hence, it is assumed that increasing temperatures affected larval growth negatively in the late season, i.e. under food limitation.

Eurytemora affinis was the numerically dominant copepod species found in Kiel Canal, contributing 79 % to total copepod abundance across all years investigated. *Eurytemora* is known to be dominant in brackish waters (Schnack 1972; Hirche 1992 and references therein). At a temperature of about 15°C, the abundance of *E. affinis* decreased rapidly to negligible values throughout the years. This is consistent with findings by Hirche (1992), who reported that the productivity of *E. affinis* females is directly related to their size, and the size decreases remarkably at temperatures ~15°C. *Eurytemora* is reported being outcompeted by *A. tonsa* at temperatures above 15°C (Hirche 1992, and references therein); the latter species needs relatively high temperatures for the eggs to hatch (≥13°C; Holste & Peck 2005).

High variability of individual larval growth rates was observed at given *Eurytemora* abundances. Factors causing this variability are especially temperature

(increasing metabolism with increasing temperature, see above) and size-dependent growth. The latter phenomenon was shown being dependent on prey concentrations (Paulsen et al., accepted): the higher the prey concentrations, the smaller the differences between growth rates of small and large larvae were in the Kiel Canal during the years of investigation. Additionally, genetic and behavioral components (individual growth capacity and hunting capabilities) might play a role (as seen in experiments, when single larval herring specimens grow at rates far above average compared to same-age conspecifics).

Larval G_i variability was largely explained by standardized RNA:DNA ratios (sRD; Caldarone et al. 2006). This indicates that larval growth rates were predominantly determined by the nutritional condition of the larvae rather than temperature. This was also supported by results of GAM analyses, which showed major effects of *Eurytemora* abundance on sRD of larvae < 14 mm. Between sRD and *Eurytemora* abundance an asymptotic relationship was observed. This is in line with findings from other studies, where non-linear to asymptotic relationships between larval growth rates and prey concentrations or prey ingestion rates were observed (Peck et al. 2013, and references therein). This means that especially at the lower end increasing prey abundances are strongly beneficial for larval nutrition. The relationship between sRD and temperature showed a decline at ~15°C, the temperature where *Eurytemora* abundances strongly decreased throughout the seasons investigated.

A WP2-net with 200 µm mesh-size was used to sample the prey field. Though the smallest copepodite stages might be sampled only semi-quantitatively and nauplii are missed for most parts by using 200 µm mesh size, we are confident to have sampled a representative prey field as copepodite abundance follow nauplii abundances trends (Postel et al. 1989), and a significant correlation between copepod and nauplii abundance using 100 µm mesh size was observed in the Kiel Canal in 2014 (Beckmann 2015).

Why were the slow growing larvae not removed by predators in the Kiel Canal, as observed in Japanese anchovies (Takasuka et al. 2003)? Unfortunately, information on predators in the habitat investigated is scarce. Potential predators known to occur in the Kiel Canal are pikeperch (*Stizostedion lucioperca* L.; Hansson et al. 1997), river perch (*Perca fluviatilis* L.; Lappalainen et al. 2001) and moon jellyfish (*Aurelia aurita* L.; Möller 1984). While three-spined sticklebacks (*Gasterosterus aculeatus* L.) heavily prey upon herring eggs (Kotterba et al. 2014), they only rarely feed on larval herring (Kotterba 2015). Pikeperch and river perch are occasionally caught by local anglers close to the

sampling site. However, the angler´s catches start relatively late in the year during summer, which might hint on a temporal mismatch. Similarly, moon jellyfish were observed on the sampling site, but not before mid-June to July, also leading to a temporal mismatch situation between larval herring and predators in Kiel Canal.

We conclude that our first hypothesis proposing that in coastal habitats food-limited growth of larval fish can occur regularly was supported. Larval growth observed in Kiel Fjord was substantially lower compared to the Greifswalder Bodden at temperatures ≥15°C. Furthermore, growth rates decreased simultaneously with *Eurytemora* abundance in the late season, and asymptotic relationships between *Eurytemora* and larval growth were observed. Larval growth was best explained by the abundance of the numerically dominant prey item *Eurytemora affinis*, highlighting the crucial role of this copepod concerning larval herring nutrition in the Kiel Canal. As food limitation occurs regularly at the end of the season in Kiel Canal, the optimal time point for larvae to hatch is early in the season so that larvae can take full advantage of the high copepod production in this period.

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Chapter 3

Preliminary insights into effects of protozooplankton on growth of spring-hatched Atlantic herring (*Clupea harengus* L.) larvae

To be submitted

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Abstract

Protozooplankton availability is considered to be important for growth and survival of small and first feeding larval fish. However, field studies on the effects of protozooplankton availability on larval fish vital rates are missing. We investigated ciliate biomass, the dominant protozooplankter in the system investigated, along with chlorophyll *a* concentrations, copepod abundance and female adult *Eurytemora affinis* as well as larval herring nutritional condition in Kiel Canal, western Baltic Sea. We found significant effects of ciliate biomass on female adult *Eurytemora* nutritional condition, but not on larval fish condition. We conclude that protozooplankton biomass can directly affect copepod nutritional condition in the field, and potentially indirectly larval fish growth conditions via increased copepod productivity.

Key words**:** RNA:DNA, Kiel Canal, Western Baltic Sea, *Clupea harengus*, planktonic food web

Introduction

Many studies on the planktonic food web focused on the "classic" food chain (Cushing 1975), which considers trophic interactions between phytoplankton, copepods and fish larvae (Munk et al. 2003). Ever since the 1980´s, when Azam et al. (1983) defined the 'microbial loop', the classical concept was modified now stressing the relevance of protozooplankton (PZP) as a trophic link between microbial and classical food webs (Sherr et al. 1986; Gifford 1991; Sherr & Sherr 2002). So far, studies focusing on PZP in marine food webs have primarily considered two- or three-way-interactions within the plankton (Lessard & Murrell 1998; Calbet & Saiz 2005; Irigoien et al. 2005; Calbet 2008), excluding larval fish.

Though PZP is considered being an important prey item for larval fish (Montagnes et al. 2010; Mitra et al. 2014 and references therein), studies on the effects of PZP on larval fish growth and on copepod nutritional condition in the field are still missing.

The RNA:DNA ratio is suitable as a condition index as DNA concentrations are relatively constant within the organism´s cells, and RNA concentrations depend on the nutritional status of the organism; RNA:DNA is widely used to assess the nutritional status of both larval fish and copepods (e.g. Wagner et al. 1998; Höök et al. 2008; Malzahn & Boersma 2009; Paulsen et al. 2014).

In light of the lacking knowledge of effects of PZP on copepod and larval fish nutritional condition in the field, the present study focused on the following hypotheses: (1) PZP positively affects copepod condition in the field, and (2) PZP affects the growth of larval herring in the field. In order to test these hypotheses and to obtain a comprehensive overview of the planktonic food web, chlorophyll *a* concentrations, PZP biomass, copepod abundance as well as female adult copepod and larval fish condition based on RNA:DNA analysis were investigated in Kiel Canal, western Baltic Sea, in the spring season 2014.

Material and Methods

Sampling

Samples were taken weekly at a study site in Kiel Canal ~13 km west of the Baltic Sea (54°20'45 N, 9°57'02 E). Sampling took place during the spring season from April 15 to June 17 2014. The Kiel Canal is known as an important spawning habitat for western Baltic spring spawning herring in Germany (Brandhorst 1955; Weber 1971). Main characteristics of the Kiel Canal are a low salinity (6-10) and a constantly well-mixed water column due to the heavy shipping traffic (the most frequent traffic worldwide). Vertical profiles of salinity and temperature were taken. Mesozooplankton was sampled from bottom to surface in a vertical haul with a WP-2 net of 100 µm mesh size and preserved with 4% borax buffered formalin solution. Protozooplankton (PZP) was sampled using a Niskin water sampler and preserved with acid Lugol's iodine. Additionally, water samples were taken to measure chlorophyll *a* concentrations. Larval herring were sampled using a bongo net which was retrieved in an oblique haul with a speed of 2.5 knots. Larval fish samples were taken from the 500 µm mesh-size net and cooled with crushed ice immediately. The larvae were sorted out within 30 minutes after the haul, frozen on board at -20°C until the return to the institute (about 2 to 3 hours after the haul), and were then stored at -80°C until further analysis. Adult copepod samples were obtained from the 330 µm mesh-size bongo net. The sampling material was fixed in RNAlater.

Sample analyses

Chlorophyll *a* concentrations were analysed following Jeffrey and Humphrey (1975).

PZP cell counts and identification were performed using the Utermöhl inverted microscope technique (Utermöhl 1958) and a sedimentation period of 24 hours. Depending on cell density, either a half or an entire chamber was screened for PZP. Due to neglectable numbers of heterotrophic dinoflagellates (7% over the entire investigation period), only ciliates were considered in this study. Length and diameter of maximal 30 individuals per genera (primarily Oligotrichea (70.8%) and Litostomatea (16.6%)) were measured for subsequent calculation of biovolume based on shape (HELCOM 2006). The biovolume was needed for the calculation of individual biomass of the heterotrophic ciliates according to Putt and Stoecker (1989). Ciliate biomass was chosen as a parameter as it provides the best estimate on the amount of food (carbon) available.

For mesozooplankton analyses, samples were divided into subsamples (Kott 1953) such that one subsample contained at least 100 individuals of the dominant copepod species. Species identification was performed on genus or species level according to Sars (1895). In this study, only copepodids and copepod nauplii were considered for later analysis. Those are the developmental stages reportedly contributing to the diet of small herring larvae (Schnack 1972; Busch 1993).

A previous study showed that correlations of larval herring growth rates improved when the most abundant calanoid copepod species, *Eurytemora affinis* (hereafter referred to as *Eurytemora*), was used for analyses exclusively, compared to using the sum of all copepod species (Paulsen et al., submitted).

Based on the different temporal development of ciliate biomass, decreasing in the first six weeks of sampling (April 15 to May 20) and fluctuating from May 27 to June 17, the data set was divided into two respective time frames (time window 1 and 2) for analysis.

The cellular RNA content is known to indicate the rate of active protein biosynthesis and hence the condition of an organism, e.g. copepods (Wagner et al. 2001). RNA:DNA analysis of female adult *Eurytemora* was conducted to relate PZP biomass to the condition of the copepods. Female individuals of *Eurytemora* were sorted from the samples prior to analysis. Depending on individual size and occurrence, the organisms were pooled in triplicates of appropriate size (range 8 to 15 individuals per subsample). The fluorimetric determination of the RNA:DNA ratio in adult female *Eurytemora*

followed a modified method by Clemmesen (1993) and Belchier (2004), based on the measurement of nucleic acids using ethidiumbromide. First, total nucleid acids were measured. After the usage of RNase to completely digest RNA in the sample, the remaining DNA was measured. The difference between total nucleic acids before and after RNase usage was defined as RNA. For the complete protocol, see Malzahn et al. (2003). For data analysis, the unit µg RNA copepod^{-1} was used.

Only herring larvae in the size range of 8 – 12 mm where included in the analyses because this size class was shown to feed on protists (Busch 1993). Based on the individual RNA:DNA ratio of the larvae, growth rate was calculated using the best-fit multi-species model (Buckley et al. 2008):

$$G_i = 0.0145 * sRD + 0.0044 * (sRD * T) - 0.078$$

where G_i is the instantaneous growth rate, sRD the standardized RNA:DNA ratio (Caldarone et al. 2006) and T the water temperature on the given sampling date. In order to give larval growth a concrete unit, instantaneous growth rate was converted to specific growth rate (SGR, % day-1; Caldarone et al. 2006):

$$SGR = 100 * (e^{Gi} - 1)$$

reflecting the increase in growth in percent per day.

Statistics

Using the statistic software package R, Pearson's correlation coefficient was applied for larval fish specific growth rate (SGR), ciliate biomass, copepod nauplii or *Eurytemora* copepodid abundance. Effects of two time windows were chosen according to the development of ciliate biomass. To test for significant differences of mean larval growth between sampling dates, a one-way analysis of variance (ANOVA) was performed and a Tukey honestly significant difference (HSD) test was conducted for *post hoc* comparison.

Results

Ciliate biomass was on a high level at the beginning of the investigation period and gradually decreased until May 27. After that, a second peak occurred which ended on June 17 (Fig.1a).

Copepodid/nauplii abundance was initially high (76,000 m^{-3}) and showed an increasing trend until the peak on May 13 (155,000 m^{-3}). Thereafter abundances gradually decreased until the end of the season, were relatively low abundances were observed (6,500 m^{-3} to 15,000 m^{-3}; Fig. 2).

The mean RNA content of female adult *Eurytemora* continuously decreased from April 15 until May 27 (Fig. 1a). Mean RNA content per female *Eurytemora* correlated with ciliate biomass in time window 1 (Fig. 1b, Pearson's correlation coefficient = 0.88, $p < 0.05$). In contrast, chlorophyll *a* did not correlate with copepod condition. Nauplii abundance and female *Eurytemora* condition were not correlated between April 15 and May 27. Contrary, when female *Eurytemora* were abundant (April 29 to May 27, range 1,500 m^{-3} to 6,400 m^{-3}; mean 3,700 m^{-3}), *Eurytemora* female abundance and nauplii abundance were correlated ($y = 13.406x - 15200$; $R^2 = 0.89$; $p < 0.05$).

Larval herring specific growth rate (SGR) increased continuously in time window 1, followed by a steep decline from June 11 onwards (Fig. 2). Neither nauplii nor *Eurytemora* copepodid abundance correlated with larval herring SGR (Tab. 1). The regression between larval herring SGR and ciliate biomass was negative.

Tab. 1: Pearson's correlation coefficient (abbreviated as Pearson´s) for larval fish specific growth rate (SGR) and ciliate biomass, copepod nauplii or *Eurytemora* copepodid abundance. Pearson's correlation coefficient and p–value are given for each correlation. Note that the correlation coefficients only refer to time window 1 (April 15 to May 20).

	Ciliate biomass	Nauplii abundance	*Eurytemora* copepodid abund.
Larval herring SGR	Pearson´s = -0.93 $p < 0.05$	Pearson´s = 0.31 $p > 0.05$	Pearson´s = 0.48 $p > 0.05$

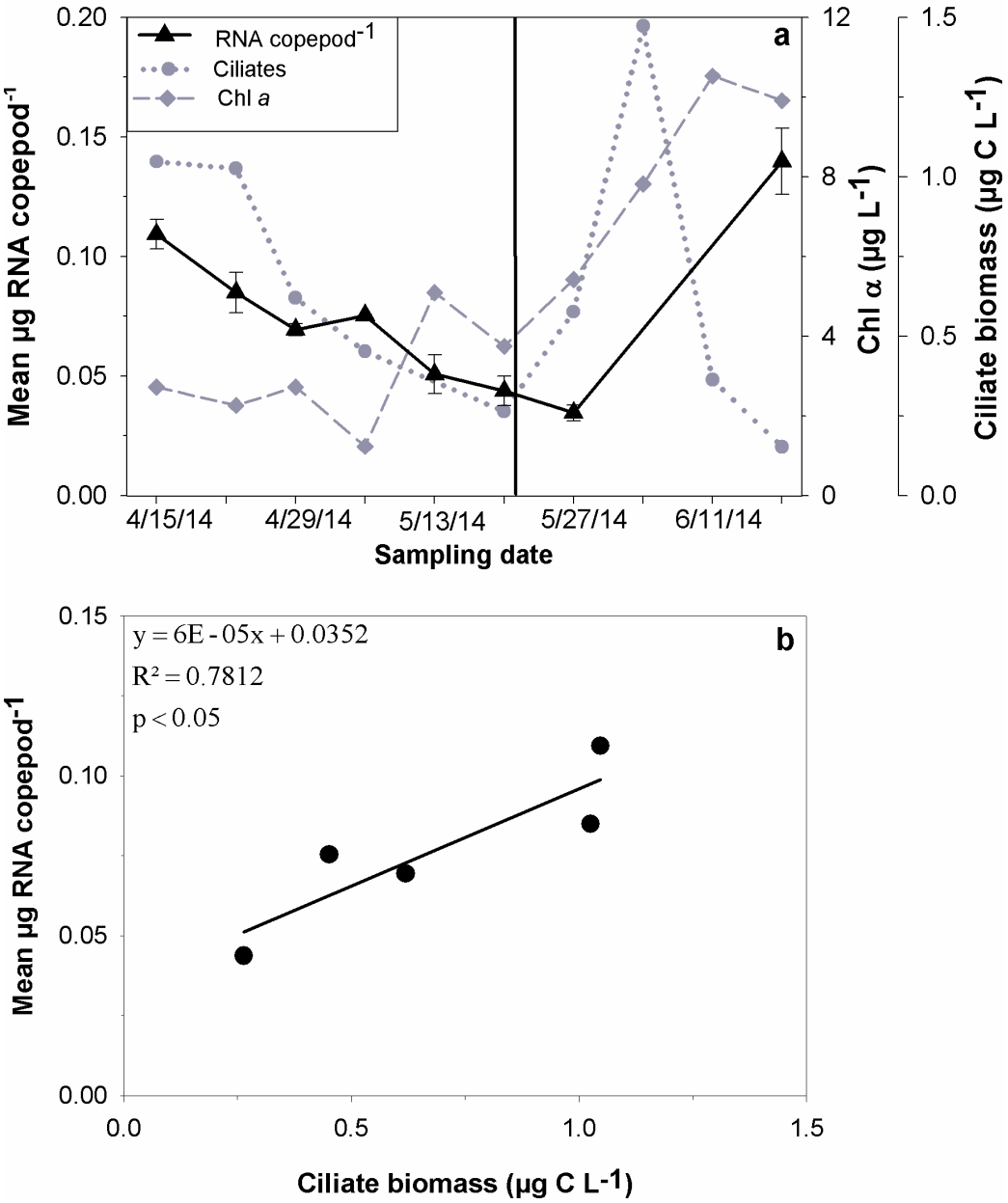

Fig. 1 a RNA/adult female *Eurytemora* (µg), Chl *a* concentration (µg L^{-1}) and ciliate biomass (µg C L^{-1}) over the sampling period. While RNA data display means of triplicates (each consisting of 8-15 *Eurytemora* females), for both chlorophyll concentrations and ciliate biomass single samples were analysed. Error bars indicate standard deviation. Note that no copepod sample material was available on June 3 and 11. No data for ciliate biomass on May 13 were available. The vertical line marks the end of the first time window (window 1: April 15 to May 20) and the start of the second time window (window 2: May 27 to June 17). **b** Regression between µg RNA copepod $^{-1}$ and ciliate biomass for time window 1 from April 15 to May 20 (Pearson's correlation coefficient = 0.88). RNA data are means of triplicates, for ciliate biomass a single sample was analysed.

