Depth-dependent nutritional condition of sprat *Sprattus sprattus* larvae in the central Bornholm Basin, Baltic Sea

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ABSTRACT: Bimodal depth distribution patterns observed for sprat *Sprattus sprattus* larvae in previous field studies conducted in the deep basins of the Baltic Sea have led researchers to hypothesise that larval sprat condition was depth-dependent. We examined this hypothesis by measuring morphological, biochemical and otolith-based proxies for nutritional condition in sprat larvae collected in discrete 5 m depth intervals from the surface to the bottom in the central Bornholm Basin. Similar to earlier studies, larval sprat were most abundant in 2 depth strata (0 to 10 and 65 to 75 m). Their nutritional condition in surface and deep waters was not uniformly expressed by the different indices. For example, sprat larvae from 0 to 10 m could not be distinguished from conspecifics caught at 65 to 75 m by a long-latency condition proxy (otolith-based growth rates). Similarly, a medium-latency proxy (RNA:DNA) did not suggest differences in condition between the depths. However, short-latency proxies (protein:standard length and DNA:dry weight) supported the depth-dependent condition hypothesis. The lack of correspondence and pitfalls associated with the use and interpretation of multiple condition indices (e.g. the influences of temperature and body size) are discussed and recommendations to strengthen these various metrics are provided.

KEY WORDS: *Sprattus sprattus* · Larvae · Baltic · Survival · Growth · Condition · RNA:DNA · Protein

INTRODUCTION

Early in life, marine fish often suffer high mortality rates, and subtle changes therein can contribute to 10-fold or higher fluctuations in year-class strength (Houde 1987). Rates of growth and survival can be positively correlated in the early life stages of a number of marine fish species (Houde 1987, Cowan & Shaw 2002), and are affected by an amalgam of factors (e.g. starvation, suboptimal hydrographic conditions, predation and disease) that vary within a season and between years. Batches of sub-cohorts can be produced on short time scales (days to weeks) and experience different environmental conditions in the course of a spawning season, making each sub-cohort's contribu-tion to overall year-class strength highly variable. Survivors probably originate from larval batches that have encountered environments favourable to fast growth early in life (Cushing 1974). Understanding the spatio–temporal dynamics of unfavourable environments (i.e. environments correlated with reduced growth rates that cause prolonged durations of developmental stages particularly vulnerable to mortality via starvation and predation: see Houde 1987), may increase our ability to predict survival of early life stages of marine fish.

In the Baltic Sea, the sprat *Sprattus sprattus* plays a key trophodynamic role as a prey resource (Nissling 2004) for a number of species (including cod *Gadus morhua* and harbour porpoise *Phocoena phocoena*)

and as a predator on both zooplankton and fish eggs (Arrhenius & Hansson 1993, Köster & Möllmann 2000). It is also an economically valuable species being, at the present time, the most important target of commercial exploitation (ICES 2004). The growth and survival of larval and early juvenile stages have been identified as the main determinants of recruitment variability in populations of sprat and cod inhabiting the Baltic Sea (Köster et al. 2003).

One of the most distinctive characteristics of the Baltic Sea is the permanent water column stratification, producing gradients in temperature, salinity, O_2 concentration and secondary production that can become particularly strong during spring and summer (Matthäus & Nausch 2001). This vertical variability in environmental conditions creates areas supportive of, as well as obstructive to, fast growth and high survival rates of larval fish. Temperature in particular has been discovered to be a key factor of sprat survival and recruitment (MacKenzie & Köster 2004). In the Baltic, survival chances may increase for larval fish that are able to distribute themselves in favourable strata of the water column. Interestingly, in sprat larvae, a bimodal depth distribution is often encountered in the Baltic basins with high concentrations of larvae close to the surface and near the seafloor. Larvae in deeper waters are often smaller, possibly suggesting that larval sprat growth and condition may be depth-dependent (Wieland & Zuzarte 1991).

In the present study, we compared the nutritional condition and growth rate of sprat larvae encountered near the surface (0 to 10 m) and bottom (65 to 75 m). Our hypothesis (H_0) was that the condition of larval sprat during the summer was similar near the surface and close to the seafloor in one of the most important spawning grounds for this species in the Baltic — the Bornholm Basin (Köster et al. 2001). Several condition indices were used: a morphometric condition factor (CF) (e.g. Hempel & Blaxter 1963), RNA:DNA (Buckley 1984, Clemmesen 1994), the daily protein growth rate Gp (Buckley 1984), DNA per unit freeze-dried weight (DW) (Bulow 1987), protein content (Ehrlich 1974, Buckley 1979, Clemmesen 1987) per unit standard length (SL), and the mean daily somatic growth rate based on otolith microstructure analysis (Fossum et al. 2000, Belchier et al. 2004). These various condition proxies are commonly used (e.g. Bulow 1987, Rè & Gonçalves 1993, Bergeron & Boulhic 1994, Clemmesen 1994, Ferron & Leggett 1994, Belchier et al. 2004) but rarely examined and compared in the same study. Therefore, the present work also attempted to evaluate whether larval condition would be expressed uniformly when multiple measures were performed on the same individual or on individuals from the same larval sub-cohort.

MATERIALS AND METHODS

Field sampling. Samples of fish larvae and zooplankton were obtained using a BIOMOC sampler, a modified 'MOCNESS' system (Wieland & Jarre-Teichmann 1997), deployed on 9 and 10 July 2000 from the RV 'Alkor' at 1 station in the central Bornholm Basin, Baltic Sea (55° 17.42′ N, 16° 00.34′ E). Maximum water depth at this station was 80 m. The BIOMOC was equipped with 9 nets each with a mouth opening of 1 m^2 and a mesh size of 330 µm with a microzooplankton liner (mesh size 50 µm) fitted into every other net opening. Over a 24 h period the whole water column was sampled every 3 h in 2 consecutive hauls. The first haul sampled 5 m depth intervals between 0 and 40 m mid-stratum (i.e. 2–3, 7–8, 12–13, … 37–38 m) and the second haul sampled between 45 and 75 m mid-stratum (i.e. 42–43, 47–48, 52–53 … 72–73 m). Due to ship movements, the exact hauldepths could not be determined and were thus given as ranges (i.e. 0–5 m, 5–10 m, ...) A total of 16 hauls was conducted, so that the complete water depth was covered 8 times (see Figs. 2 & 4).

