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Growth and condition of sprat (*Sprattus sprattus*) larvae inferred
from otolith microstructure analysis and RNA/DNA ratio
in the Bornholm Basin (Central Baltic Sea)
during spawning season 2001

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Der Dekan

Prof.Dr. W. Depmeier

To my wife Ni Wayan Diah Widhi Astuti (Didit)
and our children:
Anhari Luthvan Kamal (Ivan),
Aafini Rizqia Kamal (Fiqi),
and our incoming child

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Zusammenfassung

Wachstum und Ernährungszustand gelten als wichtige Faktoren, welche die Überlebenswahrscheinlichkeit von Fischlarven beeinflussen. Ziel der vorliegenden Arbeit war es, umweltbedingte Variabilitäten in diesen Parametern bei Sprottlarven zu beschreiben. Die Probennahme erfolgte auf Forschungsfahrten zur Leichzeit im April und Mai/Juni 2001 im Bornholm Becken. Die räumliche und zeitliche Variabilität in Wachstum und Ernährungszustand wurde an Hand von Otolithen-Mikrostrukturanalysen, der Bestimmung des RNA/DNA Verhältnisses, sowie mit Hilfe eines ‚individual-based models‘ (IBM) beschreiben.

Die Ergebnisse zeigten eine bessere Lesbarkeit der Otolithen aus dem Mai, verglichen mit, aus dem kälterem Wasser stammenden, Otolithen aus dem April. Die Lage des ‚first-feeding-checks‘, des Startpunkt zur Bestimmung von Alter und Wachstum, ließ dagegen keine Temperaturabhängigkeit erkennen.

Die Analyse der Alters/Längen-Beziehungen zeigte höhere, stark variable Wachstumsgeschwindigkeiten im Mai, besonders für jüngere Larven. Untersuchungen zur horizontalen Variabilität der Alters/Längen-Beziehung lieferten Anhaltspunkte für besseres Wachstum im nördlichen Bereich des Bornholm Becken im Mai 2001. In der Vertikalen war das Wachstum nahe der Oberfläche höher als in der tieferen Wasserschicht.

Die Längen/Gewichts-Beziehungen lieferten ein ähnliches Bild. Die für Gesamtdatensatz angepassten Wachstumsfunktionen resultierten in Exponenten von <3 im April und >3 im Mai, sowie >3 für Larven in tieferen Schichten und >3 in flacheren Schichten.

Allerdings wurden die Ergebnisse durch Unterschiede im abgedeckten Längenspektrum der Larven beeinflusst. Ein direkter Vergleich von Gewicht und Länge für kleineren Larven (4.5-6 mm) zeigte höhere Gewichte im April, während größere Larven im Mai vergleichsweise schwerer waren.

Die Ernährungszustand der Larven war zu beiden Aufnahmen größtenteils gut. Allerdings bestand ein Trend zu höheren RNA/DNA-Werten im Mai. Sowohl die RNA/DNA-Werte als auch Protein-Wachstumsraten bestätigten erneut das Bild besserer Wachstumsbedingungen in flacheren Wasserschichten. Es konnte kein Einfluss der Photoperiode auf den RNA/DNA Gehalt festgestellt werden.

Für einen Unterdatensatz konnte eine kombinierte Analyse der Otolithen-Mikrostruktur als auch RNA/DNA Verhältnisses durchgeführt werden. Larven aus flacheren

Wasserschichten zeigten dabei höhere Gewichte bezogen auf das Alter und eine größere Variabilität im RNA-Gehalt. Der Versuch, die Zuwachsbreite der beiden letzten Otolithenringe mit dem RNA/DNA-Verhältnis zu korrelieren, offenbarte eine Zeitversatz in den Reaktionszeiten. Das RNA/DNA-Verhältnis reagierte deutlich schneller auf veränderte Umweltbedingungen.

Die Anwendung eines ‚individual-based model‘, bei welchem hydrodynamisches Modell mit einem biologischem Modell kombiniert wurde, erlaubte die Rekonstruktion der Wachstumsgeschichten von Sprottlarven. Als biologische Parameter gingen Alter und Wachstum, wie aus den Otolithenstrukturen bestimmt, in das biologische Modell ein. Die Modellergebnisse zeigten eine hohe Übereinstimmung mit dem gemessenen Ernährungszustand der Larven im Mai.

Die räumliche und zeitliche Variabilität in Wachstum und Ernährungszustand der Sprottlarven war größtenteils temperatur-bedingt. Höhere Wassertemperaturen im Mai bzw in den flacheren Wasserschichten resultierten in besseren, für das Überleben vorteilhaften Larvencharakteristika. Als weitere, zur Variabilität beitragende, Faktoren sind Nahrungsverfügbarkeit, Sauerstoffgehalt des Wasser und Photoperiode zu nennen.

In künftigen Arbeiten sollte die Altersstruktur der Sprottlarven quantitative erfasst werden, so dass das Schicksal einzelner Kohorten verfolgt werden kann. Dies würde weitergehende Analyse grundlegender, das Überleben beeinflussender Prozesse in Bezug zum Alter der Larven erlauben. Eine Grundvoraussetzung hierzu ist allerdings die noch ausstehende Validierung der Tagesring-Strukturen in den Otolithen. Für die Erweiterung und Verfeinerung des Sprottlarven IBMs wären weitere biologische Grundlagendaten wünschenswert, wie z.B. Informationen zur Nahrungswahl, zur Vertikalverteilung sowie die Implementation eines Wachstumsmodells.

Summary

The aim of the study was to investigate the growth and condition of sprat (*Sprattus sprattus*) larvae as survival characteristics in relation to environmental changes at a certain spawning season. The study was conducted in the Bornholm Basin where sprat eggs and larvae were collected during two cruises in April and May/June 2001. Spatial and temporal variability in growth and condition were analysed based on information from otolith microstructures, RNA/DNA ratio and an individual-based model. The variation in growth and condition was presented both on population and individual levels.

The results revealed a better clarity and readability in the otolith microstructures of sprat larvae in May compared to April in response to warmer temperature. The first feeding check, the starting point for age and growth determination, was unaffected by temperature differences. Based on the estimated age and length relationship, somatic growth in sprat larvae was higher in May than in April in particular that of younger larvae which were highly variable in length at age. With respect to May, horizontal variability in somatic growth was found on 6 locations in the Bornholm Basin. There was a trend that the larvae encountered in the northern part of the basin experienced the highest somatic growth in comparison with the central and the southern regions. Within the vertical environment, the growth of sprat larvae tended to be higher at the upper layer than at the lower layer.

Based on the length-weight relationship, larvae showed different allometric growth with values of $b < 3$ (negative allometric) and $b > 3$ (positive allometric) for the first and second sampling, respectively. According to depth, larvae exhibited $b > 3$ on the top layer, whereas $b < 3$ was found for larvae in the deeper water. Variability in b values was influenced by different size spectra of larvae collected. Comparing weight at length, it was found that smaller larvae in a size range of 4.5 – 6 mm showed higher weight in April, whereas larger and heavier larvae were found in May.

Nutritional condition of sprat larvae was mostly good during the two months. However, the RNA/DNA ratio tended to be higher in May compared to April. Within the vertical environment, it was obtained that larvae at the upper layer were in a better nutritional condition than those in the lower layer. These findings were also confirmed by the instantaneous protein growth rates (G_{pi}) of the larvae. No diel variation in RNA/DNA ratio was found according to different photoperiods.

A joint analysis of otolith microstructure analysis and RNA/DNA ratio showed a higher weight at age exhibited by larvae from the upper layer compared to the deeper layer. Higher variation in nucleic acids, especially in RNA, was shown in the upper layer, whereas

DNA was relatively constant. A correlation between the sum of the last two increments with the RNA/DNA ratio revealed a delayed response in the otolith structures as the increase in nutritional condition was not responded with larger but with relatively constant increment widths. No effect of otolith dissection was found on the RNA/DNA ratios of the larvae.

The application of an individual-based model which combined hydrodynamic and biological modeling using age and growth estimated from otolith structures showed that the model was appropriate to reconstruct the growth trajectory of sprat larvae. The model results showed a high agreement with nutritional condition data for the May sampling.

Spatial and temporal variability in growth and condition of sprat larvae was largely due to temperature differences. A higher temperature in May than in April as well as warmer water masses in the upper than in the lower layer resulted in better survival characteristics of the larvae collected from the latter compared to the earlier sampling. Other factors that accounted for this variability were food availability, oxygen concentration, and photoperiod.

The future work for sprat larval studies in the Baltic is to quantify the age structure of sprat larvae so that cohorts can be tracked and patterns analysed based on larval age. A prerequisite for this task is the validation of the daily nature of increment formation in the otoliths of sprat larvae. More biological information, e.g. a growth model and feeding habits, is needed for the application of the individual-based model. The vertical distribution of sprat larvae needs to be resolved in the future.

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Erklärung

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Chapter 1. Introduction

1.1 Background

Baltic sprat, *Sprattus sprattus* L. (Family Clupeidae) is one of the important pelagic species in the Baltic Sea and it is assessed as one unit stock within ICES subdivisions 22-29+32 (IBSFC, 2003). In the Baltic Sea pelagic food web, the stock development of sprat is closely related to cod, *Gadus morhua* L., as cod feed to a large extent on juvenile and adult sprat and sprat prey on cod egg and early larvae (Sparholt, 1994; Köster & Möllmann, 2000^a). The biological interaction may shift either from a cod- to sprat-dominated system or *vice versa* (Rudstam et al., 1994). The shift is caused by either unfavourable hydrographic conditions for reproduction and subsequent recruitment failure of one of the species or high mortality caused by the fishery (Schnack, 1997). A corresponding shift to the sprat-dominated system occurred in the Central Baltic within the period of 1977 to 1996 (e.g. Bagge et al., 1994) resulting in a decrease of predation pressure on sprat and followed by a significant increase in sprat population size from 1988 to highest level on record in 1996 (Fig. 1a) (Parmanne et al., 1994; Köster & Möllmann, 2000^b; ICES, 2003; STORE, 2003).

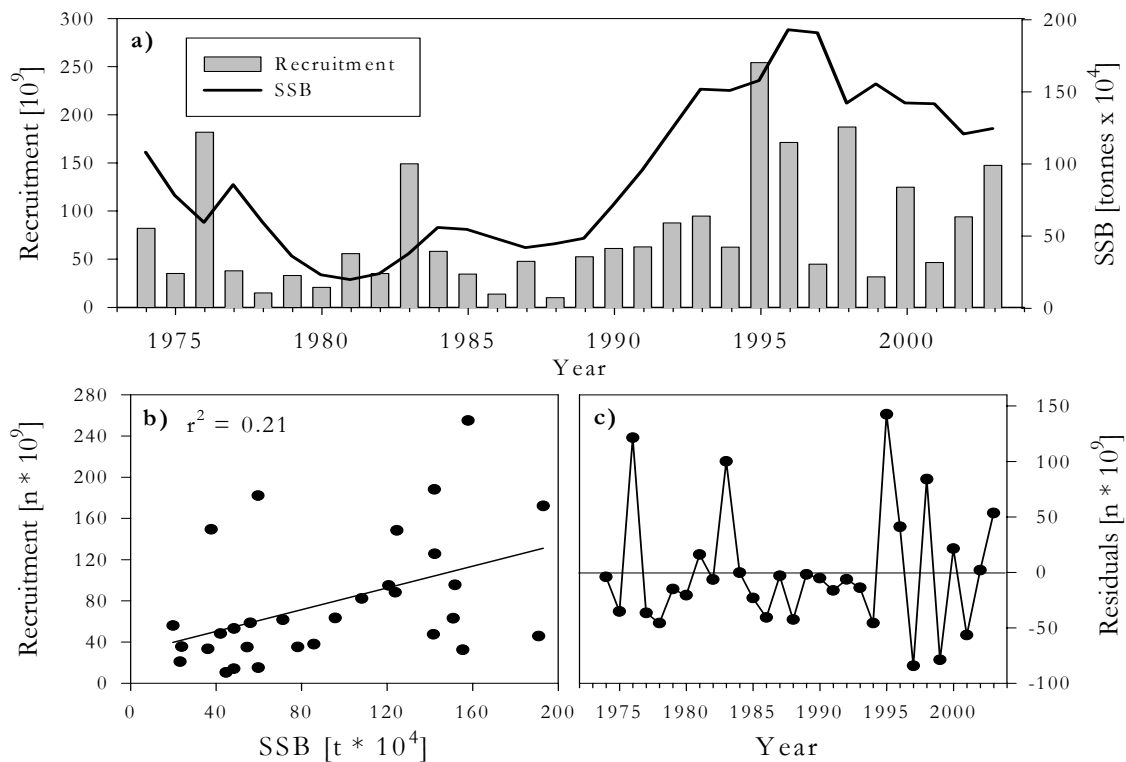


Figure 1. Recruitment (age 0+) and spawning stock biomass (SSB) of Baltic sprat (Sub-Division 22-32) established for the respective years (a). Linearity of stock-recruitment relationship and corresponding residual (b and c). Data source: ICES (2003).

Despite that sprat biomass has increased in response to lower predation from cod during the last two decades, however, density-dependent changes in sprat population show a weak relationship between spawning stock biomass and recruitment level (Fig. 1). The year class strength of Baltic sprat varies greatly; occasionally strong year classes are formed followed by a number of weaker year classes, e.g. in the 1990s a number of strong year classes was produced and the stock reached historically high levels (Parmanne et al., 1994; Cardinale et al., 2002; ICES 2003). Since 2001 the recruitment level has been increasing at relatively stable standing stock levels of 1.25×10^6 tonnes (ICES, 2003). Based on these facts, the causes of variability in year-class strength, in particular, the underlying causes of year-to-year changes in the number of young Baltic sprat surviving to enter the fishery is unclear.

High mortality due to starvation and predation during early life of marine fish is believed to be the principal agent of recruitment variability. With respect to starvation, Hjort's "critical period" hypothesis (1914, 1928) proposed that food availability for first feeding larvae was important. Cushing (1972, 1974) proposed the "match-mismatch" hypothesis stating that the timing of the spring bloom in relation to the timing of larval production was critical. This proposal implies that feeding conditions during the entire larval period have a major effect on recruitment. In the Baltic Sea, the evidence of starvation-induced mortality in regulating the year class strength of sprat has not been documented. Previous investigations confirm that the sprat's main food item, the nauplii and copepodid stages of *Temora longicornus* and *Acartia* spp., start to reproduce in spring and continues throughout the year which highest abundances found in summer (Grauman & Yula, 1989; Kalejs & Ojaveer, 1989; Kornilovs et.al., 2001; Möllmann, 2001). This coincides with the spawning season of sprat which extend from April to July (Alheit, 1988). In addition, strong eutrophication in the study area suggests that food limitation of mesozooplankton species is not very likely (e.g. Hansson and Rudstam, 1990). However, long-term analysis on mesozooplankton biomass in the Baltic during 1959-1997 revealed that a decrease in standing stock of *T. longicornis* resulted in a low growth in sprat (ICES 1999; Möllmann et.al., 2000; Kornilovs et.al., 2001; Möllmann, 2001). Possible reasons are temperature fluctuations in the upper layer (0-50 m) which are driven by meteorological forcing. Low standing stock may also be associated with high predation pressure from clupeids.

Predation can also determine the recruitment variability (Bailey & Houde, 1989). In Baltic sprat, predation occurs through cannibalism on sprat egg from adult sprat at considerable levels so that it is suggested to be a significant source of sprat egg mortality in the Bornholm Basin (Köster & Möllmann, 2000^b). The magnitude of cannibalisms is influenced by the hydrographic condition, i.e. it influences the vertical distribution of the prey and also

the dwelling depth of clupeids (Orlowski, 1991). This is especially the case during periods of low salinity and oxygen concentration in the bottom water, which result in pronounced vertical overlap of predator and prey. Whereas, favourable oxygen conditions during or after inflow periods allow the sprat to stay closer to the bottom, resulting in reduction of the predator-prey overlap (Köster & Möllmann, 2000^b). Other potential predators for sprat larvae may be jelly fish species like medusa and chaetognaths (Arndt & Stein, 1973). This is indicated by a considerable number of fish larvae including sprat found in the gut of *Aurelia aurita* and *Cyanea capillata* as by-catch from ichthyoplankton surveys in the Baltic Sea. However, Barz and Hirche (2003) found that jelly fish *A. aurita* fed largely on cladocerans, suggesting that their impact on the copepod community and on fish larvae seems to be small.

The changes in the hydrographic condition of the Baltic Sea is regulated by the replenishment of the Baltic bottom water from the North Sea. A limited-decadal inflow of rich oxygen- and higher salinity-water from the North Sea to the Baltic Sea (Krauss & Brügge, 1990; Matthäus & Franck, 1992) has caused recruitment failure in cod populations (Plikshs et al., 1993). Low salinity condition will shift cod eggs into the deeper water column where oxygen is very limited or even anoxic (Matthäus et al., 2001; Nausch et al., 2002) leading to high mortality of the early stage of this species. For sprat, oxygen and salinity are unlikely to be the limiting factor. Rather, temperature fluctuation can have a considerable effect; since sprat eggs are found in water temperature below the minimum level ≤ 4 °C (STORE, 2003), necessary for successful egg development. Temperature fluctuation also influences the long-term development of biomass of mesozooplankton which are consumed by sprat larvae (e.g. Möllmann, 2001).

A multispecies virtual population analysis (MSVPA) on the abundance of early life sprat has recently been carried out incorporated with environmental conditions to identify the critical life stages in recruitment process of sprat in the Baltic Sea (Gdanks Deep and Gotland Basin) for time series data 1976-1996 (Köster et al., 2003). The authors found that the year class strength was largely independent of larval abundance. On the other hand, the early and late egg production as well as late egg production and larval abundance were significantly related. The authors suggested that the period between the late larval and early juvenile stage appeared to be critical for sprat recruitment. Potential variables identified to affect this life stage were ambient temperature and wind stress. In order to measure at which magnitude the environment affects the survival of sprat larvae, it would be an advantage to include the information pertaining survival characteristics of the larvae.

Growth rate is one of survival characteristics of fish larvae that can be used to find a link between environmental factors and larval survival. A small change in growth rate can

have a considerable effect on recruitment success (Houde, 1987, 1989). The “stage duration” hypothesis (Cushing, 1975) states that larvae which experience better feeding conditions grow faster, and therefore experience a lower cumulative (total) mortality due to a shortened duration of earlier stages when mortalities are higher. The “bigger is better” hypothesis (Leggett & DeBlois, 1994), holds that larger larvae have increased foraging capability and are less susceptible to predation (implying that larger initial size or faster growth are critical). The present consensus about the “bigger is better” hypothesis (Cowan *et al.*, 1997) seems to be that such a simple conceptual model as decreasing vulnerability with increased size, is not widely applicable because fish are exposed to a complex and changing mixture of predators. The predators may vary in abundance, size and type, which differ in their preferences and behaviour. In some cases larger larvae may be more vulnerable than smaller larvae.

Since otolith and fish size are highly correlated for a variety of marine and freshwater species (Campana and Neilson, 1985), it is possible to estimate growth-rate histories of individual fish by measuring the widths of otolith increments. Applications specific to young fish include the determination of daily age and hatch date, growth rate and mortality rate. When these age-structured estimates are combined with independent information on population abundance, temperature, currents and spawning patterns, factors influencing recruitment can be evaluated. Particularly important in this regard are relationships between the environment and growth rate, larval drift patterns, hatch date frequencies as compared to spawning production, and the relationship between growth rate and mortality rate (Campana, 1984; Campana & Neilson, 1985; Campana *et al.*, 1989; Campana & Jones, 1992; Campana, 1996). Otolith microstructures information of sprat larvae, used as growth proxy has been reported from the North Sea (Alshuth, 1988; Ré & Gonçalves, 1993; Valenzuela & Vargas, 2002), the Irish Sea (Shields, 1989) and the Baltic Sea (Simonsen, 1996).

Another comprehensive tool for assessing the growth of fish larvae is determining larval nutritional condition expressed as RNA/DNA ratio. The amount of DNA in a cell is constant; the amount of RNA indicates how actively the cell is synthesizing proteins, and thus how healthy it is (Buckley, 1979, 1984; Clemmesen, 1988, 1993, 1994, 1996; Ferron & Leggett, 1994; Bailey *et al.*, 1995). Therefore the higher the RNA/DNA ratio the better the nutritional condition of fish larvae. A reliable indicator of the nutritional condition of larvae would allow to quantify and accordingly to estimate starvation impact on wild-caught larvae. Furthermore, RNA/DNA ratios may be used to estimate the growth of fish larvae by means of the instantaneous protein growth rate (G_{pi}) which is an expression of larval growth as a function of nutritional condition and temperature (Buckley, 1984).

The joint analysis of otolith microstructure and nucleic acids determination in a small

single larva has been difficult to apply, in particular since the analysis of RNA/DNA ratio was limited to larvae ≥ 800 mg (Buckley, 1979). Clemmesen (1993) improved the sensitivity of the analysis using a fluorimetric quantification which lead to the possibility to determine the nutritional condition on smaller larvae. By using the coupled analysis, a more powerful biological data set from a single larvae can be obtained including age, length, weight, condition, and growth giving the opportunity to elucidate the interaction between an individual as well as population with the environmental conditions.

Studies of recruitment processes have often been focused on population-level phenomena, such as correlation among environmental factors, stock factors and recruitment levels, or alternatively, on single processes occurring at the level of individual organisms or single life stages, for example predation or starvation (Hinckley, 1999). However, the idea is becoming more generally accepted that mechanisms operating on different time, space or organizational (individual, population, species) scales may be important, and that overall recruitment levels are unlikely to be controlled by one factor or process at one life stage alone (Hinckley, 1999; Rothschild, 2000). A complex set of factors are involved which may act sequentially or simultaneously through compounded interactions to affect year-class strength. The complexity of the relationship between organisms and environmental factor in studying recruitment processes has led to search a comprehensive method in that biological information and physical environment could integrally be analysed (Heath & Galego, 1997).

In this case, individual-based models (IBM) serve as a promising tool. The coupled, biophysical IBM, for example, models each individual's interaction with the environment, and preserves the unique trajectory through time and space of each individual, as well as its growth and survival. The hydrodynamic model is capable of reproducing mesoscale and larger circulation features, of advecting individual through space in a reasonably accurate manner, and of producing spatial distributions of important physical factors such as temperature and salinity (Hinckley et.al., 1996; 2001; Heath & Galego, 1997; Hermann et.al., 2001). In the Baltic Sea, a coupled hydrodynamic-trophodynamic individual-based model of drift and feeding has been developed to analyse the intra- and inter-annual variability in growth and survival of cod (*Gadus morhua*) (Hinrichsen et al., 2002). Due to still lacking biological information regarding growth and feeding habits, such a modeling results for sprat is still limited on a preliminary level (STORE, 2003).

Information available from otolith microstructure analysis may be applied to study the influence of ambient factors on growth of individuals. It is known that environmental conditions, mainly temperature and food availability, can produce differences in the increment widths and in the distance to the hatch check in the otoliths (Campana & Neilson, 1982, 1985;

Garcia et al., 1998). Based on age information estimated by increment number one can use a reverse Lagrangian model trajectory to construct larval's movement before capture. At the same time the hydrodynamic model gives information on environmental condition of the Baltic Sea. Thus, it is possible to construct the otolith-based growth history of sprat larvae from their hatching site to the captured-position.

1.2 Hypothesis

The present study tests the general hypothesis that growth of sprat larvae depends on environmental conditions. The changes in the otolith microstructures, length-weight relationship, and nutritional condition express, accordingly, the growth variability is regulated by the environmental regimes. Within the spawning season, sprat larvae exhibit different survival characteristics through underlying mechanisms which operate during early life stages.

The specific hypothesis addressed by the present work are:

- (1) Somatic and daily growth of increments in sprat larvae is coupled, therefore the number of increments is a reliable tool for larval ageing as well as a proxy for growth. Increment widths can be used as growth proxy of sprat larvae; the narrower the distance between adjacent increments the slower the growth rate.
- (2) The RNA/DNA ratio reflects nutritional condition i.e. the ratio will depend on food quality and quantity as well as environmental conditions.
- (3) Otolith microstructures and nutritional condition are coupled.
- (4) Growth variability of sprat larvae expressed by otolith increments, increment widths as well as nutritional condition reflect the different environmental conditions experienced by sprat larvae before capture.
- (5) Since the hydrodynamic model for the Baltic Sea has been established and verified, information from otolith increment can be used as a model input in developing a preliminary individual-based model for sprat larvae to simulate processes between individual and its environment during early life in the Bornholm Basin.

1.3 Objectives

The general objective of the present study is to study growth of sprat larvae during the spawning season 2001. The growth will be estimated either from otolith microstructure analysis or RNA/DNA analysis or a coupled analysis between the two. In implementing this goal, the specific objective of the study are as follows:

- (a) to age sprat larvae by means of otolith increments and radius development,
- (b) to assess the spatial and temporal variability in growth,
- (c) to assess the spatial and temporal variability in nutritional condition as well as to estimate a proxy for growth of early life of sprat by means of length-weight relationship and protein growth index,
- (d) to analyse whether otolith growth and nutritional condition as well as somatic growth are coupled or uncoupled,
- (e) to analyse the environmental factor that has a major effect on growth variability of sprat larvae over the spawning season.
- (f) to construct an individual-based model of sprat larvae combining a hydrodynamic model and a proxy of growth available from otolith microstructure.

Chapter 2. Materials and methods

2.1 Field work

Study area

The spawning season of Baltic sprat is between April – July (Heidrich, 1925; Alheit, 1988), though, it occurs earlier in the Bornholm Basin, than in the Gdansk Deep and in the Gotland Basin (STORE, 2001). The study was located in the Bornholm Basin, the principal spawning ground for cod and sprat in the Baltic Sea (Herra, 1988; Plikhs et.al., 1993; Köster et.al., 2001), because the hydrographic conditions in this basin during the last two decades supported the reproductive success for these two species (e.g. Bagge & Thurow, 1993). Sampling was performed covering most of the 45 regular grid stations during surveys with RV Alkor for the Stock Recruitment Project (STORE) 1999-2001. The sampling was performed during 17-21 April and 21 May - 6 June 2001 (Fig. 2 and 3).

The primary material delivered to the study were eggs and larvae of sprat collected from two cruises in April and May-June 2001 together with hydrographic measurements on temperature, salinity, and oxygen. Sprat eggs were collected from the grid stations in the central basin where water depth exceeded 80 m in the April survey. Larval sampling was conducted on designated-grid stations at water depth ≥ 60 m. Larvae collection was carried out in both horizontal and vertical resolution. For considering horizontal variation, 6 subareas have been defined in the Bornholm Basin, in the northern, the central, and the southern part, respectively. Each subarea included 4 grid stations covering the three contour depth areas of 40-60, 60-80, and >80 m. Sampling for horizontal resolution was carried out during both surveys, while vertical resolution was achieved during the second survey only. At this time sampling was conducted at 5 m intervals down to 80 m over period of 24 hrs. with 6 hrs time interval on a permanent station on grid station no. 15, where water depth >80 m (Fig. 3).

Egg sampling

In order to obtain newly-hatched sprat larvae, egg were sampled with the Helgoländer larvae net (1.5 m mouth ring and 500 μ m mesh size). At the designated station, the net was vertically lowered to 7 m off the bottom and towed at a rate of 0.5 ms^{-1} and 0.3 ms^{-1} , respectively. Upon recovery, the net's codend was removed and its contents was gently transferred into a bucket. Rinsing the net on board is not recommended otherwise it would damage the egg. The eggs were sorted out using a pipette in a constant-temperature room (5°C) and were kept in several transparent-glass jars each filled with eggs up to about 150-200 $\text{egg}\cdot\text{l}^{-1}$. Neither

staging nor development of egg was conducted on board. The number of egg collected was approximately 2000 eggs. In the laboratory of the Institut of Marine Science (Institut für Meereskunde) in Kiel, sprat egg were kept in transparent containers and were prepared for a larval rearing experiment (see section otolith validation).

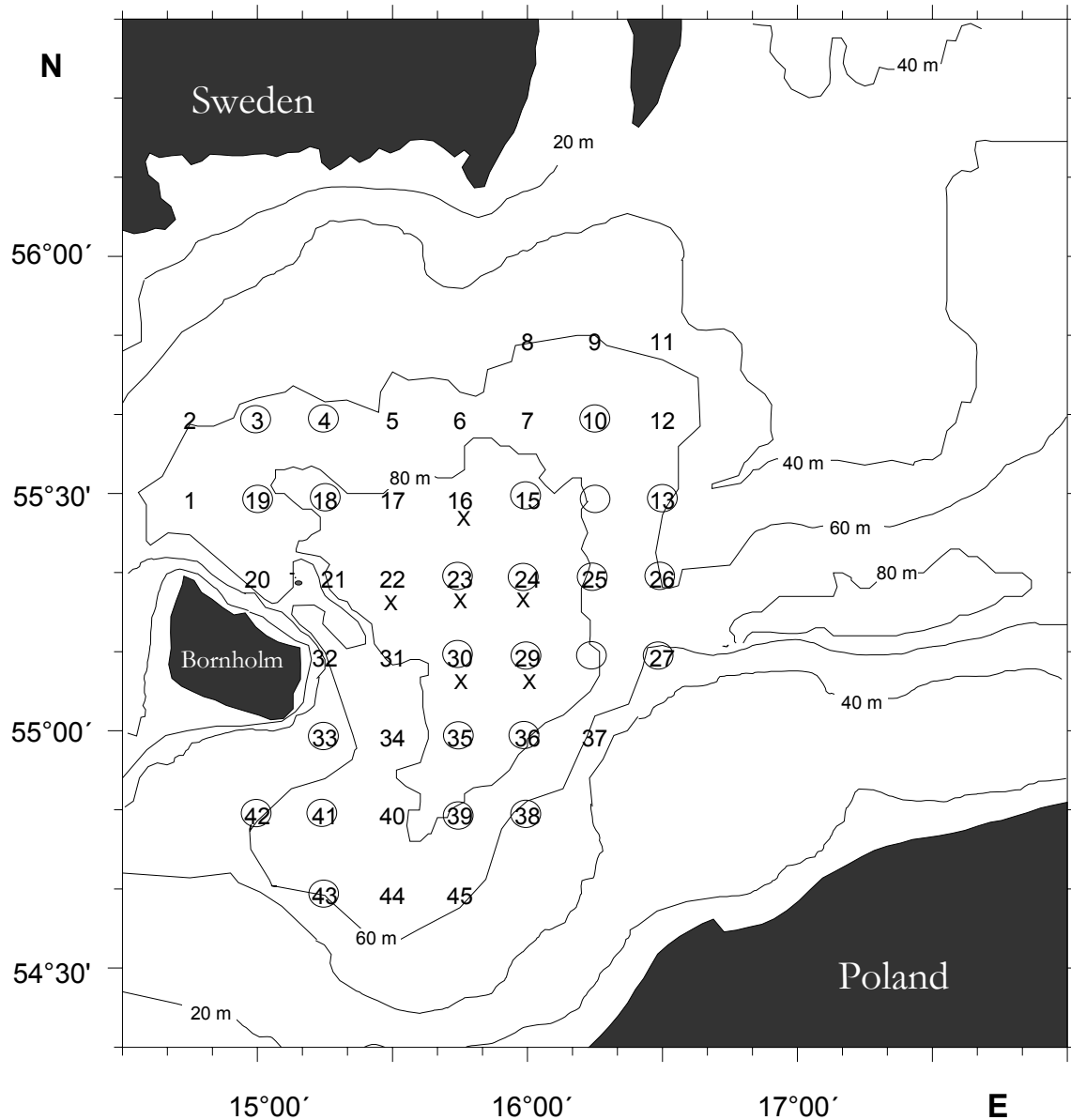


Figure 2. Study area in the Bornholm Basin, the Baltic Sea, showing 45 grid stations and sampling locations during 17-21 April 2001. Marks on the grids indicate where sample were taken for different purposes (crosses = egg, circles = otoliths)

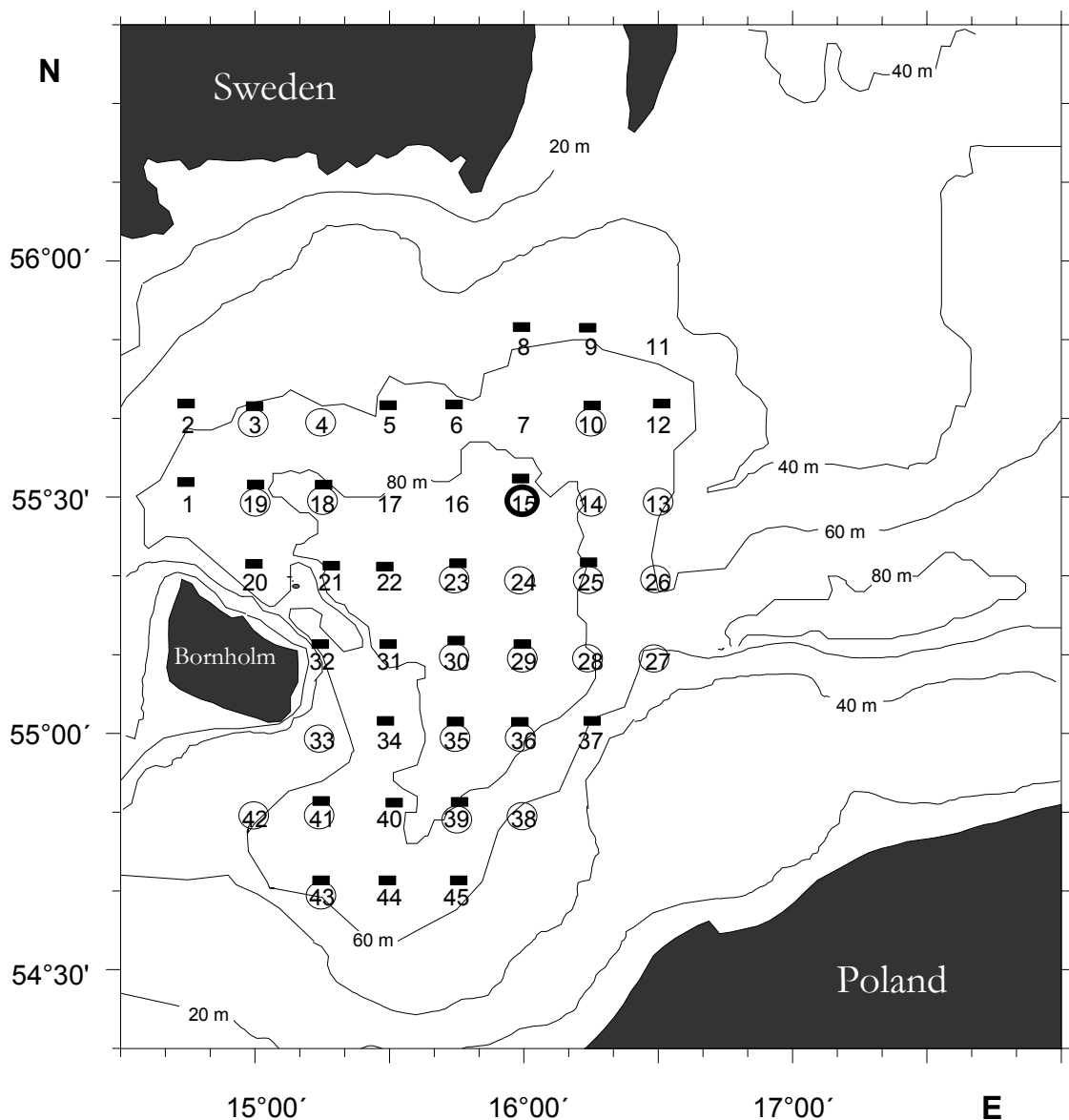


Figure 3. Study area in the Bornholm Basin, the Baltic Sea, showing 45 grid stations and sampling locations during 21 May – 6 June 2001. Marks on the grids indicate where sample were taken for different purposes (circles = otoliths, black squares = RNA/DNA, and bold circle = permanent station at grid 15/biomoc sampling).