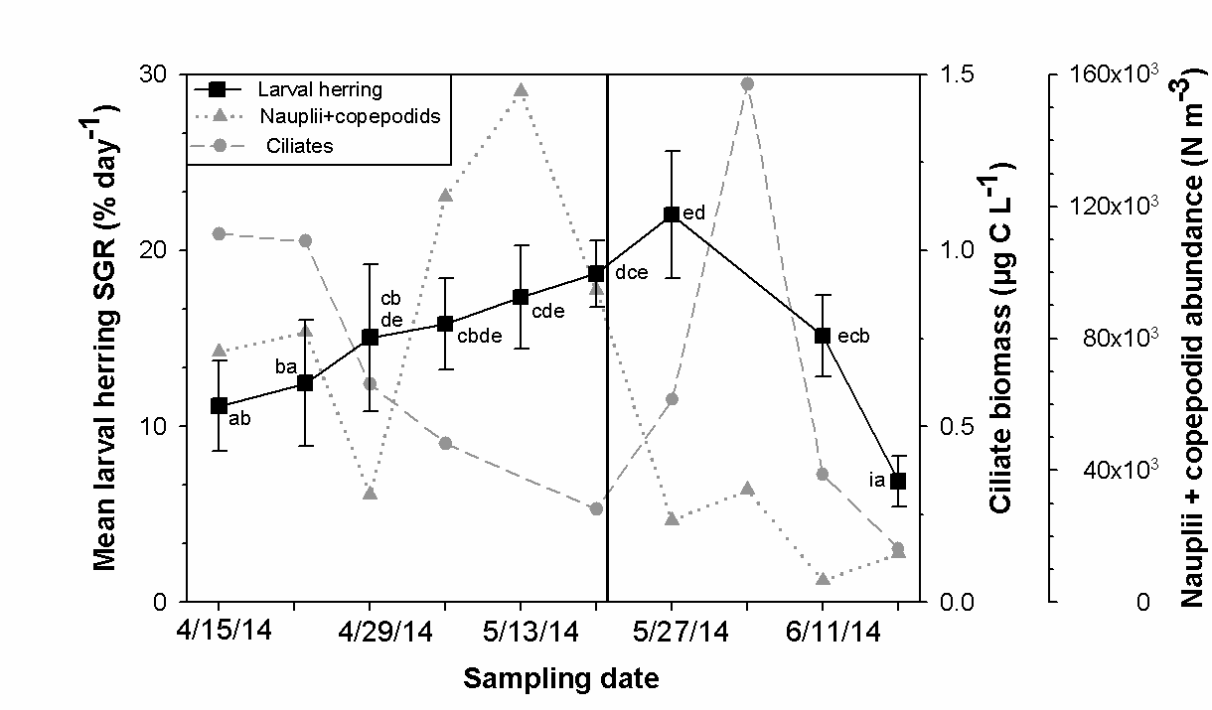

Fig. 2: Temporal development of mean specific growth rate (SGR) of larval herring, ciliate biomass and abundance of copepod nauplii and *Eurytemora* copepodids. Note that no larval herring were analyzed from June 6 due to lack of sampling material. Error bars indicate standard deviation. Different letters besides the data points denote significant differences (one-way ANOVA, $p < 0.05$). Nauplii include all copepod species, while copepodites are exclusively *Eurytemora*. No data for ciliate biomass on May 13 were available. The vertical line marks the end of the first time window (window 1: April 15 to May 20) and the start of the second time window (window 2: May 27 to June 17).

Discussion

This study addresses the effects of protozooplankton (PZP) availability on the condition of copepods and larval fish in the field, a topic which is relevant with regard to *in-situ* condition and feeding ecology of first-feeding fish larvae. We observed a positive effect of ciliates on the condition of female adult *Eurytemora*. In contrast, negative effects of PZP on larval fish growth were observed, which are discussed below.

A significant positive correlation between copepod condition and PZP was observed, but not between copepod condition and chlorophyll *a* concentrations. This stresses the relevance of ciliates as important prey of *Eurytemora* in the Kiel Canal. Traditionally, copepods were considered as the main grazers of phytoplankton, predominantly diatoms. Calbet and Saiz (2005) however, point at the trophic link between

micro- and mesozooplankton and concluded that especially ciliates are amongst the preferred prey items of mesozooplankton. Nevertheless, the availability of PZP as prey for copepods highly depends on phytoplankton standing stocks and is thus directly linked to autotroph production (Aberle et al. 2007; Löder et al. 2011). Experimental work showed that *Eurytemora affinis* is able to significantly decrease ciliate densities (Merrell & Stoecker 1998). Adult calanoid copepods (*Acartia tonsa* and *Temora longicornis*) were shown to preferentially feed on heterotrophic dinoflagellates and ciliates, rather than on diatoms (Kleppel et al. 1991; Löder et al. 2011). In addition, egg production of *A. tonsa* correlated with the occurrence of PZP, while being low on a diatom diet (Kleppel et al. 1991). Reasons given for this selective feeding behavior, meaning the imbalance between a prey's occurrence in a predator's diet relative to the prey's occurrence in the environment (Chesson 1983), are diverse, e.g. the facilitated detection of ciliates due to hydrodynamic currents created by their active movement patterns (Jonsson & Tiselius 1990; Broglio et al. 2001). In addition, the perception of favorable chemical cues e.g. a better nutritional composition of PZP and higher shares of essential components such as amino acids and fatty acids are discussed in the literature (e.g. Stoecker & Capuzzo 1990; Klein Breteler et al. 1999; Malzahn et al. 2010). However, this does not exclude phytoplankton as potential or supplementary prey item in diets of omnivorous copepods, and hence *Eurytemora* in the Kiel Canal.

While the DNA concentration per cell is constant, the amount of RNA needed in order to synthesize a certain amount of protein decreases with increasing temperature (Wagner 2001; Höök et al. 2008). A significantly negative correlation between RNA content of female adult *Eurytemora* and temperature was observed for the first time window (data not shown), supporting temperature dependence. However, the highest RNA concentration was observed at the end of the season at the highest temperature. Therefore, it seems most likely that the observed decrease in RNA in time window 1 is probably related to the decline in ciliate biomass rather than to temperature.

Studies have shown that RNA concentrations in female adult copepods correlate with egg productivity (Saiz et al. 1998; Gorokhova 2003). However, in the present study, no correlation between female *Eurytemora* RNA concentration and nauplii abundance was observed. In contrast, nauplii abundance was correlated with female *Eurytemora* abundance as long as *Eurytemora* was abundant (until May 27). This does not necessarily imply that female copepod condition does not affect productivity, but rather that high

abundances of females potentially outweigh negative effects of low female condition on productivity.

PSP biomass was observed to be relatively low in the present study. A potential reason for this might be high grazing pressure caused by the high copepod abundances observed during the study.

While a positive effect of ciliate biomass on copepod condition was observed, the regression between ciliate biomass and growth rates of herring larvae was negative. In the beginning of the season, ciliate biomass was high and decreased thereafter. This decline is most likely related to a strong top-down control of copepods, as chlorophyll concentrations were constant first and then increased during that period. Temperature was low and increased linearly over the season, while prey availability in terms of copepod nauplii and copepodids was high for larval herring until end of May. As larval fish growth is strongly affected by and increases with temperature (Houde 1989), we rather assume that larval growth increase was temperature driven than directly negative affected by PZP. Our findings are in contrast to a recent study, showing indirect positive effects of PZP prey on first-feeding herring larvae using an experimental set-up (Illing et al. 2015). However, as copepod egg productivity was shown to be correlated with RNA content in female adults (Saiz et al. 1998; Gorokhova 2003), possibly an indirect effect of ciliates on larval herring SGR through copepods as an intermediate trophic step occurred in the present study. Copepods feeding on high quality PZP (Stoecker & Capuzzo 1990; Klein Breteler et al. 1999; Malzahn et al. 2010) likely experienced a better condition thus promoting a higher number of offspring. Such an increase in copepod productivity might have promoted a higher availability of copepod nauplii and copepodids, the preferred prey items of larval herring (e.g. Lebour 1924; Schnack 1972; Busch 1993).

Although potential indirect effects of PZP on the growth of larval fish are speculative, there is a need to consider such effects in detail, especially when multiple trophic levels within a given system are addressed.

In conclusion, the results of this study support our first hypothesis that PZP, and ciliates in particular, affect the condition of copepods. A negative correlation between ciliates and small and/or first-feeding herring larvae was observed, though probably no causal relationship existed. Therefore, hypothesis (2) that PZP significantly affects the growth of larval herring in the field was neglected. The present study indicates that larval herring might have been indirectly affected by ciliate biomass through copepods as intermediate trophic level, which resulted in an increased prey availability for juvenile

stages of copepods and, in turn, higher copepod abundances promoting the growth of larval herring.

Acknowledgements

A warm thank you to the crew of RV Polarfuchs for all their efforts. Meanwhile, the captain Manfred Davids has passed away suddenly – Rest in peace, Manne! We miss your warm and friendly character. The work was partly financed by the Zooplankton Project by the State Agency for Agriculture, Environment and Rural Areas of Schleswig-Holstein given to Catriona Clemmesen.

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Chapter 4

Size dependent growth in larval fish is not an issue in a world of plenty

CIESM conference paper, accepted

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Abstract

Nutritional situation, and consequently larval fish growth rates, are of paramount importance for larval survival rates. On the basis of 6 consecutive spring seasons biochemically derived growth rates of larval herring originated from Kiel Canal (western Baltic Sea) were analyzed to evaluate size dependent growth, a phenomenon which has been observed in several species. For each season the slope of the regression line of G_i versus larval standard length was calculated and compared to seasonal mean prey abundance. We found decreasing size effects on larval growth with increasing prey abundances. We conclude that large larvae are more successful at meeting their food requirements at suboptimal prey abundances compared to small larvae, while no differences between small and large larvae at high prey abundances exist.

***Key Words*:** Growth, Ichthyoplankton, Zooplankton, Baltic Sea, Brackish Water

Introduction

Generally, it is acknowledged that the nutritional conditions fish larvae experience are of crucial importance concerning larval survival rates. Bigger larvae suffer from lower mortality rates as they are further developed and more effective to cope with suboptimal conditions, leading to lower predation rates (Houde, 2008). The larvae´s RNA:DNA ratio is a condition index with short reaction times (Clemmesen, 1994), which ensures comparability between the prey field observed during sampling and the nutritional condition of the larvae analyzed. Size dependent growth has been reported for larvae of several fish species, including Atlantic herring, Argentinean hake, Atlantic anchovy, red drum, and European pilchard (Díaz et al., 2011). In the present study, we tested the relationship between size dependent growth and prey availability for herring larvae from the field.

Material and Methods

Time series analyses of six consecutive spring seasons (2007 to 2012) in Kiel Canal at a station 13 km inland to the open Baltic Sea were conducted (54°20’45 N, 9°57’02 E). RNA and DNA concentrations of whole individual larval herring (*Clupea harengus*) were fluorimetrically measured (Malzahn et al., 2003). Instantaneous growth rates of larval herring were calculated using the best-fit multi species growth model (Buckley et al., 2008) and analyzed in relation to prey abundance (sampled with WP2-net, 200 μm mesh-size), consisting of copepods and cirriped nauplii.

Results and conclusions

Our results show a decreasing trend in size dependency in larval growth rates with increasing prey abundances (Fig. 1) reflected by a decreasing slope (Fig. 2). While at low to intermediate prey abundances a linear positive relationship (Fig. 1 a-d) was observed, at high prey abundances no relationship existed (Fig. 1 e, f). This illustrates that size dependent growth of larval fish does not occur when prey availability is high, indicating that small larvae are able to feed well in such situations, despite worse swimming and hunting capabilities compared to large larvae. Therefore, if size dependency is observed, this might point to suboptimal nutritional situations for larval fish.

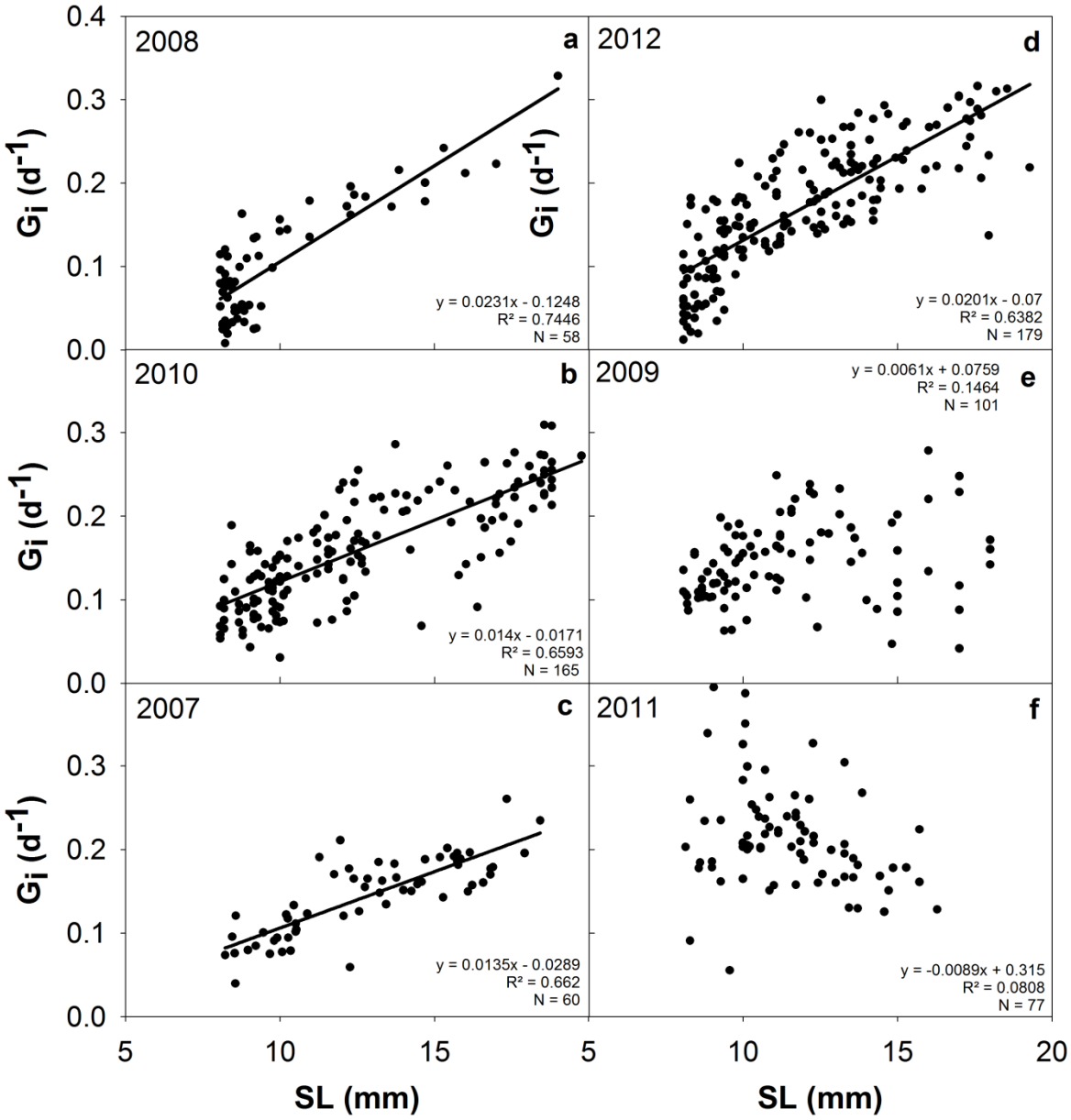

Fig. 1: Instantaneous growth rate (Gi) vs. standard length (SL). The graphs are arranged with respect to mean seasonal prey abundance from (a) lowest to (f) highest prey abundances observed.

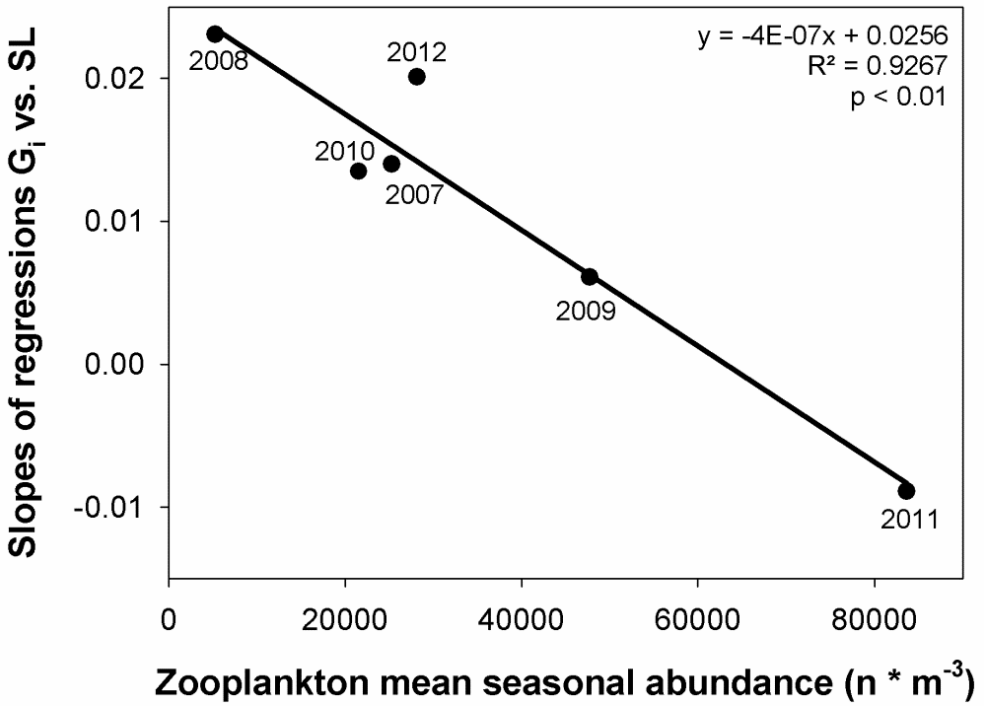

Fig. 2: Slopes of regressions (instantaneous growth rate (Gi) vs. standard length (SL) versus mean seasonal zooplankton prey abundance

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Chapter 5

Spatio-temporal variability of larval Atlantic herring (*Clupea harengus*) growth rates in the western Baltic Sea

In prep.

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Abstract

Spawning and nursery areas of marine fish species that reproduce in estuaries and lagoons are distributed along coastlines. The geographic distance between and large spatial extension of the nurseries potentially leads to a different growth conditions and, thus, different larval growth rates. Spatio-temporal variability of larval Atlantic herring growth rates was tested at 5 stations in a large nursery area (Greifswalder Bodden (GWB), 514 km^2) during one spring season. Regional variability was investigated by using seasonal mean larval growth rates from the GWB and the Kiel Canal from three consecutive years. To analyze spatio-temporal variability of the mesozooplankton, five coastal stations were sampled during three consecutive years. We show that spatial variability of larval growth was lower than temporal variability in the GWB. However, growth rates of large larvae from a station located in a channel-like part of the GWB habitat were constantly lowest towards the end of the season. Regional variability between seasonal mean growth rates of the GWB and the Kiel Canal was low compared to temporal variability. Similarly, analysis of coastal mesozooplankton showed that temporal variability was higher than spatial variability. We conclude that larval fish growth is similar in homogeneously structured nursery areas at a given date, but may change in differently structured parts of the habitats. On the regional scale, inter-annual variability of both larval growth and mesozooplankton availability is higher than spatial variability.

