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Original Article

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

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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 spring 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. While 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: DHA, EPA, essential fatty acids, food quality, 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 large 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 (Rosenthal and Hempel, 1970; Cushing, 1974; Sinclair and Tremblay, 1984; Anderson, 1988; Sinclair, 1988; Cury and Roy, 1989; Cushing, 1990; Buckley and Durbin, 2006). However, it is still not possible to reliably predict recruitment simply based on 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 affect development, growth, and survival of marine larval fish (Mourente *et al.*, 1991; Bell *et al.*, 1995; Furuita *et al.*, 1998; Copeman *et al.*, 2002; Van Anholt *et al.*,

2004; Cutts *et al.*, 2006; Copeman and Laurel, 2010). These dietary components are mainly synthesized by the phytoplankton and subsequently transferred to higher trophic levels. Therefore, the basic EFA pattern in marine foodwebs is determined by the planktonic primary producers. Two of the most important and widespread phytoplankton classes, diatoms and dinoflagellates, differ basically 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 (Morris, 1981; Kattner *et al.*, 1983; Falk-Petersen *et al.*, 1998). 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 with 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 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 Greifswalder Bodden (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 (Neb, 1952; Weber, 1971; Aneer *et al.*, 1983; Aneer, 1989; Biester, 1989a). 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 *Eurytemora affinis*, an important larval herring food source (Schnack, 1972).

In the 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 (Clemmesen, 1994; Caldarone *et al.*, 2003; Malzahn *et al.*, 2007b; Grote *et al.*, 2012; Meyer *et al.*, 2012). Standardizing 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). An 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).

Based on 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; Figure 1a), whereas the other sampling site was located in the GWB (ICES Subdivision 24; Figure 1b). All samples were collected between April and June 2011 during the seasonal occurrence of larval herring.

Herring larvae were sampled with a bongo net (60 cm diameter, 335 and 500 µm mesh size, respectively) that was heaved in an oblique haul. All larvae were frozen on board within 30 min of the haul. Before further analysis, larval standard length was measured to the lower 0.1 mm and freeze-dried for 24 h using a freeze drier (CHRIST ALPHA 1–4 LSC). Thereafter, 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
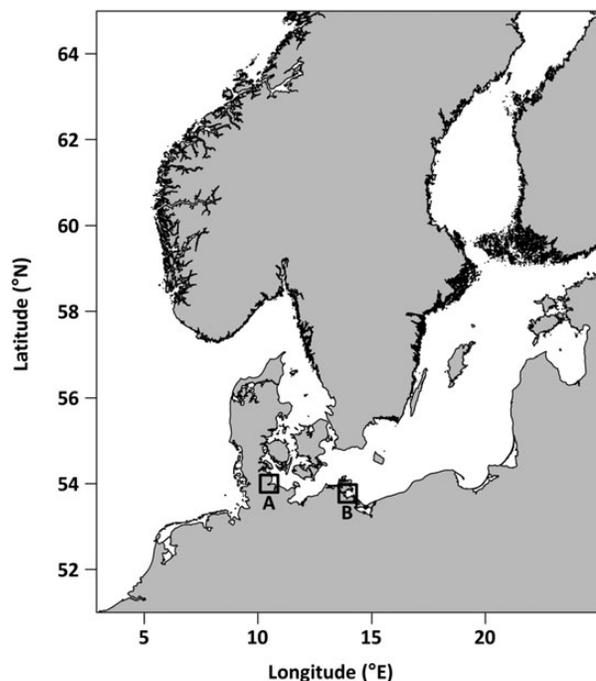

Figure 1. Sampling sites for larval herring and copepods in the western Baltic Sea during spring season. (a) KC; (b) GWB.

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 for *Acartia* species, some remained classified at the genus level.