Larvae collection

Sprat larvae were collected from the sea, afterwards selected and differently preserved on board depending on the further use for nucleic acids determination, otolith microstructure examination, or both (joint analysis). The sampling was performed both horizontally and vertically.

Horizontal sampling was made with the bongo net 500 μm , equipped with a mechanical flow meter (General Ocean) which measures the filtered-water volume. The

bongo was lowered down to 7 m off the sea bed and towed obliquely from the ship's side to the surface at a rate of 0.7 ms⁻¹ and 0.5 ms⁻¹, respectively. The flow meter counting and the duration of towing were recorded. On recovery, all plankton on the net side was gently washed down into the codend and the contents were transferred into a white-plastic tray for larvae selection in a room with constant temperature (5°C). First selection was done on larvae which were used for RNA/DNA analysis. The selection was done for a maximum time of 15 min. to prevent the possibility of changes in nucleic acids contents of the larvae during handling. Larvae were sorted out using a fine forceps and were transferred into *eppendorf* vials containing seawater. Up to 5 individuals were placed in one vial. Subsequently, the vials were transferred into liquid nitrogen, until they were stored at -71°C in the laboratory. The remaining larvae were then selected for otolith analysis and were preserved with alcohol 96%. Finally, the rest of the sample were screened with 500 µm and altogether were preserved with 4% borax-buffered formalin.

Vertical sampling was made with biomoc. The biomoc is a modified 1m² opening MOCNESS (Multiple Opening/Closing Net and Environmental Sampling System) comprised of nine nets (325 µm mesh size), and its operation has been mechanically computerised making possible to close the net automatically at certain depth. Sampling was conducted on one permanent station (Fig. 3) for 24 hrs. with 6 hrs. interval resolving different time for day, dusk, night, and morning between 5-6th June 2001. In order to sample larvae with 5 m depth interval down to 80 m, one sampling consisted of 2 hauls. The biomoc was towed behind the ship at a speed of about 3 knots. On retrieving, each net was gently rinsed and the collectors were removed, and in the following sprat larvae were sorted out and preserved similar as the bongo sample.

Hydrographic measurements

The measurement of salinity, temperature and oxygen were made by CTD casts on each of the designated stations.

2.2 Laboratory works

2.2.1 Otolith microstructure analysis

Otolith validation

The aim of validation was to investigate the time at which first increment is deposited on the otolith of post-hatched sprat, and secondly to observe the periodicity of increment formation. Validation on daily increments was directly conducted on laboratory-reared sprat larvae which

were derived from sprat egg (see egg sampling). The newly-hatched larvae were transferred into aquaria of 40 l in volume with a density of up to 5 ind.l⁻¹. Water was maintained at a temperature of 10°C and a salinity of 13 psu. Larvae of different age were kept in different aquaria, i.e each was filled with larvae of similar known age. Larvae were not fed during the experiment and about 10-15 % of water was changed daily with water from the Kiel Bay. About 5-10 individual larvae were collected daily from which the otoliths were dissected. The experiment was terminated after 10 d as total mortality of larvae occurred.

Otolith dissection

When dissecting the otoliths, the sagittae were chosen for examination (Fig. 4). No differentiation has been made between right and left sagittae as preliminary evidence showed no differences in diameter size between the two sides (Kamal, unpubl.).

Sprat larvae were mounted on object slides and the length was measured with a calibrated-ocular grid under a stereo binocular microscope (Wild Heerbrug) to the nearest 0.1 mm. The microscopes was equipped with a polarizing filter to simplify the detection of the otoliths as they reflect a bright light in the dark view field when viewed in polarized light. Before dissection take place, the larvae's trunk was cut off and in the following one little drop of water was added using a pipette to ease the removal of the sagittae from the larva's head using a pair of hand-grip needles. After the dissection was accomplished, the sagittae were allowed to dry for 15 min. prior to embedding into nail polish.

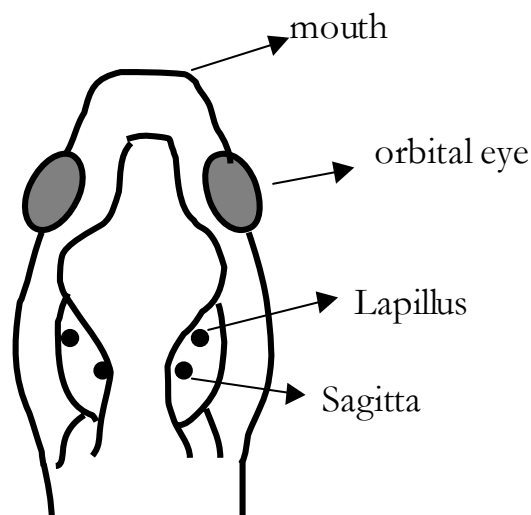


Figure 4. Illustration of otolith position in the head (ventral view) within the vestibular apparatus of typical teleost larvae. The position of the sagitta is behind the lapillus measured from the orbital eyes (redrawn from Jamieson, 2001). The trunk was cut off before dissection.

Otolith reading

The mounted otoliths were placed under the objective lens of the microscope (Leica Leitz Wetzlar). Sagittae were first located on the object slides using an objective with 10x magnification. Prior to the actual examination which was done with an objective with 100x magnification, one little drop of immersion oil (Nikon Type A $n_d=1.515$) was added on the slide. Due to a CCD camera, the picture of the otolith can be seen on a 256 gray scale monitor with a magnification of 787.5 times (100x12.5x0.63, for objective lens, ocular lens, and camera adaptor, respectively). In the following, by rotating the camera on its adaptor the position of the otolith is adjusted so that the longest axis is in a horizontal position on the monitor (Fig. 5). Data measurement on hatch check, increment growth, ring number and eventually the radius appear on a computer monitor of which all recording has been calibrated into μm unit. The data is stored as ASCII file then can be exported to EXCEL file (Heilmann, 1997). In the present study, reading was done twice and it accepted a variation <10% between the first and second reading. No sub-daily increments were considered during reading.

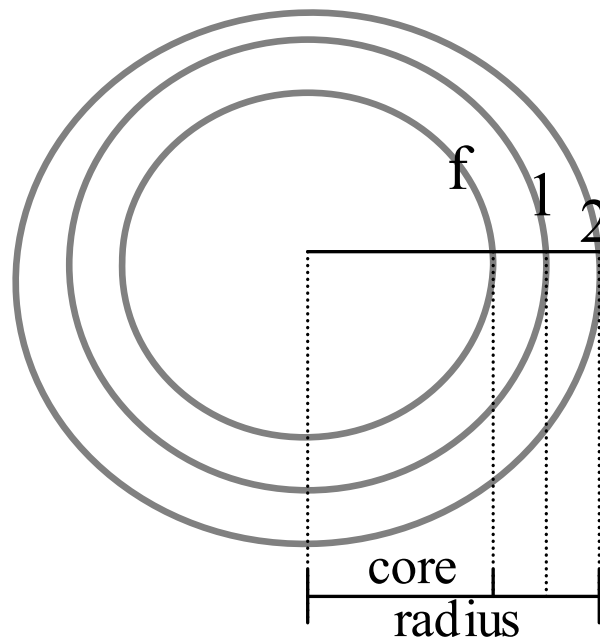


Figure 5. Illustration of otolith reading applied in this study. n = nucleus, f = first feeding check, i.e. the first clear increment formed. The horizontal distance between nucleus and first ring or first primordium (Kalish et al., 1995) is determined as core radius. Next to f , the number of increment is determined (1,2,..., i). Increment width is the distance between two successive rings. Otolith radius is the distance between the nucleus to the last increment edge, on the longest axis.

Age determination

The increment number was used to estimate larval age by adding 6 to the number of increments found (Alshuth, 1988). After correction, therefore, the youngest larvae presented in the results will be 7 d old. The increments are assumed to be formed daily, i.e. each increment is completely deposited within a 24 hrs period.

2.2.2 RNA/DNA ratio analysis

Determination of nucleic acids was based on Clemmesen (1987, 1993, and pers. comm) using the layout of Mesocosms Start Samples week 1-5 which were carried out on 32 individual larvae in one run measurement (Fig. 6). Samples were the wild-caught sprat larvae within the vials that have been stored at -71°C .

Sample preparation

The vial content was allowed to defrost, afterwards max. 32 individual larvae were taken and length measured to the nearest 0.1 mm under ocular ruler stereomicroscope (Wild Heerbrug). Each larva was then transferred into a numbered-ependorf vial that confined in a rack with ice bath beneath. Afterwards, the vials were covered with perforated parafilm and were frozen for 15 min before being placed in the precooled freeze-dryer for ≥ 14 h. On the next day, the vials were placed into a desiccator to limit rehydration of the larvae. Hence the larvae were weighed to the nearest 0.0001 mg using microbalance (Sartorius SC) and returned into their vials confined in a rack which ice bath beneath. In the following larvae were rehydrated with TE-SDS-buffer 0.01% (see next section below).

In case of joint analysis between nucleic acids and otolith microstructure, prior to rehydration of the larvae, the otoliths were dissected as quick as possible to prevent changes in RNA and DNA content of the larvae (Fig. 6).

Nucleic acids extraction

The freeze-dried larvae were rehydrated in 400 μl TE-SDS-buffer 0.01% and allowed to stabilize for 15 min. Cells were disrupted in shaking mill (Retsch Type MM2) with two different size glass beads (3 pieces of 2.0 mm and a small tip of 0.2 mm) for 15 min. Afterwards, the homogenate was centrifuged at 6000 rpm at 0°C for 8 min (Heraeus Minifuge T). Prior to fluorometric determination, a 300 μl of supernatant were taken and put into a new-numbered vials from which 2 x 130 μl of supernatant of each vial was transferred into microplate (Labsystem cliniplate 96 wells) for total nucleic acids and DNA measurement. In

addition, solution standard for RNA and DNA calibration, blank sample with and without RNase were prepared. Table 1 shown the loading map in the microplate. Dilution factor (DF) was adjusted before transferring the supernatant into the wells. It was determined that 1 unit of DF was approximately ≤ 0.15 mg of larvae. In case of larger larvae, DF was increased by adding more TE-SDS buffer into supernatant (Fig. 6).

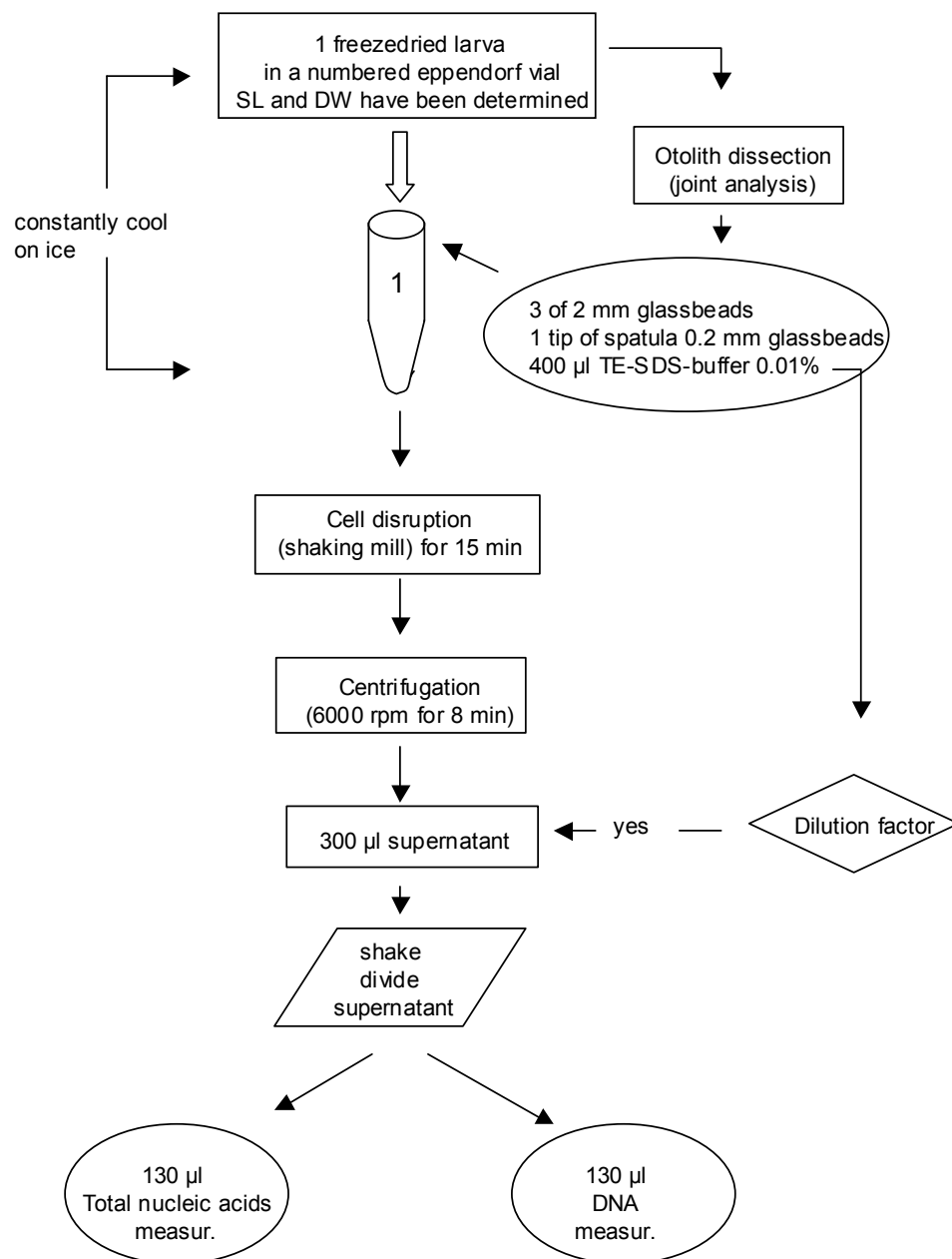


Figure. 6. Flow chart for nucleic acids determination in sprat larvae based on mesocosms start samples week 1-5. Maximum number of larvae analysed in each measurement is 32 individuals (Clemmesen, pers. comm.).

Table 1. The loading map for a microplate 96 wells prior to fluorometric determination of 130 μ l supernatant (S), RNA-DNA calibration (10-50 μ l standard with 2 replica), blank (130 μ l TE SDS buffer) with and without RNase and DNA after RNase. RNase added was 25 μ l.

	DNA calibration		RNA calibration		Total RNA + DNA				DNA after RNase			
	1	2	3	4	5	6	7	8	9	10	11	12
A	X	Blank	X	Blank+RNase	S1	S9	S17	S25	S1	S9	S17	S25
B	X	Blank	X	Blank+RNase	S2	S10	S18	S26	S2	S10	S18	S26
C	Blank	Blank	Blank+RNase	Blank+RNase	S3	S11	S19	S27	S3	S11	S19	S27
D	10 μ l	0.125 μ g	10 μ l	0.2 μ g	S4	S12	S20	S28	S4	S12	S20	S28
E	20 μ l	0.250 μ g	20 μ l	0.4 μ g	S5	S13	S21	S29	S5	S13	S21	S29
F	30 μ l	0.375 μ g	30 μ l	0.6 μ g	S6	S14	S22	S30	S6	S14	S22	S30
G	40 μ l	0.500 μ g	40 μ l	0.8 μ g	S7	S15	S23	S31	S7	S15	S23	S31
H	50 μ l	0.625 μ g	50 μ l	1.0 μ g	S8	S16	S24	S32	S8	S16	S24	S32

Fluorometric measurements and determination

Nucleic acids were fluorometrically quantified in a microtitre fluorescence (Fluoroscan LabSystems) using ethidium bromide (EB) with concentration 1:4 as fluorophor on 200 μ l total volume in each well (Table 2). EB is an intercalating reagents that reacts specifically with based-paired regions of DNA and RNA at the wave length of 355 nm extinction and 550 nm emission (Le Pecq and Paoletti 1966; 1967; cited by Malzahn (2001)). The fluorescence signal depends on the concentration of nucleic acids. The sequence analysis in nucleic acids determination is programmed in the ASCENT lay out.

Table 2. The loading map showing fluorometric determination on total volume 200 μ l derive from loading map with hand pipetting (Table 1) and dispensers of microtitre fluoroscan (bold numbers).

Blank		RNA and DNA calibration					Sample		Remarks
DNA	RNA						Total	DNA	
		10	20	30	40	50			RNA&DNA standard
							130	130	Supernatant
	25							25	RNase
130	130	130	130	130	130	130			TE-SDS-buffer
20	20	20	20	20	20	20	20	20	EB 1:4 (dispenser 2)
50	25	40	30	20	10	0	50	25	TE-buffer (dispenser 1)
200	200	200	200	200	200	200	200	200	Total volume

To measure DNA fluorescence after treatment of RNase, the 130 μl supernatant + 25 μl RNase and 25 μl TE-buffer were incubated at 37°C for 30 min eliminating RNA content. In the following, 20 μl EB were added to measure the self fluorescence and the total fluorescence.

The quantity of the total nucleic acids fluorescence was determined by mixed 130 μl supernatant with 50 μl Tris-buffer as compensating volume for RNase in DNA measurement. After the value of the self fluorescence was determined, 20 μl EB was dispensed to determine the total fluorescence.

DNA fluorescence is determined by subtracting the total fluorescence of the sample with the self fluorescence of the sample and EB. RNA fluorescence is known from subtraction of the total fluorescence with DNA fluorescence and the self fluorescence of the sample as well as EB.

Calibration curve

The concentration of nucleic acids is determined numerically based on a calibrated value of RNA and DNA. Calibration takes into account the slope and intercept from the relationship between the relative fluorescence value and μg DNA or μg RNA (Table 3). Phagus of lambda DNA (Boehringer Mannheim GmbH, Germany, 745782) was used in DNA calibration in the range of 0.125 – 0.625 μg , whereas RNA was calibrated with RNA standard (16s, 23s- ribosomal of *E. coli*, Boehringer Mannheim GmbH, Germany, 206938) in the range of 0.2 – 1.0 μg . The calibration was done in two replica (Table 1).

The slope and intercept obtained of each measurement were averaged. There were 541 larvae analysed. However, DNA and RNA standard used for calibration were derived from different date, therefore the integrated regression was split into two groups of larvae (Table 3). The slope proportion of RNA/DNA shown by Table 3 is between 0.466 – 0.470 which is close enough to ratio 0.46 of LePecq and Paoletti (1966; 1967).

Table 3. The averaged slope and intercept of two different DNA and RNA standards used in analysis of larvae numbers 1-139 and 140-541, respectively (rf = relative fluorescence, coefficient variation of both were less than 5%).

No. Larvae (n)	DNA calibration		Slope Ratio	RNA calibration	
	Regression	r^2		Regression	r^2
001 – 139	rf = 76.7333 μg + 0.8903	0.99	0.466	rf = 35.7858 μg + 0.9668	0.99
140 – 541	rf = 60.2303 μg + 1.2614	0.99	0.470	rf = 28.3159 μg + 0.9868	0.99

RNA and DNA calculation

The results of fluorometric measurement consist of the self fluorescence, the total fluorescence of nucleic acids, and the fluorescence of DNA after RNase treatment values. The data is in ascent file (*.sef) which could be imported into excel file (*.xls) for further calculation. Briefly, the calculation of DNA and RNA are as follows (RF = relative fluorescence, SF = self fluorescence, and TF = total fluorescence):

- (a) Define intercept and slope from relationship between $\mu\text{g DNA}$ and RF_{DNA} (Table 3)
- (b) Define intercept and slope from relationship between $\mu\text{g RNA}$ and RF_{RNA} (Table 3)
- (c) $\text{RF}_{\text{RNA+DNA}} = [(\text{TF}_{\text{RNA+DNA}} - \text{SF}_{\text{RNA+DNA}})] - \text{average RF}_{\text{DNA(blank)}}$
- (d) $\text{RF}_{\text{DNA(RNase)}} = [(\text{TF}_{\text{DNA(RNase)}} - \text{SF}_{\text{DNA}})] - \text{average RF}_{\text{RNase(blank)}}$
- (e) $\mu\text{g DNA} = (\text{d}) - \text{intercept}_{(a)} / \text{slope}_{(a)}$
- (f) $\text{RF}_{\text{RNA}} = (\text{c}) - (\text{d})$
- (g) $\mu\text{g RNA} = (\text{f}) - \text{intercept}_{(b)} / \text{slope}_{(b)}$
- (h) $\mu\text{g DNA}_{\text{total}} = (\text{e}) * \text{dilution factor}$
- (i) $\mu\text{g RNA}_{\text{total}} = (\text{g}) * \text{dilution factor}$
- (j) $\text{RNA}_{\text{LePecq/Paoletti}} = [(\text{f}) - \text{intercept}_{(a)} / \text{slope}_{(a)}] * 2.2$ (2.2 is an inverse of 0.46)

Out of all larvae analysed, there were approximately 1% small larvae ($\leq 5 \text{ mm}$, $\leq 0.0030 \mu\text{g}$) were excluded from analysis due to low DNA content to be quantified resulting in negative values of RNA/DNA ratio.

Instantaneous protein growth rates (G_{pi})

The growth rates of sprat larvae was also determined as instantaneous protein growth (G_{pi}) values by simply multiplying the RNA/DNA ratios with temperature. The method was originally derived from Buckley (1984) based on his experiment on 8 marine fish larvae. However, using his method the sample tissue for analysis is limited to $\geq 800 \mu\text{g}$ fish larvae. To measure the G_{pi} value of small larvae which its RNA/DNA ratio analysed by fluorimetric method (Clemmesen, 1993), therefore, Clemmesen (unpublish.) made a calibration factor for the ratio that is appropriate for Buckley's formula by multiplying the calculated RNA/DNA ratio with factor 1.3.

2.3 Joint analysis between otolith and RNA/DNA ratio in a single larvae

The analysis were done on 89 sprat larvae ranging from 6.95 – 18.78 mm and 0.0164 – 0.502 mg selected from biomoc sample representing various depths of two different day times, night and morning. Since otolith dissection was time consuming on larvae less than 6 mm in size

which may cause changes in nucleic acids content, therefore, coupled analysis has been restricted to larger larvae.

2.4 Determination of abundance and size distribution

The number and size of sprat larvae were combined from both analysed individuals mentioned above as well as non-analysed larvae selected from formalin preservation. The larvae were all counted and length measured. The larval length was defined with 1 mm interval. The abundance is the number of larvae divided by filtered-water volume recorded during bongo/biomoc operation.

2.5 Application of biophysical modelling

A coupled biophysical model has been developed which consists of 2 components: a 3-dimensional Baltic Sea Model circulation and an individual-based biological model (IBM) based on the otolith increment of sprat used to estimate age and otolith growth.

Hydrodynamic model

The hydrodynamic model of the Baltic Sea was constructed by Lehmann (1995). To date, the model has been proven to be suitable to simulate the major features of the Baltic Sea, these include the general circulation, mixed layer dynamics, water mass exchange between the North Sea and the Baltic, exchange between deep basins as well as major Baltic inflows (Hinrichsen et al., 1997). A verification by comparing model simulation and measurement in the field confirms a high agreement between the two (e.g. Hinrichsen et al., 1997, 2001, 2002; Lehmann & Hinrichsen, 2000).

Briefly, the model domain consists of the entire Baltic Sea with horizontal resolution is 5 km and 41 vertical levels specified, taking into consideration the different sill depth in the Baltic. Simulated three-dimensional velocity fields were extracted to develop a database for a Lagrangian particle tracking exercise on larval sprat. This data sets offer the possibility to derive Lagrangian drift routes by calculating the advection of “marked” water particles representing individual larvae. Vertical velocities were calculated from the divergence of horizontal velocity fields. Three-dimensional trajectories of the simulated drifters were computed using a 4th-order Runge-Kutta scheme. The drifters were allowed to leave layers from which they were initially released. The position of the drifters varied over time as a result of three-dimensional velocities that they experienced. Furthermore, the data contain information on the temporal evolution of the hydrographic property fields (temperature,

salinity, oxygen, current velocity, etc.) along trajectories. The initial launch positions can be chosen independently from the vertical resolution of the model's grid.

Biological model

The number of otolith increments was assumed to reflect a daily pattern so that the age of sprat larvae could be determined, accordingly, from the beginning of first feeding date in the Bornholm Basin during April and May 2001. Otolith growth is temperature dependent (e.g. Campana, 1997), accordingly the fluctuation of increment widths reflect the change in temperature condition and food availability experienced by the larvae before capture. The information on age, hatching date, and the mean of increment width allow to back-calculate the spawning site as well as the growth history of the larvae by means of a Lagrangian particle-tracking model (Hinrichsen et al, 1997). The assumption in applying the model is that somatic and otolith growth is a couple process which implies that increment number is a reliable estimate of age.

Model simulations

The coupled hydrodynamic and IBM models were run for April and May 2001 to obtain the intra-seasonal variability in otolith growth of sprat larvae. Therefore, a total of 424 Lagrangian drifters were released consisting of 122 and 312 drifters in April and May, respectively. The depth was chosen at 10 m on three-dimensional spaced grid enclosed by the 60-m isodepth, the area where sprat larvae were most abundant (Voß, 2001, but not in the present study). Each larval drifter was released from the locations where they were captured. In this case, sprat larvae with known age and position were traced back to its hatching date using a backward calculation of drift trajectories by reversing the temporal sequence of the three-dimensional flow fields and by inverting the sign of velocity vectors. A detailed description of the method is given in Hinrichsen et al., (1997). The growth of sprat larvae, therefore, was estimated from the mean of increment widths divided by temperature condition that experienced by the larvae within their trajectory in the Bornholm Basin.

Model validation with RNA/DNA ratio

A coupled biophysical model was verified with RNA/DNA ratio of sprat larvae derived from sampling in May 2001 (see Fig. 3). Each station consists of 5-10 individual larvae of which the mean value of their nutritional conditions was used to illustrate the horizontal distribution of sprat larval condition. The data were compared with the modeling result from corresponding sampling dates.

2.6 Data analysis and statistical tests

Data were categorized by different sampling periods April and May for horizontal sampling and June for vertical sampling. Further grouping was done for the May survey according to different contour areas. Larvae were assigned to size classes with the aim of analysing their individual variability in abundance and distribution, length-weight relationship, RNA/DNA ratios, and instantaneous protein growth rates. Following normality data using the Kolmogorov-Smirnov method or simply from box plot analysis, the difference between categories/size class was tested using Student's t-test (comparing mean) or Mann-Whitney U test (comparing median). Overall differences in categories which comprise from several groups was tested using one-way anova, followed by post-hoc comparison of honest-significant-difference (HSD) Tukey for unequal sample. Alternatively, the difference in the slope of the linear regression was compared using ANCOVA (Zar, 1984). Differences between groups were considered significant at probability levels below 0.05. All statistical analyses were carried out with STATISTICA 5.5 for Windows (StatSoft Inc., 1995).

Growth protein index (Gpi) based on RNA/DNA ratio values was calculated using the formula of Buckley (1984) with correction factor of 1.3 from Clemmesen (unpublish.) as follows:

$$\text{Gpi}[\text{d}^{-1}] = [0.93 \text{ T} + 4.75 \text{ RNA-DNA}(1.3) - 18.18]$$

Larval densities was determined as number per m^3 (N) by dividing the number of larvae counted (n) with filtered-water volume, as follows:

$$N = [n / (C / (36.053 \times \pi \times 0.3^2))]$$
 for bongo, where C is flowmeter revolution. The value of 36.053 is flowmeter revolution/m. Abundance data are converted to nm^{-2} by multiplying the calculation using formula above with corresponding depth.

In addition to power function and regression analysis, the probability distribution function was used in order to explore the relationship between variable (x) and variable (y). The statistical analysis is described in detail in Pepin et al. (1999) and Evans (2000) originally used to examine the probability distribution of nutritional condition based on RNA/DNA ratio to size in marine fish larvae. The basic idea of applying this method is to analyse the probability of a random variable y (sometimes called as dependent or response variable) in dependency to some other variable x. In this case I extended the analysis of response variable dry weight, condition, etc in relation to size, age as independent predictor. The dependency of each y value to x, the local influence of response to x variable is generalised CDF (cumulative density function) by kernell smoothing. The assumption is that observation to the target x are more relevant for estimating the distribution at x. The application of this method, however, was restricted only to percentile distribution from CDF, i.e percentiles 10th, 90th, and

50th (median). The closeness of percentile lines to data points is determined by bandwidth which depends on euclidean distance and ordinal points in the x-y plane. The smaller the bandwidth the closer the lines. However, small data and highly scattered data may contract the function distribution roughly from data points. In this case the bandwidths were then set at the point which limit distribution fit to the data point. The analysis was carried out with SIGNIF.EXE software for Windows 98/Windows Millennium (Evans, 2000).

Chapter 3. Results

3.1 Hydrographic condition

The changes in hydrographic condition in the Bornholm Basin during April and May 2001 are presented in Fig. 7. In May, water temperature was higher in the upper layer which was characterised by a thermocline layer between 10-50 m depth as the season progressed from late spring to early summer. Highest temperature differences up to 4.5°C were found at the surface between 0-15 m depth. On the contrary, a relatively stable temperature condition was found below the thermocline (50-90 m). Salinity profiles shows unaltered in all depths, whereas oxygen slightly declined by approximately 1 ml.l⁻¹ throughout the water column in May. A sharp decline in oxygen started at depth 50 m and at the beginning of 70 m depth water masses were characterised by a poor oxygen condition (< 2 ml.l⁻¹). A relatively stable condition in water temperature as well as salinity and to a lesser extent a slight drop in oxygen indicated an absence of water exchange between the North Sea and the Baltic Sea replacing bottom water masses of the Bornholm Basin.

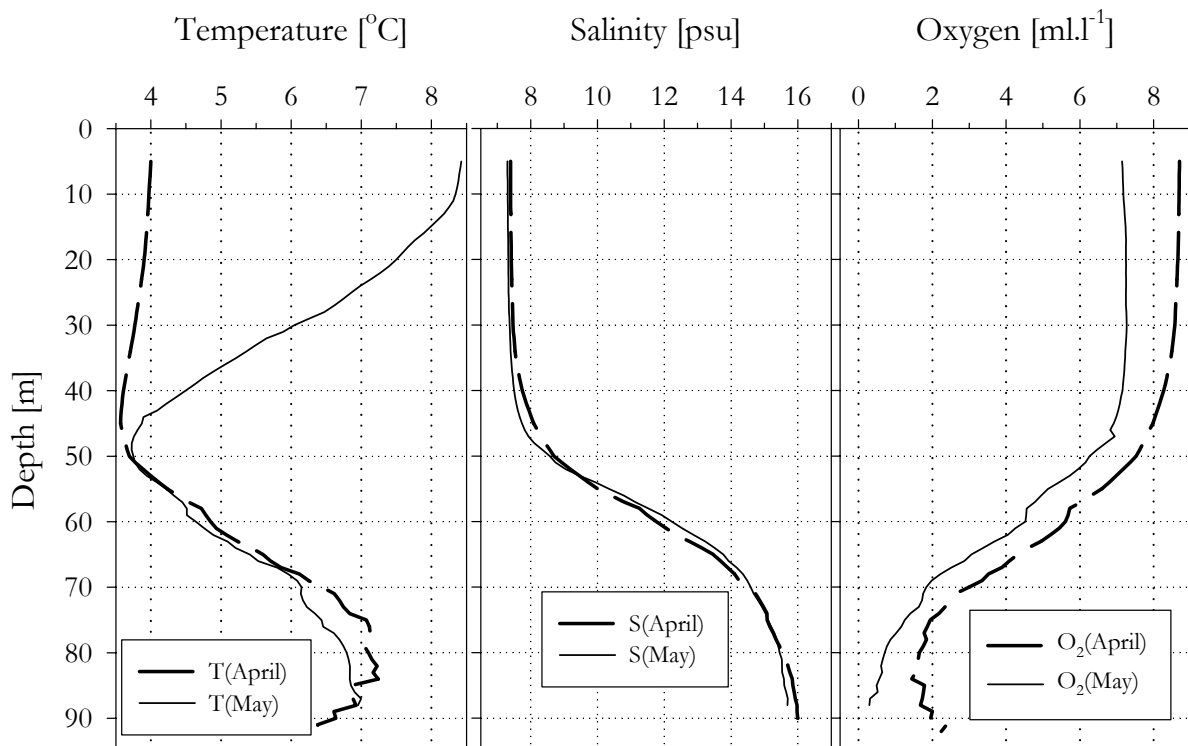


Figure 7. Average vertical profiles of salinity, temperature, and oxygen in the Bornholm Basin during April and May 2001. The profiles were depicted from measurements with 1 m depth intervals.

3.2 Abundance and size distribution

Figure 8 and 9 show the horizontal abundance and distribution of sprat larvae sampled with bongo 500 μm over the Bornholm Basin during two consecutive surveys. These are shown by the histogram frequencies which represent 6 locations in the northern, central, and southern regions in the basin. Each location was an aggregation of 4 grid stations.