***Key words*:** RNA:DNA ratio, Greifswalder Bodden, Kiel Canal, nursery area

Introduction

Growth conditions in larval fish habitats can differ spatially. This can be true for biotic conditions, e.g., when phytoplankton production varies due to different nutrient availability (e.g., near-shore vs. off-shore) or patchy distribution of zooplankton (Klais et al., 2016, and references therein). The most prominent example of spatially varying abiotic factors affecting larval growth rates is temperature, which can change rapidly in shallow waters compared to deeper ones. Following gradients from coast to off-shore habitats showed that larvae grew at higher rates near-shore compared to off-shore (Höök et al., 2008; Sponaugle et al., 2009). A comparison of RNA:DNA ratios of larval red drum (*Sciaenops ocellatus*) originated from several coastal habitats showed no significant differences (Rooker et al., 1997). Information on spatial larval growth variability within

large habitats is lacking, though this is essential for a representative and cost-effective sampling design.

In order to investigate spatial variability of larval Atlantic herring (*Clupea harengus* L.) growth rates within a large and important nursery area for western Baltic spring-spawning herring (WBSSH), larval herring growth rates were investigated during the course of a season at 5 sampling stations in the Greifswalder Bodden. It is a nursery area of wide extent (514 km^2) and potentially one of the most important nursery areas for WBSSH (Biester, 1989; Oeberst et al., 2009). Furthermore, seasonal mean larval growth rates of herring larvae originating from the Greifswalder Bodden and the Kiel Canal were compared during three consecutive years with the aim to compare spatio-temporal larval growth variability on the regional scale. Finally, mesozooplankton was sampled at five stations during three consecutive years in order to analyze spatial and temporal variability of the larvae´s potential prey.

Material and Methods

Sampling sites

The Greifswalder Bodden is a large but shallow bay in eastern Germany, with a spatial extension of 514 km^2 and restricted access to the Baltic Sea. Due to the low average depth (5.6 m), its water column is constantly well mixed. As the Greifswalder Bodden is surrounded by low saline Baltic Sea water (~8), the salinity within the Bodden is low and ranges from 6 to 8. Similarly, the Kiel Canal (northern Germany) is characterized by a low salinity (5.5 to 11.5), but is comparatively deep with an average depth of 11 m. However, due to the frequent shipping traffic, the water column is also constantly well mixed. Both the Greifswalder Bodden and the Kiel Canal are retention areas for larval herring. For the Greifswalder Bodden this was shown via model-simulations (Bauer et al., 2013), while in the Kiel Canal herring spawn far inland (13 km – 45 km distance to the Baltic Sea; Brandhorst, 1955) and water exchange to the Baltic Sea is low as the water level is kept fairly constant (± 10 cm; Rieper, 2001), which leads to only weak and wind driven currents at the spawning sites.

Sampling

To analyze spatial variability of larval growth rates within a large retention area, Atlantic herring larvae (*Clupea harengus* L.) as well as temperature and salinity data were sampled at 5 different stations in the Greifswalder Bodden in 2010 (Fig. 1 *right*). In order to compare seasonal mean larval growth between different retention areas, herring larvae were sampled at station 306 in the Greifswalder Bodden and in the Kiel Canal at a station 13 km inland to the open Baltic Sea (Fig. 1 *left*) from 2010 to 2012. To test for hydrological homogeneity within the Greifswalder Bodden, water samples for nutrient (NO_3, PO_4, Si) and chlorophyll *a* analyses were taken at 5 stations (same as in 2010) in 2011. All samples were taken between April and June on a weekly basis in the years of investigation. To test for spatial and temporal variability of the mesozooplankton, data from 5 stations along the coast of the federal state of Schleswig-Holstein, Germany (beeline between the northern- and southernmost stations ~90 km) were investigated between 2013 and 2015. As the mesozooplankton is characterized by short life cycles, only samples taken within a temporal range of maximum two weeks were used for this comparative approach.

A bongo net (60 cm diameter, 335 µm and 500 µm mesh size respectively) that was heaved in an oblique haul was used for the sampling of the herring larvae. The larvae were frozen on board within 30 minutes after the haul and stored at -80°C for later processing. The larvae were measured to the lower 0.1mm and then freeze-dried for 24 hours (freeze drier: CHRIST ALPHA 1-4 LSC). Then, the larvae´s dry weight was weighed to the nearest 0.1µg (SARTORIUS microbalance SC2).

Mesozooplankton was sampled along the coast of the German federal state Schleswig-Holstein by using a WP2-nets with 100 µm (spatial and temporal comparison at the coast of Schleswig-Holstein) mesh-size that was heaved in a vertical haul from near-bottom to the surface, and fixed with 4 % borax buffered formalin solution.

Nutrient and chlorophyll a *analyses*

Nutrients were analyzed according to Grasshoff (1999) and chlorophyll *a* concentrations after Jeffrey and Humphrey (1975). Per station and sampling date only individual samples were analyzed.

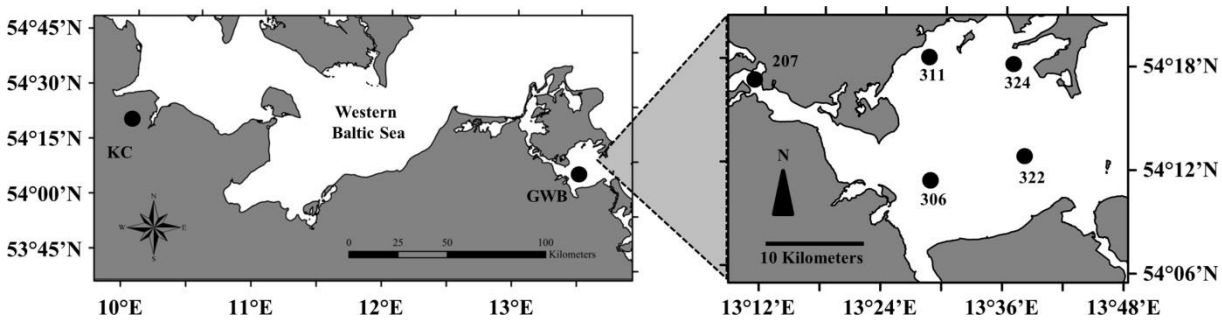

Fig. 1: Sampling stations for *left* the regional comparison of seasonal mean larval growth rates and *right* for the spatial comparison within the larval herring retention area Greifswalder Bodden

RNA:DNA analysis and instantaneous growth rates (G_i)

The RNA:DNA ratio is a short term larval condition indicator with a response time of several days up to a week (Clemmesen, 1994; Clemmesen, 1996). A weekly sampling design was performed accordingly.

To ensure exclusion of yolk-sac larvae, only larvae ≥ 8 mm were used for analyses. To ensure complete homogenization of the larvae and extraction of nucleic acids, the upper limit of larvae analyzed was 20 mm.

RNA and DNA concentrations of whole individual larvae were measured. A detailed description of the analysis is given by Clemmesen (1989; 1994). Larvae caught in 2011 however, were defatted before analyzing RNA:DNA ratios in order to determine effects of essential fatty acids on larval growth and analyzed according to Malzahn et al. (2007) and Paulsen et al. (2014a; 2014b). Validation with the classical approach by Clemmesen (1989; 1994) showed no significant differences. To appropriately include individual variability, at least 5 larvae individuals per size class (for definition of the size classes, see below) were analyzed. Accordingly, we analyzed a minimum of 5 larvae but on average 12 larvae (standard deviation ± 5.1) per sampling day and size class. For the comparison of seasonal growth rates between habitats (sampling station 306 in the Greifswalder Bodden; Kiel Canal), all larvae available per size class and year were used. In total, 945 larvae from the Greifswalder Bodden were analyzed, and 594 from the Kiel Canal. The resulting RNA:DNA ratios were then used to calculate dry weight related instantaneous growth rates (G_i) of the larvae (Buckley et al., 2008).

Categorization of larval size classes

Normally, herring larvae growth increases with larval size (Clemmesen, 1994). To make sure that observed changes in larval growth are not primarily driven by changes in larval size during the course of the season, we defined two size classes: larvae smaller and larger than 14 mm. This categorization was chosen because the dorsal fin was clearly protruded at 14 mm (Doyle, 1977). Fin differentiation was assumed as an indicator for an ethological change, due to enhanced swimming and therefore hunting capabilities (see Moyano et al., 2016).

Statistics

Generally, data were only used when at least 5 individuals per size class were available to appropriately cover individual variability. For all statistics, the significance level was set to $\alpha = 0.05$. Shapiro-Wilk-test and Levene-test were used to test data for normal distribution and homogeneity of variance, respectively. To check for spatial larval growth variability within one size class (i.e., < 14 mm and > 14 mm) in the Greifswalder Bodden at a given sampling date, one-way analyses of variance (ANOVA) were conducted and Tukey honestly significant difference (HSD) tests were performed for *post hoc* comparison. The same statistics were applied for the regional comparison of seasonal mean larval growth rates between the Greifswalder Bodden and the Kiel Canal. However, to receive information on inter-annual variability additionally to the spatial component, data from all years of investigation were incorporated into the analyses. To test for size-dependent larval growth rates, t-tests between large and small larvae per station and sampling date were performed for pairwise comparisons.

Results

In the Greifswalder Bodden, spatial variability of growth rates in larvae < 14 mm standard length was relatively low. In 50% of the sampling dates significant differences in larval growth rates between some of the sampling stations could be detected. In absolute terms, however, the spatial difference of larval growth ($G_i(d^{-1})$) ranged only between 0.01 and 0.06 (mean: 0.03) per sampling date. In contrast to this, temporal variability was higher, ranging from 0.05 to 0.19 (Fig. 2) during the course of the season. Only in SW 13 clear spatial differences in growth rates were observed, but it has to be noted that two of the four sampling station are missing, which potentially confounds this result.

Spatial variability in growth rates of large larvae (> 14 mm standard length) followed a similar pattern. Here, in 60% of the sampling dates no significant difference in growth rates between stations could be detected (SW 9, 13, 14; SW of consideration: 9, 10, 11, 13 and 14). Station 207 formed an exception, where in comparison to the other stations a strong negative trend in larval growth rates was observed from SW 12 onward (Fig. 3). Apart from this exception, the sampling stations did not show a ranking order in terms of low or high larval growth rates. Generally, regardless of station, growth rates of large larvae were higher than of small larvae at the beginning of the season, but this size-dependency of larval growth rates decreased with time (Tab. 1).

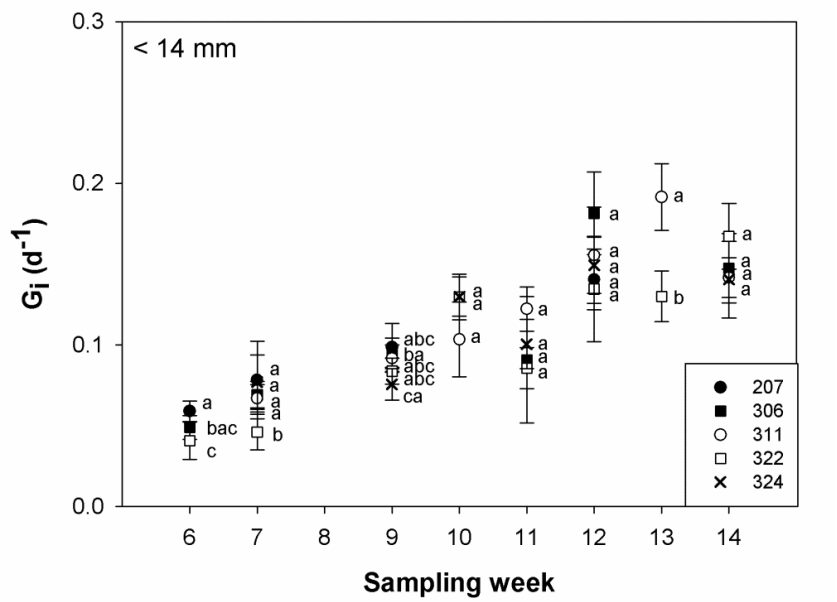

Fig. 2: Spatial and seasonal variability of small larval herring (< 14mm) growth rates (Gi) at 5 different sampling stations. Error bars indicate 95% confidence interval. Total number of analyzed larvae: 405. Mean of analyzed larvae per data point: 14 ± 5.6.

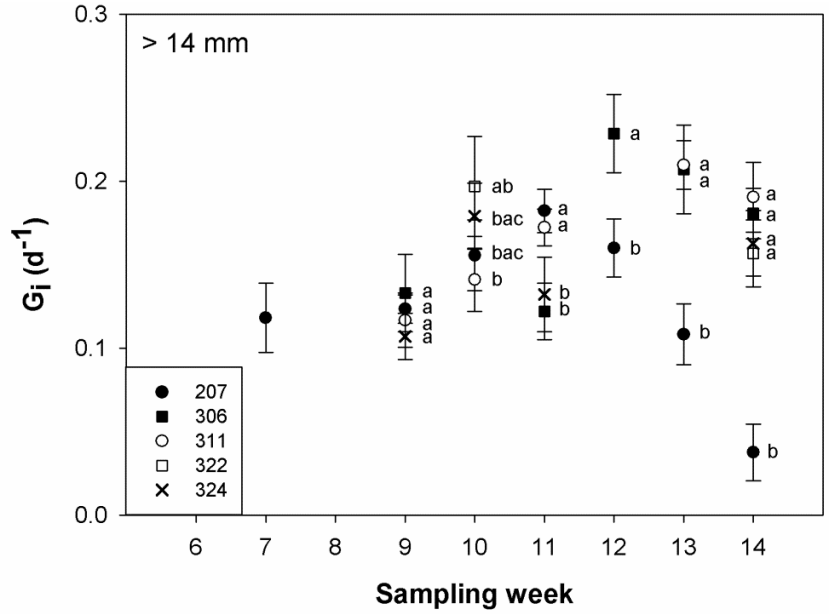

Fig. 3: Spatial variability of large larval herring (> 14mm) growth rates (Gi) over time at 5 different sampling stations. Error bars indicate 95% confidence interval. Different letters besides the data points denote significant differences between stations at a given date. Total number of analyzed larvae: 221. Mean of analyzed larvae per data point: 10 ± 3.6.

Tab. 1: Results from t-tests between small (< 14 mm) and large larvae (> 14 mm) to test for size-dependent growth at a given sampling station (upper row) and sampling week (SW). "+" and "-" indicate significant and non-significant differences, respectively. Empty cells denote that sample size *N* was too low for statistical analyses (≤ 4) or that larvae were not available from both size classes.

	207	**306**	**311**	**322**	**324**
SW 7	+				
SW 9	+	+	+		+
SW 10			+	+	+
SW 11		-	-		+
SW 12	-	+			
SW 13			-		
SW 14	-	+	-	-	-

Since spatial differences in larval growth variability in 2010 were minor, nutrient (NO_3, PO_4, Si) and chlorophyll *a* (chl *a*) concentrations were analyzed. This was done in

order to check for hydrological homogeneity in the Greifswalder Bodden in 2011 with the assumption that the spatial variability found in 2011 should generally reflect the situation in 2010, when the larval herring growth analyses was performed. While phosphate was not limiting throughout the whole season (Fig. 4), nitrate was depleted at all 5 stations in the beginning of May (Fig. 5). At the same time, silicate concentrations started to increase (Fig. 6). When silicate concentrations were on a relatively constant level from 17 May onwards, chl *a* concentrations were on a constantly low level until end of June (Fig. 7). Only at station 207 chl *a* concentrations were sometimes higher.

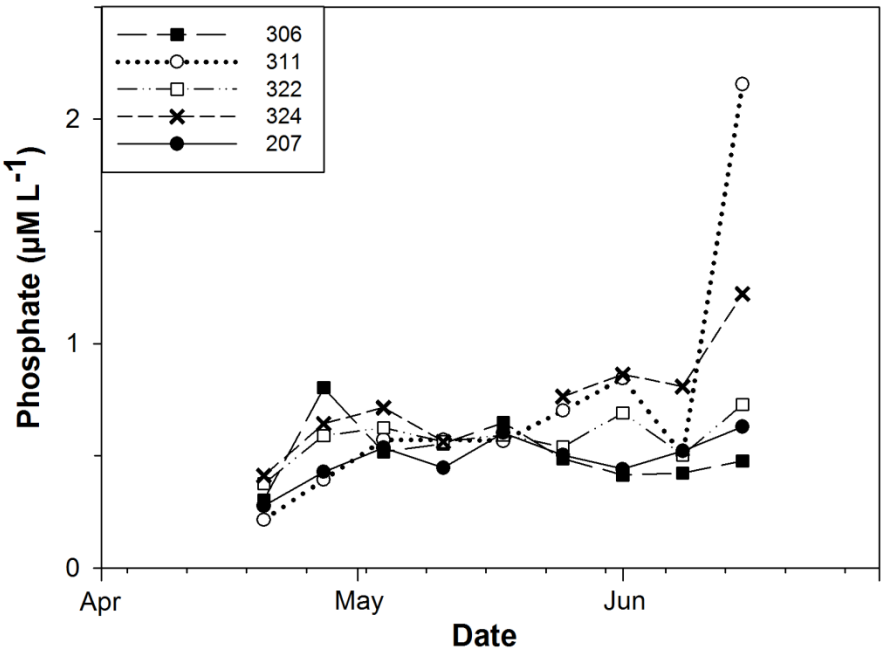

Fig. 4: Phosphate concentrations at 5 stations in the Greifswalder Bodden during the spring season 2011