The gear was towed horizontally at a speed of 3 knots. Each net was kept open for 3 to 5 min. The filtered water volume was calculated by multiplying the area of the net opening by the distance towed as tracked by an on-board GPS device. A vertical profile of temperature, salinity, pressure, oxygen and chlorophyll a was obtained using a CTD cast. Water density was calculated from temperature, salinity and pressure (Fofonoff 1985) and the maximum change in density within a 5 m depth interval was calculated to evaluate the degree of stratification (Coombs et al. 1992).

At the completion of each haul, the nets were carefully rinsed and sprat larvae were removed and preserved in a 4% buffered formaldehyde/seawater solution within 15 min. In the laboratory the solution was replaced with a formaldehyde-free preservation fluid (Steedman 1976) prior to analysis. Immediately after recovery from the nets, subsamples of sprat larvae (total n = 127) were randomly selected across hauls and stored in liquid nitrogen for biochemical and otolith microstructure analyses.

Gut contents were examined under a stereomicroscope (Leica MZ 16) and identified to the lowest possible taxonomic level.

Zooplankton samples were preserved as described above for the sprat larvae, determined to the lowest possible taxonomic level, and counted in a Bogoroff tray under a stereomicroscope (Leica MZ 16). Where necessary, subsamples were taken using a plankton splitter.

Morphological measurements. SL (±0.5 mm, stereomicroscope Leica MZ 16 with object micrometer) and DW (±0.1 µg, Christ Alpha freeze-drier, Sartorius

microbalance SE2-0CE) of all individuals captured (n = 1121) were measured. Values were not corrected for larval shrinkage induced by sampling and preservation. In order to ensure comparability among specimens and depth ranges, larvae of a size spectrum that was adequately represented in both shallow and deep samples (>20 µg DW and between 8 and 12 mm SL), were divided into 1-mm standard length-classes. Only larvae of the same length-classes were compared.

Many larvae were damaged during catch (e.g. eyes and/or intestines lost). The degree of damage may influence the proxies measured and is quantitatively assessed elsewhere (A. Dänhardt & A. Temming unpubl.). For the present study, we compared only larvae with similar degrees of damage between depths.

The relationship between DW and SL was determined from:

$$\log DW = \log a + b (\log SL) \tag{1}$$

for individuals from both depth strata combined, where DW = mg, SL = mm, and a and b are constants.

A morphometric condition factor (CF), a normalised measure of body weight-at-length (Hempel & Blaxter 1963) was then calculated as:

$$CF = 100\,000\ DW\ SL^{-b} \tag{2}$$

A decrease in DW (and thus in CF) is expected within a few hours of starvation (Ferron & Leggett 1994). In order to exclude size effects on CF (Peck et al. 2005), the comparisons between deep and surface strata were confined to the same length range of sprat larvae.

Biochemical measurements. For biochemical analyses only undamaged larvae were used. The concentration of nucleic acids was measured in 127 individuals using procedures outlined by Clemmesen (1993) and Belchier et al. (2004). Briefly, the amount of DNA and RNA was measured fluorometrically in a microtitre fluorescence reader (Labsystems, Fluoroscan Ascent) using the fluorophor ethidium bromide. The DNA content was determined after treatment with RNase (ribonuclease A, Serva). RNA- and DNA concentrations were determined based on calibration curves using lambda DNA and 16S/23S ribosomal RNA (Boehringer Mannheim). The RNA:DNA ratio was used as a proxy for the cells' metabolic activity (Buckley 1984, Clemmesen 1994).

Protein growth rates (Gp, % d^{-1}) were then calculated from RNA:DNA and water temperature (T, °C) using an equation in Buckley (1984) calibrated to Clemmesen's (1993) analytical procedure by dividing RNA:DNA by 1.3 (C. Clemmesen unpubl.):

$$Gp = 0.93\ T + 4.75\ (RNA{:}DNA)\ (1.3)^{-1} - 18.1 \tag{3}$$

At a given temperature, RNA:DNA and Gp often exhibit a significant decrease after 2 to 4 d of reduced food intake in larval and post-larval marine fish (Buckley 1984), including sprat (Peck et al. 2004b). The ratio DNA:DW was calculated as a proxy for cell size (response time <12 h, Ferron & Leggett 1994).

The protein content (µg larva^{-1}) of each larva was determined using the Bio Rad assay, based on the spectrophotometric method of Bradford (1976) and expressed per unit SL. It is expected to increase with improving nutritional condition (Ehrlich 1974, Buckley 1979, Clemmesen 1987). Protein content and DW are positively correlated in larval fish and thus both respond to starvation on the same time scale (<12 h, Ferron & Leggett 1994).

Otolith measurements. The sagittae of 61 larvae between 9 and 17 mm SL were extracted with fine insect needles under a stereomicroscope and fixed in conventional nail polish on a glass slide. The otoliths were magnified (400 to 1000×) under a microscope and digital images were taken. A computer image-analysis system (Image Pro 4.5) was used to count the number of rings. Mean daily somatic growth rates (e.g. Fossum et al. 2000) for larvae from 0 to 10 and 65 to 75 m were calculated as:

$$(SL - SL_1) \times RC^{-1} \tag{4}$$

where SL_1 is the size at first feeding (5.5 mm SL, Shields 1989) when the first otolith rings are formed in sprat (Alshuth 1988), and RC is the ring count (n). Growth rates derived from counts of otolith daily rings integrate the whole lifespan of an individual.

Statistics. Non-parametric Mann-Whitney U-statistics were used to test the null hypothesis of no difference in nutritional condition between top- and bottom-caught sprat larvae. Since multiple comparisons were made, the significance level was adjusted using a modified Bonferroni test (Keppel 1991) across length classes. Differences were considered significant at $p < 0.01$.