Fatty acids

Fatty acids (FA) were measured as FA methyl esters by gas chromatography slightly modified after Malzahn *et al.* (2007a, b). Lipids were extracted with dichlormethane/methanol (vol. 2:1) for a minimum of 72 h at $-80°C$. After the extraction, larvae were removed and stored in a desiccator to vaporize 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 EFA concentration of the larvae, RNA/DNA ratio and FA were measured in the same larvae individuals. This was possible by first defatting the herring larvae and then homogenizing 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 homogenized in three steps: (i) freeze-dried larvae were placed in a cell mill for 15 min together with different sized glass beads (diameter 2.0 and $0.17-0.34$ mm), (ii) supersonic treatment in Tris−sodium dodecyl sulphate (SDS) buffer (Tris 0.05 mol l^{-1}, NaCl 0.01 mol l^{-1}, ethylenediaminetetracetic acid 0.01 mol l^{-1}, SDS 0.01%), and (iii) larvae together with buffer and glass beads were placed in the cell mill for 15 min. Then, the homogenate was centrifuged at $3829g$ (6800 rpm) at $0°C$ for 8 min (Sigma Laboratories Centrifuge $3-18$k). 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 min 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 multispecies growth model that was chosen for further calculation was:

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

where G_i is the instantaneous growth rate, sRD the standardized 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 chlorophyll *a* data from four stations in the GWB have shown limited spatial variability (Paulsen *et al.*, in prep.), indicating comparable conditions within the system. Owing to logistic
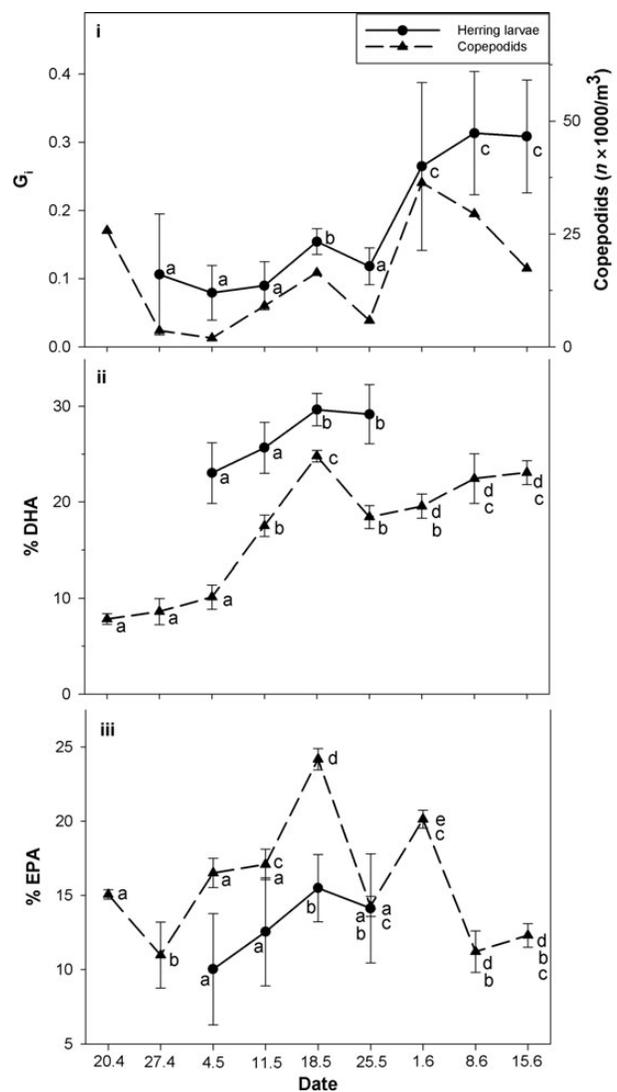
Figure 2. GWB. Error bars indicate standard deviations. (i) Instantaneous growth rate (G_i) of larval herring and copepodid abundance per cubic metre over time. (ii) DHA concentration of larval herring and copepods over time. (iii) EPA concentration in larval herring and in copepods over time. Different letters besides the data points denote significant differences.

Table 1. Average standard length per date in the GWB.

Date	27 April	4 May	11 May	18 May	25 May	1 June	8 June	15 June
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

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 ~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 tests. When variances were heterogeneous, data were transformed by extracting the cube and fourth root, respectively. To check between sampling days, a one-way analysis of variance (ANOVA) was conducted and a Tukey honestly significant difference (HSD) 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. Thereafter, the different parameters were tested with a *t*-test between the two regions.