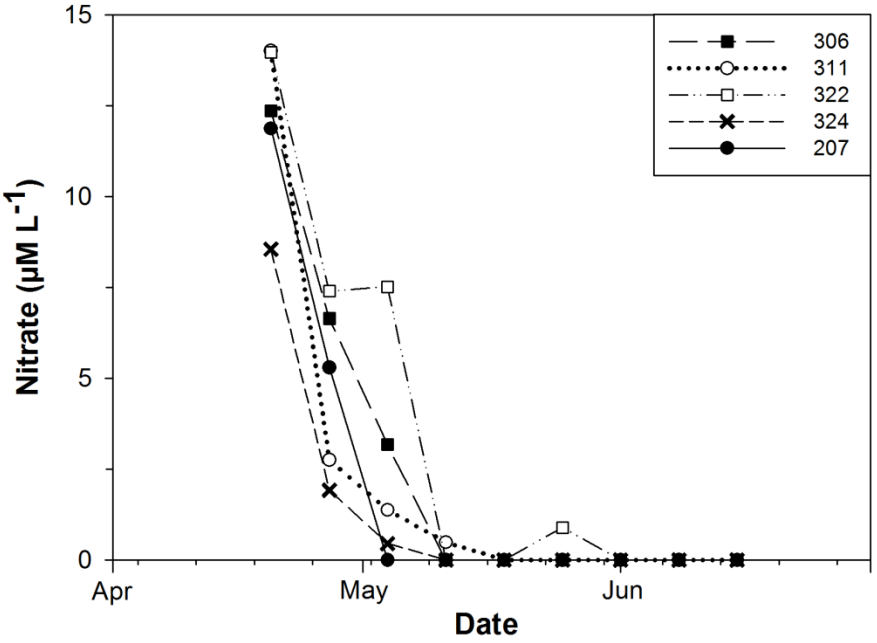

Fig. 5: Nitrate concentrations at 5 stations in the Greifswalder Bodden during the spring season 2011

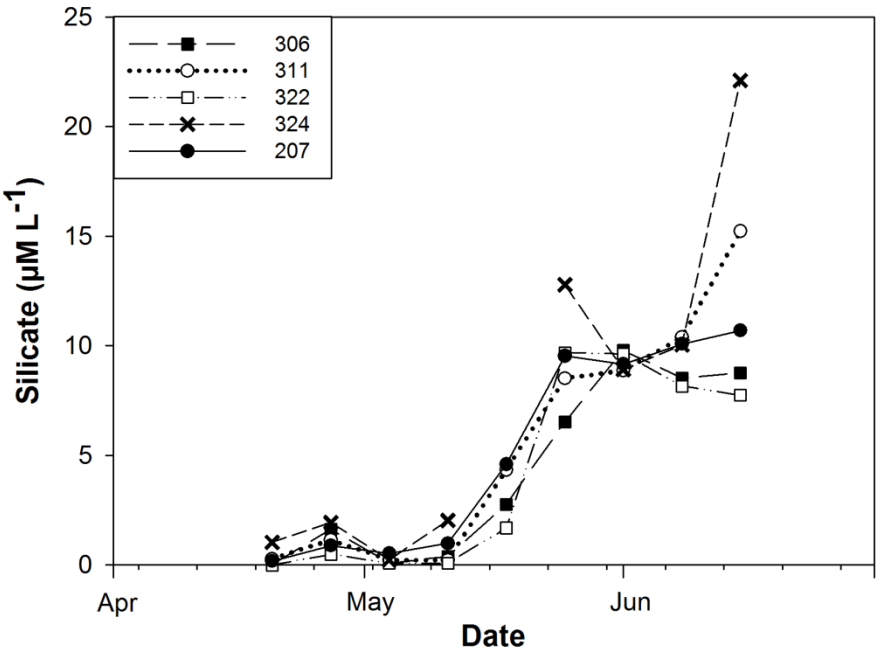

Fig. 6: Silicate concentrations at 5 stations in the Greifswalder Bodden during the spring season 2011

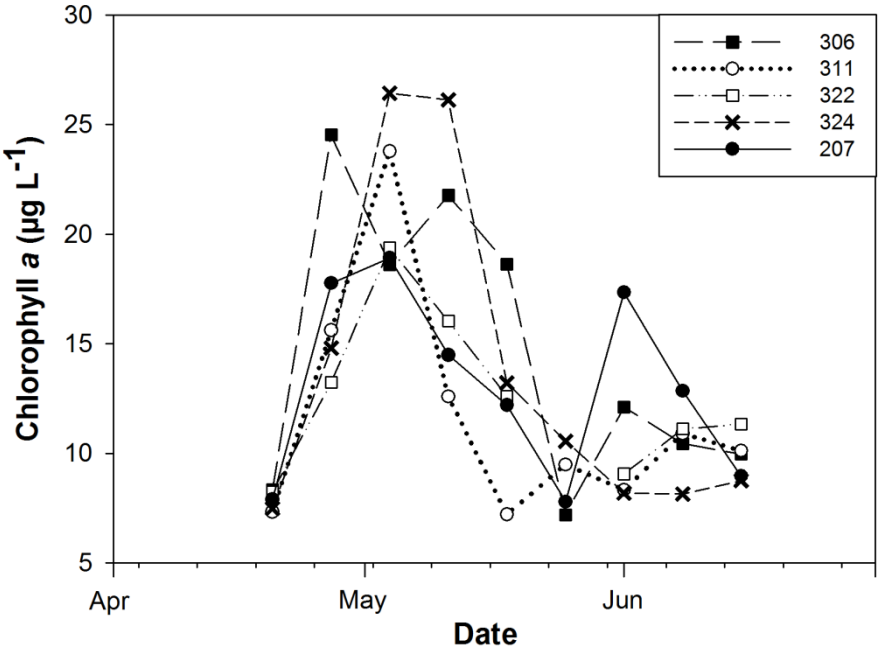

Fig. 7: Chlorophyll *a* concentrations at 5 stations in the Greifswalder Bodden during the spring season 2011

On the regional scale, seasonal mean larval growth was significantly different but showed the same temporal trends, between Greifswalder Bodden and Kiel Canal from 2010 to 2012 (Fig. 8). This was true for both larvae smaller and larger than 14 mm. Only in 2011, growth rates from large larvae the Kiel Canal were considerably lower compared to those from the Greifswalder Bodden. Generally, small larvae grew at higher rates in the Kiel Canal, while large larvae grew faster in the Greifswalder Bodden. Similarly, temporal variability in mesozooplankton mean size and total abundance was higher along the coast of Schleswig-Holstein, Germany, compared to spatial variability (Fig. 9).

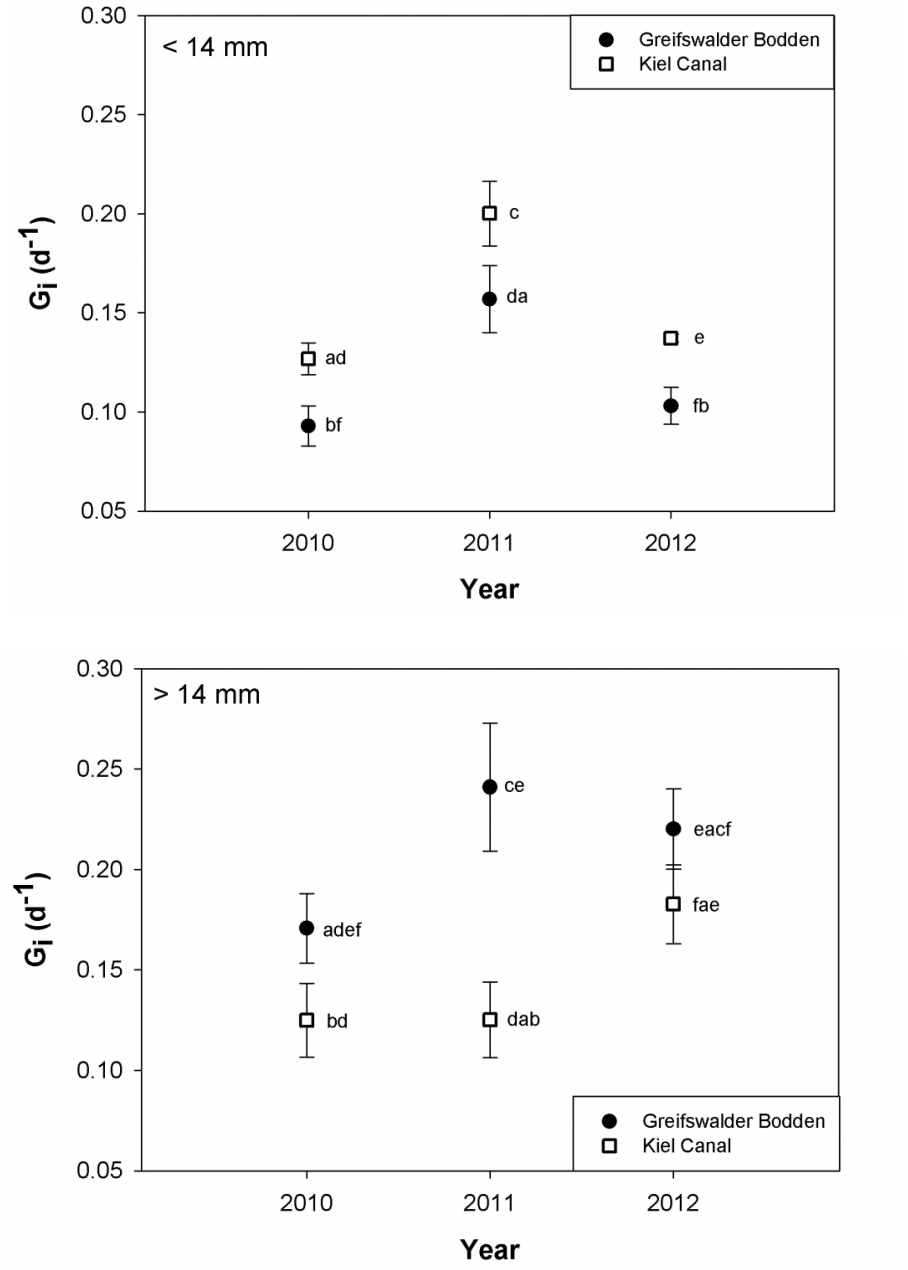

Fig. 8: Seasonal mean larval herring growth rates (G_i) in Greifswalder Bodden (Station 306) and Kiel Canal during the spring seasons 2010 to 2012. Error bars indicate 95% confidence interval. Different letters besides the data points denote significant differences. Total N of analyzed larvae < 14 mm Greifswalder Bodden: 331; Kiel Canal: 371. Mean of analyzed larvae per data point < 14 mm: 117 ± 27.8. Total N of analyzed larvae > 14 mm Greifswalder Bodden: 123; Kiel Canal: 223. Mean of analyzed larvae per data point > 14 mm: 58 ± 31.9.

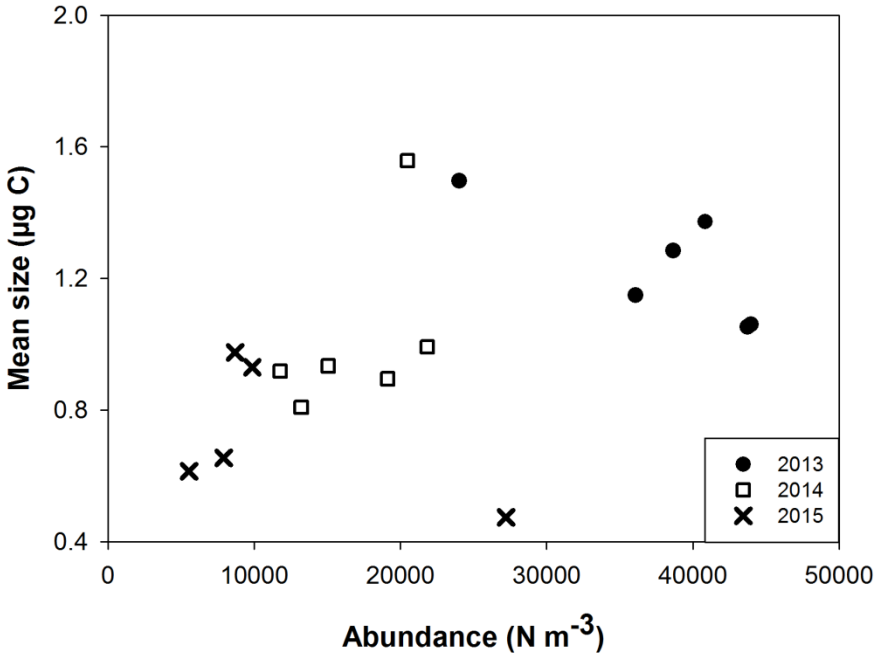

Fig. 9: Temporal and spatial variability of the mesozooplankton along the coast of Schleswig-Holstein, Germany. The beeline between the northern- and southernmost stations is ~ 90 km.

Discussion

In this study, the observed spatial variability of larval Atlantic herring growth rates was relatively low compared to the temporal variability within the widely extended nursery area Greifswalder Bodden. Further, the sampling stations did not show a ranking order in terms of low or high larval growth rates. Similarly, the spatial comparison on a regional scale (Greifswalder Bodden compared with Kiel Canal) using seasonal mean larval growth rates demonstrated overall higher temporal than spatial variability. Further, mesozooplankton data originated from 5 different sampling stations along the coast of the German federal state of Schleswig-Holstein support this assumption, showing that mesozooplankton mean size and total stock vary stronger temporally than spatially. Overall, dynamics affecting larval growth vary stronger in time than in space. Klais et al. (2016) analyzed long-term mesozooplankton data and came up with the same conclusion for mesozooplankton dynamics, though on small spatial scales (< 1 km) mesozooplankton tends to patchy distribution (Folt and Burns, 1999).

These results indicate that the fate of a larva stronger depends on its position in time than in space. However, there was an exception: large larvae from the station in the

Strelasund (sampling station 207) grew at significantly lower rates towards the end of the season compared to larvae from the bay-like structured Bodden itself. A possible explanation might be the channel-like structure of the Strelasund, leading to concentrating effects of herring larvae. Oeberst described that larval abundances were extraordinary high in this area, and supposed density-dependent mortality in the larvae (Oeberst et al., 2008). The density-dependence might be transferable to the similarly structured Kiel Canal: exclusively in 2011 growth rates of large larvae were remarkably lower than in the Greifswalder Bodden. In that year, copepod abundances and consequently larval growth rates were highest during six years of investigation (chapter 2). High survival rates of small larvae might have been the consequence, as high quantities of large larvae were observed at the end of the season (Hesse, 2012). This might have led to density-dependent effects on larval growth.

Size-dependent growth in larval fish is a phenomenon observed in several marine fish species (e.g., Clemmesen, 1994; Clemmesen et al., 1997; Rooker et al., 1997; Diaz et al., 2008). However, it seems that size-dependent growth is primarily driven by food availability, i.e., that size dependency is the lower the more prey is available (compare chapter 3). In the present study, size-dependent growth was observed in the Greifswalder Bodden at the beginning of the season 2010, while this effect decreased with time. This suggests improving nutritional conditions over time during the investigation period.

Investigations of nutrient concentrations indicated that the phytoplankton bloom in the Greifswalder Bodden was nitrogen-limited in 2011. The spatial comparison of both nutrients (NO_3, PO_4, Si) and chl *a* concentrations showed a very similar temporal development throughout the Bodden (increase/decrease of nutrients and chl *a*), though some delay (~1 week) in reaching "end points" (onset of periods characterized by relatively constant values) was observed for NO_3 and chl *a*. The spring phytoplankton bloom from end-April to mid-May was most likely dominated by diatoms, as silicate values were extremely low during that period. Silicate is essential for diatoms and not consumed by other phytoplankton classes. Recovering silicate concentrations coincided with decreasing chl *a* concentrations mid-May, indicating the end of the spring bloom.

Unfortunately, no direct comparison between larval growth data and nutrients/chl *a* was possible due to the fact that data originated from two different seasons. However, it can be assumed that spatial variability was similar in 2010, which might be responsible for some variability observed in larval growth rates. Within the Bodden (excluding Strelasund, station 207), with only few exceptions, generally both hydrology and larval

growth rates were spatially comparable at a given time point. A possible explanation might be the well mixed water column due to the shallowness and the cyclic current within the Bodden. The Strelasund, however, should be treated separately, as both growth rates in large larvae and chl *a* concentrations sometimes strongly differed compared to the other stations.

Overall, we found evidence that largely temporal variability of larval growth, mesozooplankton and hydrological parameters is higher than spatial variability. Homogenously structured retention areas such as the Greifswalder Bodden provide relatively constant growth conditions for larval fish spatially. The differences observed at some time points between the channel-like Strelasund (207) and the other stations located in a bay-like habitat (306-324) deserve caution, i.e., it seems advisable to treat differently structured parts of habitats separately. The comparison of seasonal mean larval growth rates between the Greifswalder Bodden and the Kiel Canal demonstrated that inter-annual variability is higher than spatial variability in most cases (exception: 2011, large larvae). While small larvae grew at higher rates in the Kiel Canal compared to the Greifswalder Bodden, the opposite was true for large larvae. Most likely, this is owed to the fact that growth conditions are good in the Kiel Canal in the early season, when most small larvae occur, while in the Greifswalder Bodden growth conditions are better in the late season, when major parts of analyzed larvae are large.

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Chapter 6

Effects of a broad-scale climate process (Baltic Sea Index) on larval Atlantic herring (*Clupea harengus*) growth and copepod abundance

In Prep.

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Abstract

Recruitment variability is high in marine fish stocks, and climate forcing was shown to significantly affect population dynamics of commercially used fish stocks. The larval stage is known as a recruitment bottleneck, as mortality rates are extraordinary high. Larval fish growth is frequently used to estimate survival during the highly sensitive larval stage. In this study, effects of a broad-scale climate index (Baltic Sea Index, BSI) on biochemically derived larval Atlantic herring growth rates and copepod abundance were investigated in the western Baltic Sea. Larval growth and prey abundance data from a 6-year time-series from the Kiel Fjord, a 5-year time-series from the Kiel Canal and a 3-year time series from the Greifswalder Bodden (here prey abundance data were only available from one year) were analyzed. No significant effects of the BSI on larval growth were observed. Similarly, no significant effects of the BSI on copepod abundance could be detected. However, a trend of a negative effect on one important copepod genus (*Pseudocalanus* spp.) was observed. Moreover, the growth rate of small herring larvae (< 14 mm) correlated positively with the abundance of copepods. The results suggest that larval growth is not directly, but may be indirectly affected by the winter BSI.

Key words: BSI, western Baltic Sea, *Eurytemora*, *Pseudocalanus*, *Acartia*

Introduction

Today, evidence exists that climate forcing has the potential to strongly affect fish population dynamics. For instance, changing dominance of "wasp-waist" ecosystem-forming fish species (Bakun, 2006) such as sardine (*Sardina pilchardus*, *Sardinops* spp., *Sardinella* spp.) and anchovy (*Engraulis* spp.) is governed by strong climatic changes, as extensively described for the coast off Peru (Alheit and Niquen, 2004). In North Sea herring (*Clupea harengus*), significant effects of the North Atlantic Oscillation (NAO) on recruitment were observed (Gröger et al., 2010). The NAO describes the difference in atmospheric pressure at sea level between Azores high and Icelandic low. Historically, massive fluctuations of herring populations along the Swedish west coast correlated with strong NAO fluctuations, with periods of negative NAO characterized by severe winters in Europe, leading to decades of highly productive herring fisheries (*Bohuslän periods*), while the opposite was true for decades characterized by mild winters, caused by positive NAO (Alheit and Hagen, 1997).