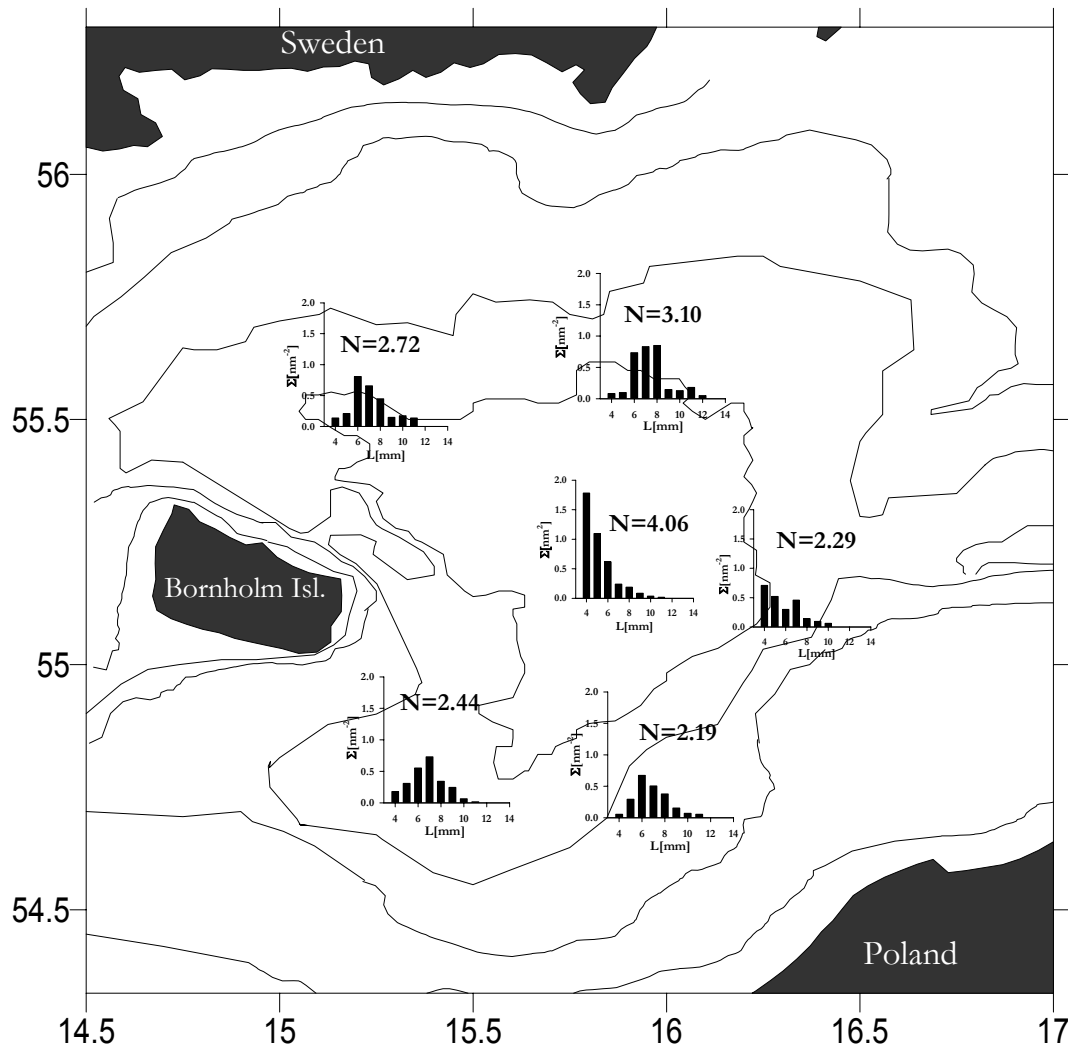


Figure 8. Abundance and size distribution of sprat larvae collected with bongo 500 μm in the Bornholm Basin during April 2001. For more explanation see Fig. 9.

The larval abundance were all found higher in May compared to April reflecting that the latter sampling was probably close to the peak spawning season. Both figures confirmed the highest abundance was found in the central part of the basin which may be an indication that the central part of the basin is an important spawning ground of this species. The abundance of larvae in the northern part was higher compared to the southern part. Likewise, with respect to size distribution, the central part was dominated by smallest larvae supporting

the believe that sprat larvae spawn primarily in the deeper water. The histograms of size distribution were all non-normally distributed (Kolmogorov-Smirnov test). It is clearly shown in particular that larvae sized 6-8 mm were found in a higher abundance in the coastal areas as well as the shallower part of the eastern basin.

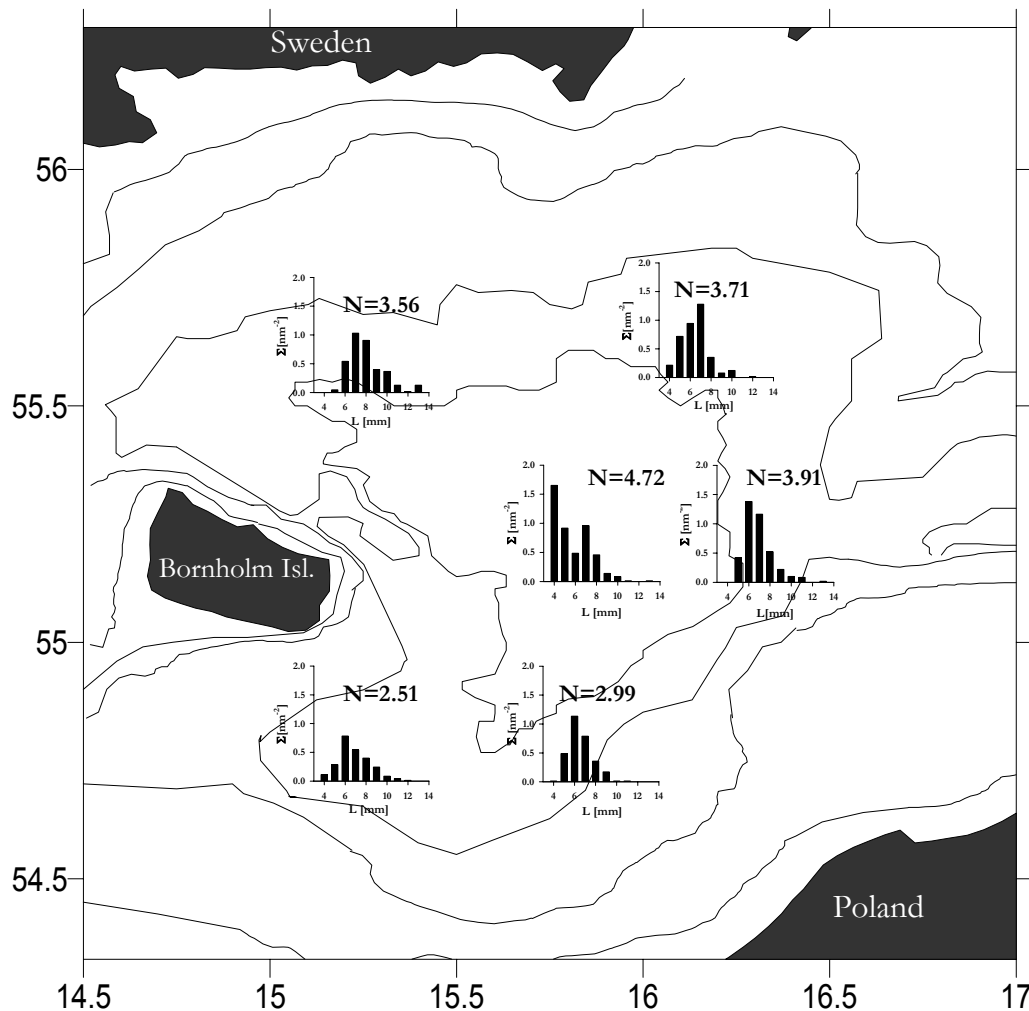


Figure 9. Abundance and size distribution of sprat larvae collected with bongo 500 μm in the Bornholm Basin during May 2001. The histograms are representing larval abundance and distribution which has been aggregated from 6 locations that covered contour area of 60-98 m (shown by contour lines). Symbols N, Σ , and L stand for nm^{-2} , abundance at size, and length, respectively.

A daily pattern in abundance and size distribution of sprat larvae within vertical column was compared between night and morning on 5-6th June 2001 (Fig. 10a-f). The results of dusk and day sampling were not included due to the low number of larvae collected. In the absence of light, sprat larvae were predominantly found in the upper as well as in the bottom layer with a lesser variation in between (Fig. 10a). During morning, contrarily, the highest abundance was found in the intermediate water column then in the bottom, whereas

the lowest abundance occurred at the surface (Fig. 10b). By grouping the larval size into small (5-10 mm) and large (>10 mm), it is shown that small larvae expressed a higher variability in abundance than the larger ones in particular during dusk (Fig. 10c-f). Larger larvae occurred predominantly at 5 m depth and abruptly decreased at depth 10 m down to the bottom during night, whereas smaller larvae seemed to exhibit an opposite pattern in distribution to the larger ones during night (Fig. 10, 10e). In contrast to smaller larvae, little variation in abundance was shown by bigger larvae during dawn (10d, 10f).

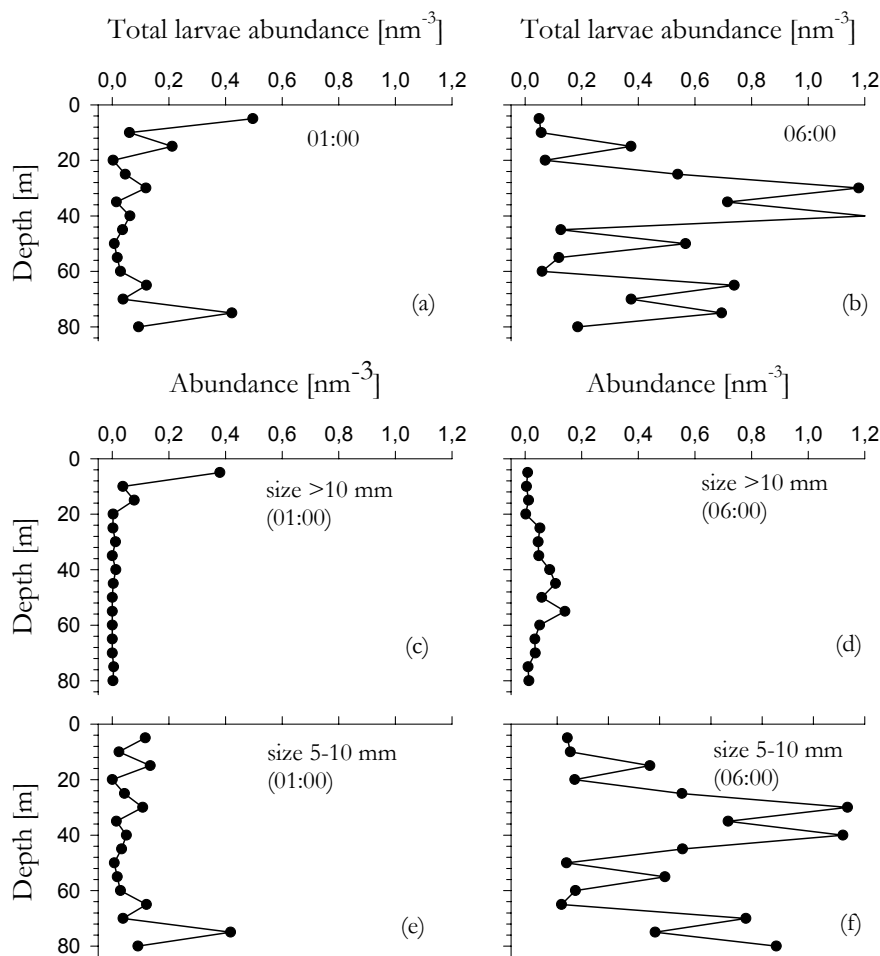


Figure 10. Vertical distribution of sprat larvae of two different size groups in the Bornholm Basin during night and dusk in May 2001. Note larval abundance are in nm^{-3} .

3.3 Otolith microstructure analysis

Otolith validation (laboratory experiment)

There were 51 survivors (3.05 – 5.12 mm) obtained from the rearing experiment, of which 43 individuals have been sacrificed for otolith dissection. Out of all otoliths examined, only 6 larvae had formed increments (Table 4).

Table 4. Data on age, length and otolith microstructure obtained from validation on daily growth increments.

Age (d)	Length (mm)	Radius (μm)	Core (μm)	No. Incr.	Inc. 1 (μm)	Inc.2 (μm)	Inc.3 (μm)	Σ incr. widths(μm)
1	3.11	5.6239	-	-	-	-	-	-
1	3.07	5.2724	-	-	-	-	-	-
1	3.05	6.1511	-	-	-	-	-	-
1	3.26	5.6239	-	-	-	-	-	-
1	3.48	5.2724	-	-	-	-	-	-
2	3.76	6.3269	-	-	-	-	-	-
2	3.72	6.5026	-	-	-	-	-	-
2	3.98	6.8541	-	-	-	-	-	-
3	4.16	8.3601	-	-	-	-	-	-
3	4.16	7.2056	-	-	-	-	-	-
3	4.4	7.3814	-	-	-	-	-	-
3	4.4	8.0844	-	-	-	-	-	-
3	3.84	7.7329	-	-	-	-	-	-
4	4.64	8.4359	-	-	-	-	-	-
4	4.28	8.9631	-	-	-	-	-	-
4	4.08	8.0844	-	-	-	-	-	-
4	4.08	7.9086	-	-	-	-	-	-
4	4.4	8.9631	-	-	-	-	-	-
5	4.4	9.1388	8.331	1	1.0544	-	-	1.0544
5	4.4	9.3146	-	-	-	-	-	-
5	4.12	8.6116	-	-	-	-	-	-
5	4.32	8.6116	-	-	-	-	-	-
6	4.4	10.0176	-	-	-	-	-	-
6	4.4	10.8963	10.018	1	0.8787	-	-	0.8787
6	4.4	9.4903	-	-	-	-	-	-
6	4.16	9.6661	-	-	-	-	-	-
7	4.56	10.5448	-	-	-	-	-	-
7	4.61	10.8963	-	-	-	-	-	-
7	4.4	10.8963	-	-	-	-	-	-
7	4.72	10.1933	-	-	-	-	-	-
7	4.72	10.0176	8.612	2	0.703	0.703	-	1.406
8	4.72	10.8963	-	-	-	-	-	-
8	4.8	10.3691	-	-	-	-	-	-
8	4.8	11.2478	-	-	-	-	-	-
8	4.8	10.7208	9.3149	2	0.5272	0.8787	-	1.406
9	4.8	11.9507	9.3149	3	0.8787	0.8787	0.8787	2.6361
9	4.8	10.5448	-	-	-	-	-	-
9	5.12	11.4236	-	-	-	-	-	-
10	4.96	11.9508	-	-	-	-	-	-
10	4.96	10.5448	-	-	-	-	-	-
10	4.96	10.8963	-	-	-	-	-	-
10	4.96	12.3023	8.0844	3	1.2302	1.0545	0.8787	3.1634
10	4.64	10.7206	-	-	-	-	-	-

From Table 4 above, it is shown that at a rearing temperature of 10°C (± 0.23) and in the absence of exogenous food, the otolith increments of sprat larvae were mostly indiscernible. After the core ($8.401 \pm 0.85 \mu\text{m}$) being determined, the first increments occurred 5-6 d post hatch at 4.4 mm in size (Fig. 11). It was presumably one day after yolk-sac larval period since it occurred on larvae with functional mouths, pigmented eyes and no remaining endogenous food. The narrowest detectable increment was $0.527 \mu\text{m}$ which was still in the range of the light microscope resolution, whereas the broadest was $1.230 \mu\text{m}$. The increasing pattern of increment number was inconsistent with the elapsed time of experiment (Table 4).

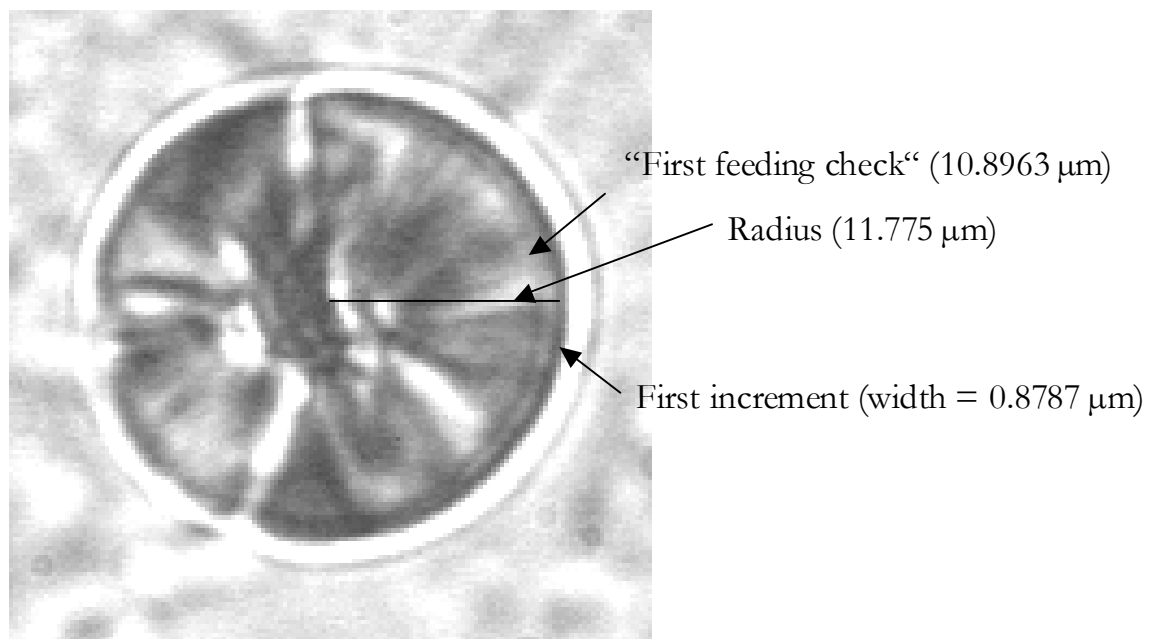


Figure 11. Sagittae of sprat larva 6 day old with first increment. Note that “first feeding check” was formed during starving condition. The size of larva was 4.4 mm.

From 1 d old larvae, the initial length at hatching is determined by $3.19 \pm 0.16 \text{ mm}$ and the corresponding otolith radius was $5.59 \pm 0.322 \mu\text{m}$ ($n=5$). Larval size increased considerably during the yolk-sac period up to 4-5 days posthatch. Afterwards the growth rate decelerated in particular to that of somatic in response to the absence of the food required by first feeding larvae. The daily changes both in body length and otolith radius at age were best fitted with semi logarithmic regression (Fig. 12 and 13) which gave rise to the assumption that initial size at hatching was 3.26 mm and $5.12 \mu\text{m}$ for length and otolith radius, respectively.

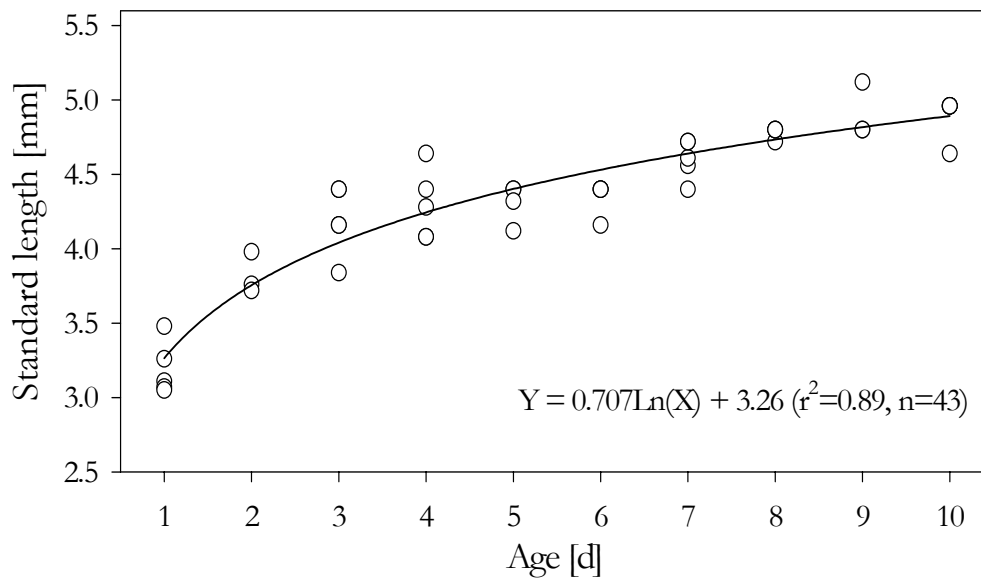


Figure 12. Changes in length on age of laboratory-reared sprat larvae for 10 d experiment.

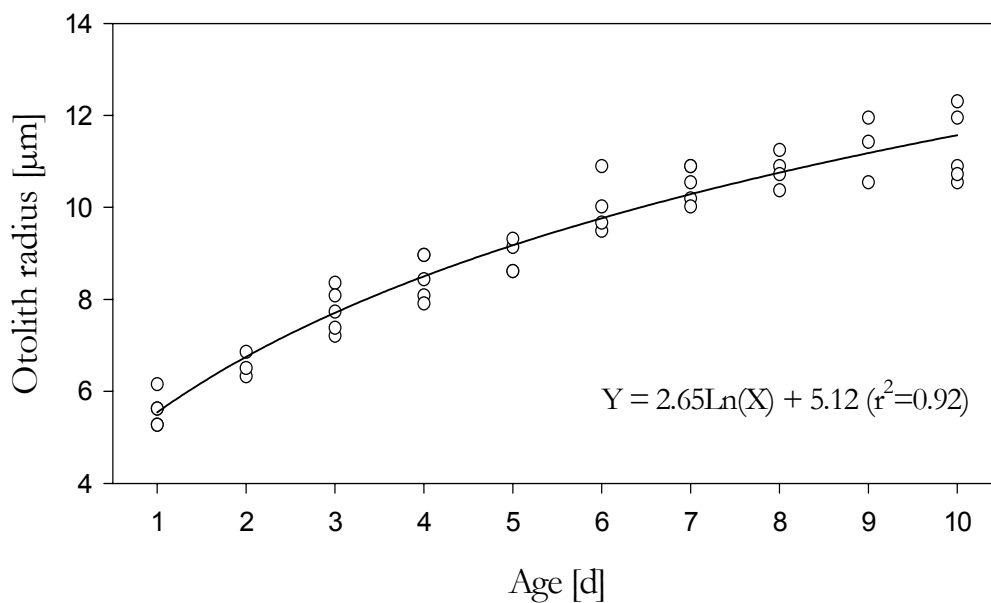


Figure 13. Changes in the otolith radius on age of laboratory-reared sprat larvae for 10 d experiment.

Taking into account the intercept values obtained from logarithmic relationships above (Fig. 12 and 13), hence the cumulative growth was determined by subtracting the measured length(radius) with initial length(radius) at hatching divided by age. By summing up the daily cumulative growth and divided by day of experiment, the average cumulative growth was $0.204 \text{ mm}\cdot\text{d}^{-1}$ and $0.727 \text{ }\mu\text{m}\cdot\text{d}^{-1}$ for somatic and otolith growth, respectively. Furthermore, the changes in cumulative growth at age from laboratory-reared sprat larvae are presented for the 10 d experiment (Fig. 14). The cumulative growth was relatively constant in all but in the

lower percentile during the first three days.. A considerable decrease occurred in somatic growth at yolk-sac exhaustion, whereas otolith showed a subtle response. In the following, the cumulative growth decreased continuously in the absence of exogenous food.

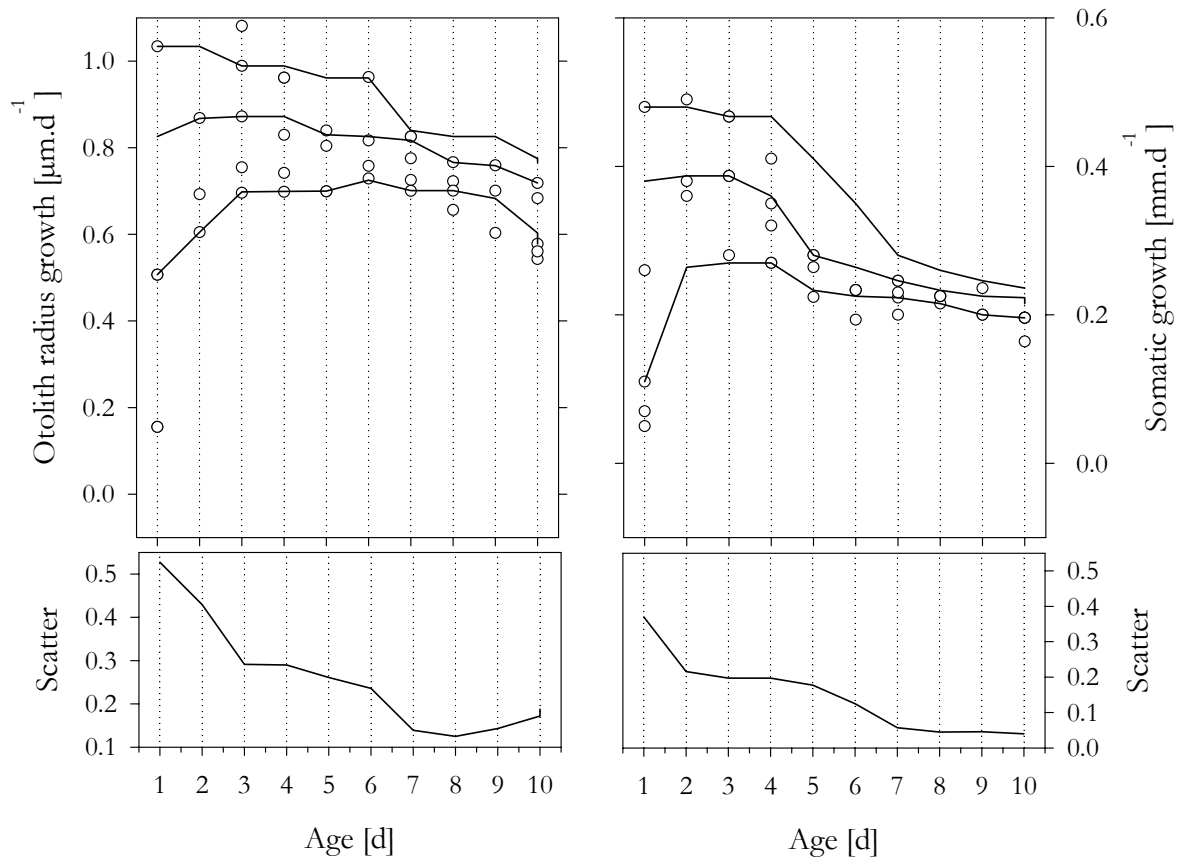


Figure 14. Changes in the cumulative otolith (left panels) and somatic (right panels) growth of laboratory-reared sprat larvae during 10 d experiment. See text for explanation of the points. The scatters show the difference between 90th and 10th percentiles. Note the different scales of the two y axes.

Otolith microstructures of wild-caught sprat larvae

The sagittal otoliths of sprat larvae within the size range observed showed a relatively round form and were relatively symmetrical from the nucleus (Fig. 15). The clarity of the otolith's structures was better in specimens collected in May than in April. Table 5 presents the meristic data of sprat larvae chiefly in relation to otolith microstructures during the first ring formation. According to different sampling periods, the size spectrum of larvae collected was increasing from late spring to early summer. With respect to otoliths, the first feeding check is relatively constant over different months (t-test, $P > 0.5$) which is $\sim 10 \mu\text{m}$ in distance from the nucleus (centrum). Observed from sagittae with one increment (n was 66, 87, and 23 for three

successive months), it was obtained that the first ring was deposited on smaller larvae in April (t-test, $P < 0.001$), whereas, such differences were not found between May and June.

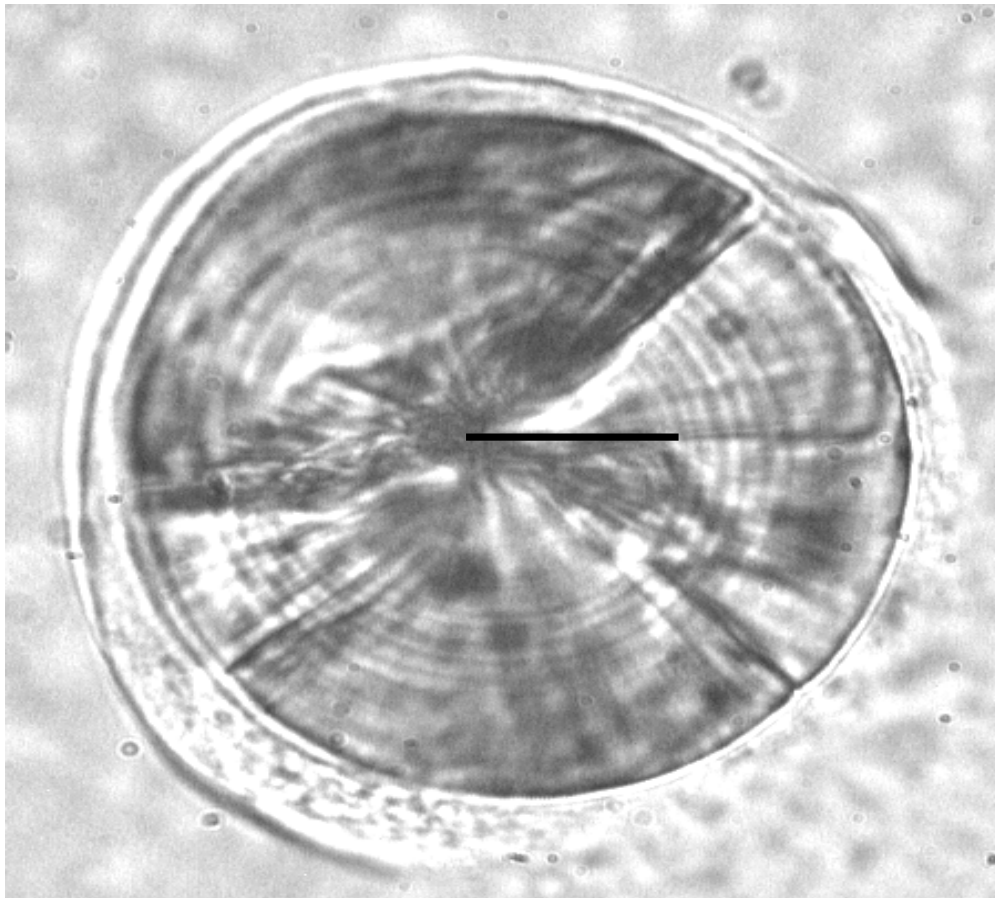


Figure 15. Otolith of wild-caught sprat larvae with the distance to first feeding check (horizontal black line) $\sim 8.9631 \mu\text{m}$ and radius size $\sim 28.8648 \mu\text{m}$. By adjusting the focus lens there are 9 successive rings after first feeding check (FFC). The ring-like picture next to the otolith edge is an optical artifact.

Table 5. Information on number of examined otoliths, size spectrum, size at first feeding check (FFC) deposition and radius of FFC from three different sampling times. Note that April and May were derived from bongo sampling, whereas June was from biomoc sampling.

sampling 2001	otoliths viewed (n)	size range (mm)	Size at FFC deposition \pm SD (mm)	FFC \pm SD (μm)
April	122	4.06 – 12.03	7.99 ± 1.27	9.88 ± 0.57
May	206	4.72 – 14.50	8.72 ± 0.84	9.90 ± 0.52
June	96	4.74 – 16.07	8.74 ± 1.70	9.51 ± 0.99

The fraction of sagittae with clear increments increased by approximately >30% from April to June and 20% from May to June 2001 (Fig. 16). This is in accordance with the ascending water temperature from spring to summer. Using information on size at first increment provided in Table 5, there were sagittae without visible structures that might be already bearing increments. However, the structures were unable to be seen due to either that the increments formed are beyond the optical resolution of the light microscope or in fact that rings may have not been formed. These specimens were categorised as “supposed incremented sagittae” (Fig. 16). The percent of such specimens was decreasing over the sampling time.

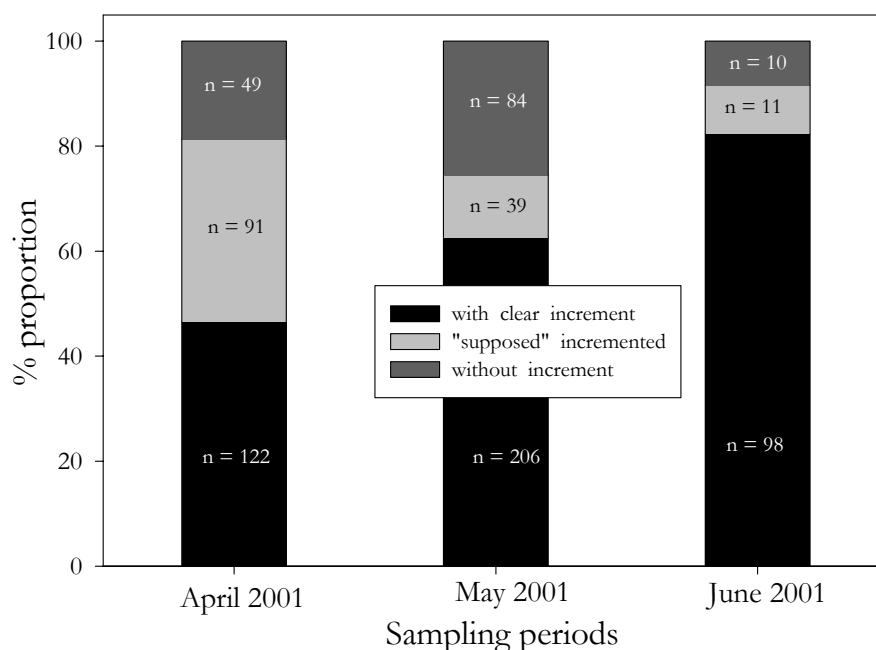


Figure 16. The composition of sagittae with and without increments intersected with specimens which were supposed to had increments. Term “supposed” in the middle part of histogram box is based a size relationship at a certain size increments are expected.

The changes in radius at length in sprat larval otoliths collected from various sampling periods is shown in Fig. 17. Otolith radius varied in size with increasing body size. Comparing between April and May, it is shown that larvae between 4-8 mm were bigger in radius during the first compared to the second sampling. There were no clear differences in radius size observed in larvae between 8-12 mm from these two months. Whereas, larvae with smaller radius were found in June. The upper limits distribution in otolith radius at length show that larvae sized 4-10 mm were largest in radius during April in comparison to May and June. On the contrary, the lower limit distribution exhibit a condition that at the beginning otolith radii were relatively similar up to 8 mm, in the following otolith radii tended to be

largest in the second month compared to the others. However, the differences could only be regarded up to 12 mm as no larger larvae were found in 10th percentile distribution in May.