Results

Greifswalder Bodden

The mesozooplankton assemblage of the GWB consisted mainly of copepods. *Acartia* spp. was the dominant genus until 25 May, contributing 60–80% 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 (Figure 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 and 18 May (ANOVA, Tukey HSD, $p < 0.05$), which was reflected in the significantly increasing DHA concentration of the larvae (ANOVA, Tukey HSD, $p < 0.05$, Figure 2ii). Growth rates increased significantly on 1 June (ANOVA, Tukey HSD, $p < 0.01$) when prey abundance increased sixfold. However, not only growth rates increased, but also average larval standard length (Table 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
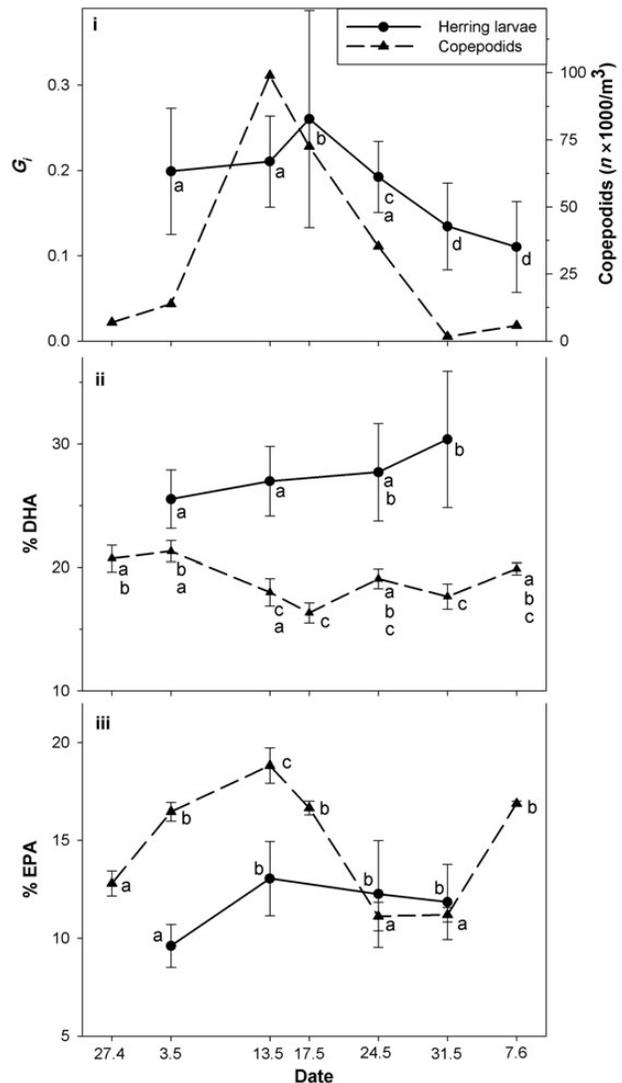
Figure 3. KC. Error bars indicate standard deviations. (i) Instantaneous growth rate (G_i) of larval herring and copepodid abundance per cubic meter over time. (ii) DHA concentration of larval herring and copepods over time. (iii) EPA content in larval herring and in copepods over time. Different letters besides the data points denote significant differences.

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 (Figure 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 (Figure 2iii).

Table 2. Average standard length per date in the KC.

Date	3 May	13 May	17 May	24 May	31 May	7 June
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

Kiel Canal

Eurytemora dominated the copepodite assemblage during late April and throughout May (90–100% of all copepodites sampled) in the KC. From late May on, *Acartia* dominated (70–80% of all copepodids). Similar to the GWB, growth rates of larval herring followed prey abundance in the KC (Figure 3i). However, growth rates remained constant, even when prey abundance increased eightfold on 13 May. This occurred when DHA concentration of the copepods decreased significantly (ANOVA, Tukey HSD, $p < 0.05$) and DHA of the larvae showed an increasing trend (Figure 3ii). Since larvae were larger on 13 May compared with 3 May (Table 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 (Figure 3ii), EPA remained constant after an initial increase (Figure 3iii).