Biological processes governed by climate forcing seem to start at the planktonic level (Beaugrand et al., 2003; Alheit et al., 2005), thereby affecting fish recruitment as

larval fish nearly exclusively feed on zooplankton. Larval fish nutrition is assumed to be a major driver of recruitment variability (e.g., Hjort, 1914; Cushing, 1974). Typically, its potential effects on recruitment are linked to larval growth rates, as the early life stages are highly vulnerable and suffer from immense mortalities (Houde, 1994; Le Pape and Bonhommeau, 2015). Based on the "bigger is better" or the "stage-duration" hypotheses (Houde, 1987; Anderson, 1988), it is assumed to be beneficial when larvae remain as short as possible in this sensitive stage. Although indication exists on the relation between climate and marine fish stock recruitment and between larval growth and recruitment, studies are lacking that investigate the effects of broad-scale climate processes on biochemically derived larval fish growth rates.

In light of the ongoing low recruitment of western Baltic spring spawning herring (WBSSH) we set out to test the effects of the Baltic Sea Index (BSI) on larval herring growth and prey availability in the three important nursery areas Kiel Fjord, Kiel Canal, Greifswalder Bodden. The BSI indicates the difference of the atmospheric pressure at sea level between southern Norway and the Baltic Sea coast off western Poland. Its effects on WBSSH recruitment (here: larval abundance) in the Greifswalder Bodden were shown by Gröger et al. (2014). Using larval growth data from five, six, and three spring seasons from the Kiel Canal, the Kiel Fjord, and the Greifswalder Bodden, respectively, we tested the hypotheses that 1) broad scale climate processes such as the BSI affect larval fish growth. Using Copepod abundance data that were available for all but one season investigated in the Kiel Canal and the Kiel Fjord, and only for one season in the Greifswalder Bodden we tested the hypothesis that 2) the BSI affects copepod abundance.

Material and methods

Sampling

To analyze effects of the Baltic Sea Index (BSI) on larval herring growth rates, Atlantic herring larvae (*Clupea harengus* L.) as well as temperature and mesozooplankton were sampled in the Kiel Canal at a station ~13 km inland to the open Baltic Sea (54°20'45 N, 9°57'02 E) in 2007, 2008 and 2010 to 2012. Similarly, both larval herring (2007-2012) , temperature and mesozooplankton (the latter only from 2007-2011) were sampled at a station in the inner Kiel Fjord, western Baltic Sea (N 54°19.69, E 10°09.06). Additionally, herring larvae, temperature and mesozooplankton (the latter only in 2011) were sampled at one station (No. 306; 54°11'10 N, 13°28'80 E; part of the Rügen Herring

Larvae Survey) in the Greifswalder Bodden from 2010 to 2012. Previous investigations showed that larval herring growth rates were often not significantly different at different stations within the Greifswalder Bodden at a given sampling date, and that temporal variability was higher than spatial variability (Chapter 5). This is in line with previous findings, which showed that zooplankton abundance does not differ significantly at a given date within the Greifswalder Bodden (Hesse, 2010). Samples at all sites were taken on a weekly basis between April and June in the years of investigation. To test for spatial and temporal variability of the mesozooplankton at a larger scale, data from 5 stations along the coast of the federal state of Schleswig-Holstein, Germany (beeline between the northern- and southernmost stations ~90 km) were investigated between 2013 and 2015. As the mesozooplankton is characterized by short life cycles, only samples taken within a temporal range of maximum two weeks were used for this comparative approach.

A bongo net (60 cm diameter, 335 µm and 500 µm mesh size respectively) that was heaved in an oblique haul was used for the sampling of the herring larvae. The larvae were frozen on board within 30 minutes after the haul and stored at -80°C for later processing. The larvae were measured to the lower millimeter and then freeze-dried for 24 hours (freeze drier: CHRIST ALPHA 1-4 LSC). Then, the larvae´s dry weight was weighed to the nearest 0.1 µg (SARTORIUS microbalance SC2).

The potential prey field was analyzed using WP2-nets with 200 µm (to sample the prey field in Kiel Canal, Kiel Fjord, Greifswalder Bodden) and 100 µm (spatial and temporal comparison at the coast of Schleswig-Holstein) mesh-size that was heaved in a vertical haul from near-bottom to the surface, and fixed with 4 % borax buffered formalin solution.

Prey field analysis

For mesozooplankton analyses, samples were divided into subsamples (Kott 1953) such that one subsample contained at least 100 individuals of the dominant copepod species. If the subsample contained less than 100 individuals of the dominant copepod species, further subsamples were analyzed until this critical number was reached. Species identification was performed on genus or species level according to Sars (1895).

For further analyses, the seasonal mean abundance of the dominant copepod species was calculated for the Kiel Canal (*Eurytemora affinis*) and the Kiel Fjord (*Pseudocalanus* spp.). It was focused on the dominant calanoid copepod species, because they correlated best with instantaneous larval growth rates (Gi (d^{-1}); see explanation below and chapter

2) . Since no species was dominant in the Greifswalder Bodden in 2011, the sum of both *Acartia* spp. and *Eurytemora affinis* was used.

RNA:DNA analysis and instantaneous growth rates (G_i)

RNA:DNA analysis is a short term larval condition indicator with a response time of several days up to a week (Clemmesen, 1994; Clemmesen, 1996). To receive representative seasonal mean data, we performed a weekly sampling design accordingly. In contrast to the Kiel Canal and the Greifswalder Bodden, in the Kiel Fjord, sprat (*Sprattus sprattus*) larvae occur additionally to herring from approximately the beginning of June onwards (depending on temperature development). In order to avoid mixing of these species, analyses in the Kiel Fjord were restricted until the week before peak hatch of the sprat larvae. Generally, no big differences in season length for respective years were observed between sampling sites, and normally the herring larvae season reached from mid/end of April until the beginning/mid of June.

To make sure that yolk-sac larvae are excluded from analyses, the lower size limit of analyzed larvae was set to 8 mm standard length. In order to ensure complete homogenization of the larvae, the upper size limit of larvae analyzed was 20 mm standard length.

RNA and DNA concentrations of whole individual larvae were measured. A detailed description of the analysis is given by Clemmesen (1989; 1994). Larvae caught in 2011 however, were defatted before analyzing RNA:DNA ratios and analyzed according to Malzahn et al. (2007) and Paulsen et al. (2014a; 2014b) to analyze effects of essential fatty acids on larval growth (Paulsen et al., 2014b). Validation with the classical approach by Clemmesen (1989; 1994) showed no significant differences. The resulting RNA:DNA ratios were then used to calculate dry weight related instantaneous growth rates (G_i (d^{-1})) of the larvae (Buckley et al., 2008):

$$G_i = 0.0145 \times sRD + 0.0044 \times (sRD \times T) - 0.078$$

where sRD is the standardized RNA:DNA ratio (Caldarone et al., 2006) and T the temperature (°C) at a given date.

In total, 945 larvae from the Kiel Canal were analyzed, and 594 from the Greifswalder Bodden and 371 from the Kiel Fjord.

Categorization of smaller and larger larvae

Under low to intermediate prey abundances (<< 50,000 m^{-3}; Peck et al., 2013; chapter 4), size-dependent growth can be observed in herring larvae (Clemmesen, 1994). To make sure that observed changes in larval growth are not primarily driven by changes in larval size during the course of the season, two size classes were defined: larvae smaller and larger than 14 mm. This categorization was chosen because the dorsal fin is clearly protruded at 14 mm (own observations and Doyle, 1977). Fin differentiation was assumed as an indicator for an ethological change, due to enhanced swimming and therefore hunting capabilities. As no sufficient numbers of larvae > 14 mm were available in the Kiel Fjord, for this sampling site only the category "small larvae" was available.

Baltic Sea Index (BSI)

According to Gröger et al. (2014), the winter BSI (Jan-Mar) has strongest effects on recruitment in the Greifswalder Bodden in the following year. This was explained by potential parental effects. In the present study, we tested the direct effect of winter BSI on larval herring growth and prey availability. For the analyses, the winter BSI (Jan-Mar) of the same year when the larvae were sampled was used.

Statistics

Seasonal mean larval growth rate was used to investigate effects of BSI on larval growth rate. To appropriately include individual variability, at least 5 larvae individuals per size class (for definition of the size classes, see below) and date were analyzed. However, only very few larvae were available per sampling date in the Kiel Fjord in 2011. Here, also dates were included were at least 4 larvae per size class were analyzed. Occasionally, large differences in the number of analyzed larvae per date existed due to changing availability caused by natural fluctuations. Hence, to avoid bias, means per sampling date were used for the calculation of seasonal means instead of data from individual larvae. Correlation analyses were performed to test for the relationship between the winter BSI and larval growth as well as copepod abundance.

Results

Generally, no effects of the winter Baltic Sea Index (BSI) on growth rates of neither small or large herring larvae, nor copepod abundance were detected (Fig. 1-3, Tab. 1). Similarly, no effects of winter temperatures on copepod abundance were detected.

Growth rates of small larvae were generally higher in the Kiel Canal than in the Greifswalder Bodden (Fig. 1). While small larvae from the Kiel Fjord grew at higher rates than in the Kiel Canal in 2007 and 2008, the opposite was true in 2010 and 2011. Similarly, the abundance of the dominant copepod species was higher in the Kiel Fjord compared to the Kiel Canal in 2007 and 2008, while it was higher in the Kiel Canal in 2010 and 2011 (Fig. 3). Generally, high inter-annual variability in copepod abundance was observed. Seasonal mean growth rates of small larvae significantly correlated with seasonal mean copepod abundance ($N = 11$; $p < 0.001$; Fig. 4), while this was not true for large larvae. Consequently, no clear trends in G_i (d^{-1}) of large larvae were observed between Kiel Canal and Greifswalder Bodden (Fig. 2).

Growth of small larvae showed similar temporal trends in the Kiel Fjord, the Kiel Canal and the Greifswalder Bodden (Fig. 1). The spatial variability of larval G_i (d^{-1}), i.e., the variability of larval growth within the same season between different sampling sites, constituted max. 0.05, while the variability over the whole investigation period, i.e., the temporal variability, constituted 0.11 (Fig. 1).

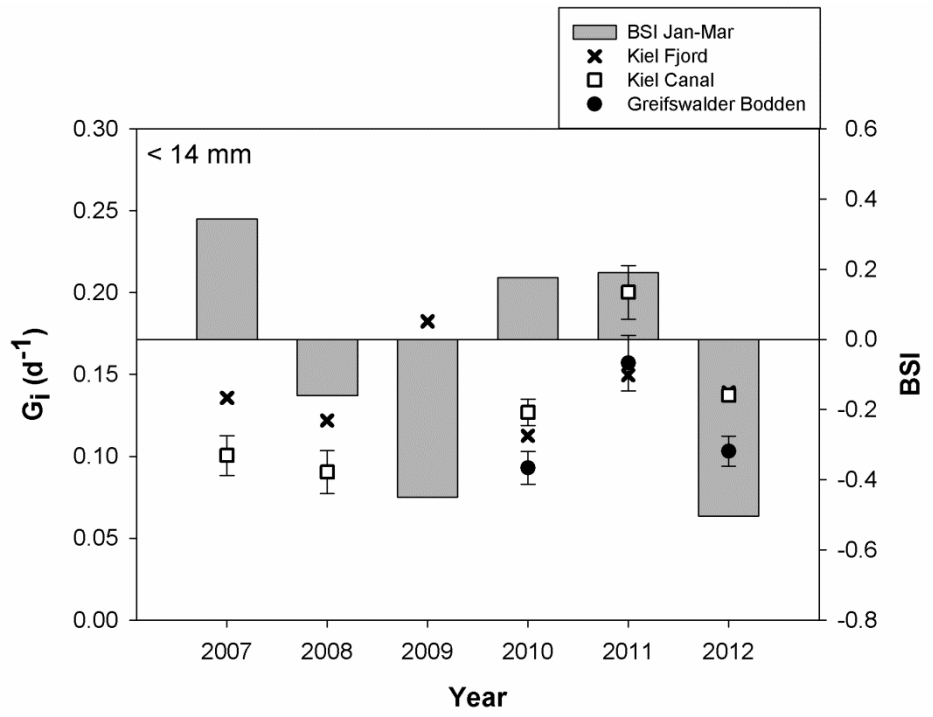

Fig. 1: Temporal course of seasonal mean larval herring growth rates (G_i (d^{-1}) for larvae < 14 mm), the abundance of the main prey item in the Kiel Canal, *Eurytemora affinis*, and the Baltic Sea Index (BSI, Jan-Mar). Error bars indicate 95% confidence interval.

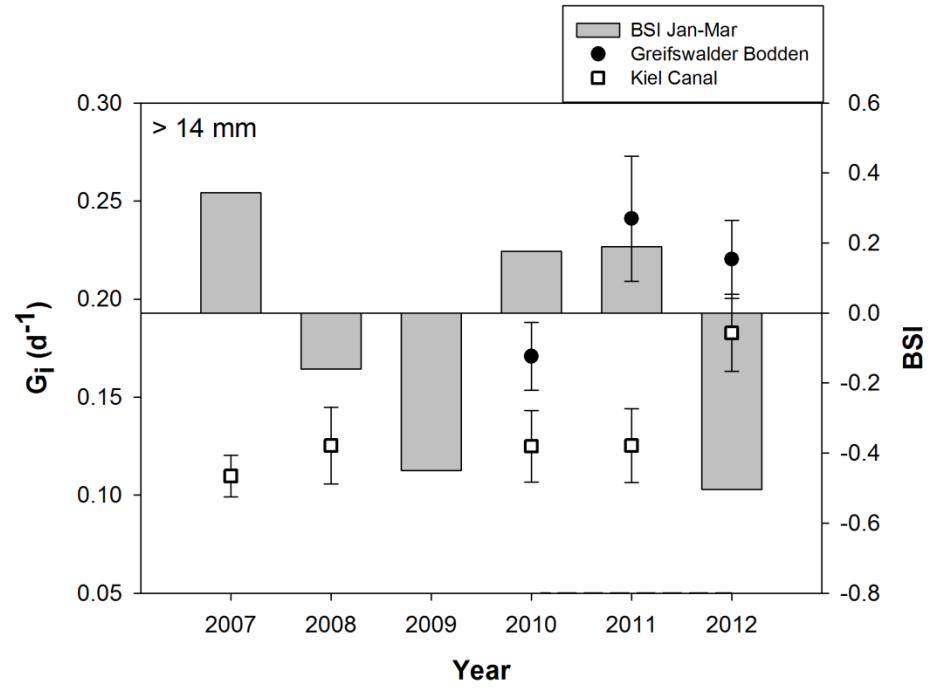

Fig. 2: Temporal course of seasonal mean larval herring growth rates (G_i (d^{-1}) for larvae > 14 mm), the abundance of the main prey item in the Kiel Canal, *Eurytemora affinis*, and the Baltic Sea Index (BSI, Jan-Mar). Error bars indicate 95% confidence interval.

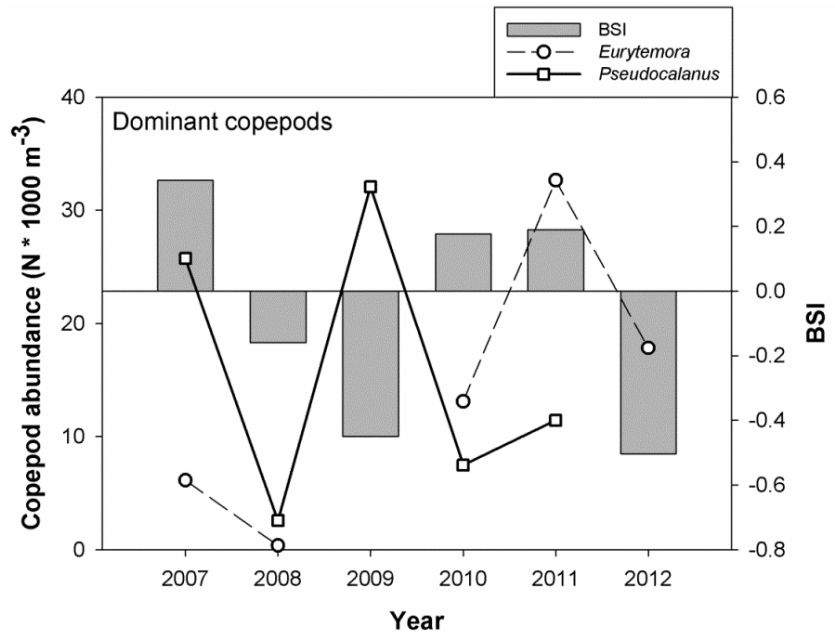

Fig. 3: Temporal course of the seasonal mean abundance of the dominant copepod species in Kiel Canal and Kiel Fjord, *Eurytemora affinis* and *Pseudocalanus* spp., and the Baltic Sea Index (BSI, Jan-Mar).

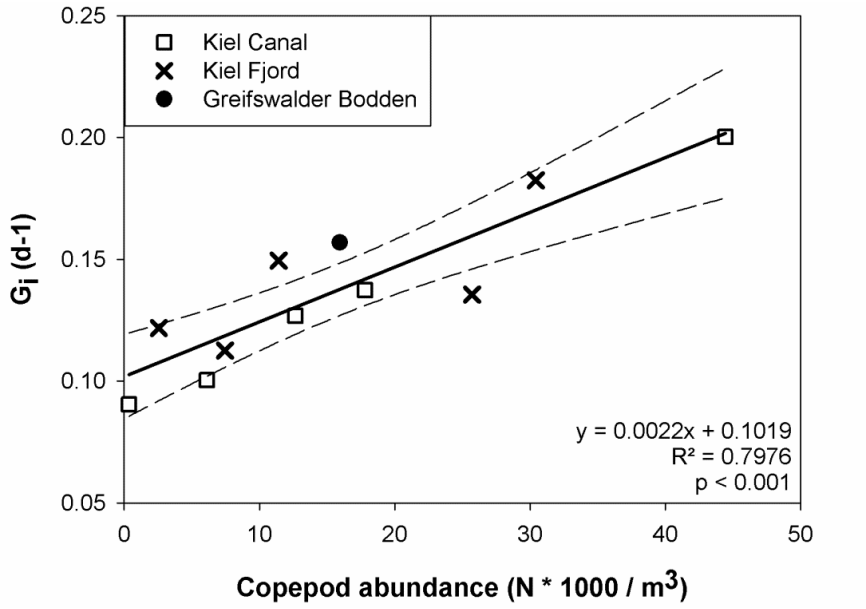

Fig. 4: Regression between seasonal mean larval instantaneous growth rates (G_i (d^{-1})) of larvae < 14 mm and the seasonal mean abundance of the respective dominant copepod species (N = 11; Kiel Canal: *Eurytemora affinis*; Kiel Fjord: *Pseudocalanus* spp., Greifswalder Bodden: no copepod species was dominant; the displayed value is the sum of *Eurytemora affinis* and *Acartia* spp.). The dashed lines indicate 95% confidence interval.