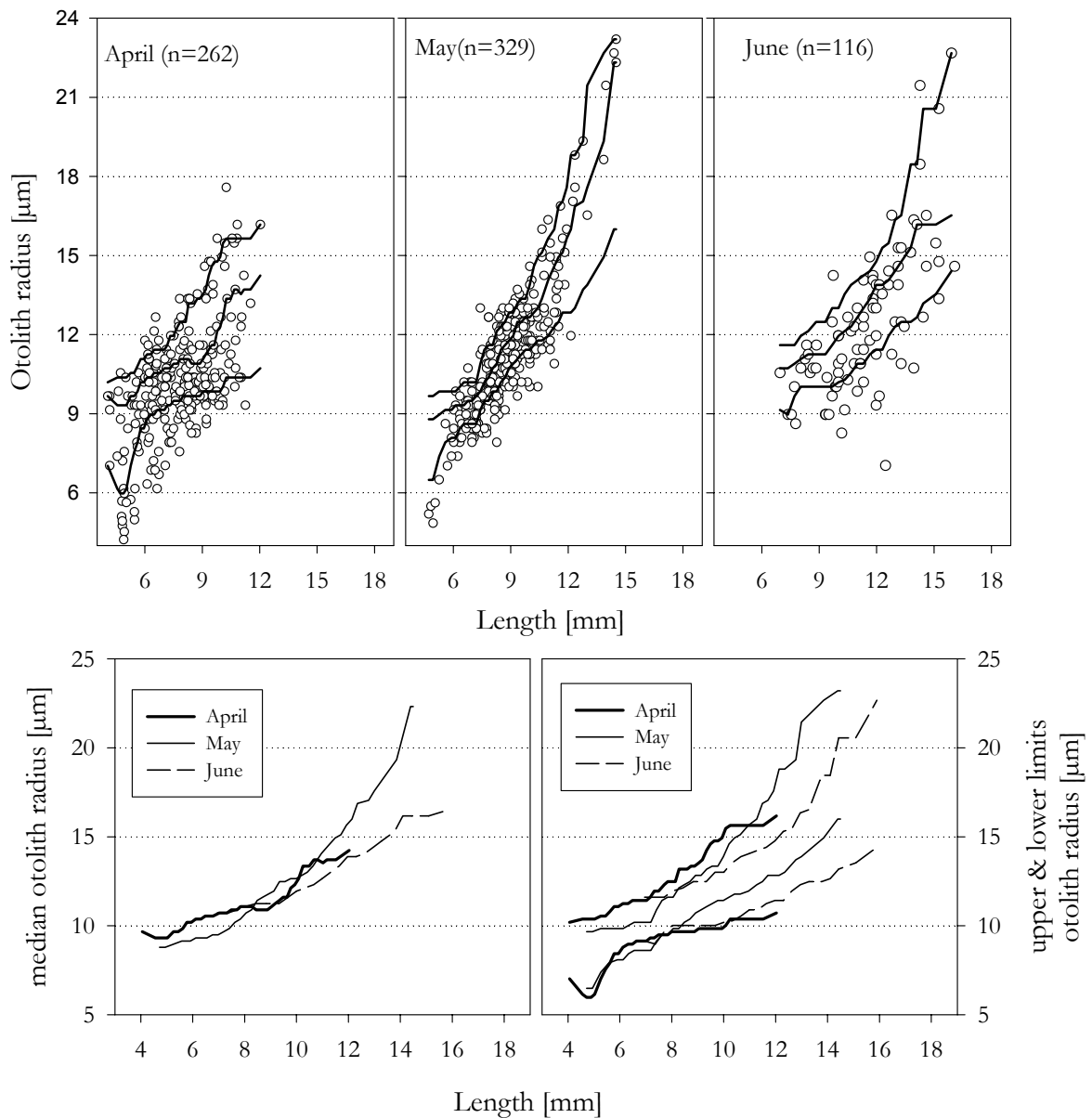


Figure 17. Measured otolith radius in relation to length of sprat larvae with the estimated 10th, 50th, and 90th percentiles from three different sampling periods (upper panels). The medians as well as upper and lower limits are shown (lower panels). The bandwidths were set at 0.1 for all panels. Larvae in April and May were collected with bongo whereas in June with biomoc.

Based on the correlation between increment number and otolith radius, the slopes were obtained between 1.025 – 1.091. Thus it confirms that one increment is proportional to an expansion of otolith size by approximately 1 µm. The explained variation varied between 78-89 % (Fig. 18). No significant difference in the slopes were found among months (ANCOVA, $P > 0.05$). This may be an indication of the existence of daily formation in the

otolith of early larval sprat in the Baltic Sea. Estimated from the intercept values, the core size ranged between 9.28 - 9.80 μm which was relatively close to validation results (Table 4).

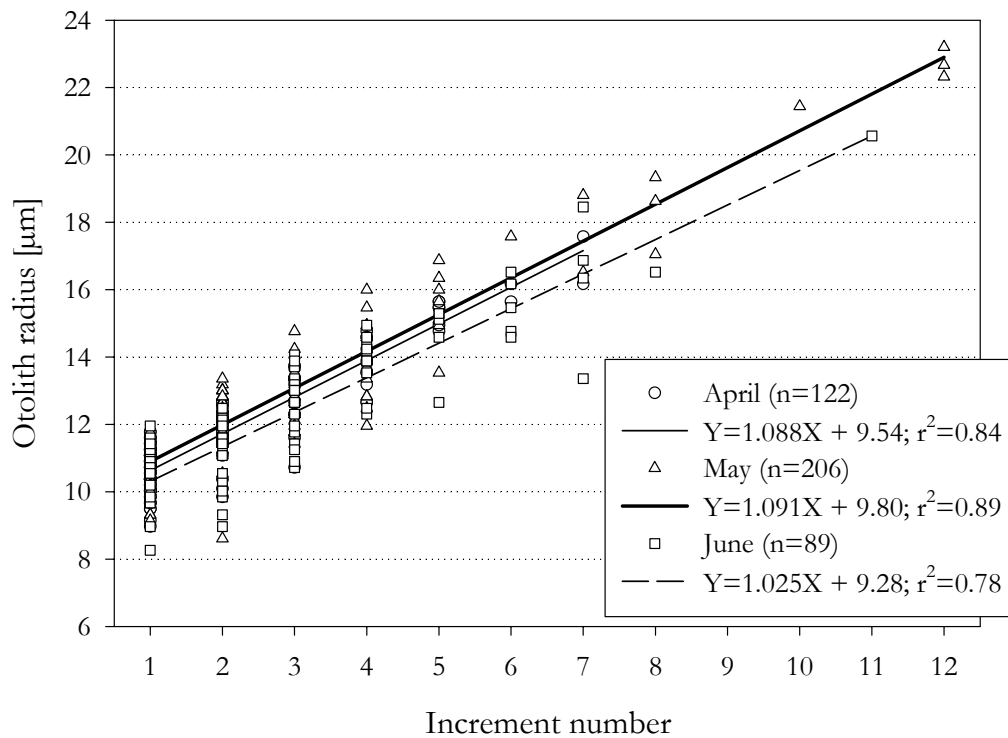


Figure 18. Otolith radius in relation to increment number of sprat larval otoliths in the Bornholm Basin during April, May, and June 2001.

The reading of otolith structures was consistently started from the first clear ring that accounted for first feeding check (Table 5) and continued with the next distinguishable ring structures. The results were presented without considering the sub-daily ring formation. Based on increment number, age and growth of sprat larvae was estimated. These results shall be described in the following section.

Growth proxy based on otolith microstructure analysis

Temporal and horizontal variability

In estimating somatic growth based on otolith structures, the age was determined by adding 6 to the observed increment number. In the following, based on the age-length relationship, the integrated somatic growth is estimated from the slope of the relationship. It was found that somatic growth was significantly higher in May compared to April (ANCOVA, $P < 0.001$). The maximum age was 13 d (~ 11.6 mm) and 18 d (~ 15 mm) in these two consecutive months. A comparison between length at age revealed that larvae aged 7, 9, 10, and 11 days

old demonstrated larger length in the latter compared to the earlier sampling (t-test for unequal sample, $P < 0.05$). The percent of variation explained by the correlation was higher in May due to a larger age range (Fig. 19).

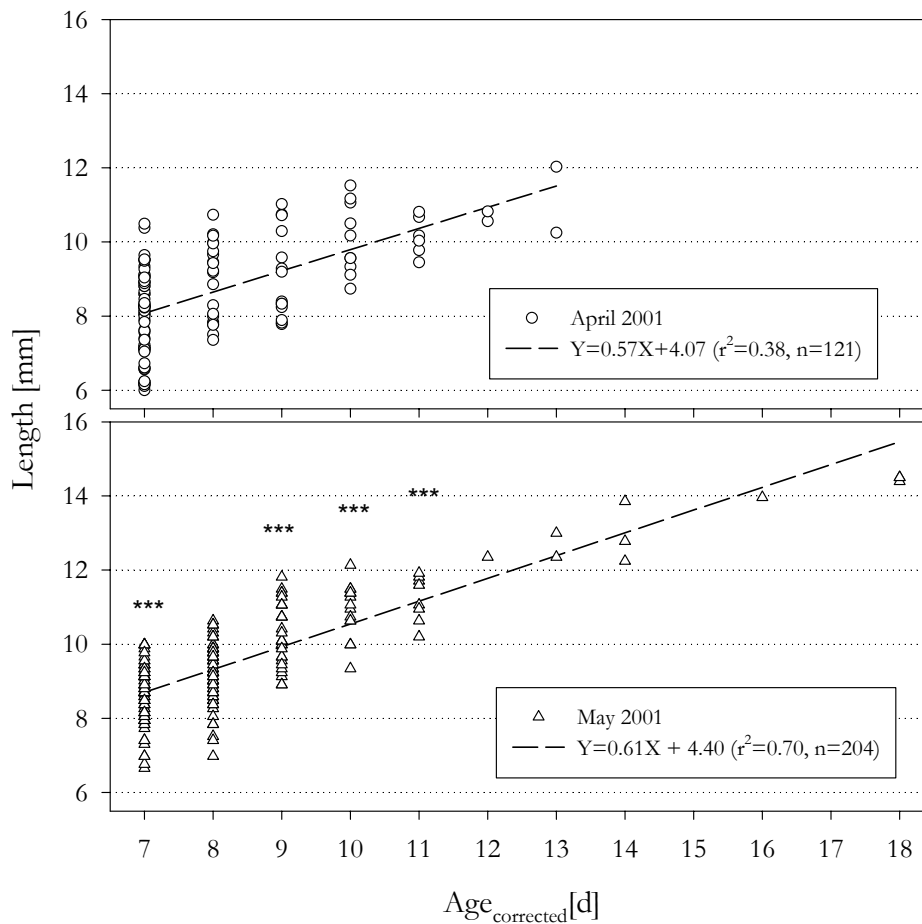


Figure 19. Length of sprat larvae in relation to age for two different bongo sampling in the Bornholm Basin during April (upper panel) and May 2001 (lower panel). Age was corrected from increment number + 6. The star symbols inside the lower panel represent significance level at $P < 0.05$.

Horizontal variability in integrated somatic growth from 6 locations in the Bornholm Basin is summarised in Table 6 and illustrated in Fig. 20. Based on the slopes, the growth of sprat larvae varied among locations and sampling. In the north east and central basin, larval growth increased from April to May, whereas in the north west and south east it decreased. In the south west and central east growth was relatively similar. However, the differences in somatic growth between similar location at different time was found significant only in the north eastern part of the basin (ANCOVA, $P < 0.05$; Table 6). In addition, by combining each two locations into northern, central, and southern regions, there was a tendency that larvae encountered in the northern part had highest somatic growth, whereas the lowest was found in the southern region (Fig. 20).

Table 6. Age-length relationship of sprat larvae over 6 locations in the Bornholm Basin during May 2001. Numbers in the brackets under location column indicate stations. *** (highly significant, $P < 0.05$), NS (not significant).

Locations	Months	Age-length relationship	n	R ²	ANCOVA test
North West (3,4,18,19)	April	$Y = 0.67X + 4.35$	13	0.39	NS
	May	$Y = 0.57X + 4.45$	49	0.79	
North East (10,13,14,15)	April	$Y = 0.59X + 4.77$	20	0.74	***
	May	$Y = 0.85X + 2.74$	24	0.61	
Central (23,24,29,30)	April	$Y = 0.57X + 3.09$	15	0.40	NS
	May	$Y = 0.68X + 3.83$	39	0.71	
Central East (25,26,27,28)	April	$Y = 0.59X + 3.22$	20	0.67	NS
	May	$Y = 0.58X + 4.99$	32	0.70	
South West (33, 41,42,43)	April	$Y = 0.64X + 2.50$	23	0.68	NS
	May	$Y = 0.63X + 4.46$	42	0.60	
South East (36,38,39,45)	April	$Y = 0.54X + 4.90$	31	0.56	NS
	May	$Y = 0.45X + 5.79$	20	0.20	

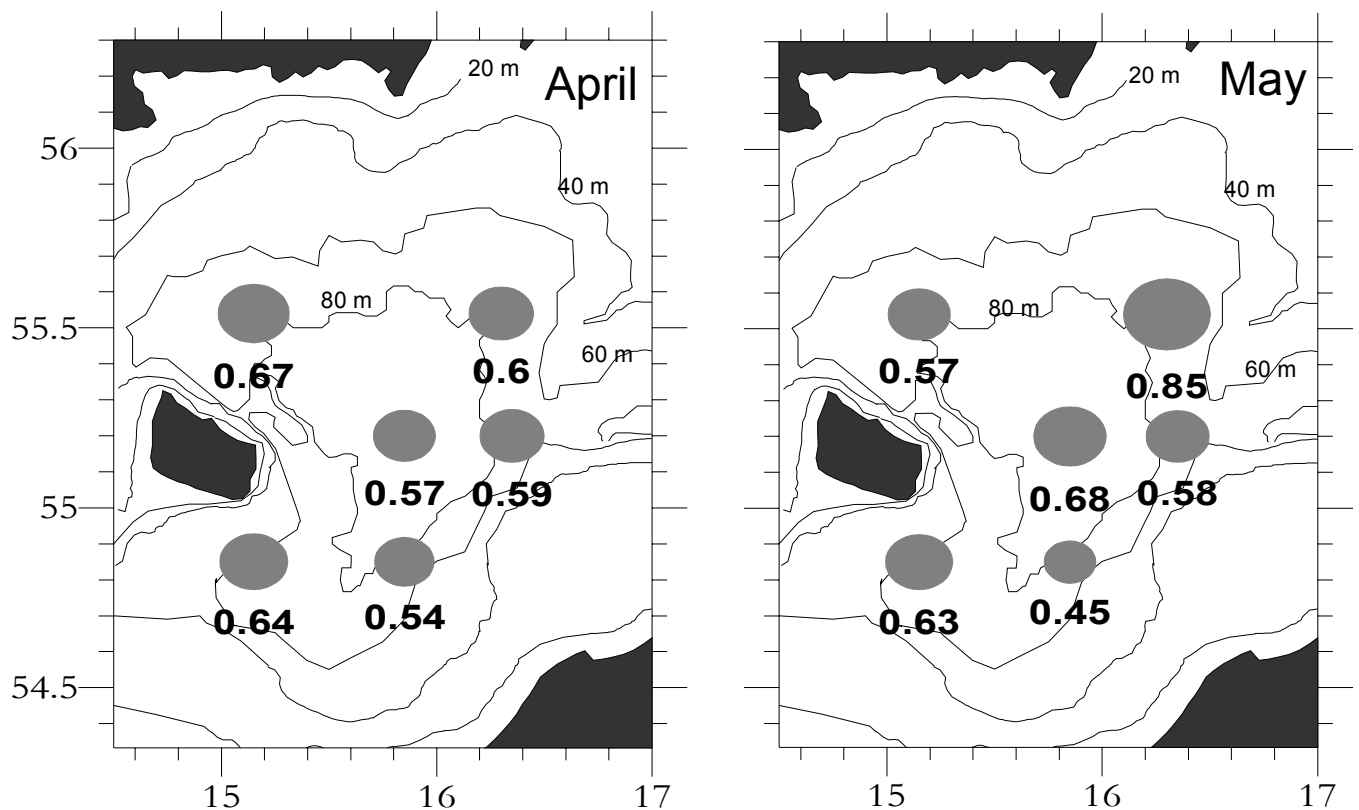


Figure 20. Horizontal distribution of integrated somatic growth of sprat larvae based on age-length relationships over 6 sub-locations in the Bornholm Basin during April (left panel) and May (right panel). All data values are showing the slopes of the relationships in $\mu\text{m}\cdot\text{d}^{-1}$.

Further analysis of change in length in relation to age is depicted in Fig. 21. It is clearly shown that the average somatic growth rate was always higher in May compared to April. Accordingly, the lower and upper limit of length at age distribution in the latter month was always above the earlier month. Both figures show that a higher variation in length at age was found on smaller larvae.

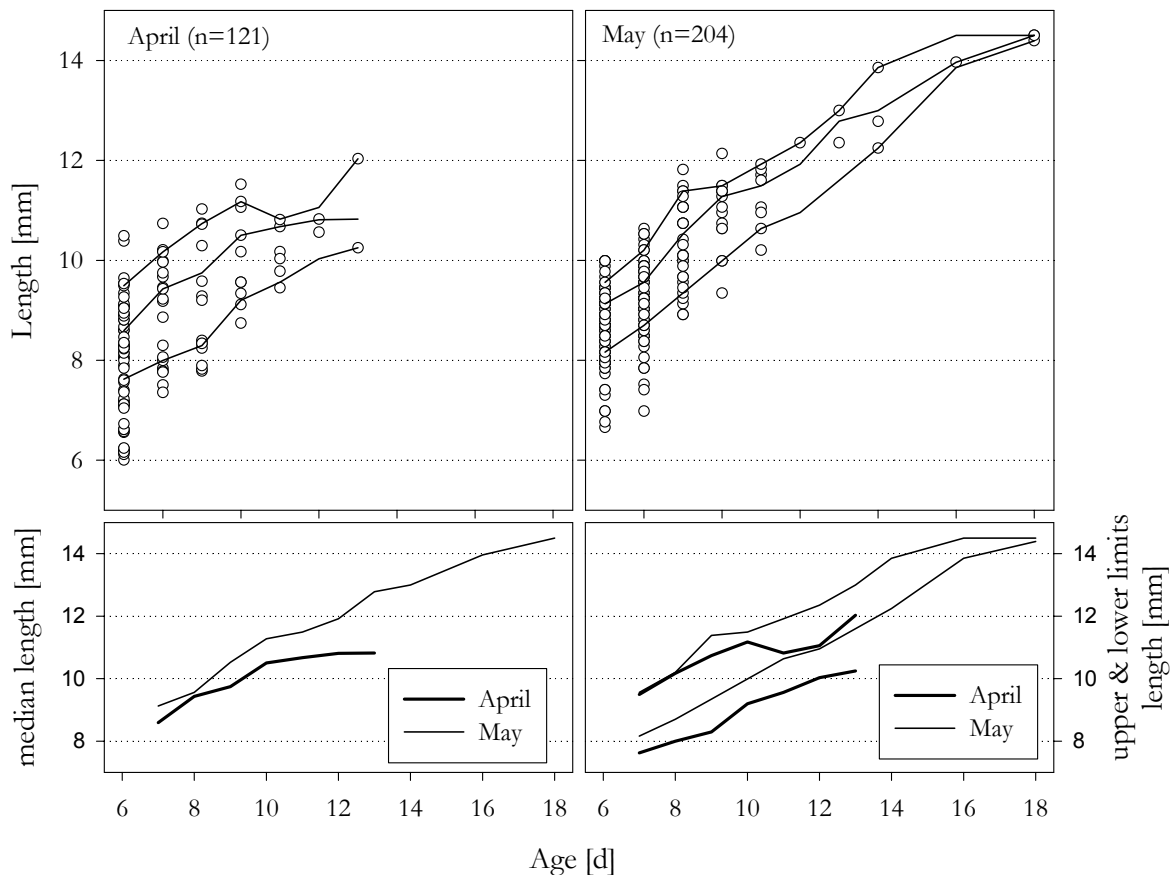


Figure 21. Measured length in relation to age of sprat larvae with the estimated 10th, 50th, and 90th percentiles for April and May 2001 (upper panels). Comparison in median and upper as well as lower limit distribution are shown (lower panels). Bandwidths for each panels were set at 0.1.

Higher variation in length of earlier larval stages, is correspondingly expressed by the increment widths (Fig. 22). The narrowest and broadest increments were 0.527-1.406 μm and 0.527-1.5817 μm with an average of 0.9 μm and 1.1 μm for April and May, respectively. Although no significant difference was found between the two (ANOVA), the comparison of mean widths in each increment resulted in highly significant differences in the first four increments (t-test, $P < 0.05$). This indicated that wider increments had been formed in the otoliths of early sprat larvae collected in May. Unfortunately further comparison could not be done as the numbers of larvae with 5 and 6 increments in April larvae were limited.

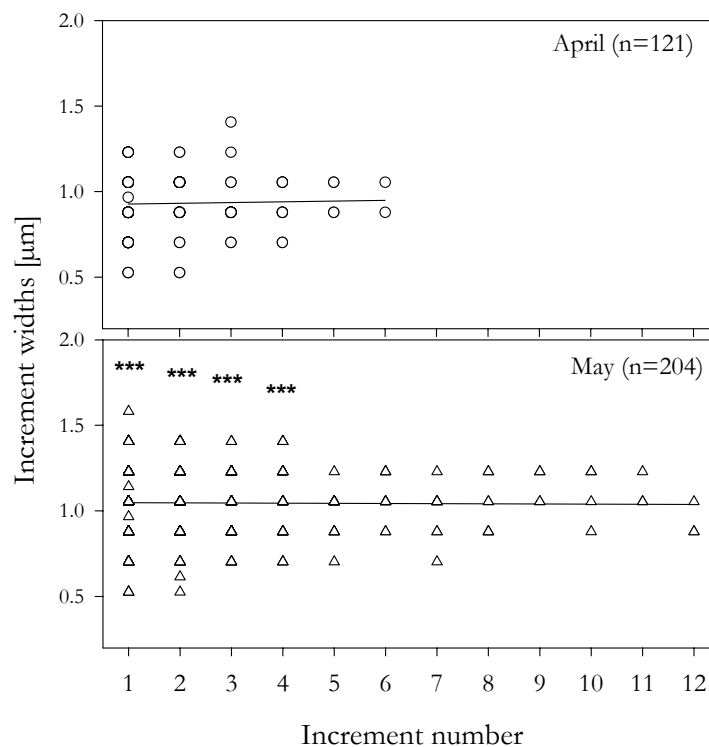


Figure 22. Increment widths of sprat larvae showing a relatively constant otolith growth during their early life stages. The lines are regression line. Stars in the lower panel are showing significant difference at $P < 0.05$.

Vertical variability

In order to examine the vertical variability in somatic growth of sprat larvae based on the age-length relationship, the larvae collected from different water columns were grouped into 5-30 m and 35-80 m. Such grouping was due to small sample sizes collected from 5 m depth intervals. The changes in length at age of sprat larvae from upper and lower water columns is presented in Fig. 23. Comparing the median lines between the two water columns, it is shown that larvae in the upper layer were slightly higher in length at age compare to those in the lower layer. A relatively clear difference was found on the upper limit distribution. Temperature condition was 7.39°C and 5.38°C in the surface and bottom, respectively (Fig. 7).

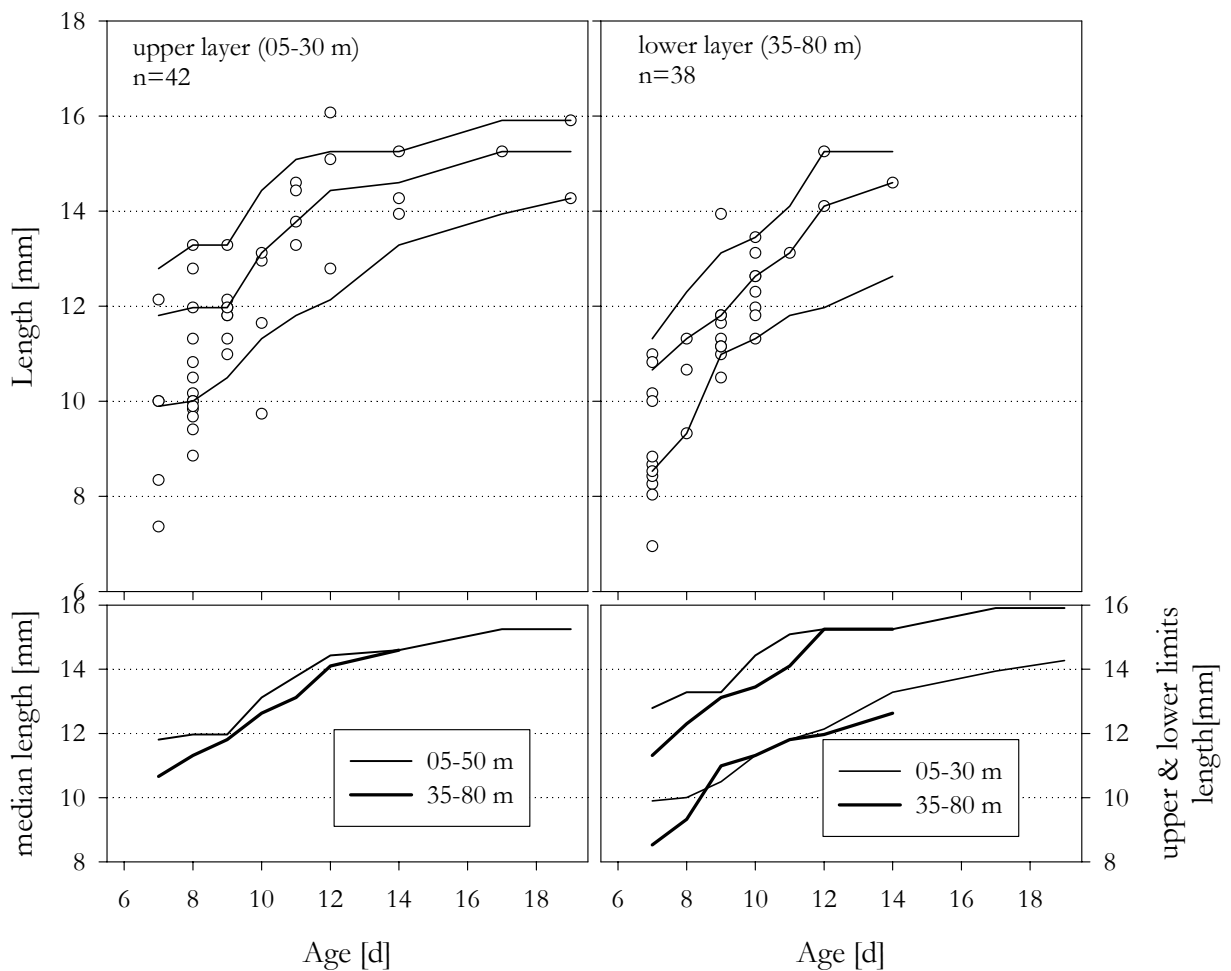


Figure 23. Measured length in relation to age of sprat larvae within two water columns with the estimated 10th, 50th, and 90th percentiles in the Bornholm Basin during May 2001. Bandwidths were set at 0.4. Medians comparison as well as upper and lower limits distribution is shown in the lower panels.

The increment widths were compared within the vertical water column (Fig. 24). The range of the distance between two successive rings was 0.703 – 1.932 μm and 0.703 - 1.582 μm in the surface and bottom respectively. There was no significant difference in increment widths compared between upper and lower layer (ANCOVA). A comparison in increment widths at each increment between the two water columns resulted in no significant difference (t-test, $P > 0.05$). Higher variation in increment widths were found in the surface layer.

Age distribution of larvae depicted in Fig. 25 shows that age spectrum was bigger in the surface than in the bottom. Interestingly, larvae up to 10 d old were found in almost any depth, whereas larger individuals were more concentrated in the surface. However such distribution was aggregated from various sampling time within 24 hrs, therefore it can not be regarded whether it is representing the vertical distribution in the water column.

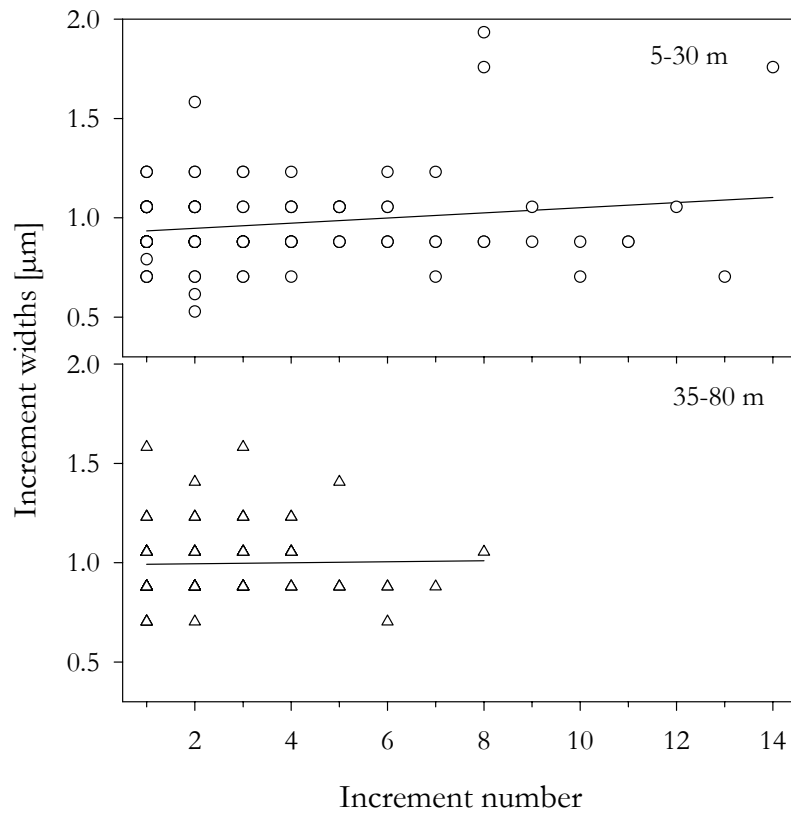


Figure 24. Variation in increment widths of sprat larvae from two different water columns in the Bornholm Basin. The lines indicate the regression line.

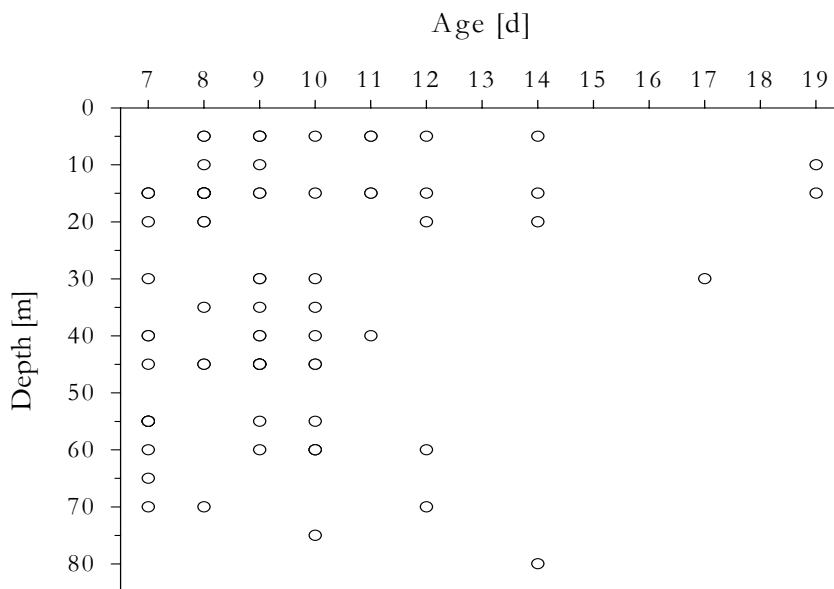


Figure 25. Estimated age distribution in relation to depth for sprat larvae in the vertical water column during sampling in May 2001.

Growth proxy based on length-weight relationship

Temporal and horizontal variability

A power function of the length-weight relationship in sprat larvae showed an allometric growth with b values differing from $b < 3$ in April to $b > 3$ in May. This was due to a different length and weight spectrum of larvae collected. Correspondingly, maximum weight at length was ~ 0.12 mg at ~ 11 mm and ~ 0.33 mg at ~ 15 mm, respectively. Logarithmic transformation resulted in a linear relationship which was higher in slope of the latter (0.141) compared to earlier sampling (0.120) (Fig 26). Slope comparison by means ANCOVA analysis revealed that larvae overall were higher in weight at length in May than in April ($P < 0.001$). However, it is clearly shown in Fig. 26 that larvae were heavier at 4-8 mm in April compared to May. Therefore, a statistical inference on dry weight of the larvae at corresponding length is strongly influenced by a different size range between the two months as well as to a lesser extent different sample size.

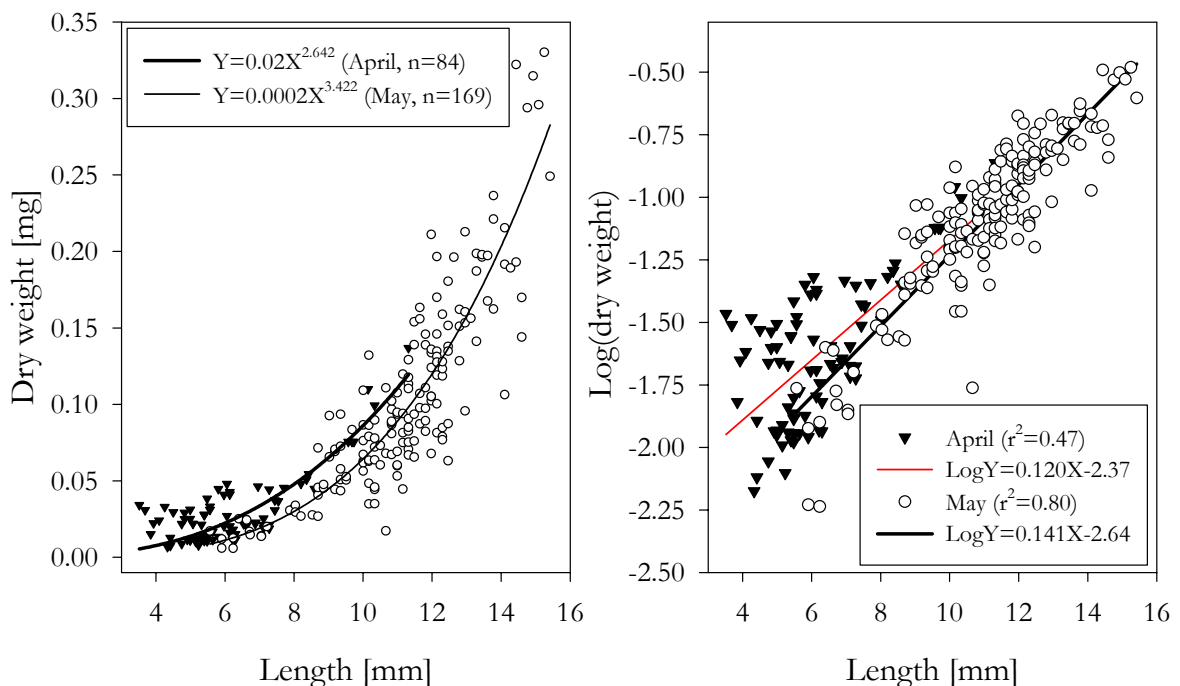


Figure 26. A power function between dry weight and length (left panel) and after logarithmic transformation (right panel) of sprat larvae in the Bornholm Basin during April and May 2001.