Comparison of both areas

In the GWB, larval growth was significantly correlated with the DHA and EPA concentration in the larvae ($p < 0.01$, Figures 4 and 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 (Figure 6), though one between larval growth and EPA was found (Figure 7).

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

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 made up of two or three samplings from 31 May to 15 June, depending on the habitat. In contrast to significantly different prey abundances in both time windows ($p < 0.05$, Table 3), prey DHA concentration was only significantly different in the second time window ($p < 0.05$, GWB > KC, Table 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 (Table 4). This was true for all ontogenetic stages. Larval FA 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 (Table 4). 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 were significantly higher in the GWB compared with the KC irrespective of the bad growth conditions in terms of prey abundance and prey EFA concentration during that time window.
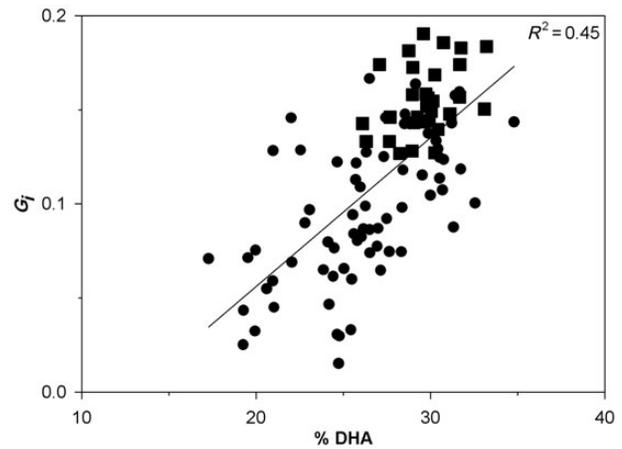

Figure 4. *GWB.* Significant correlation ($p < 0.01$) between instantaneous growth rates (G_i) of larval herring and their DHA concentration. Dots indicate all available data, and squares show data from 18 May, when DHA concentration in copepods was highest.
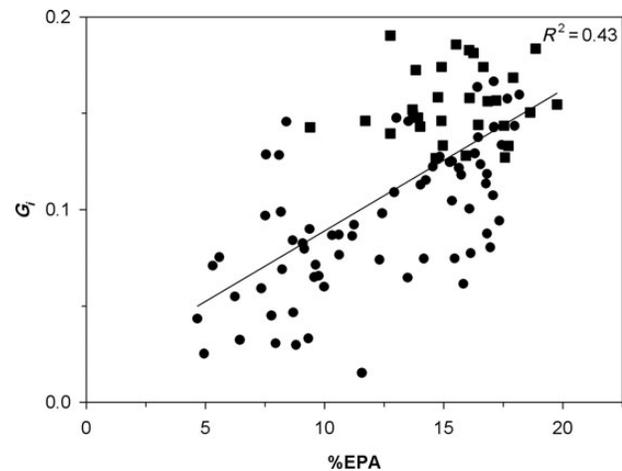

Figure 5. *GWB.* Significant correlation ($p < 0.01$) between instantaneous growth rates (G_i) of larval herring and their EPA concentration.

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 with the 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
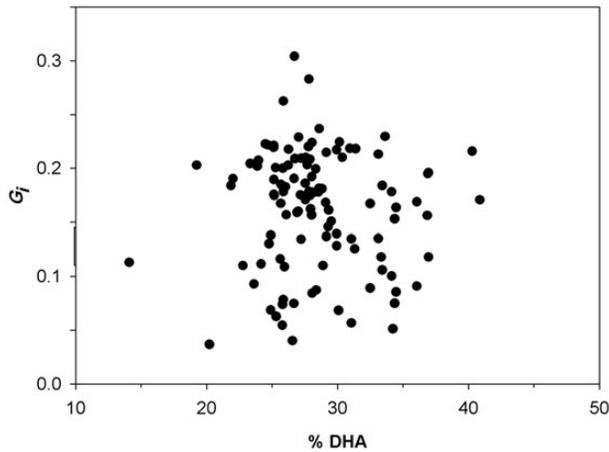
Figure 6. *KC.* Correlation between instantaneous growth rates (G_i) of larval herring and their DHA content. The correlation is not significant.
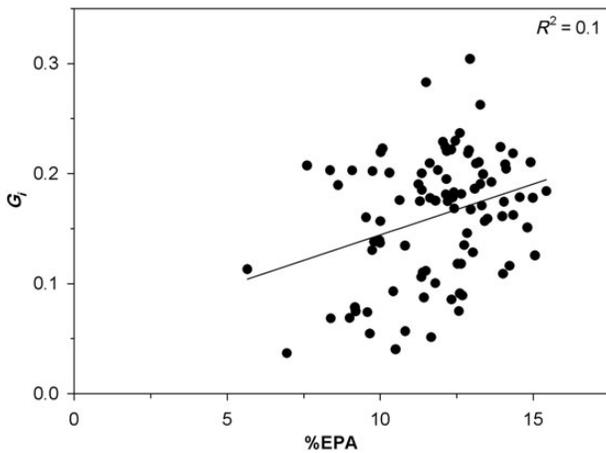