Tab. 1: Relationships between the Baltic Sea Index (Jan-Mar) and larval growth rates (G_i (d^{-1}); N = 6), *Eurytemora* abundance (N = 5), *Pseudocalanus* abundance (N = 5), winter temperature (Jan-Mar; N = 6) and western Baltic spring spawning herring stock recruitment (N = 6).

	Baltic Sea Index
G_i (d^{-1}) larvae < 14 mm	p = 0.7; R^2 = 0.06
G_i (d^{-1}) larvae > 14 mm	p = 0.3; R^2 = 0.34
Eurytemora	p = 0.7; R^2 = 0.06
Pseudocalanus	p = 0.33; R^2 = 0.31
Temperature	p = 0.87; R^2 = 0.008
Stock recruitment	p = 0.35; R^2 = 0.22

Discussion

In the present study, no effects of the Baltic Sea Index (BSI) neither on larval growth rates nor copepod abundance or western Baltic spring spawning herring (WBSSH) stock recruitment were observed. Further, no effects of both winter and spring-season temperature were observed. Gröger et al. (2014) found a significant correlation between winter BSI and larval recruitment in the Greifswalder Bodden, a major nursery area for WBSSH (Biester, 1989; Oeberst et al., 2009). However, this correlation only existed between winter BSI (Jan-Mar) and larval recruitment of the following year. Concerning the nutritional situation of the larvae, it is unlikely that reproduction of the larvae´s main prey organisms, copepods which are characterized by short generation times, is directly related to winter temperatures more than a year ago. Therefore, parental nutrition might be a possible explanation for the observed effects. Parental nourishment during gonad development can affect egg quality and beyond that the performance of the larvae (Perez and Fuiman, 2015). Consequently, parental effects of the BSI on herring recruitment were suggested rather than direct effects on the larvae (Gröger et al., 2014). In contrast to Gröger et al., in the present study direct effects of the BSI on prey availability and larval growth were investigated. Consequently, winter BSI data were related to larval growth, copepod abundance and stock recruitment of the same year.

The Kattegat and Skagerrak region is the feeding ground for WBSSH. Historically, catches of Swedish herring (Kattegat/Skagerrak) were low during periods of mild winters, while fishery was highly productive during periods characterized by severe winters

(Bohuslän periods; Alheit and Hagen, 1997). The calanoid copepod *Pseudocalanus acuspes* occurs in the central Baltic Sea and the northern North Sea but is of arctic origin and therefore prefers colder temperatures (Renz et al., 2008, and references therein). It is known from both experimental and field studies that *Pseudocalanus* is a preferred prey item for both larval and juvenile herring (Hardy, 1924; Checkley, 1982; Bernreuther, 2007). Further, Baltic herring condition was demonstrated to positively correlate with the share of *Pseudocalanus* in the diet (Fetter and Davidyuk, 1993). This might explain the observed effects of climate indices on historic herring catches (Alheit and Hagen, 1997) and potential parental effects on recruitment (Gröger et al., 2014). On the contrary, no consistent temperature effects on the copepods *Acartia bifilosa* and *Eurytemora affinis* were observed in the northern Baltic Sea (Viitasalo et al., 1994). Both species are known to be dominant in the investigation area Greifswalder Bodden (Postel et al., 1989; Busch, 1993; Hesse, 2010; Paulsen et al., 2014b) and *Eurytemora* in the Kiel Canal (chapter 2), while in the Kiel Fjord *Pseudocalanus* spp. was the numerically dominant copepod genus (unpublished data). In line with these findings, no temperature effect on seasonal mean *Eurytemora* abundance was observed in the present study, whereas a negative, though not significant, trend between *Pseudocalanus* abundance and temperature existed.

Classical recruitment hypotheses such as the critical period (Hjort, 1914) or the match-mismatch (Cushing, 1974; Cushing, 1990) hypotheses pronounce the importance of the nutritional situation especially for the very early life stages. In the present study, a highly significant positive correlation between larval herring growth rates and copepod abundance was observed. However, this was only true for small larvae (< 14 mm), while for the larger larvae (14 mm – 20 mm) no significant correlation was found. This finding highlights the importance of the nutritional situation especially for small fish larvae which need to learn to successfully hunt and additionally are in a low development stage. The latter impairs successful feeding, as swimming capabilities are restricted. For this regression, only abundances of the dominant copepod species were used, as it was shown previously that larval herring tend to feed selectively (e.g., Checkley, 1982; Arula et al., 2012). The dominant copepod species/genera included were *Pseudocalanus* spp. (*P. acuspes* and *P. elongatus*; Kiel Fjord), *Eurytemora affinis* (Kiel Canal and Greifswalder Bodden) and *Acartia* spp. (*A. bifilosa* and *A. tonsa*; Greifswalder Bodden). As different species were included in that significant model, this suggests that herring larvae are plastic though selective with respect to the choice of the preferred prey item.

Generally, temporal trends in growth rates of the small larvae were akin over the years. Temporal variability was twice as high as temporal variability, suggesting that inter-annual differences in larval growth conditions are larger than growth conditions regionally. This can be true for both major larval growth driving factors, which are food availability and temperature (Jones, 2002). It was previously shown that explained variability of the regression between larval growth rates and standardized RNA:DNA ratios (sRD) is high (82%; chapter 2), suggesting that calculated larval instantaneous growth rates (see Buckley et al., 2008) are primarily driven by larval nourishment and not temperature as sRD displays the nutritional condition of the larvae. In turn, the temporal course of larval growth data in the present study suggests that prey field varies stronger inter-annually than spatially.

To conclude, no significant effects of the BSI on larval growth, stock recruitment or copepod abundance were observed. However, a negative trend between the abundance of *Pseudocalanus* and winter BSI existed which might suggest at least modifying effects of climatic factors on the abundance of this key copepod genus. It has to be kept in mind that though a time-series was analyzed, the data basis was restricted (5 to 6 yrs). Longer periods might strengthen effects by smoothing data variability and reducing the effect of single data points. A highly significant correlation between growth rates of small but not large herring larvae and copepod abundance was observed, which highlights the importance of high prey availability for larval growth and, hence, probably survival.

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General Discussion

The recruitment of marine fish stocks is to a great extent bottom-up controlled. Larval nutrition affects larval survival rates due to starvation and, indirectly, by modifying predation rates. In literature, the interaction of both larval nutrition and predation is frequently discussed. For example, unfavorable feeding conditions lead to a more active behavior of the larvae which are therefore easier detected by predators. Furthermore, inadequate feeding conditions lead to a bad physical condition of the larvae, which increases their risk to be caught. Low growth rates cause long duration times in the highly vulnerable larval stage. Due to this, larval growth rates are used as a proxy for feeding conditions, duration time in the larval stage and therefore, indirectly, larval survival rates. Related to this, variability in growth rate potentially is a major factor responsible for recruitment variability (Houde, 1987). Since 2000, a steep decline in western Baltic spring spawning herring (WBSSH) recruitment numbers was observed, which reached a plateau in ~2005 with low-level recruitment ever since. Reasons for this decline, however, are unknown. It was hypothesized in this thesis that insufficient larval nutrition is a factor contributing to the low recruitment observed at present. Based on this, the nutritional situation and the growth rate of larval Atlantic herring were investigated in three important nursery areas of the WBSSH, the Kiel Canal, the Greifswalder Bodden and the Kiel Fjord in this thesis. Special emphasis was set on food availability, but also food quality aspects were included; biochemically derived larval growth rates based on RNA:DNA ratios served as response variable. Food availability investigations were not restricted to the larvae´s main prey items, different life stages of calanoid copepods, but examined different aspects of the planktonic food web including nutrient concentrations, protozooplankton and/or phytoplankton biomass, in order to receive a more holistic picture of the planktonic food web in western Baltic Sea watersheds. Additionally, more general ecological phenomena such as food-limited and size-dependent growth in larval fish were investigated. Finally, as significant effects of the Baltic Sea Index (BSI; a small scale climate index) on WBSSH recruitment were recently observed (Gröger et al., 2014), potential effects of the BSI on prey availability and larval growth were analyzed.

Effects of food quantity and food quality on larval growth

It is scientific consensus that the ability of larvae to find adequate prey resources is one of the main drivers for inter-annual recruitment variability (e.g., Hjort, 1914; Cushing, 1974a; Houde, 2008; Jørgensen et al., 2014). In the past, emphasis was set on food availability in relation to larval growth and survival. Lately, food quality aspects are increasingly in the focus of larval fish research.

Food quantity

In this thesis, significant relationships between prey availability and larval growth rates were observed (chapter 2 and chapter 5). Only seasonal mean larval growth rates of small herring larvae (< 14 mm) significantly correlated with prey availability, while this was not true for the large larvae (chapter 5). Additionally, the highest seasonal mean prey abundances (≥ 50,000 *Eurytemora* m^{-3}) showed no significant relationship between larval growth and larval size, i.e., small and large larvae grew at the same rates (chapter 4).

As zooplankton abundance strongly varies both during the season and inter-annually, and patchy distribution of zooplankton is common on small spatial scales (Klais et al., 2016, and references therein), the larvae´s fate is determined by their position in space and time. Especially small, low developed larvae depend on high prey availability for different reasons. First, they need to learn to successfully hunt. To do so, sufficient prey for practicing needs to be available, as success rate is very low in the beginning (3% to 10 %, Blaxter 1969). Due to low Reynold´s numbers, fish larvae are not able to search in large water volumes for prey. Additionally, low suction flow hampers feeding (China and Holzman, 2014; Holzman et al., 2015). If the larvae are not able to meet their energy demand, after some time (varying and depending on temperature) they reach the point-of-no-return where the larvae become incapable of feeding (Blaxter, 1965). Another aspect is that low prey abundances lead to a more active behavior of fish larvae, which might increase predations rates (Jørgensen et al., 2014). Keeping in mind all the difficulties fish larvae have to face and tackle concerning their nourishment might help to explain and understand their extremely low survival rates. Estimates of survival rates of marine fish larvae range from 1 per 1,000 (Houde, 1994) up to 1 per 100,000 (Le Pape and Bonhommeau, 2015). This can explain the high recruitment variability in marine fish stocks: even slightly increasing relative survival rates (e.g., 0.2 % instead of 0.1 %) can lead to strongly increasing recruitment numbers. On large scales, small relative changes are equivalent to large changes in absolute numbers (e.g., 2 billion compared to 1 billion).

Food quality

Though food quantity issues are frequently investigated in larval fish ecology, food quality was mostly neglected in field studies until recently (see Paulsen et al. 2014). In this dissertation, significant differences of larval fish food quality were observed over the season with significant effects on larval growth rates (chapter 1; Paulsen et al., 2014). Consequently, the question arises in how far this might affect recruitment. Larval development is characterized by a high neurosomatical index and high growth rates (Sargent et al., 1997; Tocher, 2003), which leads to a high demand for essential components in the food. Hence, early life stages might be most susceptible to essential fatty acid (EFA) limitation (Bell and Sargent, 1996; Litzow et al., 2006). Three fatty acids are known to be essential for marine fish: docosahexaeonic acid (DHA), eicosapentaeonic acid (EPA) and arachidonic acid (ArA). The food organisms commonly used for larval nourishment under aquaculture settings, *Brachionus* spp. and *Artemia* spp., are insufficient in terms of EFA concentrations for marine fish larvae. Consequently, this issue was extensively investigated in aquaculture research in the past (e.g., Sargent et al., 1999). In the meantime, several experiments were conducted investigating effects of EFAs on larvae of fish species that are not or rarely used for aquaculture, but are of high interest for the fisheries (e.g., Atlantic cod (*Gadus morhua*) and Pacific cod (*Gadus macrocephalus*) (Cutts et al. 2006; Copeman et al. 2010); yellowtail *Seriola quinqueradiata* (Ishizaki et al. 2001) or Atlantic herring (Bell et al. 1995)).

The quality of the fish larvae´s natural prey items also varies. Changes in relative concentrations of fatty acids in the phytoplankton are reflected in the fatty acid profiles of their grazers (Dalsgaard et al., 2003 and references therein). Furthermore, it was shown that phytoplankton fatty acids are incorporated by zooplankton and ultimately reflected in larval herring fatty acids (Fraser et al. 1989). Apart from differences of EFAs in the phytoplankton, some protists are able to synthesize EFAs from fatty acid precursors, thereby performing a qualitative trophic upgrading (e.g., Klein Breteler et al., 1999). As a consequence from varying EFA concentrations in the phytoplankton, it was observed that EFA concentrations in the same copepod species fluctuate in space and time (El-Sabaawi et al., 2009). Further, it was shown that taxonomic differences in terms of the EFA concentrations in copepods exist, resulting in significant changes in larval growth rates (Paulsen et al., 2014). Larval cod fed with copepod nauplii based on a DHA-rich dinoflagellate-diet grew significantly faster than larvae fed with copepod nauplii based on DHA-poor diatoms (St. John et al., 2001). Similarly, larval herring growth rates

decreased significantly when plenty of EFA poor ciriped nauplii were available, displayed in significantly decreasing EFA concentrations in the larvae (Paulsen et al., 2014). Moreover, larval herring growth correlated with EFA concentrations in the larvae, and EFA concentrations in the larvae correlated with EFA concentrations in the copepods (chapter 1). Given these facts, it seems likely that food quality also affects recruitment (see Bell and Sargent, 1996; Litzow et al., 2006). However, sufficient data for testing this hypothesis are lacking to date.

Effects of protozooplankton on growth of spring-hatched herring larvae

In the past, protozooplankton (PZP) was largely ignored as potential prey for larval fish (Montagnes et al., 2010), though it might significantly contribute to larval fish nutrition (e.g., Fukami et al. 2012; Bils et al., 2016). Busch (1993) demonstrated that larval herring from the Greifswalder Bodden exposed to a natural zooplankton assemblage fed up to a size of 12 mm on synchaetes, which are large protists. Additionally, it was shown that the presence of protists potentially supports the feeding of larval herring on other taxa (Illing et al., 2015). The effect of PZP on the nutritional condition of spring-hatched larval herring in the field was investigated in this thesis (chapter 3). As copepods graze on protozooplankton, the nutritional condition of adult female copepods was investigated in order to analyze potential effects of PZP on copepod production. The data indicated that PZP did not directly affect larval growth of spring-hatched herring larvae. Similarly, no significant effects of copepod abundance on larval growth rates were observed, possibly due to the very high copepod abundance during the investigation period ($\geq$ 40,000 m^{-3}). However, significant positive effects of PZP biomass on female copepod condition were detected, which suggests that the herring larvae may have indirectly benefited from PZP biomass. As nutritional condition in female copepods was found to correlate with egg production rates (Gorokhova, 2003), resulting increases in nauplii and copepodid abundances could have been beneficial for small herring larvae.

The observed non-significant effect of PZP on larval growth rates might be explained by the high availability of food (copepods) during the bloom situation in spring. Nevertheless, inter-annual variability in copepod abundance is high in spring (e.g., copepod peak abundances were found to vary by a factor of 36 (range: 5,000 m^{-3} and 180,000 m^{-3}) in the Kiel Fjord). It is possible that when copepods are relatively scarce, PZP is of importance also for the nutrition of spring-hatched larval herring. Nutritional conditions during the spring blooms in coastal waters fundamentally differ from the

conditions in nutrient poor waters further off-shore or from the autumn/winter season. Here, copepod abundances are lower, which might lead to increasing importance of PZP as potential prey for fish larvae. For example, modelled larval herring growth was lower compared to observed growth when PZP was omitted in the model (Bils et al., 2016). Further, it was shown that small herring larvae (8 mm - 12 mm) of North Sea autumn/winter-spawning herring also feed on dinoflagellates and diatoms (Denis et al., 2016). Though the microzooplankton-ichthyoplankton link is insufficiently investigated until today (e.g., Montagnes et al., 2010; Bils et al., 2016), accumulating evidence shows that PZP can be important for larval nutrition, especially for very small larvae and during times of scarcity.

Size-dependent larval growth

Larval fish rapidly grow until metamorphosis within a few months: while a spring-herring larva´s dry weight is 50 µg – 100 µg at hatch, the larva´s dry weight increases to 4000 µg – 5000 µg at the onset of metamorphosis. During the energy consuming metamorphosis, growth is strongly reduced. Thereafter, juveniles still grow, in relative terms, much faster than adults, but never at the high rates observed during the larval stage. Size-dependent instantaneous growth of fish larvae, i.e., increasing growth rates with increasing size, is a phenomenon frequently observed in larval fish growth field studies (e.g., Clemmesen et al., 1997; Rooker et al., 1997; Diaz et al., 2008). This pattern is explained by increasing swimming and hunting capabilities with size which are assumed to increase the ability to successfully feed under conditions of low prey abundances. If this is true, size effects in larval growth must decrease with increasing prey abundance. Indeed, we found that the size effect in larval growth rates decreased with increasing prey abundance, indicated by a decreasing slope of the regression line with increasing prey abundance (chapter 4). In contrast to chapter 2, where analyses were restricted to *Eurytemora*, showing best-fit regressions with larval growth rates, effects of prey availability on size-dependent growth were best explained using total zooplankton abundance. This effect was driven by the prey field composition of 2009, where relatively low *Eurytemora* abundances, but high abundances of cirriped nauplii occurred. In the Kiel Canal, cirriped nauplii were found to be prey items of larval herring (Donner, 2006). However, it was also shown that cirriped nauplii are of low quality and affect larval growth rates negatively compared to similar abundances of copepods (Paulsen et al., 2014). Therefore, it has to be concluded that high abundances of low quality food lead to

higher larval growth rates than low abundances of high quality food. A possible reason might be that encounter rates and also feeding success are higher at higher prey abundances, which then levels out potential negative effects of lower prey quality. At prey abundances ≥ 50,000 m^{-3}, regressions between larval growth and larval standard length became insignificant. These prey abundances are comparable with upper threshold concentrations of ~50,000 copepod nauplii m^{-3} until which positive effects on larval growth rates of small pelagics can be observed (Peck et al., 2013). It has to be kept in mind that these results are not directly transferable, as Peck et al. defined nauplii threshold concentrations, whereas the prey field in our study comprised juvenile and adult copepod stages as well as cirriped nauplii (sampled with 200µm mesh size, therefore slightly underestimated). To conclude, the advantage of being larger is especially pronounced at low to intermediate prey abundances (observed up to ~30,000 m^{-3}), since under these nutritional situations size dependent growth was observed.