In order to explore the changes in weight in relation to length, the percentile analysis is presented in Fig. 27. By comparing the medians, it is demonstrated that sprat larvae sized $\sim 6 - 8.5$ mm showed a relatively similar weight in the two months. Larvae sized 4-7 mm showed a constant size at length in April. Such condition can be an expression of either a subtle or an absence of growth. Such a stable condition did not occur in May which comprised of initially

larger individuals compared to April. At length >9 mm, larval weight between the two months could not be compared as sample size was too low in April.

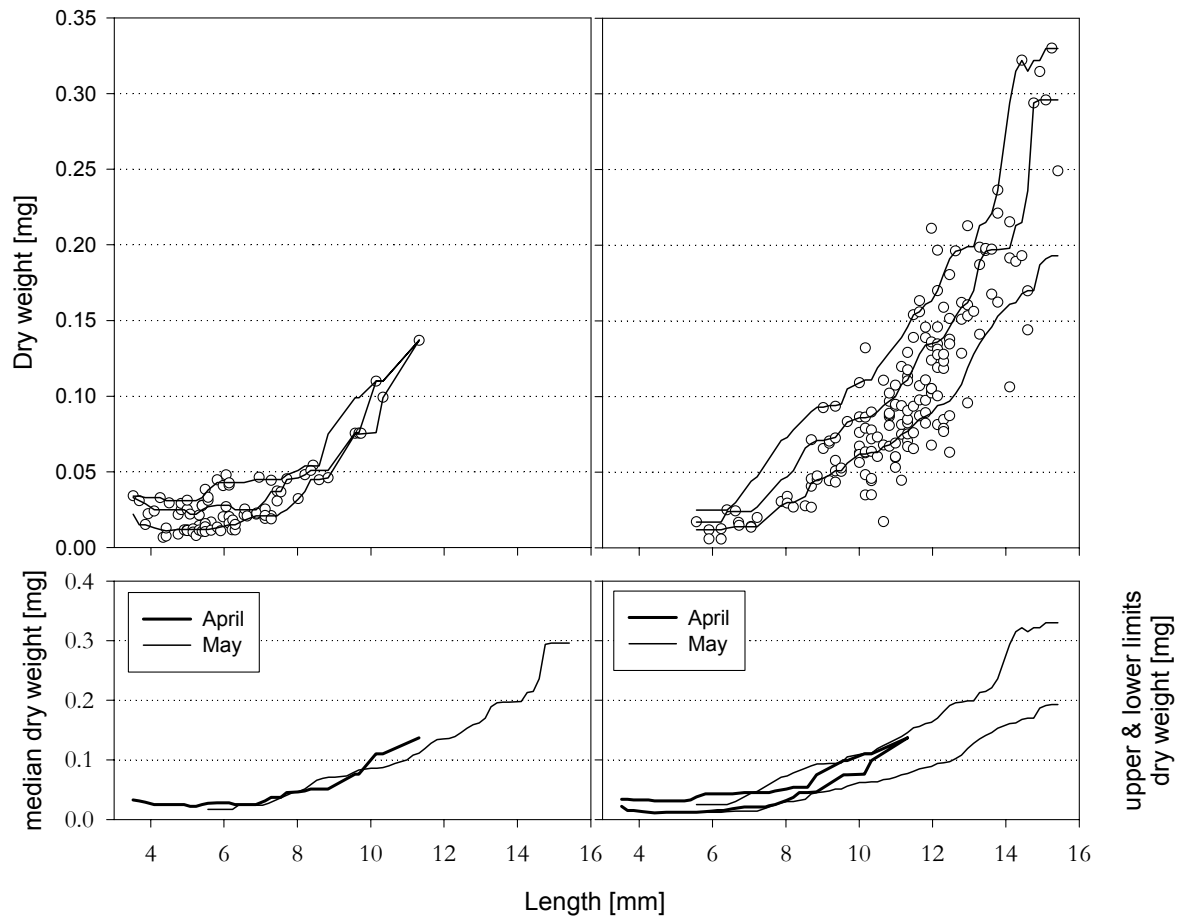


Figure 27. Dry weight in relation to length of sprat larvae with the estimated 10th, 50th, and 90th percentiles in the Bornholm Basin for April and May 2001. Bandwidths were set at 0.05.

With respect to May, sprat larvae have been grouped according to different contour depth areas of 40-60 m, 60-80 m, and 80-98 m (Fig. 2-3, p. 9-10) in order to investigate the horizontal variability in weight at length. The slope (b) resulted from length-weight relationship was close to isometric growth in the central basin, whereas allometric growth ($b > 3$) occurred in the other two shallower regions. Maximum weight at length was found in the two shallower areas (~ 0.33 mg, 15 mm), whereas smallest individuals occurred in the central basin (Fig. 28). This to some extent was in accordance with horizontal distribution shown in Fig. 9 (May 2001). Based on the slope of the relationship between length and log transformed dry weight, it was obtained that larvae had the lowest increase in weight at length in the contour area of maximum 40-60 m depth compared to the others (ANCOVA, $P < 0.0001$). Whereas, no differences were found between contours 60-80 m and >80 m.

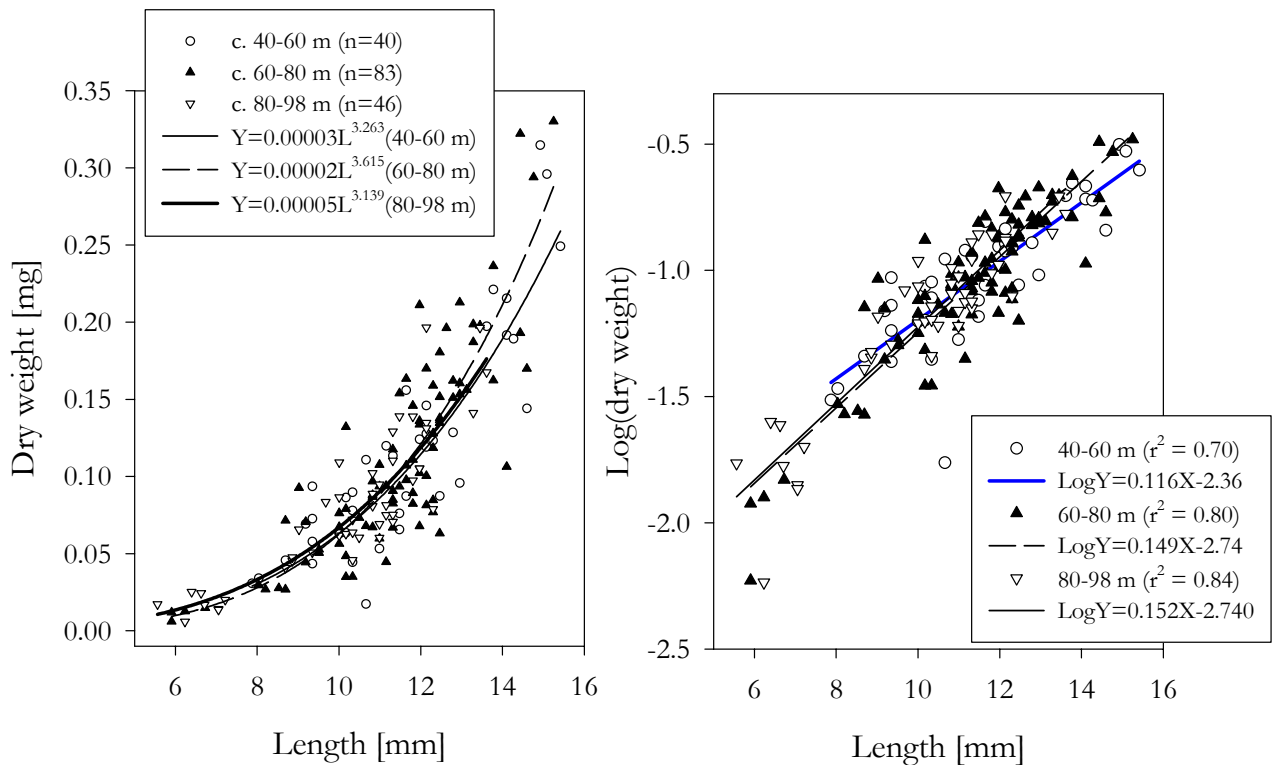


Figure 28. Dry weight (left panel) and log dry weight (right panel) in relation to length of sprat larvae of three different depth contour areas in the Bornholm Basin during May 2001. Note the different range in length and weight of the larvae.

An examination on the changes in body weight with the increasing length is illustrated by percentile distribution analysis in Fig. 29. It is shown, in fact, that the increase in weight at corresponding length is relatively similar among the contour areas. This is shown by a high overlap in median lines as well as the upper and lower percentiles. However, to compare the changes in weight at length is only appropriate in a size range with sufficient numbers of larvae. In this data, this is the case at length between 8-14 mm. The results shown by percentile analysis may explain the unexpected results of lower increase in weight per length in the shallowest areas presented by Fig. 28. In this case, the differences in the size spectrum influence the result of power function analysis.

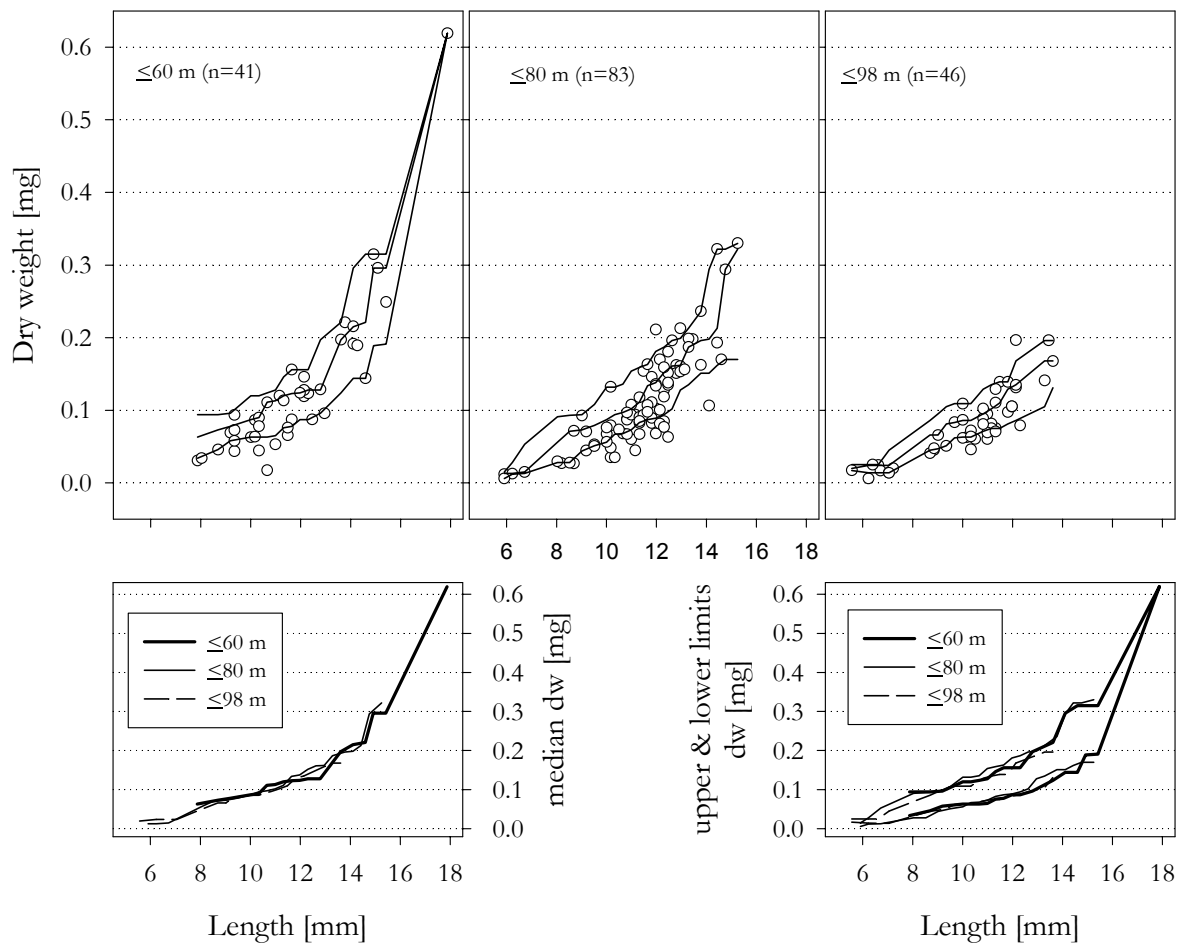


Figure 29. Dry weight in relation to length of sprat larvae with the estimated 10th, 50th, and 90th percentiles (thin solid lines) for three different contour areas in May 2001. Bandwidths were set at 0.1, 0.08, and 0.1 for the three contour areas, respectively.

Vertical variability

A length-weight relationship of sprat larvae is also presented for the vertical water column of which water depth has been grouped into 15 m depth intervals down to intermediate layer of 45 m in the Bornholm Basin (Fig. 30). The larvae were collected with the biomoc on a permanent station during June 2001. Due to highly scattered data and small sample size of larvae collected from depth 50-80 m the power function analysis could not be defined, therefore they were not included in the analysis. Power function of length-weight show the deeper the water the higher number of smaller larvae. Maximum weight and length occurred in the middle water columns, whereas the minimum was found in the deepest part. Larvae in the deepest layer demonstrated an allometric growth with $b < 3$, whereas $b > 3$ was found in the upper layer. Logarithmic transformation on dry weight revealed that overall weight increase at

length was highest at the surface between 5-15 m, followed by 20-30 m and 35-45 m depths (ANCOVA, $P < 0.05$).

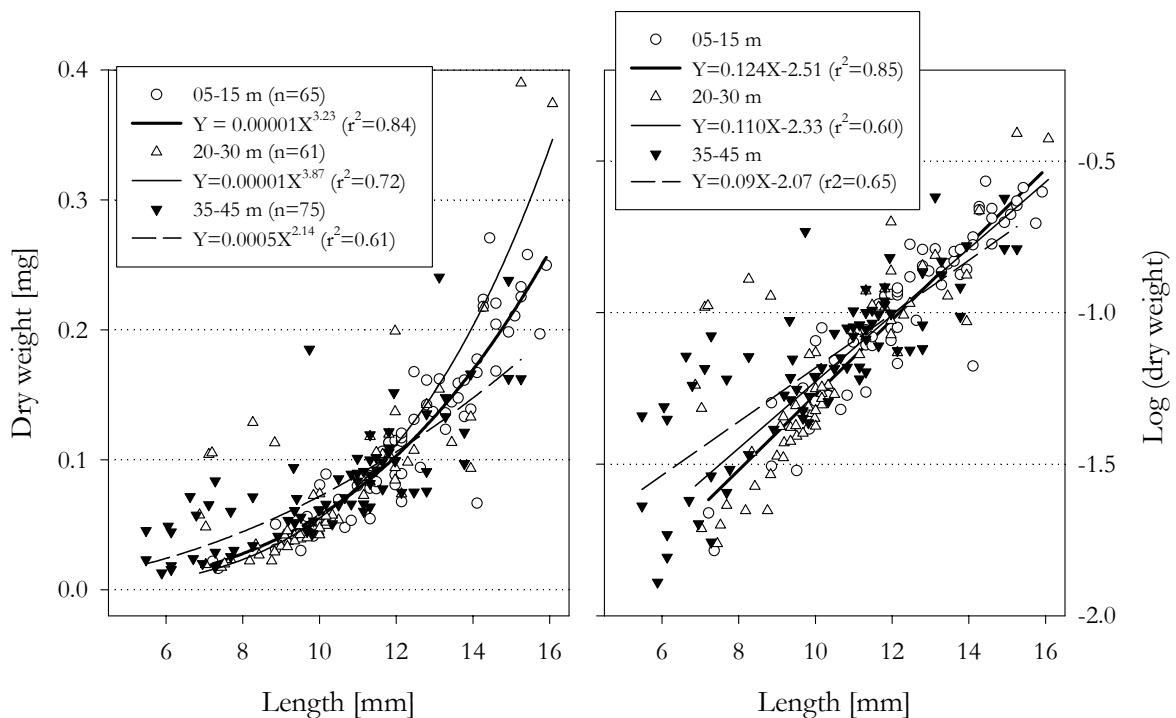


Figure 30. Length-weight relationship (left panel) of sprat larvae in the vertical water column in the Bornholm Basin during June 2001. Logarithmic transformation is showing (right panel). Note that individual larvae in the same depth groups were lumped together regardless sampling time.

Regardless of photoperiod during sampling, the vertical changes in weight with length are presented in Fig. 31. Based on the medians, it is shown that small larvae (~8-10 mm) from the deepest layer were higher in weight. In fact, shown by upper and lower limits, very few sample were found at this size in the first two upper layers. As larvae increased in size, there was a tendency that larger larvae up to 14 mm with highest weight were found at the surface between 5-15 m. It is important to note that larvae less than 8 mm and bigger than 14 mm could not be compared due to small sample sizes.

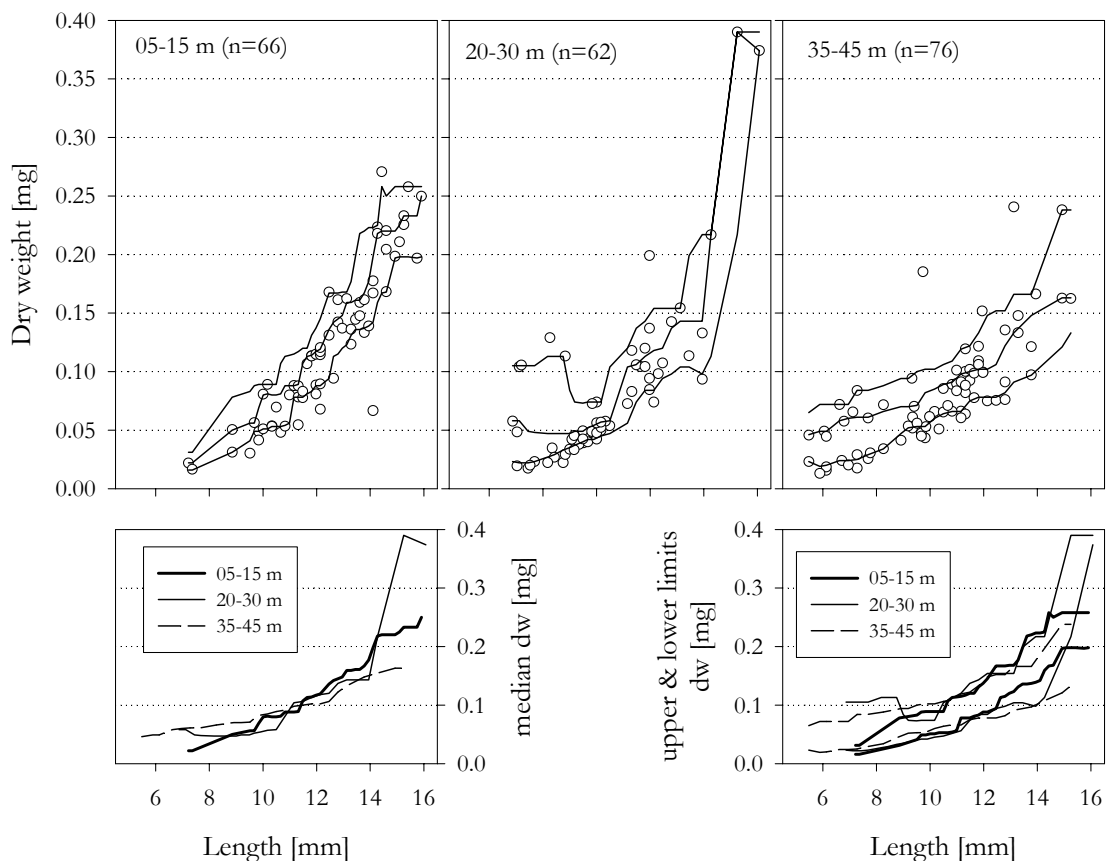


Figure 31. Dry weight in relation to length of sprat larvae with the estimated 10th, 50th, and 90th percentiles (thin solid lines) in three vertical water column in the Bornholm Basin in June. The median as well as upper and lower limit are shown (lower panels). Bandwidth were set at 0.05, 0.08, 0.1 for three different depths, respectively

3.4 RNA/DNA ratio analysis

3.4.1 Nutritional condition of sprat larvae

Temporal and horizontal variability

The concentration of nucleic acids increased by a power of length and linearly by dry weight (Fig. 32). Indicated by r^2 coefficient of determination, the variation in nucleic acids was higher by length than by weight, in particular for April. The RNA/DNA ratio ranged between 0.68-3.83 and 1.04-4.54 measured from the first and the second surveys, respectively, of which the fraction of larvae with RNA/DNA ratios less than 1.2 were 6% and 1% consecutively from both months. Variation in nucleic acids was found to be higher on smaller larvae in April, on the contrary on larger larvae in May.

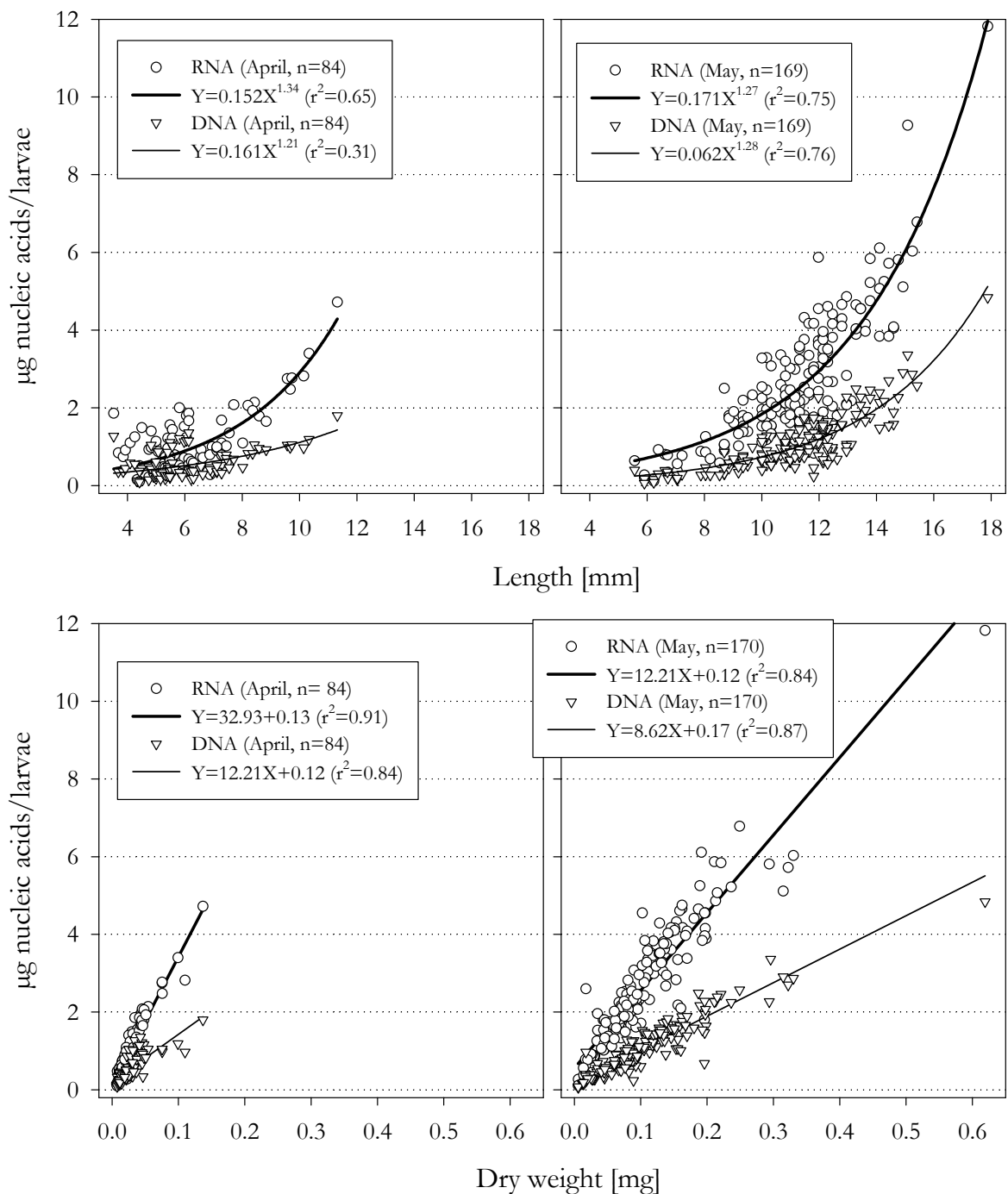


Figure 32. Nucleic acids in relation to length and dry weight of sprat larvae in the Bornholm Basin during April and May 2001.

The changes in nutritional condition with size of sprat larvae is depicted in Fig. 33. At corresponding length and weight, it is shown that smaller larvae up to 10 mm (0.1 mg) demonstrated higher RNA/DNA ratio in May compared to April. Nutritional condition of sprat larvae would be expected to be relatively similar on larger larvae for both months.

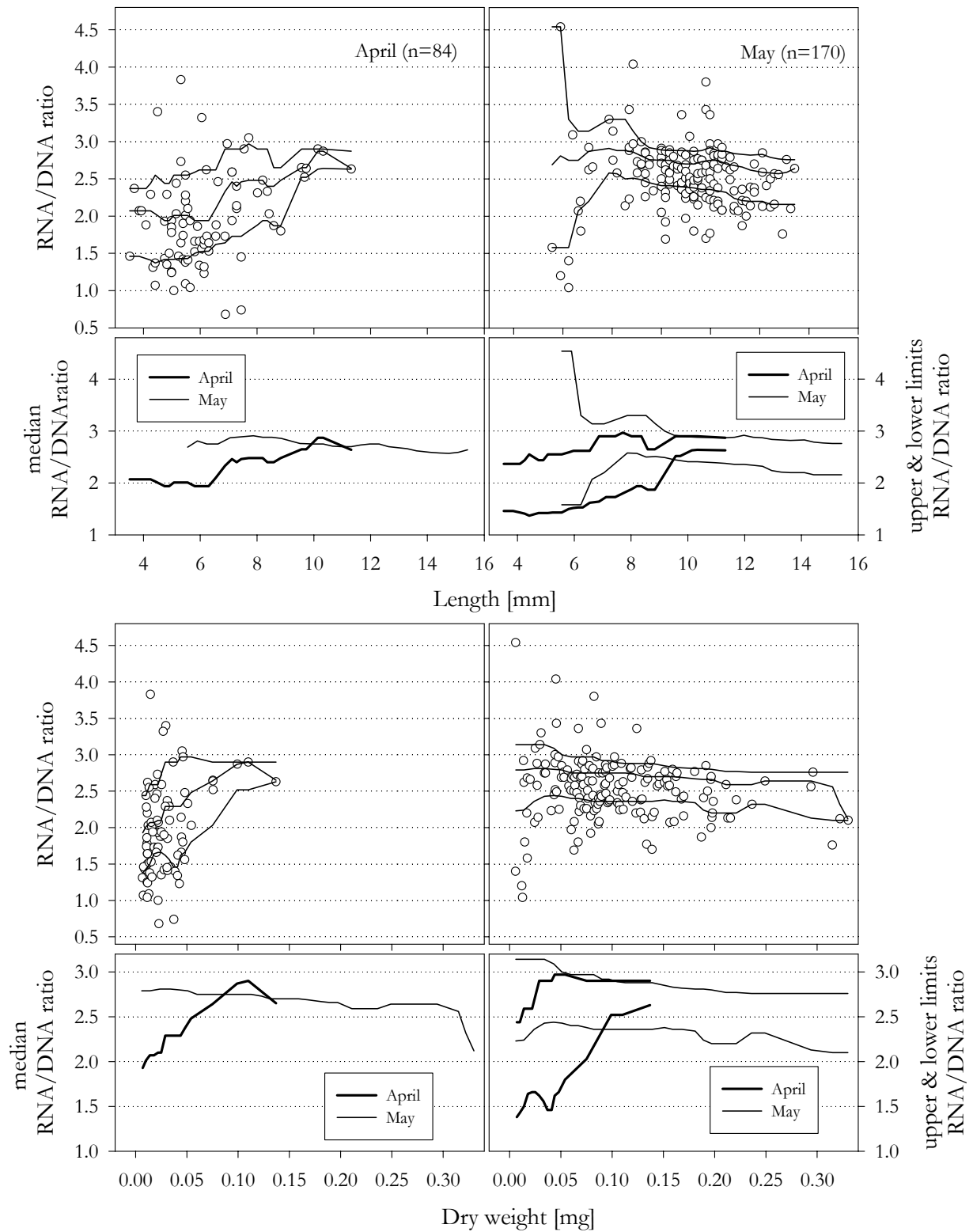


Figure 33. RNA/DNA ratio in relation to length (upper panels) and weight (lower panels) with the estimated 10th, 50th, and 90th percentiles (thin solid lines) of sprat larvae in the Bornholm Basin during April and May 2001. Medians as well as upper and lower limits of distribution are shown. Bandwidths were set at 0.08 and 0.09 for length and 0.08, 0.09 for weight.

Based on the RNA/DNA ratio, it may be stated that sprat larvae were in good nutritional condition during spawning season 2001 although there was a tendency that larvae expressed a better RNA/DNA ratio in May. This is shown by the medians which show an average between $\sim 2 - 2.5$. However, the exact RNA/DNA variability between the two months was unclear due to different size spectra of larvae collected.

According to the different sampling time in May 2001, a diel periodicity in the RNA/DNA ratio of sprat larvae was analysed. The samples were grouped into those caught at dark (20.00 – 06.00) and light (06.00 – 20.00) time of the day. Figure 34 shows that the medians of comparable size classes were relatively similar to confirm that a diel variation in nutritional condition of sprat larvae did not occur during dark and night (Mann-Whitney U test).

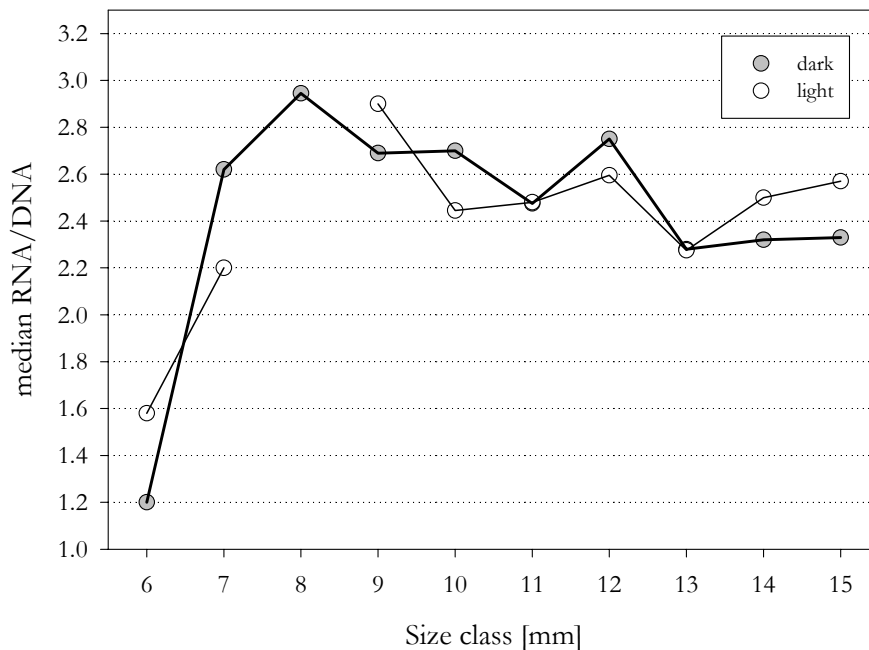


Figure 34. Median values of RNA/DNA of sprat larvae in the Bornholm Basin comparing between dark and light sampling. Sizeclass 8 mm of light sampling was unavailable.

Larval condition was also analysed based on the proportion between DNA and dry weight (DNA/DW). According to this analysis, larvae with lower DNA/DW ratio are in better condition because larvae had larger cell expressed by higher dry weight on relatively constant DNA content within the cells. The results presented in Fig. 35 show that the ratio was obviously higher in larvae collected during April than in May which means a better condition of larvae from the latter sampling. This is in accordance to the previous results on RNA/DNA ratio. The information presented in Fig. 35 may also be interpreted as the

different growth patterns of larvae from different size spectra. Smaller larvae showed hyperplasia, whereas the larger ones showed hypoplasia.