RESULTS

Hydrographic conditions

Water temperature was highest (14.4°C) within 10 m of the surface, decreased rapidly within a thermocline starting at 15 m, and was lowest (4.2°C) at 40 m. Between 65 and 75 m the temperature was 7.2°C. From the surface down to 40 m salinity remained constant at 7.5 to 8.0 psu, then steadily increased to 16.0 psu at the seafloor. The oxygen content declined from 6.8 ml l^{-1} at the surface to 0.7 ml l^{-1} at 80 m. Chlorophyll *a*

concentration was highest in the top 15 m (0.95 µg l^{-1}) and decreased to 0.16 µg l^{-1} at 60 m. Temperature and chlorophyll a concentration developed similarly with increasing water depth. The strongest stratification was at depths between 60 and 65 m with a density difference of 1.94 kg m^{-3} (Fig. 1).
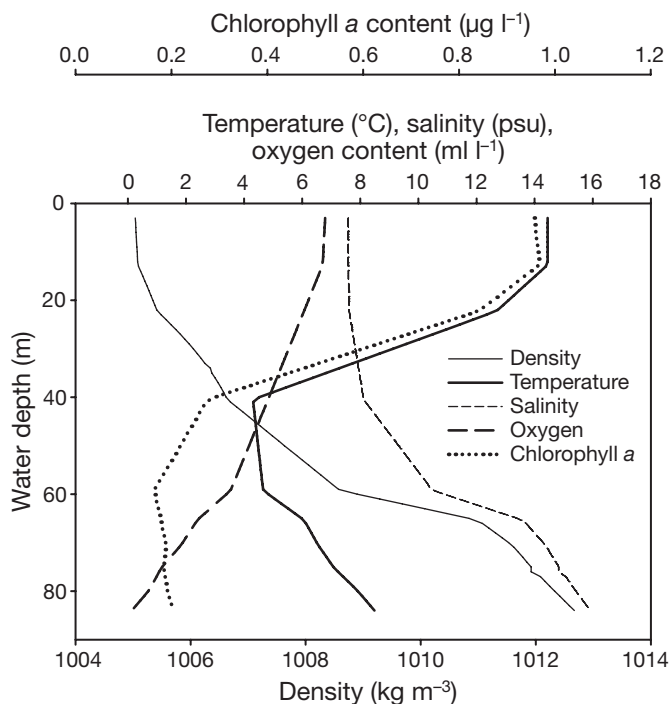

Fig. 1. Vertical profiles of physical variables and chlorophyll *a* content during collection of larval sprat *Sprattus sprattus* at 1 sampling station in the Bornholm Basin, Baltic Sea
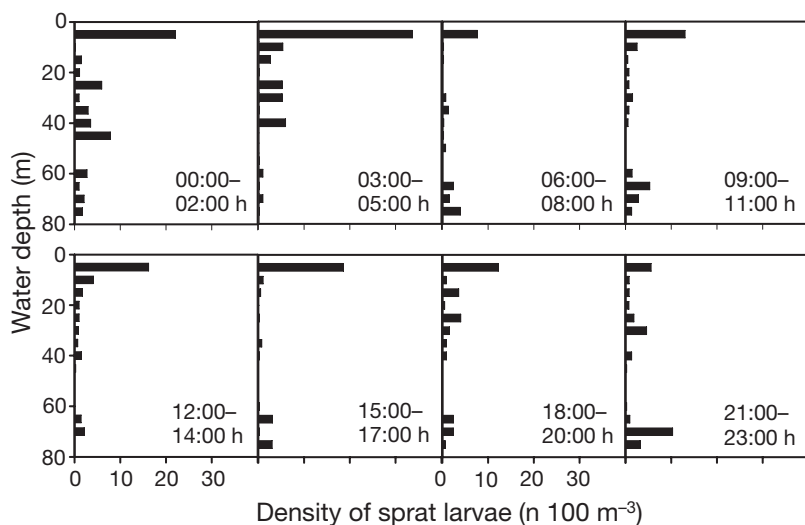
Vertical distribution

The density of sprat larvae caught varied among local sampling times and depths. Larval abundance was highest in the upper 10 m except for 06:00 to 08:00 h and 21:00 to 23:00 h. At each sampling time, a smaller peak in total number occurred at 65 to 75 m while very few larvae were caught between 45 and 60 m. Abundance peaks were most pronounced during daytime, whereas larval distribution in the water column was more even between dusk and dawn (Fig. 2).

Larvae caught near the surface were longer on average (mean ± SD, 0 to 10 m: 12.1 ± 2.4 mm) than their conspecifics caught at depth (65 to 75 m: 9.9 ± 2.1 mm). The longest larvae (>20 mm) occurred in low abundances at 5 m depth and below the thermocline (Fig. 3).

The cladoceran *Bosmina corigoni maritima* was the most abundant organism in the plankton samples at all depths (Fig. 4) as well as in larval guts (35% by number). Larval gut contents appeared to be biased due to the catching procedure and could not be assessed quantitatively. They were excluded from subsequent considerations. During daylight hours, zooplankton occurred at highest concentrations near the surface and had a variable depth distribution below 15 m. At night and in the early morning, the peak in zooplankton near the surface was absent. During the course of the sampling period the concentrations of zooplankton changed by 2 orders of magnitude and was lowest between 00:00 and 08:00 h and subsequently increased from 09:00 to 20:00 h Central European Summer Time (CEST).