Overall, the results of the studies in this dissertation highlight the importance of the nutritional situation especially for the very early life stages and the transition phase from internal to external feeding for larval herring, which is in line with classical larval fish ecology hypotheses such as the critical period (Hjort, 1914) and match-mismatch hypotheses (Cushing, 1974b). Due to the current debate of food quality effects on larval fish growth and recruitment, uncertainty exists on relative contributions of both food quantity and food quality on larval growth and survival. The outcome of the presented studies suggests, that particularly for the most sensitive stage of first feeding high prey availability in itself is of paramount importance especially for small larvae and might be more important than food quality. This can be explained by the fact that the larvae need to learn to successfully forage first and that high prey abundances ensure sufficiently high ingestion rates, so that even larvae with initially low feeding success may be able to better meet the demand of essential components in the food (e.g., essential amino and fatty acids) and, hence, survive. Logically, one can expect that conditions of combined high prey quantity and quality lead to highest larval survival rates. Generally, times of plenty (spring) and times of scarcity (autumn/winter) can be distinguished which also influence the importance of food quality. The usability of PZP as prey for larval depends on the larva's size: the smaller the larvae are at hatch, the more important PZP potentially is. Results from these studies concerning larval growth in relation to prey availability are expected to be globally transferable, as marine fish larvae in general face the problem of being less developed and having a small size when they start first feeding.

Comparison of WBSSH nursery areas

The WBSSH spawns in coastal waters of the Belt Sea of Denmark and Germany in spring. Common features of the spawning and larval nursery areas are a low salinity (but not lower than 4; Brandhorst, 1955) and a shallow water depth. Further, these waters are typically enclosed, such as fjords, bays, estuaries or canals. In this dissertation, three different habitat types were included for analyses of nutritional situation and larval growth rates: an artificial canal (Kiel Canal), an inner fjord (Kiel Fjord) and a large but shallow bay (Greifswalder Bodden). The latter is considered to be one of the most important nursery areas of WBSSH (Biester, 1989; Oeberst et al., 2009).

In light of the ongoing low recruitment of WBSSH, the prey field for larval herring in the Greifswalder Bodden was investigated in 2008 and 2009 (Hesse, 2010). The author suggests that the larvae were presumably not food-limited, i.e., larvae potentially found sufficient prey in the Greifswalder Bodden in May, while prey abundances were rather low earlier in the season during the years of investigation. As prey quantity did not seem to be limited in the Greifswalder Bodden, it was hypothesized that food quality might be an issue for larval herring in the western Baltic Sea. Further, it was assumed that the Greifswalder Bodden provides paramount qualitative nutritional conditions compared to other nursery areas, explaining its potential importance for recruitment. In the dissertation at hand, the Greifswalder Bodden and the Kiel Canal were compared with respect to their qualitative and quantitative nutritional conditions provided to larval herring. Furthermore, it was assessed if food quality determined as EFA concentrations in the prey can significantly affect larval herring growth rates and thereby potentially survival rates (chapter 1). Overall, the results indicated that EFA concentrations in the prey can significantly affect larval herring growth rates in the investigation areas. Further, larval growth rates correlated significantly with DHA and EPA concentrations in the larvae. Contrasting to former assumptions, it was not observed that the Greifswalder Bodden provides qualitatively better food than the Kiel Canal.

Given that growth conditions for small herring larvae did not differ between the Greifswalder Bodden and the Kiel Canal, the question arose of what might explain the importance of the Greifswalder Bodden as a nursery area? When larval abundance data from the Kiel Canal and the Greifswalder Bodden from 2011 were compared, the Kiel Canal was relatively (N m^{-3}) more productive. The combination of both high prey availability for small larvae and high larval abundance suggested that the Kiel Canal

might generally be more suitable as a nursery area for larval herring. However, when total production was estimated, the large habitat of the Greifswalder Bodden produced more than 20fold larval herring than the Kiel Canal (chapter 1).

Larval herring growth patterns observed over the season in the three investigated habitats were not uniform: while larval growth rates increased in the Greifswalder Bodden and the Kiel Fjord with the proceeding season, larval growth in the Kiel Canal increased in the first part of the season and decreased thereafter. High larval growth rates in the late season in the Greifswalder Bodden coincided with high larval survival at that time: results from cohort analyses showed that recruits in the Greifswalder Bodden predominantly stem from the second cohort, which hatched between mid-May and beginning of June (Polte et al., 2014). Similarly, using otolith analyses it was shown that the survivors stem from the early season in Kiel Canal, when the nutritional situation is most favorable (Hesse, 2012). Both examples suggest that prey availability and growth are crucial in determining larval herring survival rates.

It was observed that abundances of the numerically dominant copepod species, *Eurytemora affinis*, strongly decreased at 15°C and that a similar pattern was observed in larval growth rates (chapter 2). Similar observations towards the prey field´s phenology have been made in the Schlei Fjord (Hirche, 1992), which is also described as an important nursery area for WBSSH (Weber, 1971). Hence, the findings might be transferable to other brackish water nursery areas in the western Baltic Sea with *E. affinis* being the major prey item for the larvae. In the Greifswalder Bodden, however, *Eurytemora* seems to be frequently outcompeted by *Acartia* spp. . While Postel et al. (1989) found *Eurytemora* being the dominant copepod species in the Greifswalder Bodden, subsequent studies found *Acartia* spp. being the numerically dominant species (Busch, 1993; Hesse, 2010). Results from both Busch and Hesse indicated that *Eurytemora* might be negatively selected by the herring larvae. In contrast to this, herring larvae were found to feed on calanoid copepods in the Schlei Fjord and the Kiel Canal, where *Eurytemora* is the dominant copepod species.(Schnack, 1972; Donner, 2006). Similarly, larval growth rates in the Kiel Canal were high at times of *Eurytemora* dominance (chapter 2) and very low when abundances of *Acartia* spp. dominated. Additionally, it was observed in feeding experiments that *Acartia* spp. was negatively selected, an effect supposed to be caused by high escape burst speeds of Acartia (Checkley, 1982). This finding is supported by own observations of both *Eurytemora* and *Acartia* spp., with *Acartia* being much more mobile and faster than *Eurytemora*.

The relationship of seasonal mean growth rates of small larvae from the Kiel Canal, the Greifswalder Bodden and the Kiel Fjord was found to be linear with only low variability (chapter 5). While in the Kiel Canal *Eurytemora* was the dominant calanoid copepod species, *Pseudocalanus* was dominant in the Kiel Fjord, and in the Greifswalder Bodden a combination of both *Acartia* and *Eurytemora* was found with varying dominance during the course of the season. This result suggests that larval herring seem to be plastic and are able to adjust to different dominant prey items and do not depend on the occurrence of a certain species.

To conclude, despite the different hydrographical features of the investigated nursery areas (varying depths, different salinities), no habitat offered consistently high quality habitat supporting relatively high larval growth rates, indicating that none of the three habitats, including the Greifswalder Bodden, continuously provides paramount nutritional conditions. However, different time windows during the season can be of importance for survival windows in the different habitats, e.g., end of April to end of May in the Kiel Canal and mid-May to beginning of June in the Greifswalder Bodden. Generally, inter-annual variability of mean growth rates of small larvae was higher than spatial variability, and temporal trends were akin (chapter 5).

Fish larvae in artificial waterways

Major parts of the investigations of the dissertation at hand have been conducted in the Kiel Canal, which is an artificial waterway with the highest ship traffic frequency in the world (www.kiel-canal.org). During the investigation period (2007 to 2012), the number of ship passages ranged between ~80 and ~115 per day (container ships of up to ~130 m length and 9 m draught at 11 m water depth). Due to this, general considerations about larval fish in artificial seaways might be transferable to others, such as Panama- or Suez-Canal, but also small interior canals. Three aspects might be generalized for artificial seaways: 1) locks inhibit permanent currents and 2) passing ships cause temporally occurring, extraordinary strong currents 3) passing ships cause temporally occurring underwater noise. Considering turbulence effects on larval nutrition (e.g., Cury and Roy, 1989), it is worth to highlight the special conditions caused by the shipping traffic in artificial seaways. Normally, permanent currents are inhibited by the locks. During a ship passage, however, both larvae and prey experience very strong currents. Due to this, prey patches and larvae are scattered and re-organized. As the ship passes by and the current weakens, encounter rates between both larvae and prey increase by moderate water

movements. Due to frequent shipping traffic and consequent mixing of the water column, feeding conditions are probably quite homogenous over time. Therefore, observations on individual growth variability of herring larvae in the Kiel Canal might be largely caused by differences in individual foraging skills rather than the spatial location of the larvae themselves, i.e., within a prey patch (leading to high growth rates) or between prey patches (starvation). Apart from this it is remarkable that fish larvae, normally referred to as highly sensitive organisms, can be resistant against the strong physical forces they are facing in terms of strong currents or extreme underwater noise. In line with that it was previously experimentally shown that larval common sole (*Solea solea*) were non-sensitive concerning noise exposure in the range of pile-driving sound normally occurring during the construction of wind parks (Bolle et al., 2012). The example of the Kiel Canal shows, that artificial canals can successfully contribute to recruitment (Brandhorst, 1955; own observations/catches of juvenile herring shoals) despite frequent noise exposure and strong currents caused by passing container ships.

Food limitation, density dependence and their effects on WBSSH recruitment

In the Kiel Canal, larval herring growth rates were food-limited at the end of the season in each year of investigation (chapter 2). Similar zooplankton phenology was observed in other nursery areas of WBSSH, e.g., the Schlei Fjord (Hirche, 1992), most likely with comparable consequences on larval herring growth rates. In light of this finding and combined with the classical assumption that food limitation is critical for recruitment the question arises why these habitats are established spawning and nursery areas for WBSSH? First of all, herring is a very plastic species, and exact homing, i.e., that herring return to spawn to exactly the site they hatched in, is unlikely. As the nutritional situation is beneficial in some of the other nursery areas (e.g., Greifswalder Bodden, Kiel Fjord) late in the season, selection pressure is not uniform. In the Kiel Canal the nutritional situation was recurrently found being favorable in the first part of the season when peak hatch occurs, the probability to survive might be relatively high during this highly sensitive phase. Due to the small water body of the canal and the frequent mixing by the shipping traffic, the water warms quickly in spring. The combination of extraordinary high prey abundances and relatively warm water provides ideal growth conditions for the larvae, at least temporally. As food limitation occurs towards the end of the season, when at least the first cohort has grown to large larvae, it is expected that this is not as critical for recruitment as food limitation in the beginning of the season.

Consequently, results from otolith analyses and length frequency distributions indicated that the larvae either need to hatch early or need to grow at high rates to reach a size that is of certain robustness against unfavorable growth conditions when food limitation occurs (Hesse, 2012). According to the results of the study, this length might be between 14 mm - 18 mm.

Stock recruitment is potentially affected by various factors, including maternal nutrition, egg mortality, larval and juvenile nourishment and predation (for references, see introduction). For WBSSH, recruitment bottlenecks are unknown at present. Predation can significantly affect recruitment variability (Houde, 2008, and references therein). Unfortunately, effects of predators on larval herring mortality are rarely investigated in the western Baltic Sea. It was shown that moon jellyfish (*Aurelia aurita*) can significantly decrease larval abundance in the Kiel Fjord (Möller, 1984). However, jellyfish occurrence was not found to temporally match with larval herring occurrence in the Kiel Canal (own observations) and the Greifswalder Bodden (Kotterba, 2015). Potential predators known to occur in the enclosed nursery areas are pikeperch (*Stizostedion lucioperca* L.; Hansson et al. 1997) and river perch (*Perca fluviatilis* L.; Lappalainen et al. 2001). Recently, potentially strong grazing pressure on herring eggs by three-spined sticklebacks (*Gasterosteus aculeatus*) and river perch was observed in the Greifswalder Bodden (Kotterba et al., 2014). Though three-spined sticklebacks strongly prey upon herring eggs (Kotterba et al. 2014), larval herring was rarely eaten (Kotterba 2015). Total egg mortality caused by grazing pressure is difficult to estimate, but might have significant effects on the number of hatchlings.

Apart from predation, food availability is considered as major recruitment driver (e.g., Hjort, 1914; Jørgensen et al., 2014; Le Pape and Bonhommeau, 2015). A significant linear relationship between growth rates of small larvae (< 14 mm) and prey availability was observed in this dissertation (chapter 5). The N20-index, which is the total number of herring larvae with 20 mm caught during a season in Greifswald Bay (Oeberst et al., 2009) and used to estimate recruitment strength before catch data of respective year classes exist, was twice as high in the 1990´s compared to 2000-2015, which corresponds to the observed recruitment development. Similarly to the larvae´s preferred prey, the diet of juvenile herring is primarily based on zooplankton, especially calanoid copepods and cladocerans (e.g., Flinkman et al., 1992; Bernreuther, 2007; Arula et al., 2012). Peak zooplankton abundance (i.e., cladocerans and adult copepods) in German Baltic Sea offshore waters were twice as high from 1991-1995 and 1999 compared to the period

between 2000 and 2012 (Wasmund et al., 2013), which is in line with the temporal development of WBSSH recruitment (including the order of magnitude). Further, weight-at-age 1 is significantly lower in WBSSH compared to same-age conspecifics from the Kattegat/Skagerrak area (Tab.1). This is true for periods of both low and high WBSSH recruitment (compare Fig. 1; Tab.1). Le Pape and Bonhommeau (2015) hypothesized that density-dependent mortality in juvenile fish is common, while no density effects in the egg and larval phases are expected. Food limitation is assumed to be of major importance in determining the carrying capacity of nursery areas (Le Pape and Bonhommeau, 2015). However, such evidence was found only in groundfish, but not in pelagic fish species (Iles and Beverton, 2000). A major reason for this finding probably is the limited habitat space available for bottom dwellers. Similarly, in restricted waterbodies such as bights and basins density-dependent effects may also be possible for pelagic fish species, e.g., due to limited food resources and restricted possibilities to migrate. For example, condition of clupeids in the Bornholm Basin in the central Baltic Sea was shown to be density-dependent (Casini et al., 2011). Similarly to the clupeid stocks in the Bornholm Basin, juveniles of WBSSH inhabit comparatively small waterbodies in the Belt and Arkona Seas. Iles and Beverton (2000) showed that recruitment variability is lower in populations with large density-dependent effects (*concentration hypothesis*). Compared to marine fish stocks spawning in the open seas (e.g., Norwegian spring spawning herring, Barents Sea cod (skrei) and saithe (*Pollachius virens*)), inter-annual recruitment variability in WBSSH is remarkably low (Fig. 1; compare www.ices.dk), which additionally hints to density-dependent effects on recruitment.

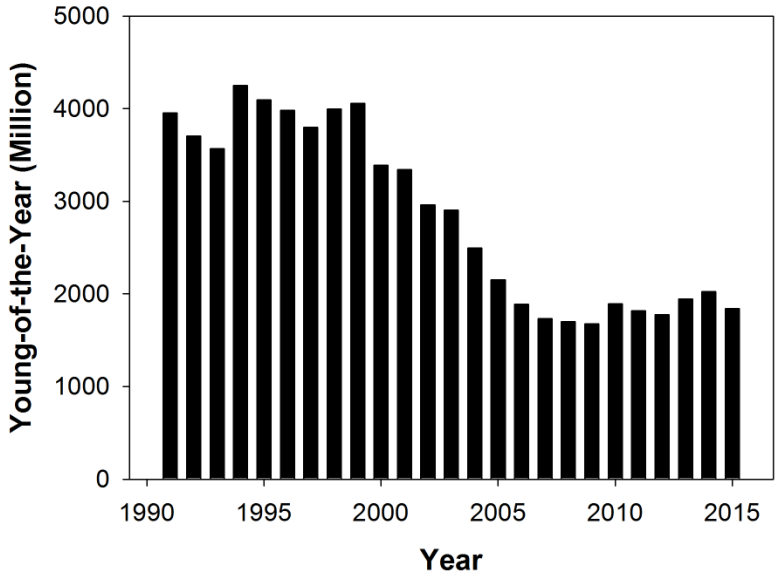

Fig. 1: Recruitment of western Baltic spring spawning herring

Tab. 1: (**a)** Period in the 1990´s, where herring recruitment was high. (**b**) Period from 2001 on, were recruitment decline started. Weight-at-age (WAA) is mean individual weight (g) of the respective period. Recruitment is number of individuals * 1,000. ± indicates standard deviation. Data derived by ICES.

(a) 1993-2000

	Subd. 22-24	Div. IV-IIIa	pairwise comparison
WAA 0	15.2 ± 6.6	18.1 ± 5.7	$p > 0.05$
WAA 1	24.0 ± 3.7	40.2 ± 10.5	$p < 0.01$
recruitment	3,931,742 ± 226,173		

(b) 2001-2013

	Subd. 22-24	Div. IV-IIIa	pairwise comparison
WAA 0	13.8 ± 4.9	14.5 ± 6.4	$p > 0.05$
WAA 1	24.8 ± 9.5	48.7 ± 14.2	$p < 0.001$
recruitment	2,288,433 ± 630,018		

In combination, the low inter-annual recruitment variability, changing prey availability with following recruitment numbers and the recruitment independent low weight-at-age 1 point to density-dependent food limitation, and recruitment close to carrying capacity in WBSSH. A significant regression between yearly mean abundance of the numerically dominant copepod genus in Kiel Fjord, *Pseudocalanus* spp. (*Pseudocalanus* abundance explained 73 % of total copepod abundance between 2005 and 2013), and WBSSH recruitment was observed (Fig. 2). Regressions with other copepod species as independent variable were insignificant. Explained variability was the higher the more months per year were included into the analyses, leading to a best-fit regression when yearly means of *Pseudocalanus* abundance were taken as independent variable. Yearly means of copepod abundance integrate prey effects on both larval and juvenile stages. This result further supports the assumption that both early larval and juvenile stages are important in determining recruitment variability in WBSSH, which is potentially strongly affected by prey availability.