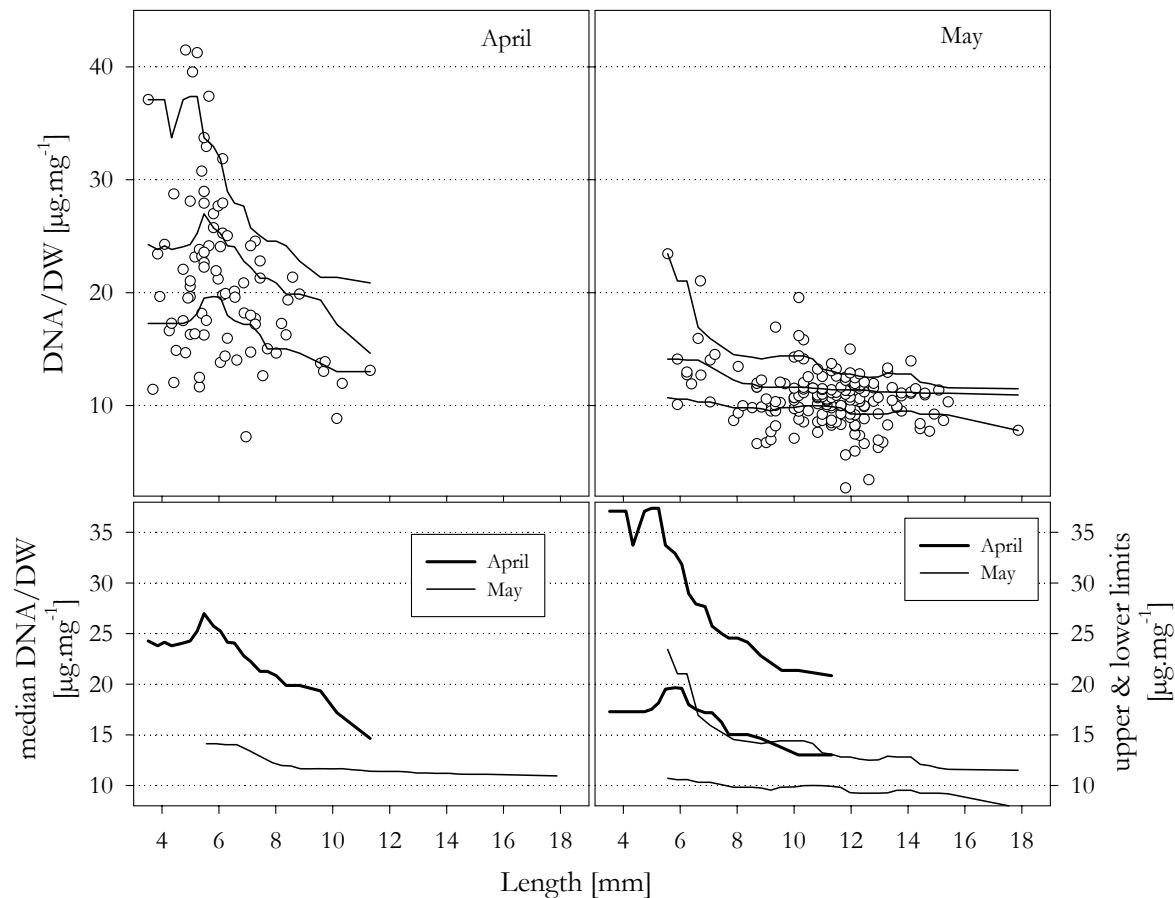


Figure 35. DNA/DW ratio in relation to length of sprat larvae with the estimated 10th, 50th, and 90th percentiles in the Bornholm Basin during April and May 2001. Median as well as upper and lower distribution limits are shown. Bandwidths were set at 0.12 and 0.084.

Vertical variability

Vertical variability in nutritional condition of sprat larvae was analysed according to water depths between 05-30 m, 35-55 m, and 60-80 m. Similar to that of the horizontal profile, the concentration of nucleic acids in sprat larvae increased exponentially by length and linearly by weight measured from different depths (Fig. 36). According to body length, the larger the larvae the higher the variation in RNA. Highest variation in RNA was found in the surface layer whereas the lowest occurred in the bottom. Contrarily, DNA content in sprat larvae were less variable shown in all water column. Small variation in nucleic acids of sprat larvae collected from the bottom was due to a smaller sample size.

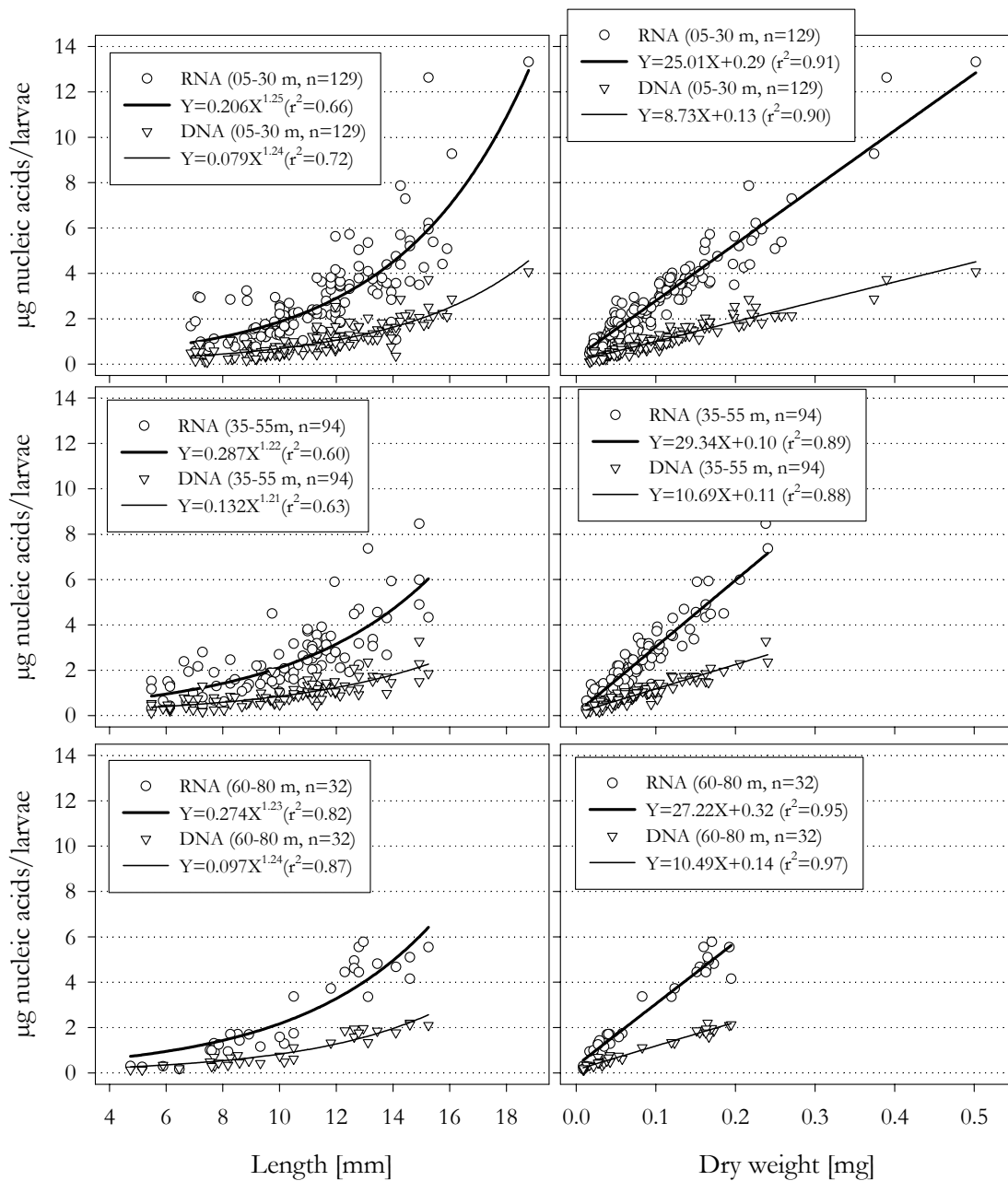


Figure 36. Concentration of nucleic acids by length (left panels) and by dry weight (right panels) of sprat larvae in three different water depth in the Bornholm Basin in June 2001.

Shown by percentiles distribution (Fig. 37), larvae at depth 05-30 m had a better nutritional condition compared to other depths in particular that of larvae between ~ 6 -10 mm. Larvae with nutritional condition less than 1 were found at depth 60-80 m. Despite the fact that smallest larvae were initially higher in condition in the middle water column, it is shown that larvae at 8-12 mm were then slightly lower in RNA/DNA ratio than larvae in the bottom layer. A relatively similar pattern is shown by changes in nutritional condition with dry weight.

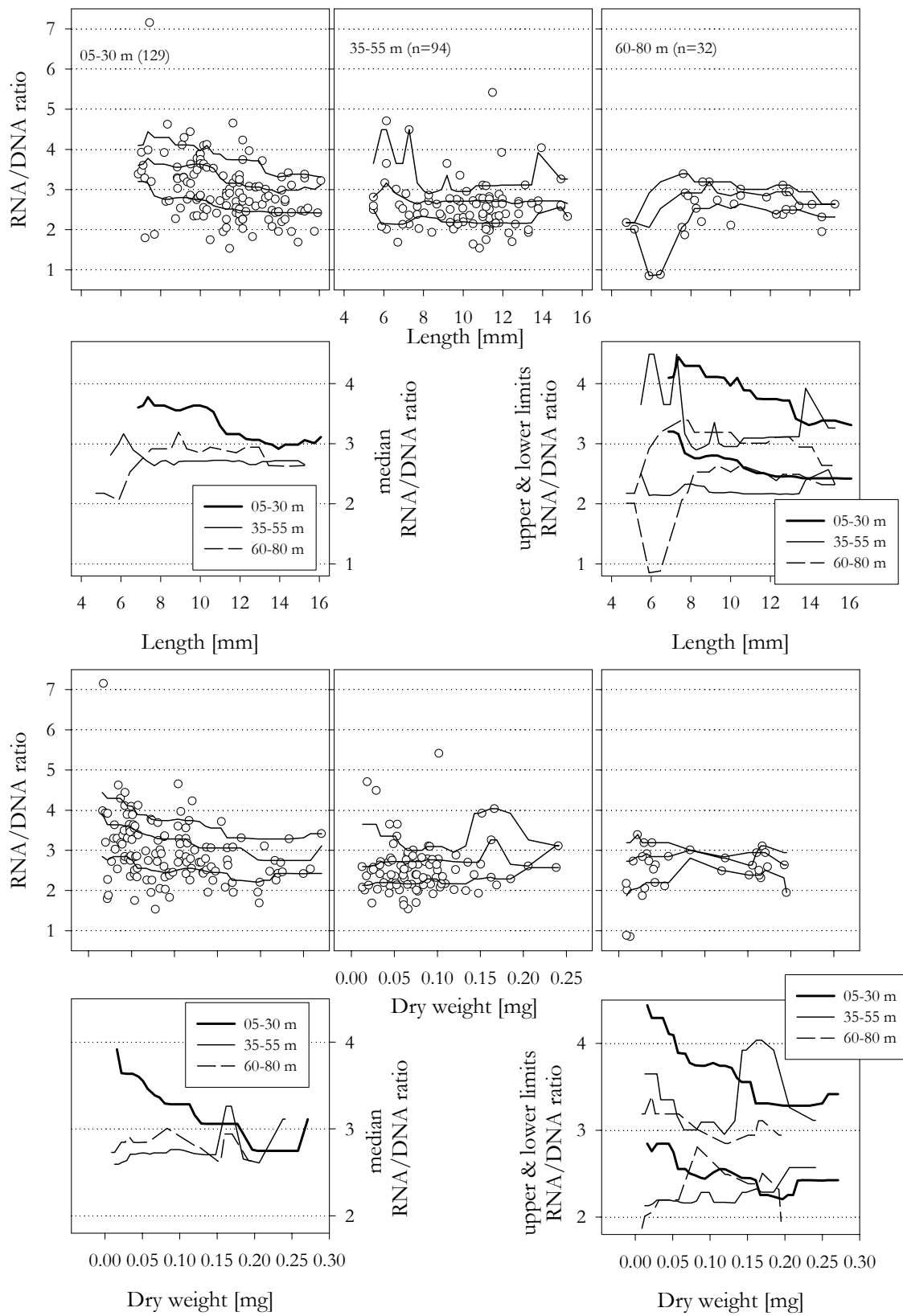


Figure 37. RNA/DNA ratio in relation to length (upper panels) and dry weight (lower panels) of sprat larvae in three different water column with the estimated 10th, 50th, and 90th percentiles in the Bornholm Basin during May 2001. Bandwidths were set at 0.09 for all panels.

The overall distribution of RNA/DNA ratio in sprat larvae is depicted each 5 m depth intervals (Fig. 38). It is shown that larvae in the upper layer were better in nutritional condition compared to the lower layer. The pattern in RNA/DNA ratio values was relatively in agreement with temperature changes throughout the water column. A serial t-test was conducted to observe variation in nutritional condition among depth which summarised in Table 7. Based on this analysis, it is obviously that variability in nutritional condition of sprat larvae was virtually unaffected by depth differences. However, this may be due to a different size spectrum as well as number of larvae analysed in the present study (Fig 38).

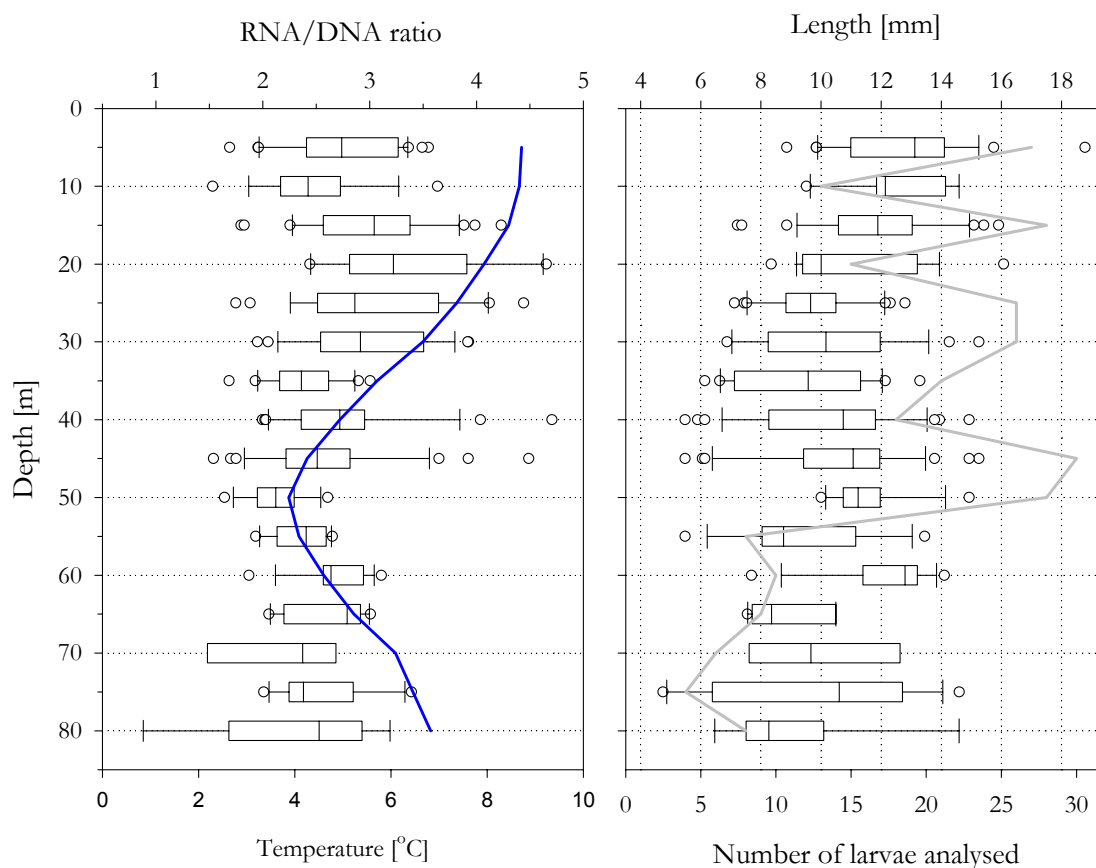


Figure 38. RNA/DNA ratio distribution of sprat larvae in each 5 m depth intervals in the Bornholm Basin in May 2001. A thick vertical line is showing the temperature average at each 5 m depth intervals (left panel). Box plots show length distribution of larvae with a gray vertical line show the number of larvae analysed (right panel).

Furthermore, by grouping larvae into five different depth at 15 m intervals (Fig. 39), it is shown that regardless of the size, larvae collected from depth between 20-30 m were apparently better in nutritional condition compared to other larvae from the surface and two deeper water layers (t-test for unequal sample, $P < 0.05$). The second group of better condition was found on larvae at depth 05-15 m followed by the last three bottom layers (Fig. 39 and Table 8).

Table 7. T-tests results comparing the mean differences in RNA/DNA ratio of sprat larvae collected in the vertical water column with 5 m depth intervals in the Bornholm Basin. Means (*italics*) and significant differences at P<0.05 (**bold**) are shown.

Depth (m)	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	0.999	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	0.939	0.463	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	0.048	0.009	0.819	-	-	-	-	-	-	-	-	-	-	-	-	-
25	0.870	0.361	1.000	0.926	-	-	-	-	-	-	-	-	-	-	-	-
30	0.979	0.607	1.000	0.840	1.000	-	-	-	-	-	-	-	-	-	-	-
35	0.930	1.000	0.064	0.000	0.042	0.138	-	-	-	-	-	-	-	-	-	-
40	1.000	0.948	1.000	0.206	0.997	1.000	0.503	-	-	-	-	-	-	-	-	-
45	1.000	1.000	0.497	0.005	0.376	0.695	0.999	0.988	-	-	-	-	-	-	-	-
50	0.540	0.994	0.027	0.000	0.018	0.050	1.000	0.199	0.846	-	-	-	-	-	-	-
55	0.957	1.000	0.188	0.002	0.136	0.284	1.000	0.684	0.999	1.000	-	-	-	-	-	-
60	1.000	1.000	0.987	0.241	0.969	0.994	0.999	1.000	1.000	0.907	0.999	-	-	-	-	-
65	1.000	1.000	0.991	0.374	0.980	0.996	1.000	1.000	1.000	0.981	1.000	1.000	-	-	-	-
70	0.860	0.999	0.254	0.012	0.202	0.314	1.000	0.614	0.970	1.000	1.000	0.971	0.993	-	-	-
75	1.000	1.000	0.876	0.096	0.806	0.925	1.000	0.998	1.000	0.993	1.000	1.000	1.000	0.998	-	-
80	0.985	1.000	0.504	0.033	0.422	0.586	1.000	0.880	0.999	1.000	1.000	0.999	1.000	1.000	1.000	-

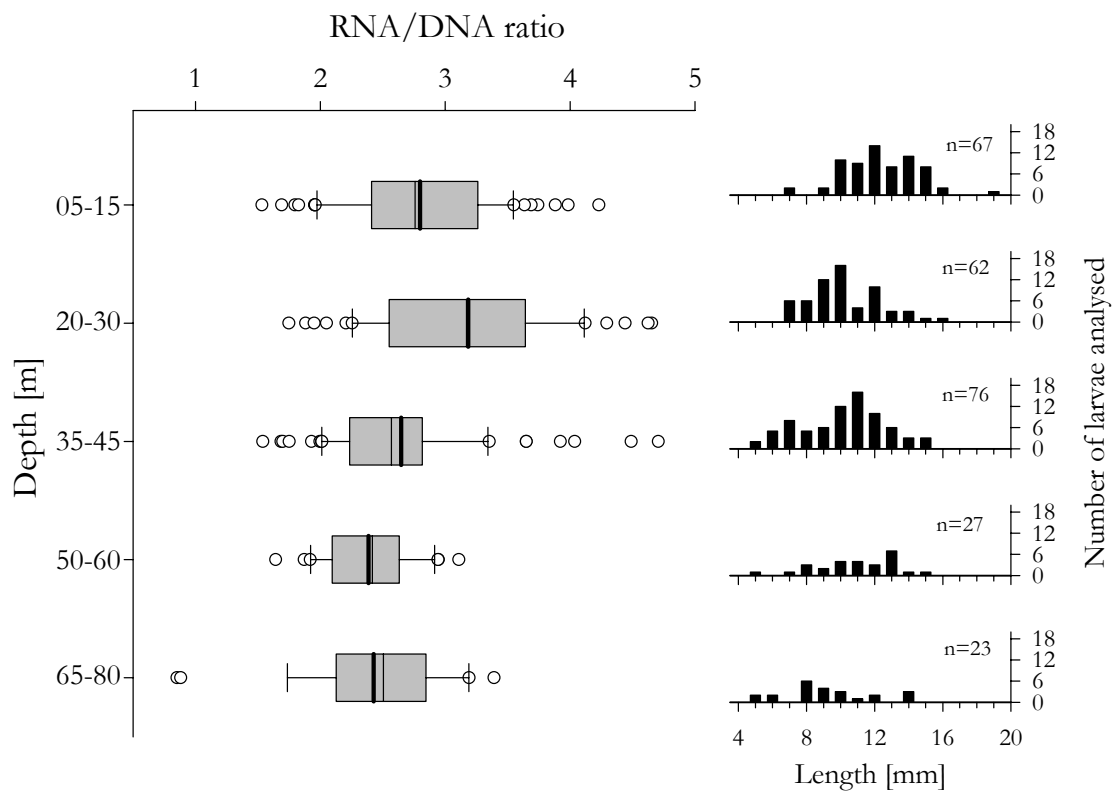


Figure 39. RNA/DNA distribution of sprat larvae plotted for 15 m depth intervals in the Bornholm Basin. The number of larvae in each size class analysed is presented on the right showing a slightly different size spectrum. Median (thick vertical line) and mean (thin vertical line) are shown.

Table 8. T-tests results comparing the mean differences in RNA/DNA ratio of sprat larvae collected in the vertical water column with 15 m depth intervals in the Bornholm Basin. Means (*italics*) and significant differences at $P < 0.05$ (**bold**) are shown.

Depth (m)	05-15	20-30	35-45	50-60	65-80
	<i>2.799</i>	<i>3.186</i>	<i>2.648</i>	<i>2.387</i>	<i>2.428</i>
05-15	-	-	-	-	-
20-35	0.013	-	-	-	-
40-55	0.696	0.000	-	-	-
50-60	0.166	0.000	0.617	-	-
65-80	0.340	0.001	0.805	1.000	-

The pattern of RNA/DNA ratio of each size class with respect to depth is presented in Fig. 40. According to coefficient determination (r^2), it was only larvae of 5-6 mm that clearly showed a decrease in nutritional condition through increasing water depth. The others showed a relatively weak relationship despite there was a tendency that the ratio decreased against depth.

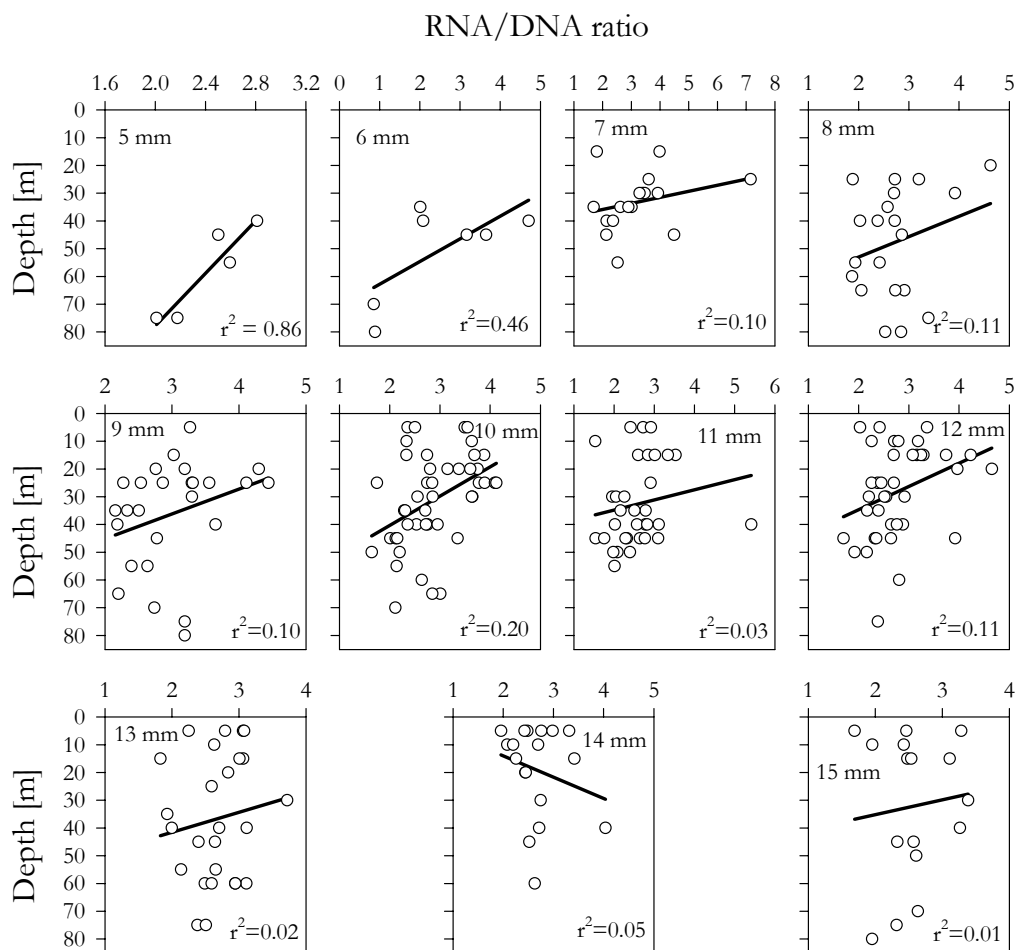


Figure 40. Nutritional condition of sprat larvae in size class against depth. Coefficient of determination (r^2) is diminishing as larvae grow larger.

A further analysis for vertical variability in RNA/DNA ratio of sprat larvae was done by comparing the medians ratio within similar size classes between two different depth group. It is shown that sprat larvae at size class 7, 9, and 10 mm demonstrated better condition at the surface compared to the bottom (Mann-Whitney U test, $P < 0.05$) (Fig. 41). There was a tendency that smaller larvae up to 13 mm of the upper water were higher in RNA/DNA ratio.

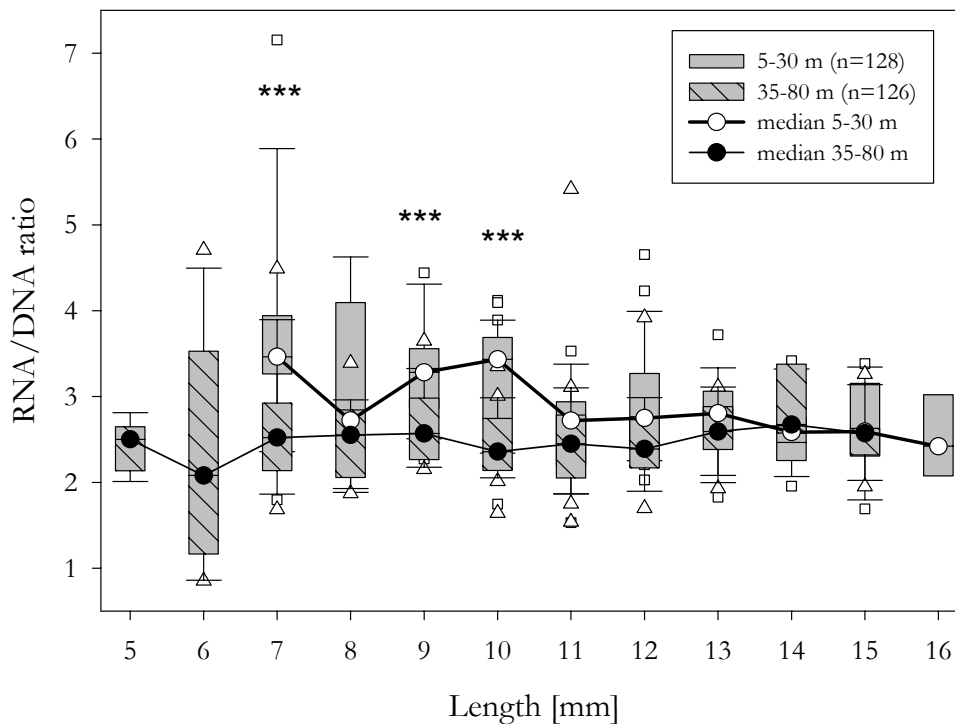


Figure 41. Box plots showing RNA/DNA ratio distribution of sprat larvae at each size class from two depth groups in the Bornholm Basin during May 2001. Box plots without pattern indicated surface, whereas those with coarse pattern are bottom. Outliers: squares (0-30 m), triangle up (35-80 m).

As the biomoc sampling was conducted with 6 hrs intervals, it is possible to analyse the diel vertical variability in nutritional condition of sprat larvae. By grouping each size class according to sampling time in day (09:00-13:00), noon (19:00-21:00), night (22:00-02:00) and morning (05:00-07:00), the medians differences in RNA/DNA ratio were analysed and presented (Fig. 42). The results showed no significant difference in RNA/DNA ratio in each size class according to sampling time (Mann-Whitney U Test, $P > 0.05$) except in the 7 mm group whose nutritional condition was higher during morning compared to those collected during day and noon (Mann-Whitney U test, $P < 0.05$).

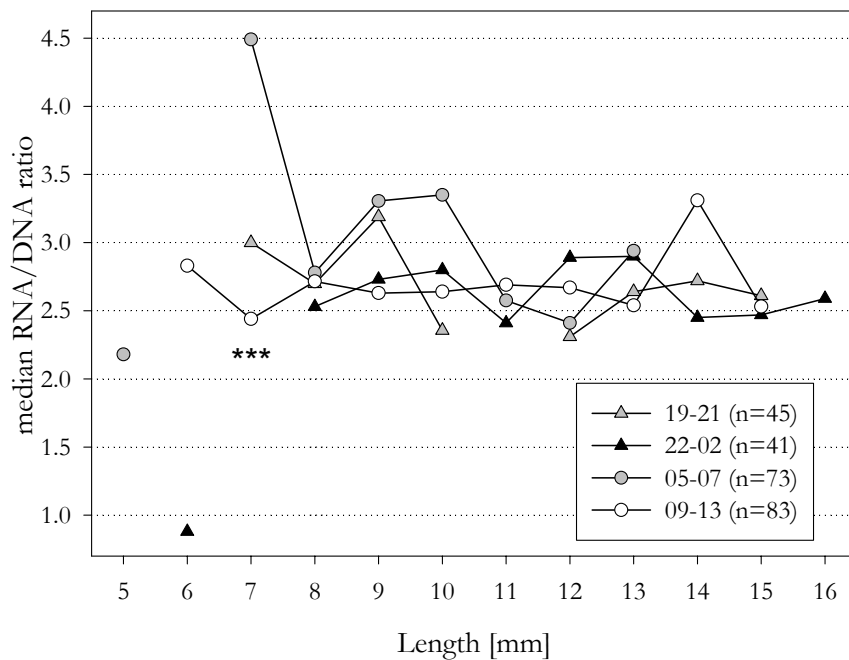


Figure 42. Median values of RNA/DNA ratio of each length class comparing different sampling times in the vertical water column in the Bornholm Basin. Mann-Whitney U test was conducted by comparing the RNA/DNA ratio in each size class. Stars indicate significant differences at $P < 0.05$.

Diel variation in nutritional condition was also not found as the median RNA/DNA ratios were compared within two different sizes at ≤ 10 mm and > 10 mm in both water depth categories for 6 hrs interval (Mann-Whitney U test, $P > 0.05$), despite a slight variation was found within the groups (Fig. 43). From Fig. 43 it is also shown that a tendency of higher RNA/DNA ratio in the upper water column (Mann-Whitney U test, $P < 0.1$) was mainly derived from smaller larvae (Fig. 43 a, c). Whereas, larger larvae were less variable in nucleic acids compared within and between the group (Fig. 43 b, d). Based on these facts, it is unlikely that feeding time and daily vertical migration pattern caused variation in nutritional condition of larvae.

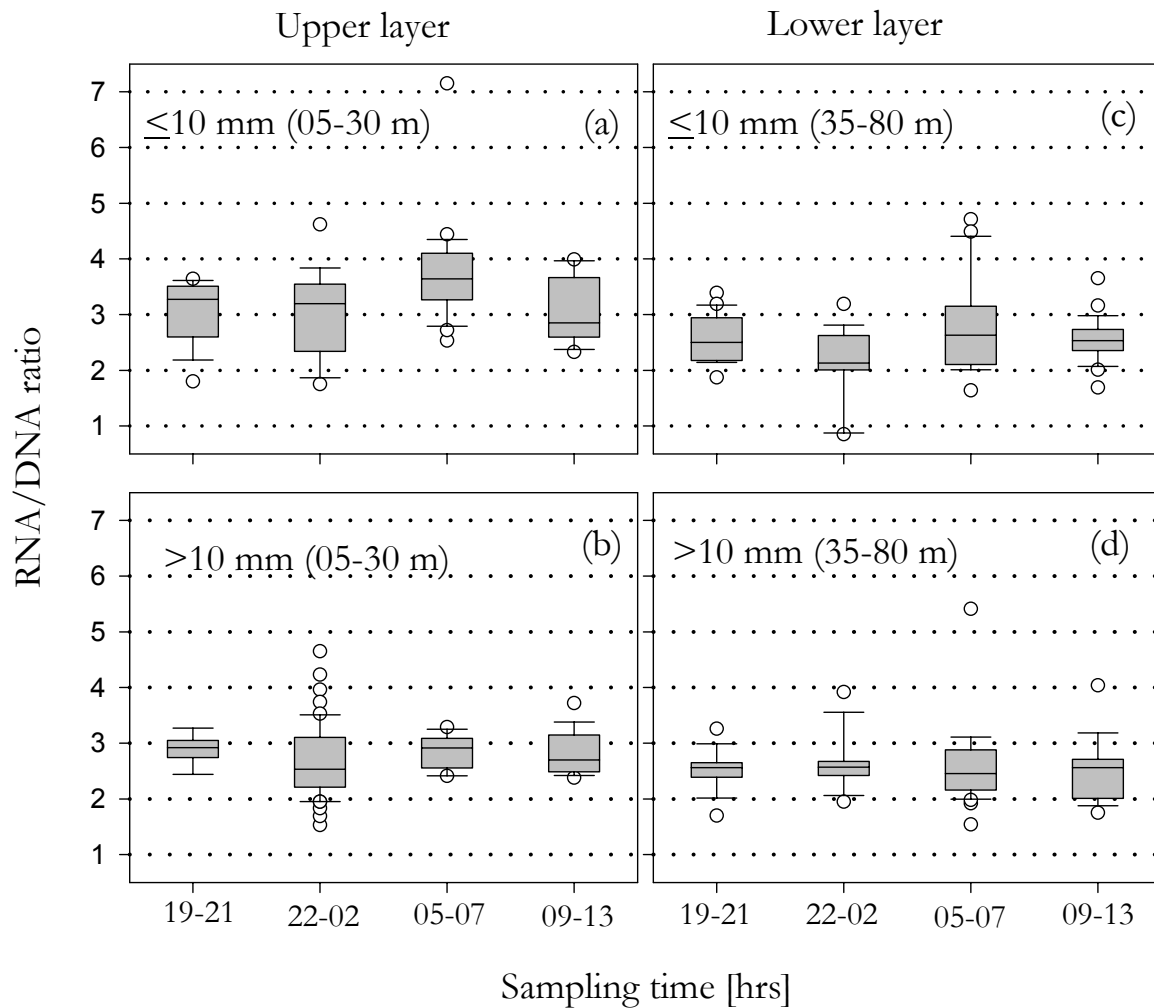


Figure 43. Distribution of RNA/DNA ratios of two different size groups at two different water columns in relation to sampling time within 24 hrs.