Morphometric proxies

For larvae within the length range encountered in sufficient quantities for comparison between shallow and deep samples (8 to 12 mm SL), larval SL (±SE) and DW (±SE) were related by:

$$\log DW = 3.76(\pm 0.07) \log SL - 4.97(\pm 0.07) \quad (5)$$

where DW = mg and SL = mm (n = 501, r^2 = 0.86, p < 0.001). Larvae grouped within 1 mm SL Classes 9, 10, 11 and 12 mm caught at 65 to 75 m had statistically significantly higher values of CF (Eq. 2) (range 1.3 to 8.1) than individuals in the same SL classes from 0 to 10 m (range 0.6 to 7.1, p < 0.01). No statistically significant differences in CF were observed in larvae of SL Class 8 mm. When all SL-classes were pooled, differences between
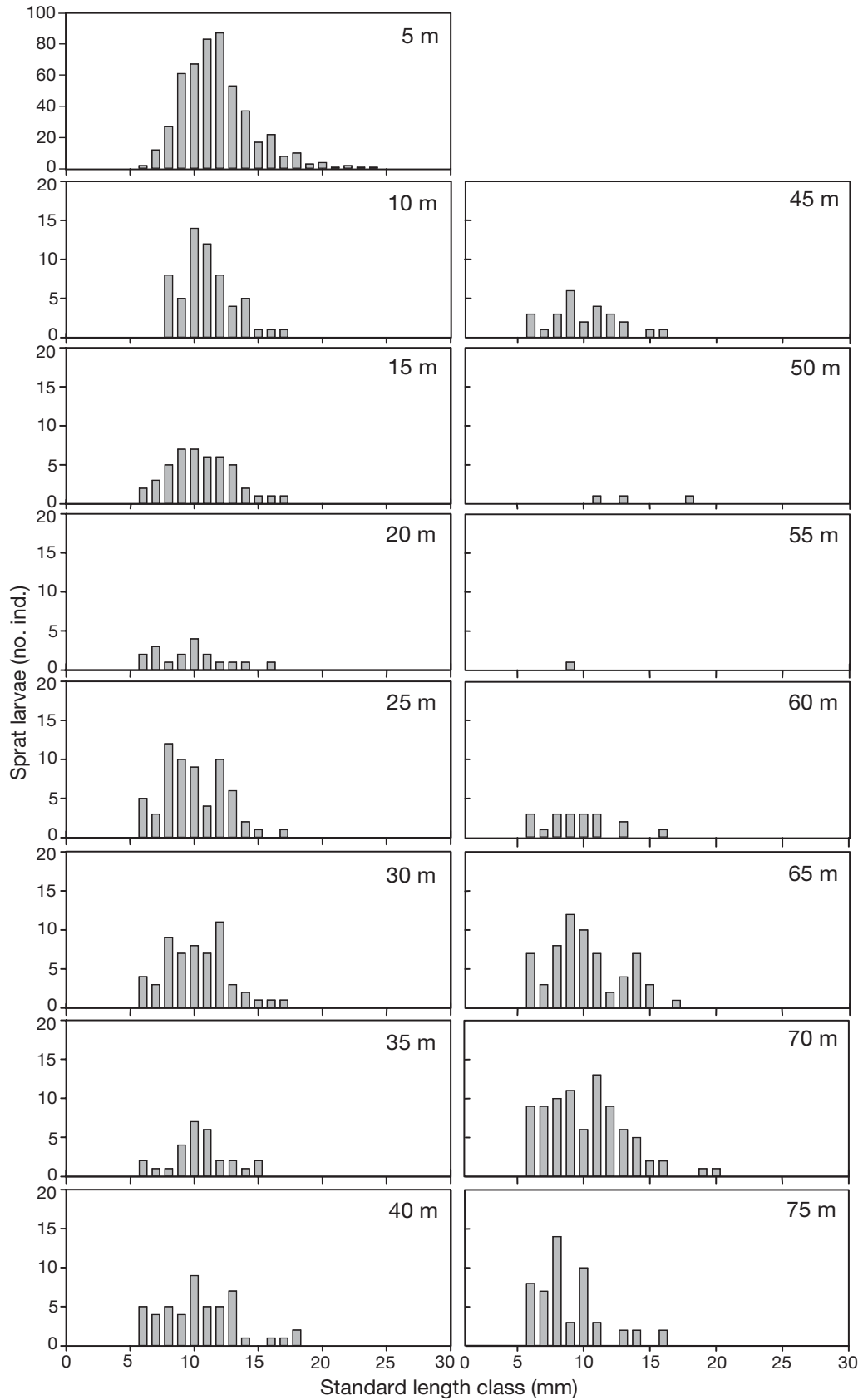

Fig. 2. *Sprattus sprattus.* Density (n 100 m^{-3}) of sprat larvae (all length classes combined) caught at different times of the day (Central European Summer Time, CEST) at discrete water depths

Fig. 3. *Sprattus sprattus.* Absolute number of sprat larvae caught at different water depths as a function of length class
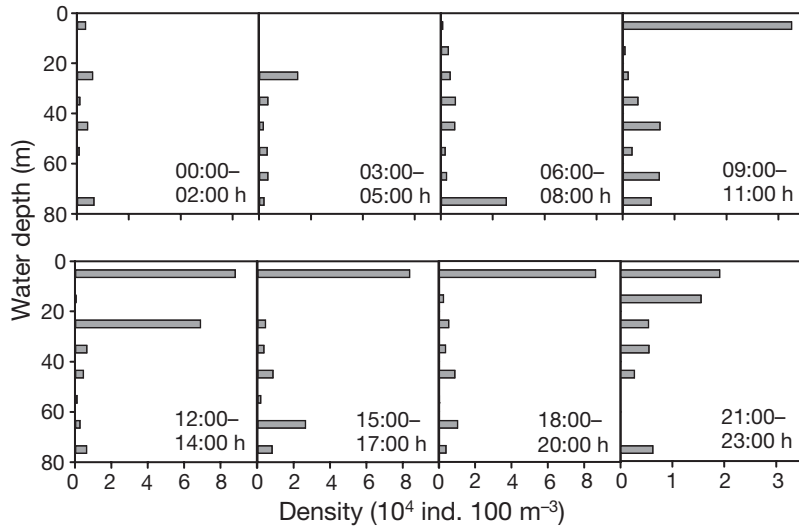
Fig. 4. *Bosmina corigoni maritima*. Density (n 100 m^{-3}) at different times of day (CEST) at discrete water depths

top- and bottom-caught larvae remained statistically significant (Fig. 5). CF minima increased with increasing SL only in larvae from a depth of 65 to 75 m. The variability (expressed as the coefficient of variation, CV) of CF decreased steadily with increasing length in larvae caught at 65 to 75 m, while in individuals from 0 to 10 m no such trend was observed (Fig. 5). Together with protein:SL, the variability of CF was highest among proxies.