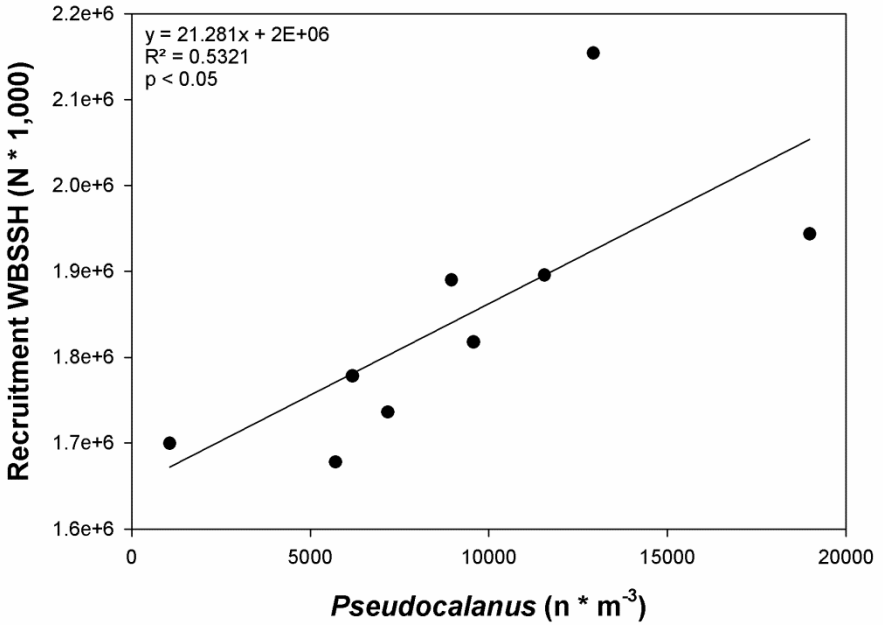

Fig. 2: Relationship between western Baltic spring spawning herring recruitment and the yearly mean abundance of *Pseudocalanus* spp., which was the numerically dominant copepod genus (*Pseudocalanus* abundance explained 73 % of total copepod abundance) between 2005 and 2013

Mature WBSSH conduct extensive feeding migrations into the Kattegat/Skagerrak area during summer. Migration behavior is common and wide spread throughout the animal kingdom, ranging from insects (e.g., monarch butterfly (*Danaus plexippus*; Urquhart and Urquhart, 1978) or bogong moth (*Agrotis infusa*; Common, 1954)) and fishes (e.g., bluefin tuna (*Thunnus thynnus*; Aranda et al., 2013) or white sharks (*Carcharodon carcharias*; Block et al., 2011)) over birds (e.g., Arctic tern (*Sterna paradisaea*; Egevang et al., 2010) or white stork (*Ciconia ciconia*; Berthold et al., 2004)) to mammals (e.g., wildebeest (*Connochaetes* spp.; Musiega and Kazadi, 2004) or whales (Block et al., 2011)), both in terrestrial (Berger, 2004) and aquatic (Block et al., 2011) ecosystems. The two major drivers for migration behavior are food availability and reproduction (see references above). This is also true for WBSSH, were feeding and spawning are described to be responsible for the migration behavior (Klinkhardt, 1996; Muus and Nielsen, 1999). In light of the findings described above, food limitation in the western Baltic Sea is possibly the main driver for the extended feeding migrations of the WBSSH stock into the Kattegat/Skagerrak areas.

Climate and recruitment variability

Mid to large-scale climate indices were shown to correlate with marine fish stock development/recruitment in Europe and Latin America, which was especially, but not exclusively, demonstrated for clupeid fish (e.g., Alheit and Hagen, 1997; Beaugrand et al., 2003; Alheit and Niquen, 2004; Gröger et al., 2010; Gröger et al., 2014). Historically, strong fluctuations in stock size of Atlantic herring occurred at the Swedish coast. Periods with exceptionally high herring catches, called *Bohuslän periods* and lasting several decades, were characterized by severe winters with extremely cold air and water temperatures (Alheit and Hagen, 1997). In contrast, the interim-periods, where herring fishery played economically only a minor role, were climatologically mild and lasted for 50 or more years. However, at least in recent decades winter temperatures did not play a significant role as recruitment driver for WBSSH, as no differences between the two periods of high (1991-2000) and low recruitment (2001-2013) existed (2.9°C vs. 2.8°C; data basis: means of the respective years calculated from three values: 1 Jan, 1 Feb, 1 Mar; data derived from www.bsh.de). Further, stock recruitment and winter temperatures did not correlate (1992-2013). Similarly, neither differences in summer temperatures between low and high recruitment periods (17.0°C vs. 17.6°C; data basis: means of 1 Jul,

1 Aug, 1 Sep of the respective years; data derived from www.bsh.de), nor a correlation between summer temperature and recruitment (1992-2013) were observed.

Moreover, effects of the Baltic Sea index on larval growth rates and copepod abundances were investigated (chapter 5). The Baltic Sea Index (BSI) is a broad-scale climate index (for further information, please see Gröger et al., 2014). However, neither effects of winter BSI (Jan-Mar), nor spring-BSI (Apr-Jun; larvae season) on larval growth and prey availability were observed. This contradicts results by Gröger et al. (2014), who found significant effects of the winter-BSI (Jan-Mar) on recruitment in the Greifswalder Bodden, though with one year delay.

It was hypothesized that climate-mediated changes in phytoplankton EFA production contribute to community transitions by lipid-rich pelagic and lipid-poor demersal fishes (EFA limitation hypothesis; Litzow et al., 2006), which were observed in ecosystems of the boreal Pacific and Atlantic Oceans (Cushing, 1980; Anderson and Piatt, 1999; Hunt et al., 2002; Choi et al., 2004). Here, especially the importance of EFAs on larval fish growth and survival is highlighted as a potential reason driving such regime shifts (Litzow et al., 2006). EFA concentrations correlate with total lipids in marine fishes (Litzow et al., 2006). Consequently, larvae of lipid-rich pelagic fishes have a higher demand for EFAs, as their total lipids are higher compared to larvae of lipid-poor demersal fishes. For example, total lipids are mostly in the order of 25% to 40% of dry weight in larval Atlantic herring (Paulsen, 2010), while total lipids are ~5% to 9% of dry weight in larval Pacific cod (*Gadus macrocephalus*) (Copeman and Laurel, 2010). Thereby, EFA availability might contribute to the observed regime shifts. As EFA availability is based upon phytoplankton production, changes on the level of unicellular organisms such as phytoplankton might affect entire ecosystems up to the level of piscivores. For example, low availability of lipid-rich forage fish reduces growth rates of seabird nestlings and reproductive success (Wanless et al., 2005).

In light of climate change, potential changes in the planktonic food web are a matter of debate. Both pCO2 and temperature are predicted to increase in the future (IPCC 2013). Simple 2-trophic level systems showed strong negative responses of elevated pCO2 concentrations (750 ppm) on both total fatty acids and long-chained polyunsaturated fatty acids (with major contributions by EFAs) in diatoms and copepods (Rossoll et al., 2012). Contrary, more complex experimental designs demonstrated that species richness and complex trophic interactions as well as natural fluctuations in pCO2 strongly dampen such effects (Rossoll et al., 2013). Temperature increase under global

warming may affect the planktonic food web in different ways. For instance, experimental studies have shown that phytoplankton mean size may decrease under warming (Peter and Sommer, 2012), which could affect copepod nutrition. Moreover, warming could induce shifts in zooplankton phenology with subsequent effects on recruitment (Edwards and Richardson, 2004). Furthermore, temperature increase has already been shown to cause shifts in the geographic range of species, particularly at the margins of their thermal tolerance range (Perry et al., 2005). Consequently, in a given habitat dominant copepod species might change and therefore copepod phenology might change as a consequence. For example, a northward shift in the distribution range of *Calanus finmarchicus* and its replacement by *Calanus helgolandicus* was shown to increase the probability of a mis-match situation between *Calanus* and larval cod occurrence in the North Sea, with implications for recruitment (Beaugrand et al., 2003).

According to the basic research questions of this thesis, it can be concluded that the potential importance of the Greifswalder Bodden for WBSSH recruitment cannot be explained by constantly qualitatively better food compared to other nursery areas. During a three-week period at the end of the season, DHA concentrations in the prey were significantly higher in the Greifswalder Bodden compared to the Kiel Canal. However, prey quantity was significantly higher in the Kiel Canal compared to the Greifswalder Bodden in the early season. Overall, none of the two compared habitats provided constantly better growth conditions. Results from chapter 2 demonstrated that food-limited growth can be a recurrent issue for larval herring in coastal habitats; in the Kiel Canal, herring larvae were food-limited by the end of the season in each year of investigation. Results from an otolith study gave support to the assumption that this food limitation affects survival of the larvae. Chapter 3 showed that protozooplankton does not significantly affect larval herring growth rates when large quantities of larger prey, like copepods, are available. Food availability strongly affects the phenomenon of size-dependent growth, as shown by the study in chapter 4. Here, size-dependent growth exclusively occurred at low to intermediate prey abundances. Chapter 5 investigated spatial variability of larval growth rates. With one exception (the channel-like Strelasund area of Greifswalder Bodden), larval growth variability was higher temporally than spatially. Finally, no significant effects of the BSI on larval growth rates were observed.

Outlook

This dissertation investigated effects of the nutritional situation on larval herring growth rates. Significant effects of both prey availability and prey quality on larval growth were found. Furthermore, especially small larvae seemed to be affected by prey availability. Additionally, evidence was found that recruitment of WBSSH is significantly affected by prey availability. This highlights the importance of a regularly conducted mesozooplankton monitoring to explain WBSSH stock development. Best-fit regression was achieved by using yearly means of the dominant copepod species. Therefore, a higher temporal resolution (monthly) is more important than a monitoring of high spatial resolution, but poor temporal resolution. This is due to the fact that zooplankton variability is higher in time than in space (Klais et al., 2016).

Individual based models (IBM) have the potential to help explaining recruitment variability by predicting the development of single individuals under given biotic and abiotic conditions. However, the quality of IBM´s significantly depends on their parametrization. In this dissertation, large data sets of field-based larval herring growth rates along with biotic and abiotic concomitant parameters were generated. As such field data are essential for IBM-validation, they are of value beyond the analyses so far conducted.

Growth models are calculated on basis of experimental and/or field data and are available for larval stages of some marine fish species, e.g., Atlantic cod (Folkvord, 2005). Unfortunately, this is not the case for Atlantic herring. This is why in the dissertation at hand a multi-species model (Buckley et al., 2008) had to be used. However, multi-species growth models struggle with a larger error compared to single-species growth models. Therefore, in a next step field-based larval herring standardized RNA:DNA ratios will be used along with prey field, temperature, photoperiod and larval size in order to calculate a general additive model (GAM)-based larval herring growth model. Cumulative size distributions are available and will be used to validate the model.

The practical application of larval fish growth rates and survival windows

Currently, the European Union aims to protect the marine environment across Europe more effectively than in the past. The goal is to achieve a good environmental status (GES) of European coastal waters until 2020 (Marine Strategy Framework Directive). Various parameters are evaluated in order to determine the GES, ranging from water quality (eutrophication, oxygen) over low trophic levels (plankton) to high trophic levels

(sea birds, marine mammals). One of the parameters to be evaluated is the mesozooplankton´s mean size and total stock. The mesozooplankton is the crucial link ensuring the energy flow from primary production to higher trophic levels. In order to define good and bad conditions with respect to the mesozooplankton community, the nutritional condition of adult zooplanktivorous fish was suggested as a response variable (Gorokhova et al., 2013). Adult WBSSH are highly migratory and conduct feeding migrations into the Kattegat and Skagerrak area. Consequently, they are not suitable as a response variable for the mesozooplankton occurring at the German Baltic Sea coast. Further, though juvenile WBSSH remain in the investigation area, weight-at-age 1 shows only little fluctuations and is therefore not suitable. As both the condition of adult and juvenile WBSSH are inappropriate as response variables to copepod abundances in order to determine the mesozooplankton GES, it was decided to choose larval herring growth rates and survival in order to determine critical thresholds of copepod abundance that might ensure high survival rates. Larvae and early juveniles are a suitable indicator for the local zooplankton situation, as their mobility is restricted. To test the general suitability of this approach, data from the Kiel Canal were used. Here, sampling of herring larvae over the whole season is ensured due to the enclosed water body. Larval growth rates were determined biochemically (RNA:DNA), while survival time windows were detected by conducting otolith analyses. Generally, an asymptotic relationship between the abundance of the dominant copepod species (*Eurytemora affinis*) and larval herring growth rates was observed, with increasingly positive effects on larval growth rates up to 10,000 *Eurytemora* m^{-3}. Furthermore, only below 10,000 *Eurytemora* m^{-3} negative growth rates were observed, suggesting that above 10,000 *Eurytemora* m^{-3} all analyzed larvae were able to successfully feed (Fig. 4). Similarly, seasonal mean larval growth was constantly low below 10,000 *Eurytemora* m^{-3} but increased with further increasing prey abundance (Fig. 3). These results suggest a critical lower prey abundance threshold for larval herring of ~10,000 prey items m^{-3}. In line with these findings, otolith analyses demonstrated that ~11,000 *Eurytemora* m^{-3} were sufficient to ensure larval survival (2007), while ~10,000 copepodids m^{-3} comprising all copepod species was not sufficient (2009 and 2014) (Tab. 2). Based on results from the Kiel Canal it is concluded that abundances of > 10,000 m^{-3} of the dominant calanoid copepod species are advantageous for larval herring. The next step which is currently under way is to test for these relationships in the Kiel Fjord. An additional validation might be politically

necessary in the Greifswalder Bodden in order to ensure general validity of the determined threshold value to serve as a national zooplankton GES-indicator.

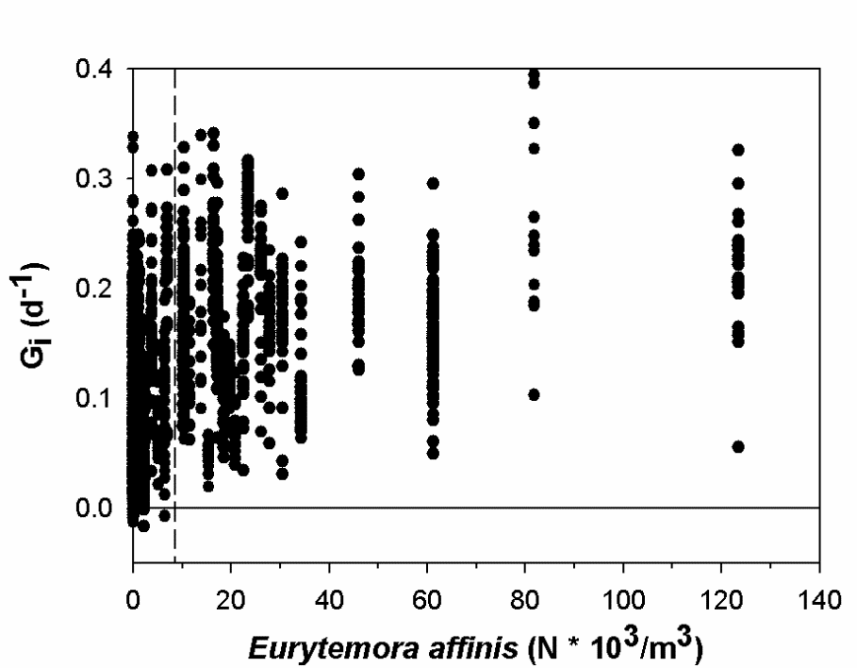

Fig. 3: Asymptotic relationship between larval growth and the abundance of the dominant calanoid copepod in the Kiel Canal, *Eurytemora affinis*. Larval growth increases up to ~10,000 *Eurytemora* individuals m^{-3}. Negative larval growth rates exclusively occurred at *Eurytemora* abundances < 10,000 m^{-3}.

Tab. 2: Average copepod abundances during survival and non-survival time windows for herring. Survival dates were determined by otolith analysis of juvenile herring caught in the Kiel Canal. While average copepodid abundance of ~11,500 *Eurytemora* were sufficient to allow larval survival (2007), ~10,000 total copepodid abundance was insufficient to support survival (2009, 2014).

Year (time window)	Avg. total copepodid abundance [N m^{-3}]	Min. total copepodid abundance [N m^{-3}]	Avg. *Eurytemora*-copepodid abundance [N m^{-3}]	Min. *Eurytemora*-copepodid abundance [N m^{-3}]
2007 (survivors)	11,945	8,839	11,625	8,316
2007 (non-surving individ.)	2,160	361	59	13
2009 (survivors)	33,433	33,065	32,674	32,330
2009 (non-surving individ.)	9,858	3,526	392	98
2011 (survivors)	39,891	6,907	37,246	6,172
2014 (survivors)	75,438	72,499	73,968	71,519
2014 (non-surving individ.)	9,915	1,089	3,788	0

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Declaration on oath

I hereby declare, on oath, that I have written the present dissertation

"**Prey quantity and quality effects on larval Atlantic herring (*Clupea harengus* L.) growth in the western Baltic Sea"**

by my own and have not used other than the acknowledged resources and aids.

Kiel, 12/08/2016 Matthias Paulsen

Declaration of individual scientific contributions to the manuscripts included in this dissertation

The chapters of this thesis are partly published (chapter 1, chapter 2) or in preparation to be published in scientific journals and contain multiple authorships. The list below serves as a clarification of my personal contributions to the publications.

Chapter 1

*Nutritional situation for larval Atlantic herring (*Clupea harengus *L.) in two nursery areas in the western Baltic Sea.* -Published in ICES Journal of Marine Sciences

The study was developed by Matthias Paulsen (MP), Dr. Arne M. Malzahn (AMM), Dr. Catriona Clemmesen (CC), and Prof. Dr. Cornelius Hammer (CH). Dr. Christian von Dorrien provided funding. MP and Dr. Patrick Polte (PP) conducted sampling. MP performed the analyses of all samples as well as the statistical analyses of the data. MP, CC, AMM and CH discussed the results. The manuscript was written by MP, CC, AMM with support from PP and CH.

Chapter 2

Food-limited growth of larval Atlantic herring Clupea harengus *recurrently observed in a coastal nursery area.* -Published in Helgoland Marine Research

The study was developed by MP, AMM and CC. MP conducted sampling in 2009, 2010 and partially in the season 2011, PP conducted sampling in the Greifswalder Bodden. MP performed RNA:DNA analyses in herring larvae from the seasons 2009-2012 and analyzed mesozooplankton samples of the seasons 2007-2009 and 2011, 2012. MP, CC, AMM, PP and CH discussed the results. The manuscript was written by MP, CC, AMM with support from PP and CH.

Chapter 3

Preliminary insights into effects of protozooplankton on growth of spring-hatched herring larvae. –To be submitted

The study was developed by MP, CC and Nicole Aberle (NA). Ramona Beckmann (RB) conducted sampling, RNA:DNA and microzooplankton analyses, MP sorted the mesozooplankton. RB performed the statistical analyses. RB, MP, CC and NA discussed the results. The manuscript was written by RB, MP, NA and CC.

Chapter 4

Size-dependent growth in larval fish is not an issue in a world of plenty.-Accepted as CIESM conference paper

The study was developed by MP and CC. MP conducted sampling in 2009, 2010 and partially in the season 2011. MP performed RNA:DNA analyses in herring larvae from the seasons 2009-2012 and analyzed mesozooplankton samples of the seasons 2007-2009 and 2011, 2012. MP performed the statistical analyses. MP, CC and AMM discussed the results. The manuscript was written by MP, CC and AMM.

Chapter 5

*Spatial variability of larval Atlantic herring (*Clupea harengus *L.) growth rates in the western Baltic Sea.* –In prep.

The study was developed by MP and CC. Sampling was conducted by PP and partially MP. MP analyzed all samples and performed the statistical analyses. The manuscript was written by MP with contributions from CC.

Chapter 6

Effects of the Baltic Sea Index (BSI) on larval herring growth and copepod abundance in the western Baltic Sea. –In prep.

The study was developed by CC and MP. Sampling was conducted by MP and PP. MP analyzed RNA:DNA from larvae originated from the Kiel Canal, Kiel Fjord and Greifswalder Bodden in the years 2009-2012. MP performed the statistical analyses. MP and CC discussed the results. The manuscript was written by MP with contributions from CC.

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