Observed from larvae between ~ 6.5 -10 mm, the vertical changes in the relative DNA content (DNA/DW) were found to be highest in larvae collected from the bottom followed by those from intermediate and surface depths (Fig. 44). With respect to cell size, larvae with lowest DNA/DW are considered to be better in their condition. Therefore, based on the illustration in Fig. 44 larval condition was found to be best at the surface layer between 05-30 m. This is clearly shown by both medians as well as upper and lower distribution limits.

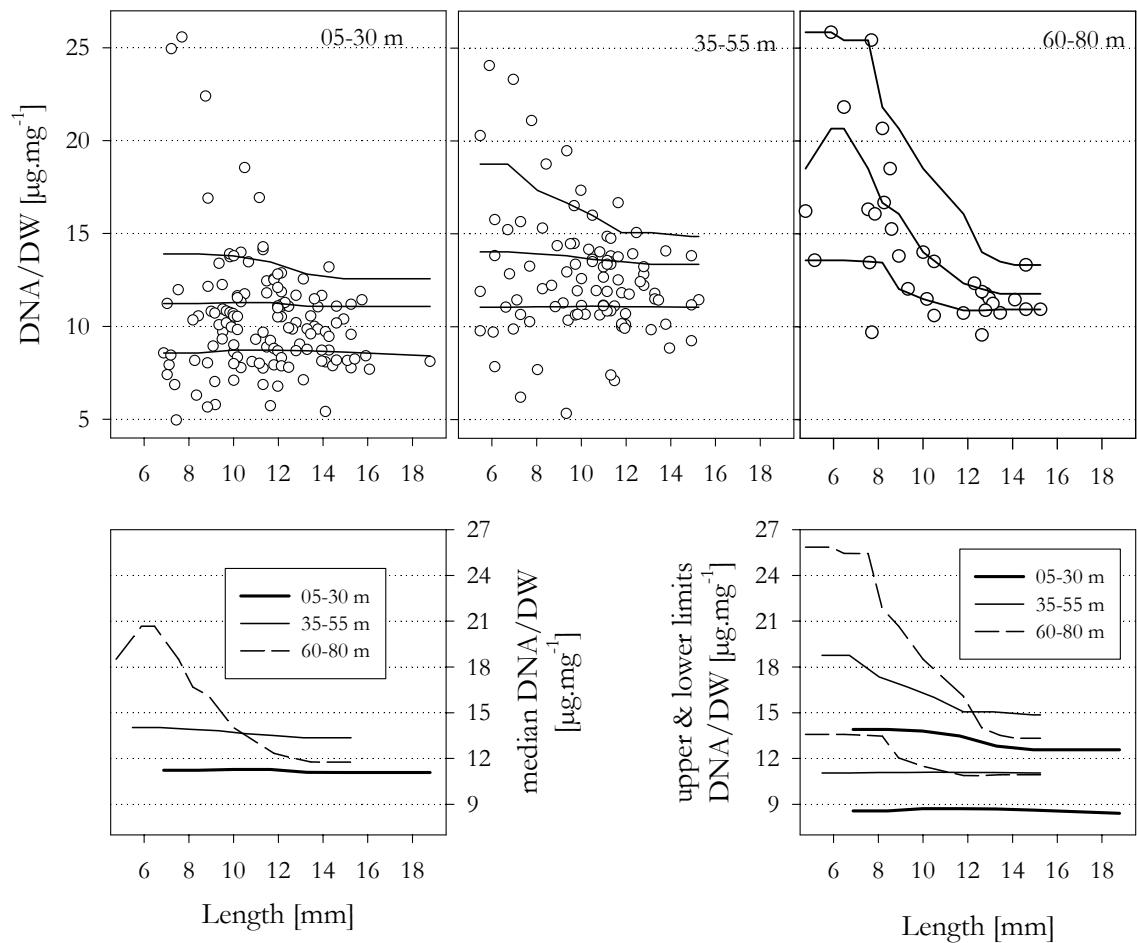


Figure 44. Vertical variability in DNA/DW ratio in relation to length with the estimated 10th, 50th, and 90th percentiles of sprat larvae from three different depths in the Bornholm Basin during May 2001. Bandwidths were set at 0.1 for both panels.

3.4.2 Instantaneous protein growth rate (G_{pi})

The growth of sprat larvae was estimated by instantaneous protein growth rate (G_{pi}) values based on RNA/DNA ratio corrected with averaged water temperature during spawning season 2001. Temperature was 4.32°C and 6.21°C in April and May, respectively. Based on G_{pi} , there was 71.4% of larvae had negative value obtained from earlier sampling whereas only 7.1% occurred on larvae from the latter sampling. This indicated a better growth and accordingly higher survival probability might be experienced by larvae collected in May. Fig. 45 shows the changes in G_{pi} at length and dry weight with a relatively similar trend. At size 6-10 mm (~0.01 – 0.10 mg) larvae were higher in G_{pi} during May compared to April in which the minimum value was found (Fig. 45). Shown by medians, the G_{pi} was in average relatively unchanged in May whereas an increase in G_{pi} could be observed at size 6-7 mm from larvae in

April. Due to small sample size obtained from the first survey, however, a comparison between two sampling time in G_{pi} of individuals larger than 7 mm may be inappropriate.

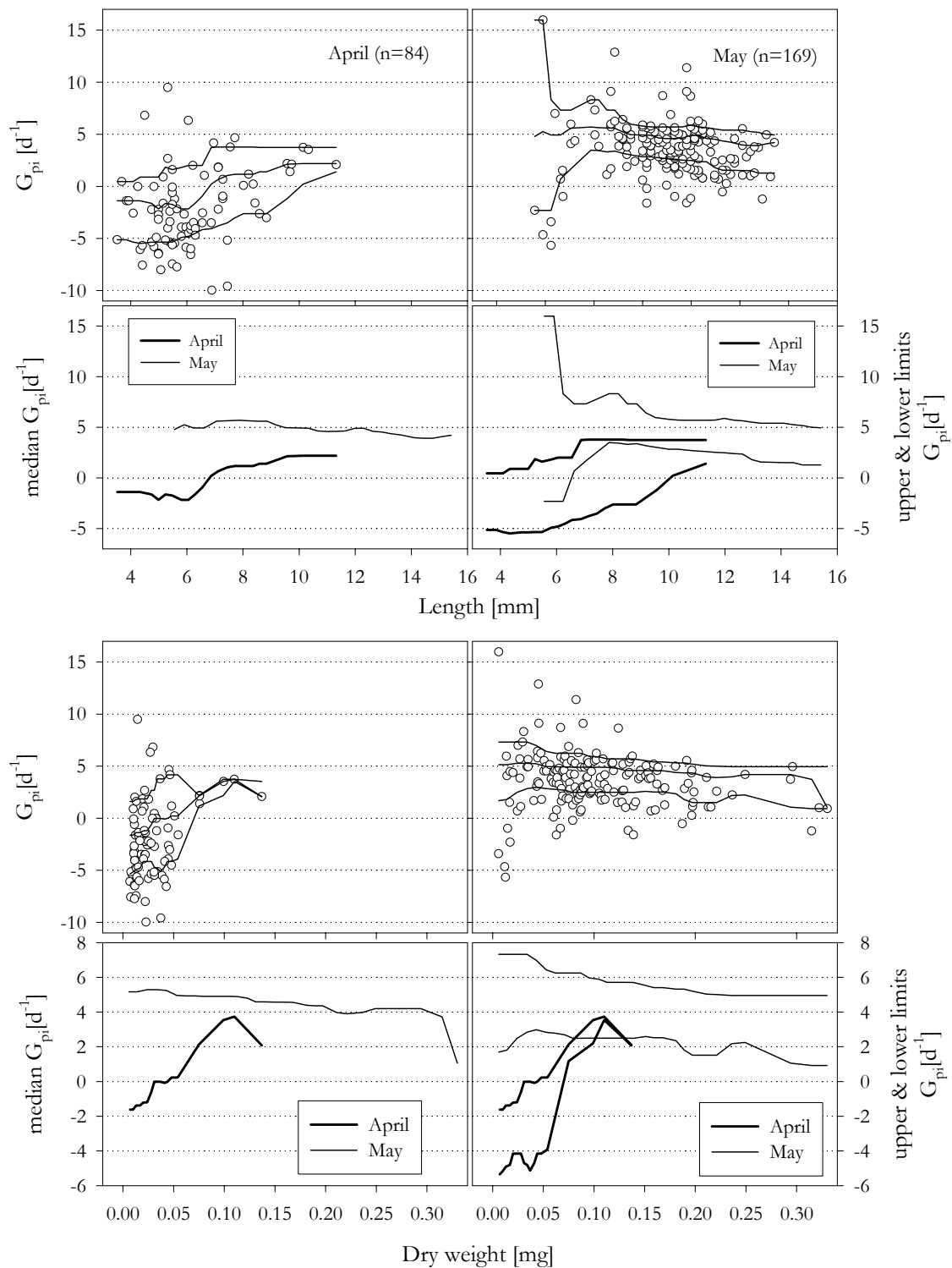


Figure 45. Instantaneous protein growth rates (G_{pi} [day^{-1}]) in relation to length (upper panels) and dry weight (lower panels) with the estimated 10th, 50th, and 90th percentiles of sprat larvae in the Bornholm Basin during April and May 2001. Bandwidths were set at 0.115 and 0.2.

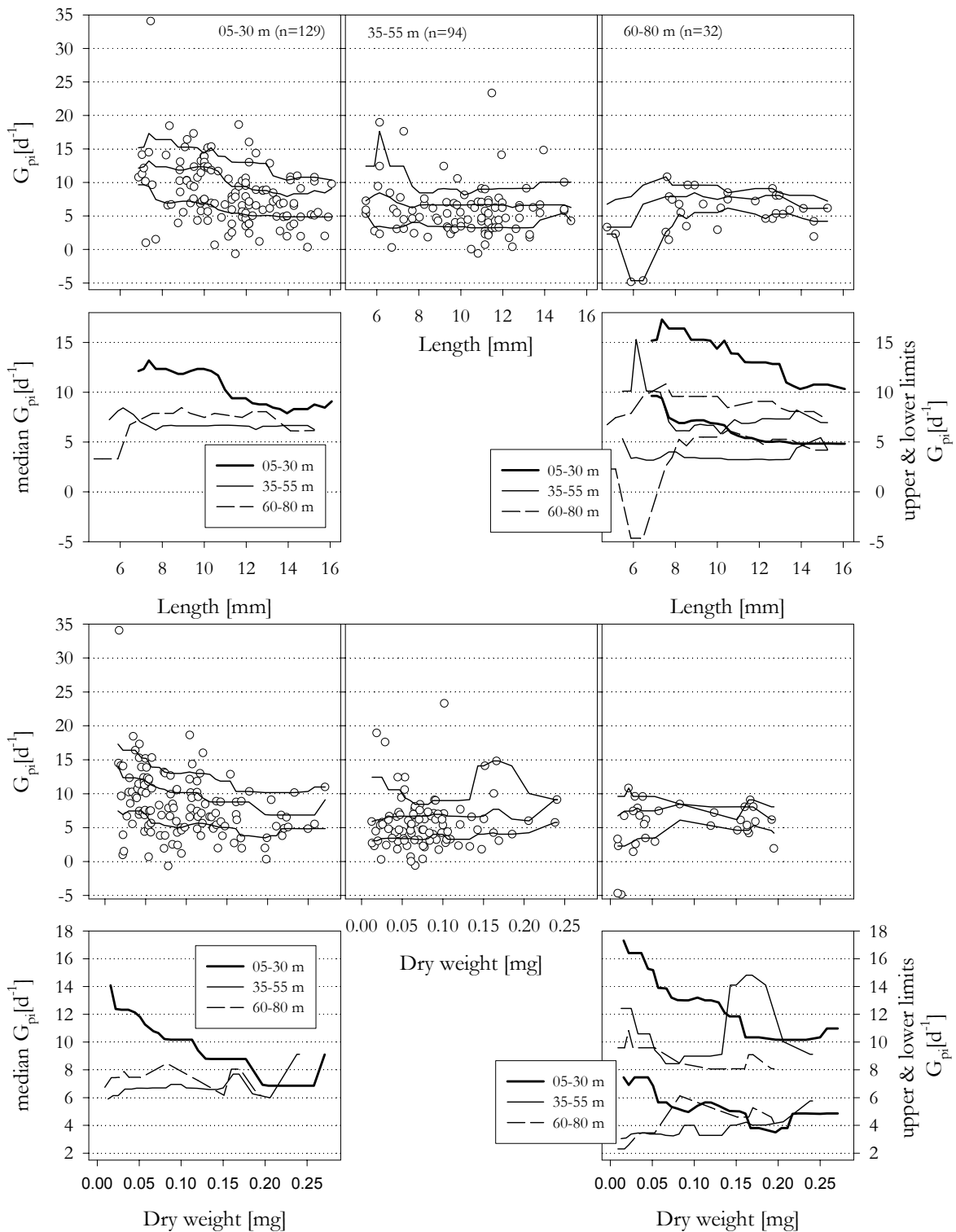


Figure 46. Instantaneous protein growth rates (G_{pi} [day^{-1}]) in relation to length (upper panels) and weight (lower panels) with the estimated 10th, 50th, and 90th percentiles of sprat larvae from vertical water column in the Bornholm Basin during May 2001. Bandwidths were set at 0.1 for each panel.

The value of G_{pi} were also compared for different water depths in May (Fig. 46). Larvae with a G_{pi} below zero were in a relatively minute fraction with <1%, 2.1%, and 6.3%

found in the surface, middle, and bottom water, respectively. The average temperature for each water column was 7.85, 4.57, and 6.37°C for the three water layers during sampling. Such differences were due to the different water masses with the Baltic water on the top and the North Sea water at the bottom during spring time. According to Fig. 46, therefore, sprat larvae at depth 05-35 m demonstrated better growth than in other depths. However, the growth was slightly higher at size 8-14 mm (~ 0.05 - 0.20 mg) in the bottom than in the middle water.

By aggregating larvae into 10 m depth intervals, the vertical distribution of G_{pi} is illustrated in Fig. 47. It is shown that larval growth at depth 60-80 increased due to higher temperature at the bottom. This would explain the higher G_{pi} value found in the bottom compared to that in the middle water column (Fig. 46). In addition, the value of G_{pi} may also be influenced by variation in sample size as well as number of larvae analysed (Fig. 47, right panel).

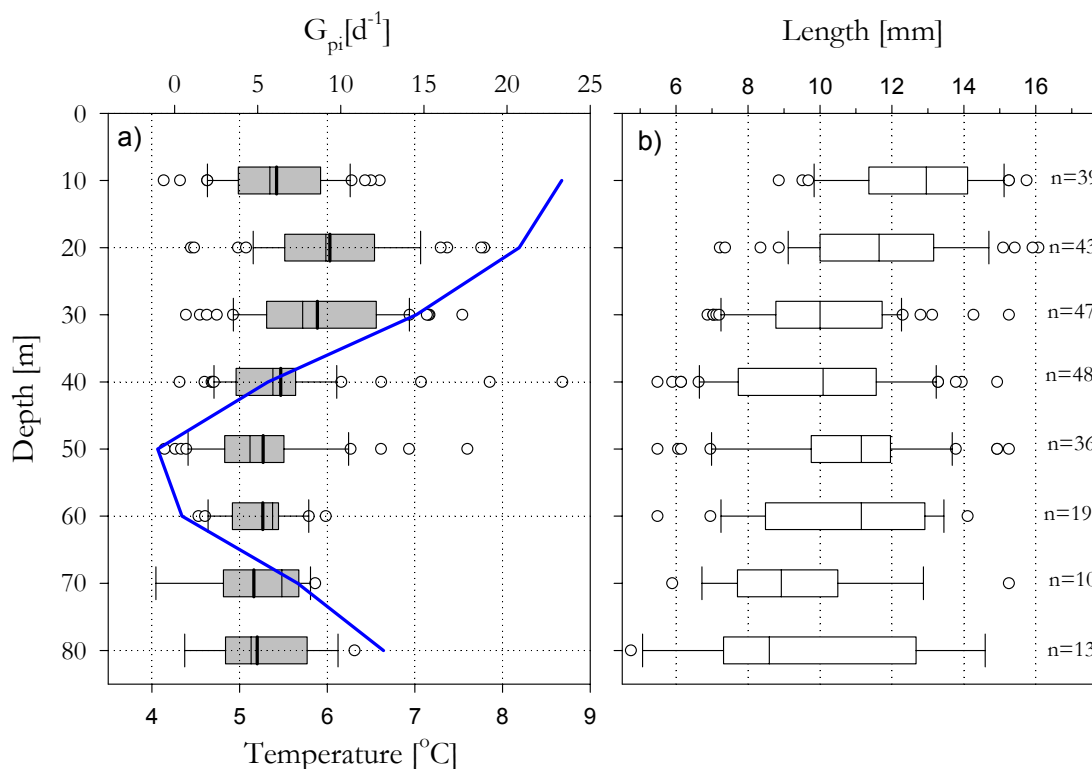


Figure 47. Instantaneous protein growth rates (G_{pi}) of sprat larvae are shown within the vertical water column at 10 m depth intervals in the Bornholm Basin during May 2001. The thick solid line in the box plots of the left panel indicate means. Size and number of larvae analysed is show in panel b.

The difference in G_{pi} with respect to depth regardless larval size is summarized in Table 9. By comparing the mean G_{pi} of larvae at each 10 m depth interval it was obtained that larvae from the first 30 m of depth had better growth compared to other deeper water columns (t-test, $P < 0.05$).

Table 9. T-test results comparing the mean differences in G_{pi} of sprat larvae aggregated from each 10 m depth intervals in the Bornholm Basin during May 2001. Means (italics) and significance different of $P < 0.05$ (bold) are shown.

Depth (m)	10	20	30	40	50	60	70	80
	<i>M=3.1944</i>	<i>M=5.7196</i>	<i>M=4.0820</i>	<i>M=-.7015</i>	<i>M=-3.285</i>	<i>M=-2.963</i>	<i>M=-1.913</i>	<i>M=-.5267</i>
10								
20	0.1346							
30	0.9826	0.6117						
40	0.0011	0.0000	0.0000					
50	0.0000	0.0000	0.0000	0.1500				
60	0.0002	0.0000	0.0000	0.7111	1.0000			
70	0.1148	0.0012	0.0300	0.9982	0.9960	0.9993		
80	0.3136	0.0036	0.0938	1.0000	0.7015	0.8173	0.9958	

Vertical variability in G_{pi} within individual larvae is best presented by dividing the sampling depth into two groups, 05-30 m and 35-80 m. By comparing the median G_{pi} of each size class, it revealed that larvae up to 13 mm (except 11 mm) were higher in G_{pi} at the upper compared to those in the lower water column (Mann-Whitney U test, $P < 0.05$). However, larvae at 14 mm showed a contradictive result (Fig. 48).

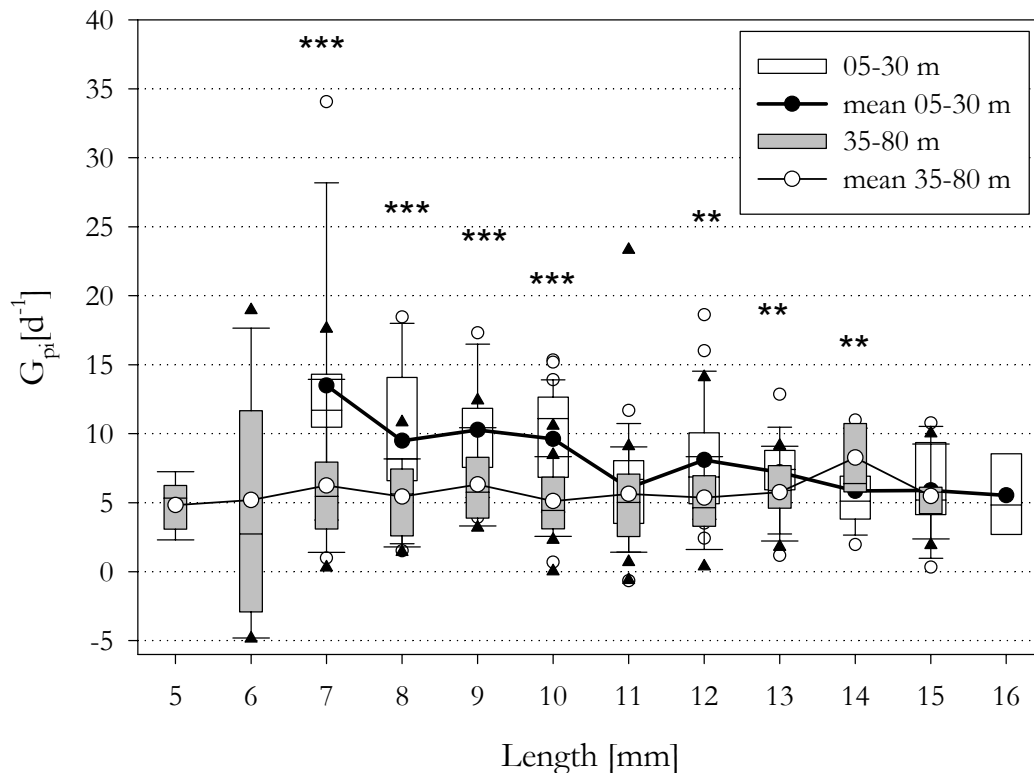


Figure 48. Instantaneous protein growth rates of sprat larvae comparing between size classes of two different water columns in the Bornholm Basin. Stars above the box plots are significance level at $P < 0.05$. Circles and triangles are outliers for surface and bottom respectively.

3.5 Joint analysis of otolith microstructure and RNA/DNA ratio

Sprat larvae which were analysed for coupled examination in otolith microstructures and RNA/DNA ratio included 89 individuals derived from vertical sampling in the Bornholm Basin during May 2001. All parameters compared below are presented according to depth 5-30 m and 35-80 m.

Based on coupled analysis, Fig. 49 shows the vertical variability in dry weight in relation to age. Sprat larvae up to ~11 mm demonstrated a small fluctuation in weight whereas a clear difference occurred on larger larvae which were showing higher weight at age for larvae collected in the upper water. Such a pattern is similarly shown by the upper limit distribution. However, the lower limit shows similar changes in weight at age for both.

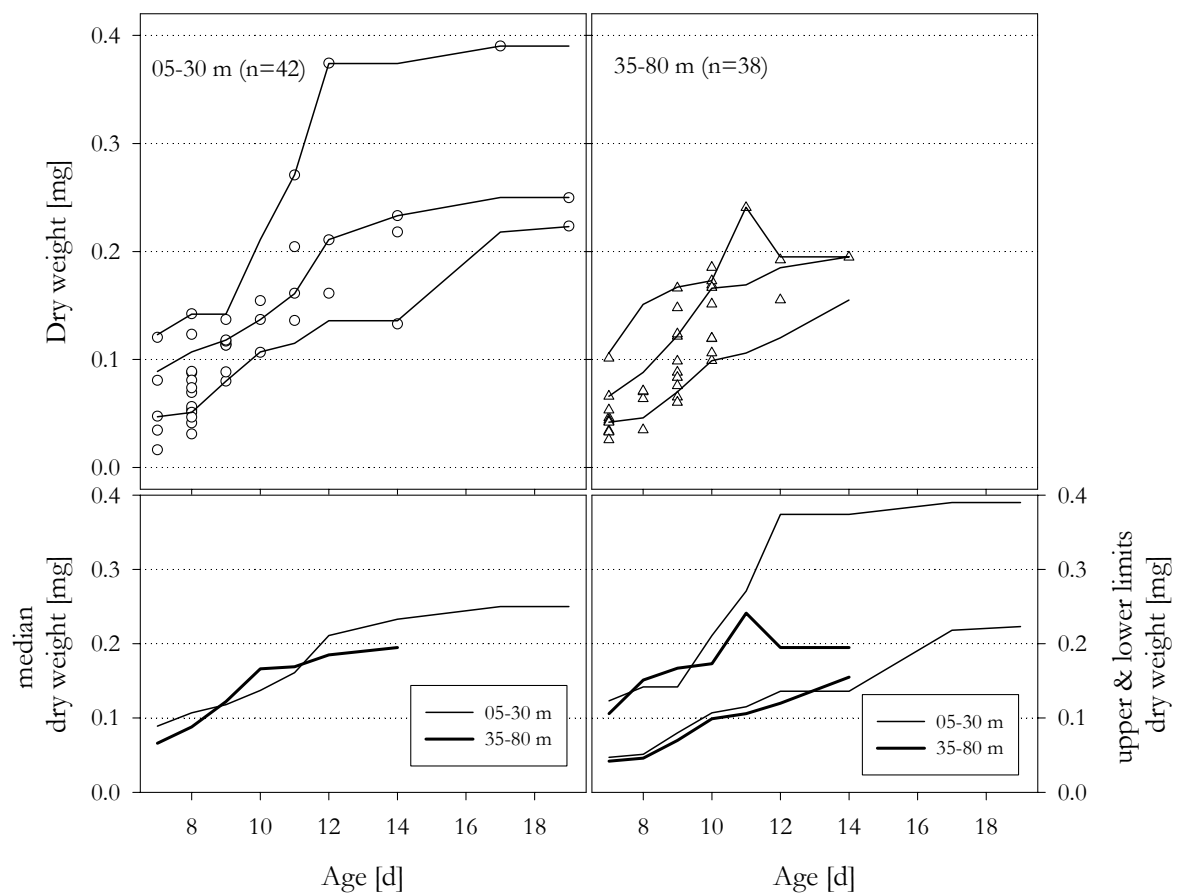


Figure 49. Observed dry weight in relation to age with the estimated 10th, 50th, and 90th percentiles of sprat larvae from the vertical water column in the Bornholm Basin during May 2001 (upper panels). Means as well as upper and lower distribution limits shown (lower panels).

The changes in nucleic acids concentration with age are presented in Fig. 50. The DNA content was slightly higher on larvae in the deeper water. Whereas RNA was initially higher at 7-9 d old larvae afterwards it was pretty close in concentration for both depth

categories. Fig. 50 also shows that RNA was more variable than DNA. Average temperature during sampling was 7.85 and 5.49°C for the two different depths.

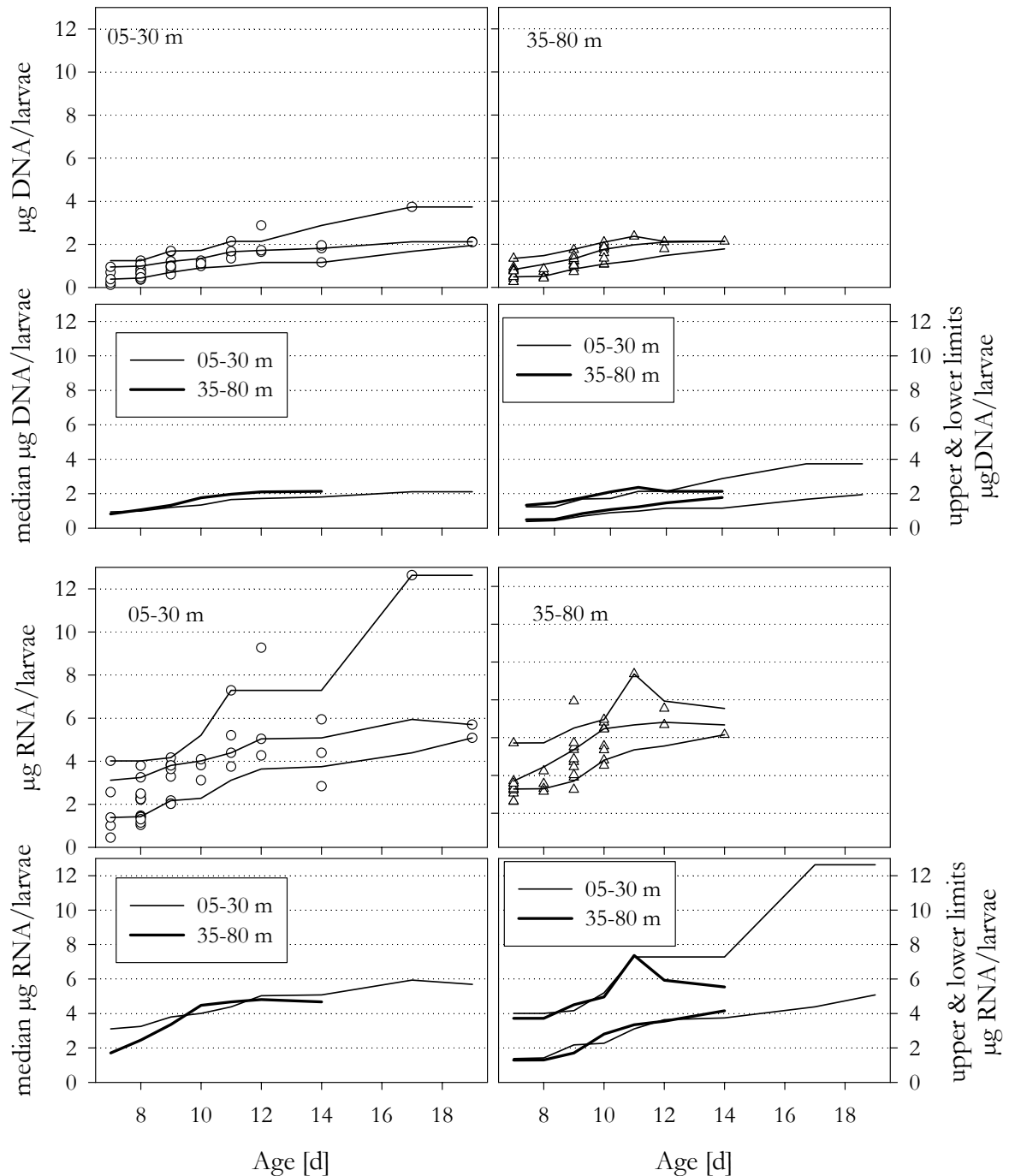


Figure 50. Nucleic acid concentration in relation to age with the estimated 10th, 50th, and 90th percentiles of sprat larvae from vertical water column in the Bornholm Basin during May 2001. Bandwidths were set at 0.2 for both panels.

In order to investigate the relationship between RNA/DNA ratio and development of otolith structures, the sum of the last two increments was calculated and plotted with

nutritional condition of larvae within corresponding depth (Fig. 51). The last two increments were chosen due to the limited sample size of larvae analysed. Shown by medians as well as upper and lower percentiles, it is obvious that increments of larvae in the upper part were wider in relation to the RNA/DNA ratio. At the upper layer, however, the increase in RNA/DNA ratio was followed by a decrease in increment widths. Similar pattern was also found for the lower water column despite of a different range in RNA/DNA ratio. This indicated a delayed response in otolith structures at changes in nutritional condition. In fact the pattern below was derived from a relatively small sample size which may not representing the actual condition.

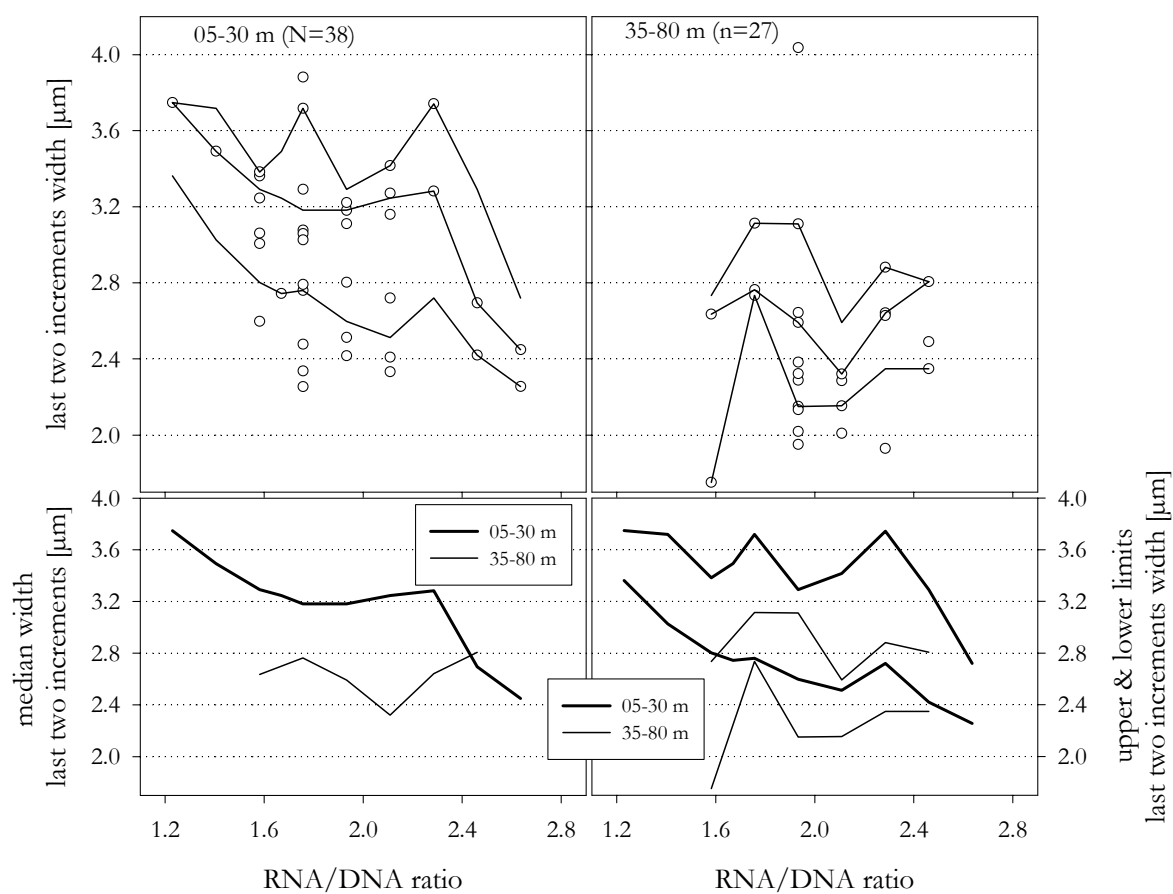


Figure 51. Increment widths in relation to RNA/DNA ratio with the estimated 10th, 50th, and 90th percentiles of sprat larvae from vertical water column in the Bornholm Basin during May 2001. Bandwidths were set at 0.1 and 0.08.

The influences of otolith dissection on RNA/DNA ratios of individual larvae was analysed. By comparing the median values of RNA/DNA ratio between larvae with and without otolith dissection on each size class, no differences were found in the ratio in both

water columns (Mann-Whitney U test, $P > 0.05$). Highest variation occurred on smallest individuals (Fig. 52).