Otolith-based somatic growth rates (mean ± SD) were 0.59 ± 0.19 mm d^{-1} and 0.66 ± 0.17 mm d^{-1} for larvae sampled at 65 to 75 and 0 to 10 m depths, respectively. The difference was not significant (p = 0.23). Another commonly used method to calculate otolith-based growth rates, using the slope of the regression of ring count (RC) on SL (e.g. Munk 1993, Rè & Gonçalves 1993, Valenzuela & Vargas 2002) yielded lower growth rates (see Table 1). However, using either method, mean daily growth rates between depths were not significantly different.

Biochemical proxies

The sub-sample of larvae taken for biochemical analyses contained sufficient numbers of larvae of 9 to 12 mm SL for a comparison between surface and deep samples in each of four 1-mm size classes. In the 10 mm SL-class RNA:DNA was significantly higher at 0 to 10 m (p < 0.01). In all other SL-classes no statistically significant difference was noted. When SL-classes 9 to 12 mm were pooled, differences were not significant. Except for SL 10 mm, the variability (CV) of RNA:DNA in individuals of all length classes caught at 0 to 10 m was
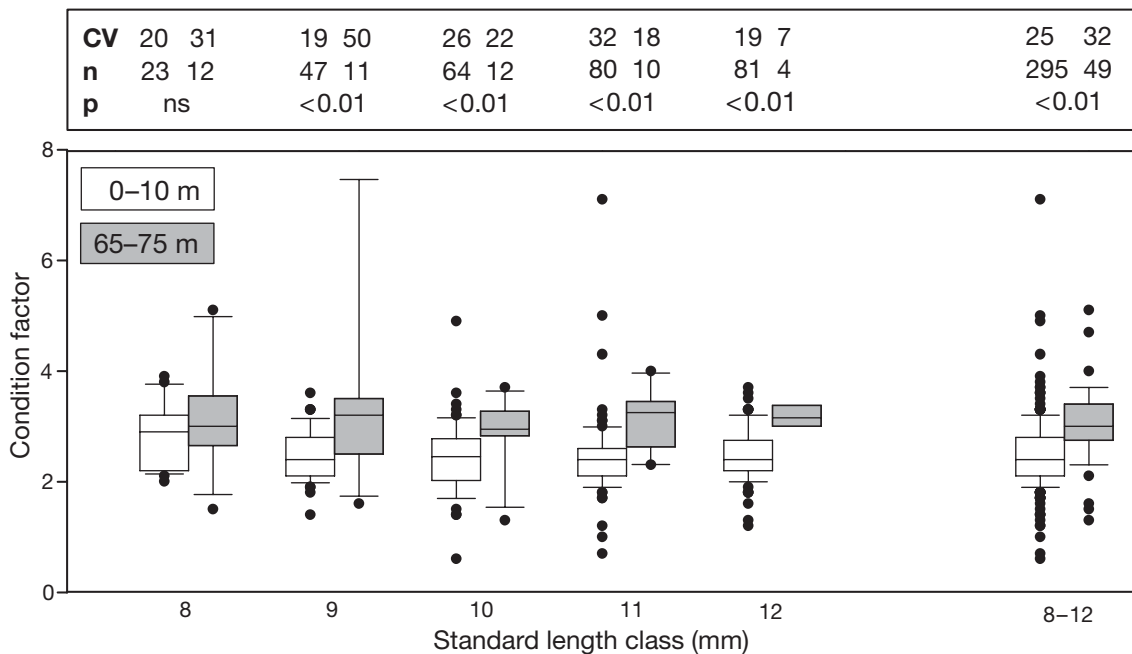

Fig. 5. *Sprattus sprattus*. Condition factor (CF) of sprat larvae obtained from 0 to 10 and 65 to 75 m, showing median (line), 10th, 25th, 75th and 90th percentiles and outliers. Variability (CV), sample size (n) and significance level (ns = not significant) are given above each plot

greater than in those from 65 to 75 m. Clear trends could not be observed in this proxy (Fig. 6).

Following Eq. (3), the Gp of larvae caught at 0 to 10 m (14.4°C) was 4.6% d^{-1}, compared to −3.5% d^{-1} (mean daily protein decrease) in individuals caught at 65 to 75 m (4.2°C). In all larval length classes, higher values (i.e. lower *RNA* efficiency) of the Gp were found at 0 to 10 m ($p < 0.01$).

Individuals from 65 to 75 m tended to have smaller cells, as expressed by greater DNA:DW in all the 1-mm SL-classes analysed, in SL-Classes 10 and 11 the differences were statistically significant (Fig. 6, $p < 0.003$ for 10 and 11 mm SL, $p < 0.016$ and 0.046 for 9 and 12 mm SL). Maximum values were measured only in larvae caught near the seafloor. In all but the 12-mm SL-class, the CV was higher in individuals caught at 0 to 10 m.

The protein content per mm SL was statistically significantly higher at 65 to 75 m for larvae in the 10, 11 and 12-mm SL classes. In the 9 mm length class, the relative protein content of larvae was similar at both depths (Fig. 6). As with CF , variability in the protein content per length unit decreased steadily with increasing length in larvae caught at 65 to 75 m, while in individuals from 0 to 10 m no clear trend could be detected. Together with CF , the variability in protein:SL was the highest among the proxies (Fig. 6).