Figure 7. *KC.* Significant correlation ($p < 0.01$) between instantaneous growth rates (G_i) of larval herring and their EPA concentration.
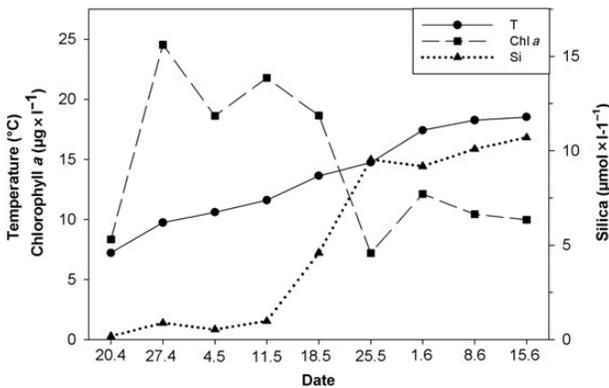

Figure 8. *GWB.* Temperature, silica, and chlorophyll *a* over time.

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
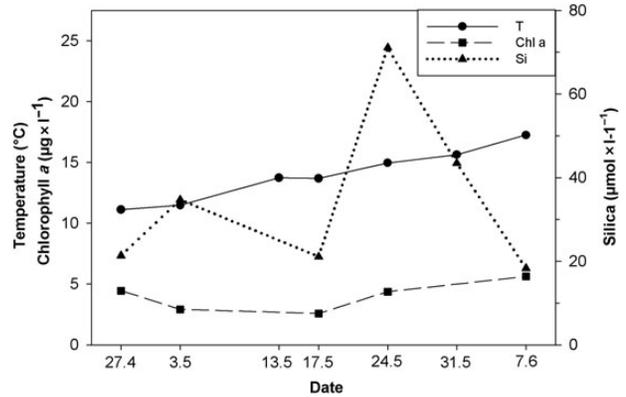

Figure 9. *KC.* Temperature, silica, and chlorophyll *a* over time.

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 with 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.

Although 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, although 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: first, food quantity increased strongly on 1 June, which enabled the larvae to take up more prey with a constant quality. Second, 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, while 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 while 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 4-week period. Nevertheless, prey DHA probably affected larval growth in the KC, for example, on 13 May, although 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 catabolize 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 ~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, and 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 at 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 thereafter 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 interannually. The prey field was sampled over the whole water

Table 3. Results from *t*-tests between prey quantity and quality of GWB and 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	$p > 0.05$	$p > 0.05$

Table 4. Results from *t*-tests of larval herring originating in GWB and KC.

	<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

The length classes reflect different ontogenetic stages of the larvae: yolk-sac (<9 mm), first feeding (9–11 mm), 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 1, reaching from 27 April to 25 May, while G_i II shows results from time window 2, 31 May to 15 June. Larval FA data to test exist only from time window 1, these results are shown

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 for 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 analysed 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 with 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; Peschutter, 2008; Paulsen, 2010), 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 most recruits in recent years (Polte *et al.*, 2014). 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. Although 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 that of the KC, this is tenable. This would also mean that while the KC may provide more recruits per cubic metre 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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