DISCUSSION

The survival of marine fish larvae is closely linked to how fast they grow through developmental stages that are particularly vulnerable to mortality via starvation and predation (Houde 1987). Growth rates largely depend on a larva's nutritional state, which can be markedly influenced by changes in food availability and hydrographic features of the environment, especially temperature (Heath 1992). Although there is always a time lag between cause (good or poor feeding conditions) and effect (fast growth or starvation), larval feeding and growth history can be reconstructed from physiological changes resulting from varying environmental conditions (Bergeron & Boulhic 1994). We measured a variety of commonly applied nutritional proxies in Baltic sprat to construct a more complete picture of whether bimodal depth distributions were associated with differences in larval condition.

Morphometric proxies

The relationship between weight and length was one of the earliest methods used to assess the condition of larval fish (Shelbourne 1957, Hempel & Blaxter 1963). It was assumed that greater weight-at-length in-

dicated a good nutritional state (e.g. von Westernhagen & Rosenthal 1981). This assumption was questioned by Ricker (1979), who ascribed a lower weight-at-length to fast-growing individuals, due to the preferential allocation of growth in length as opposed to mass. A lower weight-at-length was correlated to faster growth in juvenile Atlantic cod by Peck et al. (2004a).

In sprat larvae, the CF not only depends upon exogenous conditions (e.g. feeding and temperature) but also upon fish size, since the allometric scaling of mass and length (b, Eq. 2) changes statistically significantly during early ontogeny in this species (Peck et al. 2005). We addressed these uncertainties by comparing only similar size classes between depths. Nevertheless, the CF cannot clearly be interpreted as indicative of fast growth or bad condition and should therefore be viewed only in context with other indices.

The similarity in the otolith-based mean daily growth rates in larvae from both depths in our study suggests that specimen caught at 0 to 10 and 65 to 75 m were in similar condition, and thus, supports our null hypothesis (H_0), but this proxy integrates conditions experienced over the whole life span of the individual larva and may therefore be fairly insensitive to short- and medium-term effects. For example, a recent bout of starvation would not be represented within this integrated measure. Even though sprat larvae occur over a wide latitudinal range (e.g. Adriatic, Baltic, Irish and North Seas) and within these habitats can encounter diverse environmental conditions (e.g. surface and bottom waters of the Bornholm Basin), otolith-based average daily growth rates appear to change little in this species (Table 1). On the one hand, this observation may suggest the relative insensitivity of this integrative measure to detect changes in sprat condition; on the other hand, the correspondence of otolith-based mean growth rates among different systems for the same species may imply interesting similarities in life history traits of sprat in these different ecosystems. At the present time, evidence for the former and latter is equivocal, and more research is needed.

Biochemical proxies

The metabolic activity and other vital rates of fish larvae are closely linked to ambient water temperature (e.g. Buckley 1984, MacKenzie & Köster 2004). One value for RNA:DNA at 14.4°C (0 to 10 m) may indicate good growth potential while the same value at 7.2°C (65 to 75 m) would suggest slow growth. The minimum RNA:DNA required to maintain metabolism without growth was determined as 1.01 at 14°C in postlarval sprat (Peck et al. 2004b). Clemmesen (1994) found
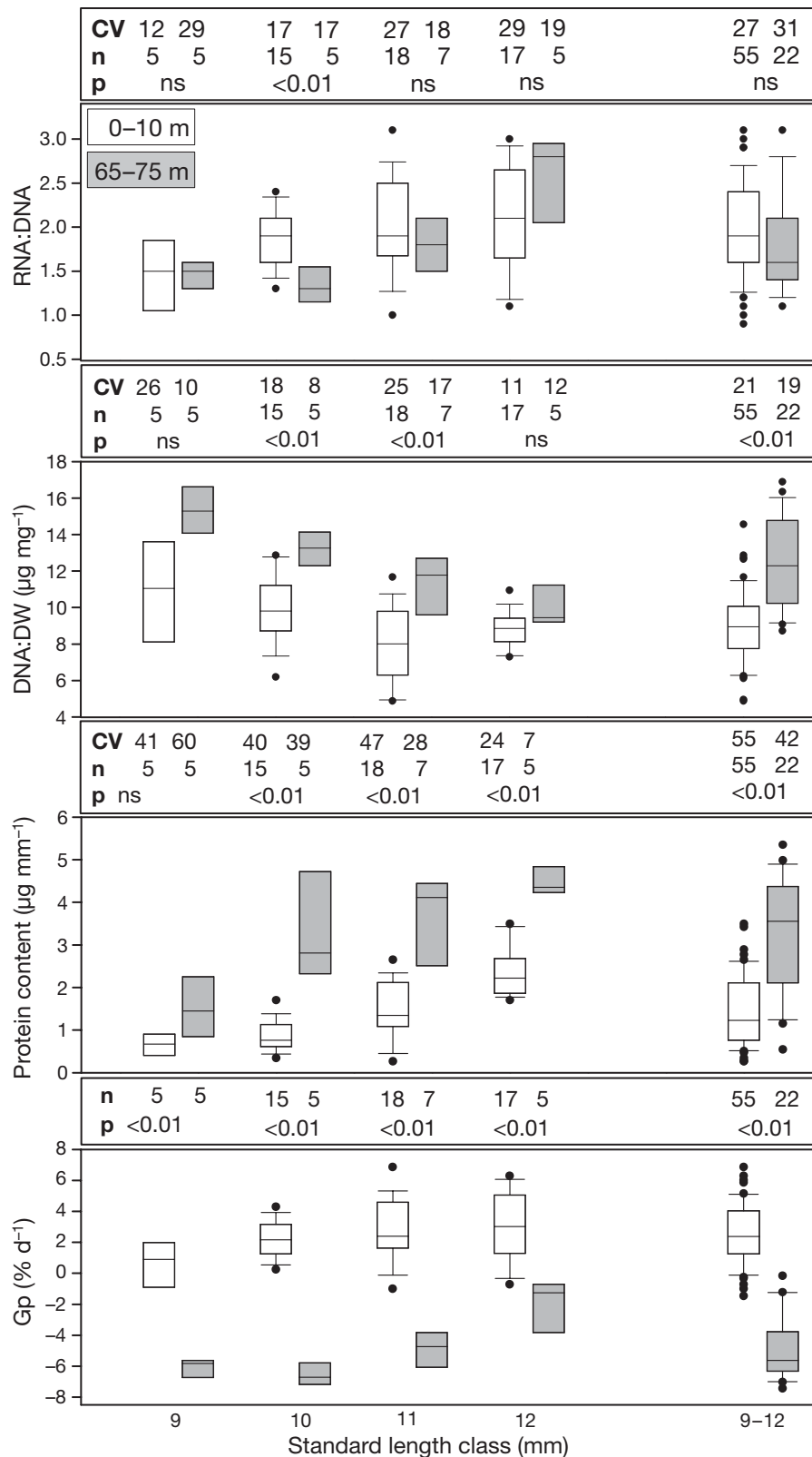
Fig. 6. *Sprattus sprattus*. Biochemical condition proxies: RNA:DNA, DNA:DW, protein content:SL (µg mm^{-1} = µg mm SL^{-1}) and daily protein growth rate (Gp) of larval length classes 9, 10, 11 and 12 mm caught at 0 to 10 and 65 to 75 m depth, showing median (line), 10th, 25th, 75th and 90th percentiles and outliers. Variability (CV), sample size (n) and significance levels (ns = not significant) are given above each plot. (CV of Gp not shown)

RNA:DNA values of around 1 to be necessary for survival in starvation experiments performed with larvae of different species reared at 12.0 to 16.7°C. According to the mean daily protein growth rate Gp (Buckley 1984, and present Eq. 3), these 2 findings suggest that larvae at 0 to 10 m (14.4°C) and 65 to 75 (7.2°C), would require RNA:DNA values of 0.99 and 2.40, respectively, to maintain metabolism (Gp = 0). Only ~1% of the sprat larvae at 0 to 10 m had an RNA:DNA value <1.0, whereas at 65 to 75 m only 13 % of the larvae had an RNA:DNA value >2.5, suggesting a restricted growth potential at the low temperature at these depths.

In contrast, the daily somatic growth rates estimated from otolith micro-structure did not show significant differences between depths, which may be explained in 3 ways: (1) larvae caught at 65 to 75 m could have experienced better growth environments earlier in life, which would be reflected in slow-responding condition proxies such as somatic growth rate rather than by more quickly responding proxies such as RNA:DNA and DNA:DW; (2) the larvae may have experienced a continuously changing temperature regime (e.g. through vertical movements of unknown amplitude and frequency, see next subsection) which affected larval physiology in a different manner than the static temperature regime used to calibrate the RNA:DNA – growth relationship presented in the model of Buckley (1984) which (3) may not be directly applicable to sprat.

Despite the good accordance between Gp-values from our study and experimentally derived RNA:DNA minimum values for metabolic maintenance (Clemmesen 1994, Peck et al. 2004b), it remains somewhat problematic to assess larval fish condition using Gp, due to its strong temperature-dependence. Therefore, this proxy should be applied with caution, especially with respect to dynamic temperature regimes that could be encountered by vertically migrating fish larvae.

A proxy less sensitive to the uncertainty imposed by temperature effects is the amount of DNA per unit dry weight, proposed as an indicator for cell size (Bulow 1987) and starvation (Bergeron 1997). Dry weight has been reported to react to food shortage within a few hours, especially in young larvae that have yet to build energy reserves (Ferron & Leggett 1994). Therefore, the higher DNA:DW ratio in larvae of all length classes at 65 to 75 m compared to conspecifics captured in surface waters suggests recent starvation at depth.

Table 1. *Sprattus sprattus*. Daily otolith-based growth rates of larval sprat sampled in different field areas and measured in the laboratory. (–) no data available

Study area	Growth rate (mm d^{-1})	T (°C)	Source
Laboratory experiment	0.31−0.37	15.0	Alshuth (1988)
Irish Sea	0.37−0.49	14.0	Shields (1989)
North Sea	0.41	11.3	Rè & Gonçalves (1993)
North Sea (mixed areas)	0.43−0.47	–	Munk (1993)
North Sea (stratified areas)	0.13−0.35	–	Munk (1993)
Adriatic Sea	0.40−0.42	–	Dulcic (1998)
North Sea	0.36−0.40	11.0−13.5	Valenzuela & Vargas (2002)
North Sea (German Bight)	0.39−0.48	11.0−14.4	Huwer (2004)
Baltic Sea, Bornholm Basin (0−10 m)	0.39/0.66[a]	14.4	Present study
Baltic Sea, Bornholm Basin (65−75 m)	0.46/0.59[a]	7.2	Present study

[a]For calculation of daily growth rates in present study see 'Materials and methods' and 'Results'

A lower protein:SL was observed in larvae captured at 0 to 10 m, and is in accordance with the lower CF (i.e. lower weight-at-length) of individuals from 0 to 10 m in that high temperatures encountered near the surface may support growth in length of sprat larvae at the expense of the protein content per unit length, which is closely linked to DW (Lied & Roselund 1984, Bulow 1987). The protein content relative to the body volume of a larva has implications for buoyancy, which is the net result of the lifting forces of water and fat and the sinking forces induced by protein structures (Blaxter & Ehrlich 1974). Prior to having a functional swim bladder, larvae with high protein contents relative to their body length (as found at 65 to 75 m) would require more energy to ascend to and remain in favourable feeding environments, which could be a disadvantage if good feeding conditions are assumed to occur close to the suface.

Vertical distribution

To put the condition indices we examined into an ecological context it is mandatory to evaluate them with respect to the vertical distribution of the sprat larvae as a function of space and time.

Voss et al. (2005) reported that maximum spawning of sprat eggs occurred in June, 6 wk prior to our sampling efforts in the Bornholm Basin. Eggs were distributed in the upper part of the halocline (typically between 45 and 55 m) in the Bornholm Basin. Nissling et al. (2003) found aggregations of sprat eggs between 45 and 60 m in the same area. The mean specific gravity of eggs decreases during the spawning season, resulting in later-spawned eggs having neutral buoy-

ancy at shallower depths in contrast to eggs released earlier in the spawning cycle (Nissling et al. 2003). The sprat larvae examined in our study can thus be assumed to have hatched between 40 and 50 m depth (Fig. 1).

After hatch, sprat larvae perform a first feeding migration into the upper water layers (Voss 2002), which results in maximum concentrations of larvae close to the surface, where prey density and light intensity are greatest and, thus, prey detection and encounter are most likely to be highest (Wieland & Zuzarte 1991, Voss et al. 2005, this study).

Vertical migration behaviour of different amplitude and temporal patterns has been commonly described for sprat larvae (>6 mm) in the German Bight (Kloppmann 1991) and in the Bornholm Basin (Wieland & Zuzarte 1991, Voss 2002). Our data revealed diel vertical movements only between the surface and 45 m (Fig. 2), a depth marking the lower limit of the thermocline (Fig. 1). The limited number of larvae between 45 and 65 m at all sampling times suggests that individuals caught below the thermocline did not perform vertical movements during our 24 h data collection. To cross this depth range undetected by depth-specific sampling with a high temporal resolution, an increase in swimming performance or in the vertical:horizontal ratio of effort would be required. An improved swimming performance appears unlikely since the low temperatures between 40 and 60 m are known to be suboptimal for this species (Nissling 2004). Furthermore, DNA:DW and protein:SL would not have shown clear differences between depths if vertical migrations over the whole water column had regularly occurred.

Alternatively, the abundance minimum of larvae between 45 and 65 m may also be attributed to increased predation by adult clupeids that were found feeding at this depth (Köster & Möllmann 1997). Other potential predators (0-group cod and medusae) occur mainly in shallow areas, but were evaluated as negligible sources of larval mortality (Voss et al. 2005).

At most times of the day, except 06:00 to 08:00 and 21:00 to 23:00 h, the highest numbers of sprat larvae were found close to the surface, whereas density peaks of *Bosmina corigoni maritima*, the most abundant organism in the zooplankton samples and larval guts, were prominent near the surface only during daylight hours, i.e. the vertical movements of the sprat larvae were not clearly linked to those of their potential prey organisms (Figs. 2 & 4).

The spatial and temporal extent and the patterns of vertical migration behaviour have been described as a compromise between sufficient light intensity for feeding (e.g. Last 1980, Munk et al. 1989), spatial overlap with prey (e.g. Lough & Cohen 1982) and predator avoidance (Köster & Möllmann 1997). Voss (2002) reported a spatial mismatch between Baltic sprat larvae and their prey in 1989 and 1990, and explained this phenomenon with larvae preferring areas of optimal water temperature over high food densities. Our findings support temperature as a key factor determining the vertical distribution and migration behaviour of larval sprat.

The selectivity of a particular environment, such as different depth strata, may become manifest by examining variability within condition indices. While the variability (CV) in condition values was generally higher at 0 to 10 m than at depth in most length classes, CV values tended to be highest for short-latency proxies (CF and protein:SL) of relatively small larvae caught at depth (Figs. 5 & 6). Higher variability within condition proxies can denote environments that exert low selective pressure in a relative sense. The CV of short-latency condition proxies decreased with increasing length in larvae from 65 to 75 m but not in larvae from shallow waters. Yolk-sac absorption occurs at 5.6 mm in sprat (Alshuth 1988) and, subsequently, the reliance on exogenous feeding increases with increasing larval length. The relatively low variability in and generally higher median values of condition indices of larger larvae at the surface could either indicate strong selection against individuals in relatively poor condition or a generally favourable environment (so that all members of the population had higher condition values). Given the observed patterns in variability and median condition values, the deep stratum could not be characterised as a favourable growing environments; however, the deep stratum also does not appear to be an area with strong selection against weak individuals.

CONCLUSIONS

Larval sprat condition was not uniformly expressed by all measures applied to test the hypothesis that nutritional state was similar between shallow and deep waters of the central Bornholm Basin. Sprat larvae from 0 to 10 m could not be distinguished from conspecifics at 65 to 75 m using slow (otolith-based daily somatic growth rates) and intermediate responding proxies (RNA:DNA), whereas short-latency proxies (protein:SL and DNA:DW) indicated superior nutritional condition and better (recent) growth of larvae in surface waters. Assessments of a larva's nutritional state using only 1 proxy may thus lead to spurious conclusions. We therefore suggest that a combination of relatively slow-, medium- and fast-responding indicators be implemented, unless sampling periods longer than the response time of all condition proxies (i.e. several days) are utilised and data on cause (good or

poor feeding conditions) and effect (fast growth or starvation) are available. In particular, for sprat residing in a given habitat, the morphometric condition factor CF and the daily protein growth rate Gp (Buckley 1984) should be applied with caution and only in addition to more unequivocal condition indices.

It is still unknown if the nutritional condition of the larvae resulted *from* or *in* the observed Bimodal depth distribution. At the time of sampling, sprat larvae found at 65 to 75 m experienced low temperatures that reduced protein growth (Gp), which may have resulted in higher DNA:DW and lower SL:DW (i.e. protein:SL). The depth-dependent differences in these short-latency proxies suggested that the observed depth distribution could have been a recent phenomenon. At 65 to 75 m, low water temperatures and a concomitant reduction in metabolic rates and other physiological processes would be expected to retain individuals in poor condition.

Successful ascent to the surface would be required for sprat larvae at 65 to 75 m to return to water layers characterised by warm temperatures, sufficient light, high food and low predator concentrations – conditions favouring growth and survival even in larvae with less than optimal nutritional condition. Larval ascents from 65 to 75 m were not observed in our study. Directed movements towards deep waters would be highly maladaptive, since these environments render fast growth and survival unlikely. The larvae we observed in the Bornholm Basin's deep strata may be interpreted as a result of a selection against individuals in a poor nutritional state at these depths (Grønkjær et al. 1997, Voss et al. 2005).

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