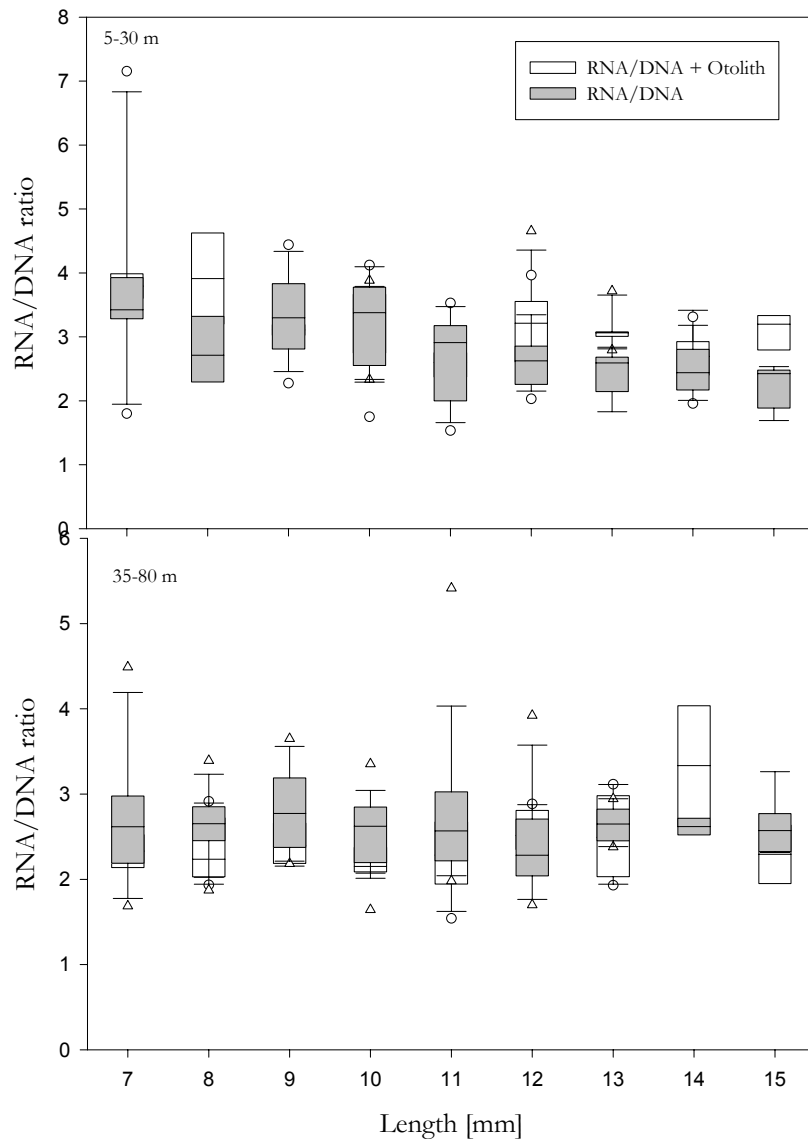


Figure 52. Box plots showing the effect of joint analysis (overlapped box plots) on RNA/DNA ratio in a single larvae. Sample was analysed from vertical sampling.

3.6 Application of biophysical modeling

Environmental condition: temperature distribution in the Bornholm Basin

Among hydrographic factors, temperature is an importance parameter which may influence the biological condition of organisms especially in the upper part of water column where the maximum changes were found between April and May (Fig. 7). Based on hydrodynamic modeling, Fig. 53 presents the temperature changes within 10 m depth in the Bornholm Basin

during 13 d (8nd - 21th) in April and 18 d (14nd May to 1st June) in May 2001. A low range in temperature was found in April which ranged between 5.6 - 5.9 °C (0.3 °C), whereas in May it changed considerably from 7.4 to 11.4 °C (4 °C), i.e temperature had been increased between 1.5 – 5.5 °C along the larval track before capture.

Lower temperature at the surface layer was due to lower insolation during the earlier compared to the latter month. It increased in response to a longer photoperiod during late spring, developing a thermocline layer which provided a barrier for bottom water to mix with upper layer in the latter month (see also Fig. 7). Fig. 53 shows that slightly warmer water was found in the south west of the basin. However the difference was only 0.3 °C. In May, the regions with lower temperatures were mostly in the northern and eastern part of the basin. Similar to April, the warmest water was found in the south western region in May. A relatively cold water in the northern and eastern parts may be associated with a minor water exchange between the North Sea and the Baltic Sea through the Bornholm Strait.

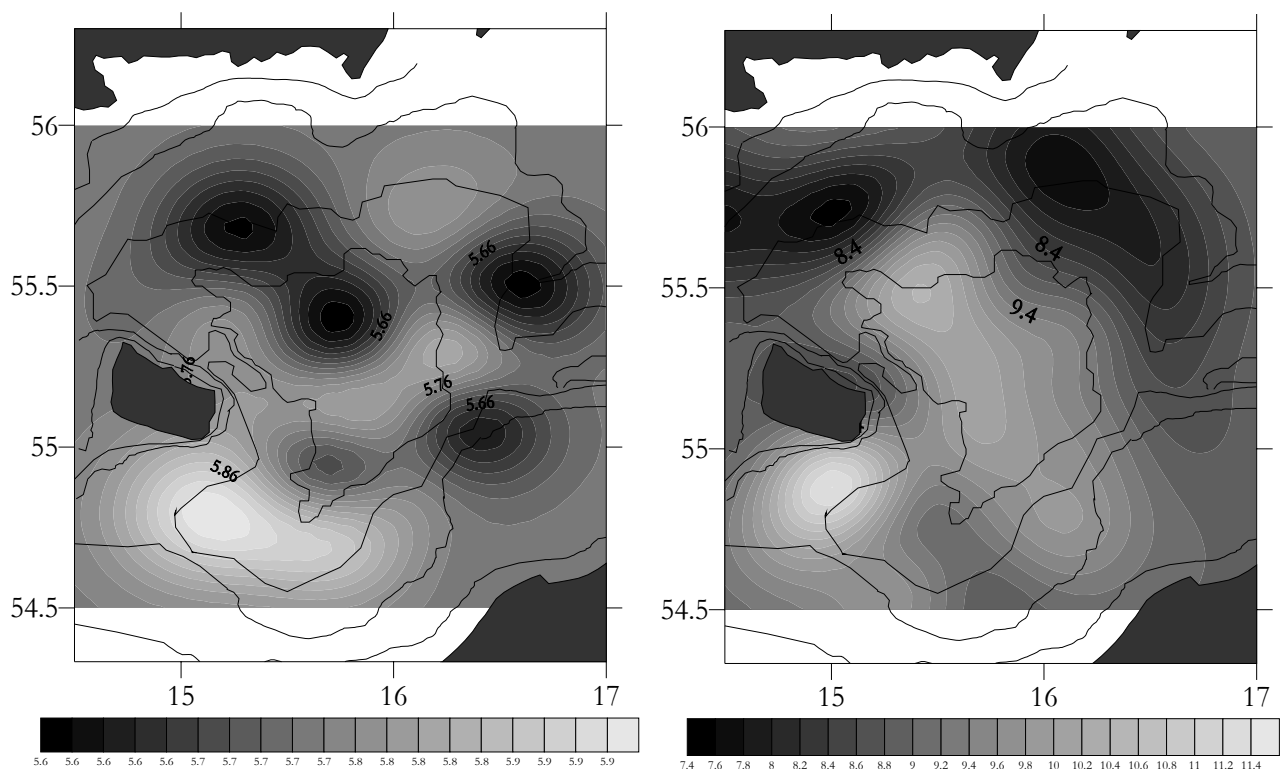


Figure 53. Temperature distribution in the upper layer (10 m depth) in the Bornholm Basin from 8th - 13th April (left) and 14th May – 01st June (right) 2001. Note the different scales in temperature.

Distribution of average increment widths

Measured from the distance between two consecutive rings in the otolith of sprat larvae collected in different months revealed that that higher temperature resulted in larger

increment widths in particular that of smaller larvae collected in May (Fig. 22, p. 38). Horizontal variability in the average distance between two consecutive rings of each increment widths, i.e. total increment widths was divided by increment number is presented (Fig. 54). The figure showed a narrower average in increment widths observed in April compared to May. The distribution of average increment widths were found to be narrower in the northern part, whereas higher value were found in the south. In the area between north and south, the average increment widths showed variations. In connection to temperature conditions during the two months (Fig. 53), this confirmed that higher temperature in May was reflected by a better development (wider increment widths).

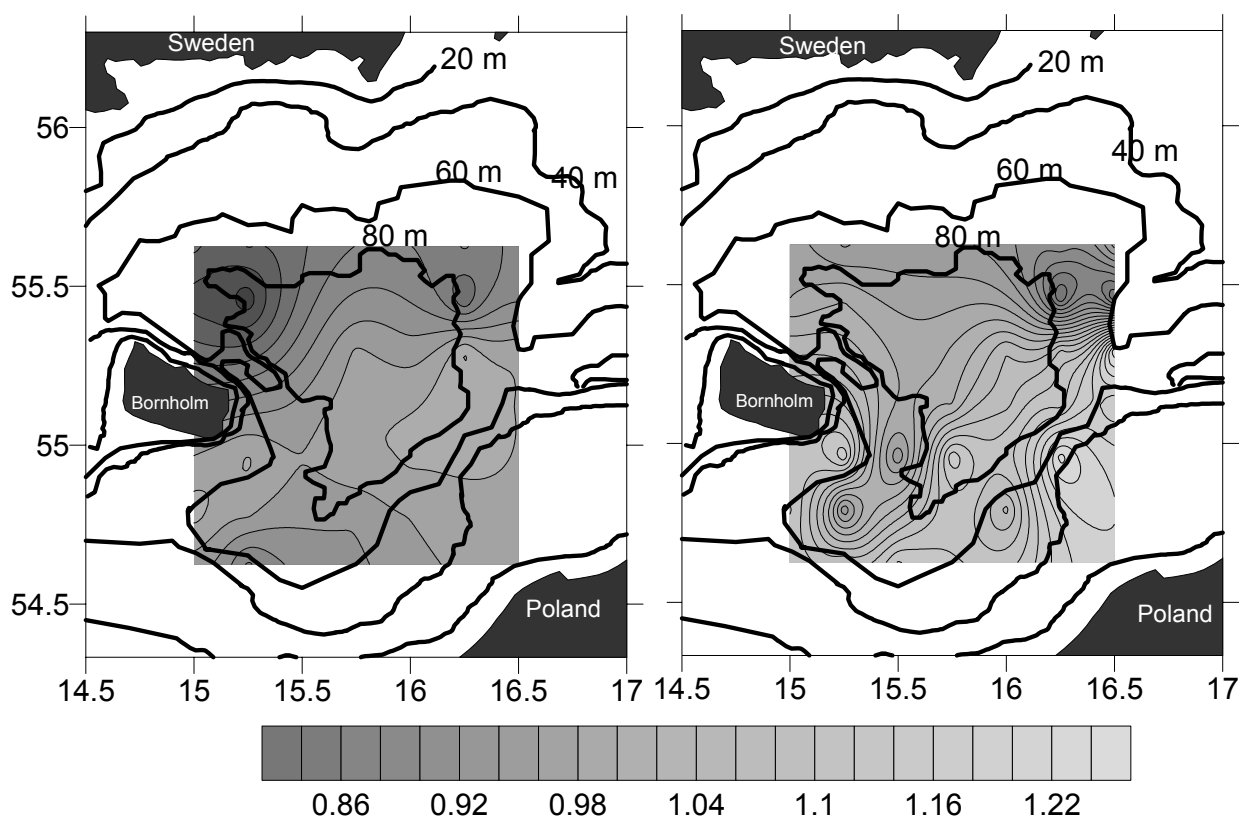


Figure 54. Horizontal distribution of average increment widths per each increment (μm) over the Bornholm Basin comparing between April (left) and May (right) 2001. The scales were at similar range.

Model results

A simulation of a reverse tracking model of sprat larvae was constructed based on otolith microstructure analysis and hydrodynamic modeling. The results is the horizontal distribution of otolith growth per 1°C calculated for 13 d in April and 18 d in May (Fig. 55). The otolith growth with respect to 1°C ranged between $0.085 - 0.180 \mu\text{m}^\circ\text{C}^{-1}\text{d}^{-1}$ in April, and $0.085 -$

0.150 $\mu\text{m}^{\circ}\text{C}^{-1}\text{d}^{-1}$ in May. In this case, though temperature was lower in April, however, the gain in increment widths contributed by each 1 °C was slightly higher in April than in May. By accepting the current assumption of coupled between otolith and somatic growth, it is therefore, the daily growth rate per 1 °C of sprat larvae were slightly higher in April compared to May.

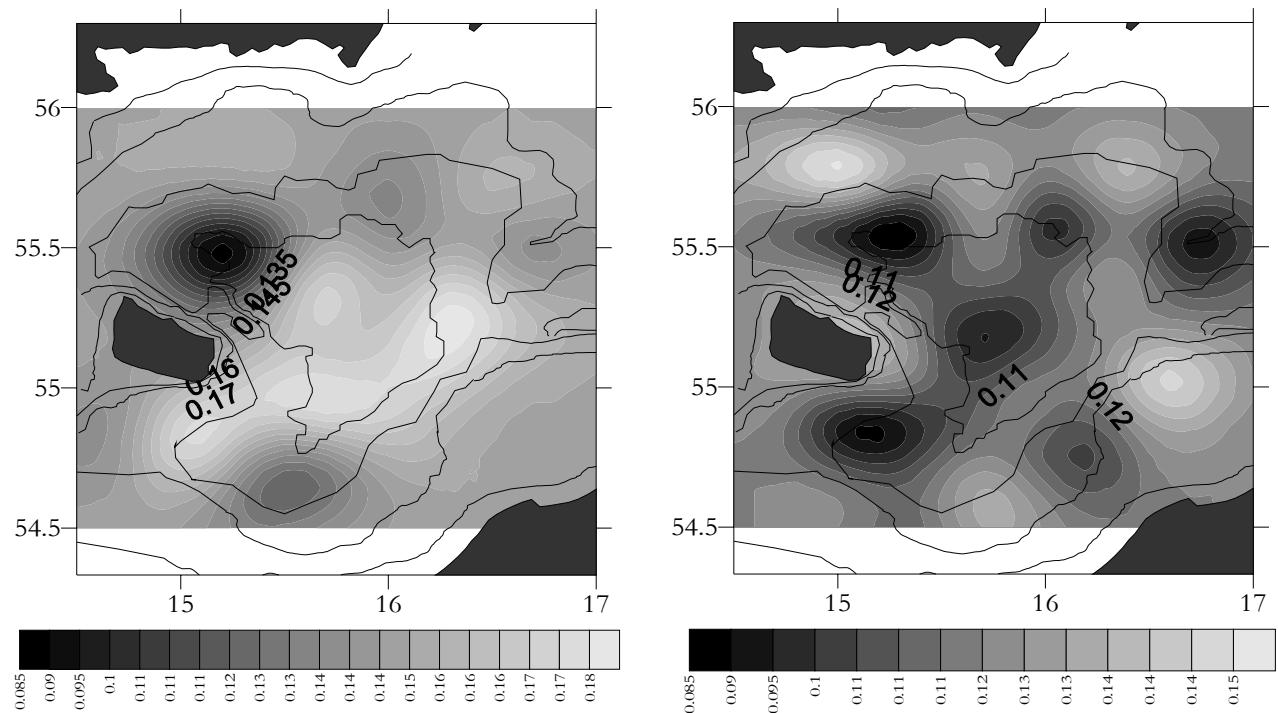


Figure 55. Horizontal distribution of otolith growth with respect to each degree Celsius of sprat larvae in the Bornholm Basin during April (left) and May (right) 2001. Note the different scales.

In April, the growth per 1°C was highest in the central and it extended to south western and eastern basin. The lowest growth was found in the north western part of the basin. Contrarily, a higher growth rate with respect to temperature changes were found in the coastal and other shallower areas of the Bornholm Basin during May (Fig. 55).

Model validation with RNA/DNA ratio

The model results was compared with the distribution of median RNA/DNA ratios of sprat larvae for May 2001. A relatively good agreement was found especially in the central, eastern, and partly southern basin where the pattern in otolith growth was reflected in nutritional condition. In the north, to the lesser extent an accordance between the two still could be found with little differences found in the middle region of the northern basin. The highest growth rate coincided with the highest RNA/DNA ratio found just below the eastern part

(Fig. 56). The existence differences between model and validation results may be caused by the existence of delay in RNA/DNA (Fig. 51, p. 66).

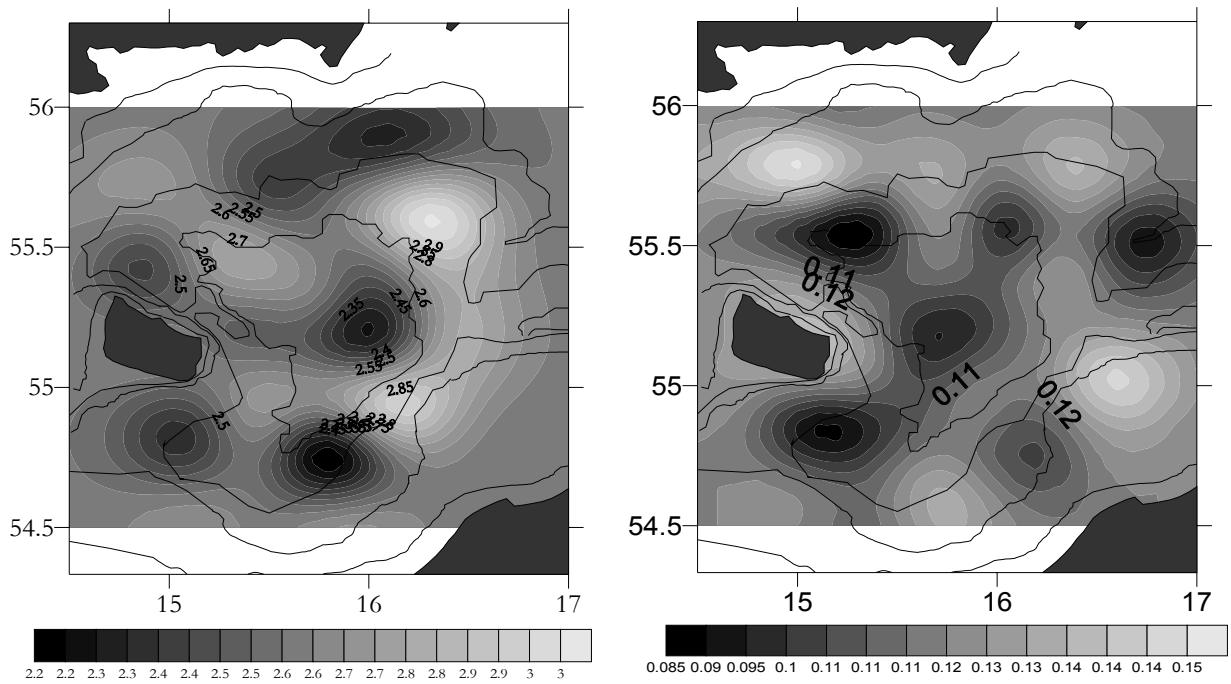


Figure 56. Validation of the model (right) with RNA/DNA ratio (left) showing a highly agreement between the two for larval condition and otolith growth per 1°C of sprat larvae in the Bornholm Basin in May 2001.

Chapter 4. Discussion

4.1 Discussion of materials and methods

Larval rearing condition for otolith validation

In the present study, the loss of an apparent daily rhythm of deposition in the otoliths of sprat larvae was probably caused by a low temperature level during the experiment (Table 4). Jones (1986) confirmed that only under optimal rearing conditions, e.g. temperature and food daily rings will be formed. In over 20 species, she found a non-daily pattern of ring deposition during low temperature and at intermittent starvation. The evidence so far showed that daily increment is predominantly influenced by temperature more than by food (e.g. Wright et al., 1991) especially over short-term experimental periods (Barber & Jenkins, 2001). Water temperature ranged between 3-5 °C during embryo collection in April 2001. It was considered that setting temperature at 10°C would be appropriate for larvae to be maintained in an experimental set up. Alshuth (1988) reported that based on various preliminary assessment the best temperature for validation of daily increment for the North Sea sprat larvae was at 15°C. She collected the embryos in summer at warmer condition than in the Baltic during spring. Unfortunately, no assessment for temperature effect was studied prior to experiment due to limited number of eggs. In fact, it was later found on wild-caught larvae, that increments were discernible at temperature level <5 °C during similar month. This lead to a suggestion that indiscernible increments may also caused by photoperiod which was not considered in the present study as well as the manipulated-environment in the experiment incomparably to natural condition.

Starvation of the larvae in the tanks lead to extremely low numbers of survivors. On which day starvation-induced mortality occurred was not observed in this study. Shields (1989) found high mortality in starved Irish Sea sprat occurring 4 d after absorption of the yolk-sac.

Increments-based ageing method

Large variations are often found in the otolith microstructures which frequently promote an inconsistent results in ageing from different laboratories and even from within a single laboratory. This is associated with a complexity of otolith's structures, numerous equipments used, and different skills and experiences of the readers (Campana, 2001). Indeed, a reliable information of larval age is crucial otherwise the otolith-based recruitment studies lead to either over- or under-estimation in assessment. A reliable ageing can be achieved by means

otolith intercalibration exercise performed with various equipments and different readers on the same species in order to eliminate bias and accordingly to standardised the technique so that reliable data can be produced. This is true, for instances, in determining the narrow increment widths less than 0.4-0.5 μm which are beyond the optical resolution of light microscope or to determine increment formation that has been either disrupted or the presence of sub-daily increments in response to sub-optimal condition e.g. food and temperature (Jones, 1986; Campana et al., 1987).

Two important information is needed in larval ageing, namely timing of first increment formation and its periodicity (Jones, 1992). Alshuth (1988) established the first increment occurrence on the North Sea sprat after 6 days and after 5 days for Irish Sea sprat. However, she mentioned nothing about the periodicity in ring formation. In the present study, the first increment occurred 5-6 d posthatch based on sagittae from two larvae. It is therefore unsafe to draw any conclusion whether or not the result is in agreement or contrast to the previously reported. Regarding increment periodicity, Ré & Gonçalves (1993) by using a marginal increment index (Tanaka et al., 1981) confirmed a daily pattern in the otoliths of North Sea sprat. Furthermore, by using a similar method, Simonsen (1996) estimated the age of Baltic sprat larvae and did confirm a daily pattern in the increment formation of Baltic sprat's otoliths. The results showed a great difference in the onset of ring deposition which occurred between 21:00 – 02:00 (dark period) for the North Sea and between 09:30 – 11:00 (light period) for the Baltic Sea sprat. In addition, the time range was much larger for initial deposition in the North Sea sprat. The explanation for such differences is unknown. This lead to the suggestion that when using such an index care should be taken in particular for small larvae, which are normally characterised by relatively constant increment widths during the first two weeks period. The marginal increment method would probably be more applicable for larger individuals.

In case that absolute age can not be determined from the whole age range, Campana (2001) recommended to: (1) Determine the age of first increment formation, and (2) Verify increment periodicity across the entire age range of interest. Regarding the latter, Peck et al. (2004) have been able to confirm a daily pattern in the otolith on starved and well-fed juvenile Baltic sprat after alizarin marking. However, exact determination on when the first increment is formed has not been resolved. Therefore it may be concluded that increment-based ageing on the Baltic sprat larvae is not well-defined yet. The present study used the ageing technique based on North Sea sprat (Alshuth, 1988). The accuracy can not be defined until validation of daily increments has been determined. A well-prepared experiment was actually intended to be

done in March-April 2003, however, ichthyoplankton surveys as well as fertilisation experiments on board were unsuccessful to obtain a sufficient number of sprat eggs.

Nucleic acids determination on first feeding larvae

The laboratory protocol for nucleic acids determination was adopted from a mesocosms experiment for 1 week old larvae applicable for cod and herring (Clemmesen, pers. comm.) using ethidium bromide (EB) as dye (Clemmesen 1993). EB is an intercalating reagent that reacts specifically with base-paired regions of DNA and RNA, therefore it is used for total nucleic acids determination. Although the fluorimetric methods offer several advantages, including the ability to analyse the smallest individual larvae (Caldarone, et al., 2001), however, it was not sensitive enough to determine DNA content in small sprat larvae ≤ 5 mm (approximately between 0.0025-0.0035 mg). Bergeron (1997) pointed out that the sensitivity of nucleic acid analysis is associated with developmental stages, i.e the presence of white muscle in more developed individuals lead to ease the analysis. However, no attempt has been made to solve the sensitivity problem by using other dyes which might have solved the problem (Clemmesen, pers. comm.).

4.2 Discussion of results

4.2.1 Otolith microstructure analysis

Otolith validation

In the absence of exogenous food, the growth rate for body length and otolith radius at age was changing (Fig. 14). At yolk-sac completion the changes in length growth rate abruptly decreased earlier than in the otolith indicating a greater influence of exogenous food on somatic than otolith growth during first feeding period. On the other hand, temperature level was too low to account for changes in otolith size. Barber & Jenkins (2001) reported from their experiment on the juveniles of Sillaginidae that short-term somatic growth was influenced by food, in contrast to short-term otolith growth being regulated by temperature. This was previously reported by Wright et al. (1991) showing that increments responded more conservatively to temperature changes than the resting metabolic rate during an absence of somatic growth did.

The duration of yolk-sac absorption is mainly influenced by temperature which associated with metabolic rate (Kamler, 1992). There has been evidence that first deposition of increment coincide with first feeding in the larval period, therefore it is also known as first feeding check (FFC). As a consequent, the timing of FFC deposition may vary within and

among species. As observed from the experiment in this study, first feeding larvae in the Baltic sprat begin 5-6 posthatch, whereas for similar species in the Irish Sea 5 days and for the North Sea 6 days posthatch have been described (Alshuth, 1988; Shields, 1989). Similar occurrence could also be found in other clupeids such as *Engraulis mordax*, *E. ringens*, and *Sardinops sagax* (all cited by Alshuth, 1988). However, in herring, *Clupea harengus*, the first increment deposition varied inconsistently either before, at time or after yolk sac absorption (Geffen, 1986). The establishment of FFC is of importance in increment-based ageing technique as a powerful tool in marine fish larvae assessment, i.e. recruitment studies.

The appearance of ring-like FFC in the Baltic sprat (Fig. 11), could not be regarded as FFC since the larvae were not fed during the experiment. In addition, larvae were exposed to lower temperature so that the formation of increments might have been impeded or disrupted. This is very much likely since there has been a considerable evidence for the influence of temperature and photoperiod combined with feeding conditions on increment deposition in otoliths in various marine species (Tanaka et al., 1981; Neilson & Geen, 1982; Geffen, 1983; Dale, 1984; Mugiya, 1987; Wright et al., 1991; Xie et al., 1999). A plausible explanation for the occurrence of increments during starvation is probably associated with the circadian rhythms, and that environmental factors like temperature and food play a role as reinforce factors (Shields, 1989). It is partly supported by evidence found by Campana & Neilson (1985) on juvenile starry flounder preconditioned to a natural environmental regime showing that the production of daily increments in this species was unaffected by photoperiod or temperature fluctuation, suggesting the presence of an internal circadian rhythm.

As a result from starvation and low temperature, the increment widths were mostly less than 1 μm per day. In other clupeids on mesocosms-reared herring, *Clupea harengus*, and laboratory-reared European anchovy, *Engraulis encrasiolus* there were indication of relatively constant widths $\sim 1 \mu\text{m d}^{-1}$ for the first 2-3 weeks (Folkvord et al., 1997; Høie, 1997; Cermeño et al., 2003).

Otolith microstructure of wild-caught sprat larvae

The otoliths of wild-caught sprat larvae demonstrated a variability in increment clarity and size at first increment over different sampling periods. As mentioned before, the increase in temperature along the progressing season in spring lead to higher development, better visibility in viewing, and broader increment widths in the otoliths of larvae collected in May (e.g. Fig. 16). Yet, the FFC was unaffected by such variation, i.e. the FFC size was independent of temperature differences within the spawning season (Table 5). This is

probably caused by a circadian rhythms in otolith ring development. Simonsen (1996) who did ageing for the first time on larval sprat in the Baltic, unfortunately, did not mention the size of the core or the FFC. The FFC of Baltic sprat larvae was $9.76 \pm 0.67 \mu\text{m}$ ($n=707$). This is smaller compared to the North Sea sprat with $10.88 \pm 0.76 \mu\text{m}$ ($n=469$) reported by Ré & Gonçalves (1993). The discrepancies in size of FFC from similar species with different location is probably due to the species-specific factor in otolith development which is strongly related to environmental characteristics of the two seas.

When FFC was available, there was an indication that larvae were larger in size at FFC (Table 5) and characterised by a broader increments in May than in April. A constant FFC lead to a possibility in assessing age and somatic growth variability in wild-caught sprat larvae using otolith microstructure analysis. With respect to otolith radius, there was a tendency that larvae sized 4.5 – 8 mm had a larger otolith radius in April (Fig. 17), despite in total size range compared was larger in May survey (Fig. 18). The discrepancies can be explained by temperature influence. As mentioned above, not only did the increase in temperature cause relatively broader increments but also enhance somatic growth rates in May. It is well known that metabolic rate depends on temperature, meaning that larvae at the same food level exposed to higher temperatures grow faster than those having experienced lower temperatures.

Growth proxy of sprat larvae based on otolith microstructure

Based on relationship between length and age (corrected from increments number), the somatic growth of sprat larvae were significantly higher in May than in April (0.61 compared to 0.57 mm d^{-1}) during spawning season 2001. The differences were mostly derived from smaller sized larvae (Fig. 19). Beside temperature, food availability may contribute to this phenomenon although it is well known that spawning season of sprat coincides with the plankton production in the Baltic (Grauman & Yula, 1989; Kalejs & Ojaveer, 1989; Kornilovs et al., 2001; Möllmann, 2001) suggesting no food limitation. However, most recently Dickmann et al. (2003) revealed different features of plankton production in the Baltic Sea. Based on stomach content analysis, they found that sprat larvae of 4-6 mm in size fed largely on microplankton, whereas larvae of 6-14 mm in size consumed Nauplii and C I-III with concomitant increase of copepod eggs in their diet as size increased. Although no plankton data are available to confirm growth variability in sprat larvae in the current study, it is possible that changes in plankton species composition would affect the growth of sprat larvae. This does not necessarily affect the quantity of the food in the Baltic Sea.

Comparing the growth rate obtained in the present study with previous results from the same region, Simonsen (1996) estimated a growth rate of $\sim 0.55 \text{ mm d}^{-1}$. In comparison to other sprat from different locations, the growth rate of the Baltic sprat was the highest. In the North Sea, Ré & Gonçalves (1993) obtained 0.406 mm d^{-1} , and recently Valenzuela & Vargas (2002) reported between 0.362 to 0.404 mm d^{-1} from various environmental condition during the reproductive peak in the North Sea. In the Irish Sea, Shields (1989) also found that integrated growth rates varied between 0.37 to 0.49 mm d^{-1} . The highest growth rate in the Baltic sprat compared to other location may be linked to species-specific and different environmental factor regardless of a potential bias in ageing technique.

Horizontal variability in growth rate was estimated over 6 locations in the Bornholm Basin during May 2001 (Table 6, Fig. 20). Simonsen (1996) revealed no differences in somatic growth among the north-eastern (frontal area), central-eastern (transition zone) and central basin (outside frontal area) in the Baltic. In the present study, on the contrary, it was shown that larvae transported to the northern part of the basin have experienced a higher growth, compared to the central and the southern regions. The explanation for highest somatic growth in the north may due to a relatively better hydrographic condition as a continuous renewal of water masses bring about oxygenated and saline-water which enhance the plankton production that is essential to feeding for sprat larvae in that region. In the absence of major inflow, a minor exchange between the North Sea and the Baltic Sea occurs continuously which is governed by the easterly and most efficiently the northerly winds. Water transport occurs along the north east and the returning flow is directed towards south east (Krauss & Brügge, 1996). In fact the northern part of the basin has been considered as frontal systems where a positive response of phyto and zooplankton to hydrographics events has been shown, e.g. in salinity/density fronts (Kahru et al., 1984). Furthermore, Raid (1989) confirmed an increase of herring and sprat larvae in temperature fronts. In this case, horizontal variability in somatic growth of sprat larvae did probably occur due to differences in food availability which are driven by hydrographic conditions. At relatively similar temperature levels, food limitation has shown to be determinant factor in larval growth in the field (Karakiri et al., 1989). However, in the North Sea it was reported that variation in oceanographical condition did not cause variation in somatic growth of sprat larvae (Ré & Gonçalves 1993; Valenzuela & Vargas, 2002).

Within the vertical environment, higher growth was encountered at the depth between 5-30 m d (Fig. 23). The explanation for this phenomenon possible is higher temperature and food availability.

With respect to the distance between successive rings, both horizontal and vertical profiles show relatively constant increment widths. Increments were wider in May/June than in April. A relatively constant otolith growth during earlier stages was also found in other clupeids, for example in herring, *Clupea harengus* (Clemmesen, pers. comm., Folkvord et al., 1997), and European anchovy, *Engraulis encrasicolus* (Cermeño et al., 2003). Higher variability in increment widths were shown from smaller larvae both temporally and spatially.

Length-weight relationship as growth proxy

Intraseasonal growth based on length-weight relationship confirmed a higher weight at length from smaller larvae (4.5 – 8 mm) collected in April (Fig. 26). An explanation for this phenomenon may come from the studies conducted by several author in the same region during 2002 (Boersma et al., 2004; Dickmann et al., 2004; van Beusekom et al., 2004). They found that the concentration of microzooplankton as well as chlorophyll were higher in April compared to May. Furthermore, Dickmann et al. (2004) confirmed from stomach analysis that microzooplankton was the main diet composition of smaller larvae. Another support was that of abundance of *Acartia* spp. which is the main food for sprat larvae. It was found that the *Acartia* nauplii showed the highest abundance in April (Voss, personal comm.).

Based on length-weight relationship it was shown that the slope value was <3 (negative allometric) in April and >3 (allometric positive) in May. Fuimann (2002) stated that allometric growth in fish larvae is caused by inproportionality in development of caudal fin and locomotive musculature. Larvae with better growth would show better appearance in pursuing their prey and to avoid the predators (Houde, 1989; Valenzuela et al., 1991). In relation to weight at length, it might be suggested that smaller larvae in April might initially have a better survival, however, larger larvae collected in May might survive better. This is in accordance with previous investigations that size and temperature is the determinant factor for survival (Pepin, 1991; Heath, 1992).

The deeper the water the lower the weight at length of sprat larvae, confirming vertical variability in growth (Fig. 30). It was shown that the increase in dry weight was slightly higher in the most upper compared to other two lower layers. Habitat superiority of surface layer is influenced by higher temperature, food availability, more oxygenated water, better light intensity as well as higher primary production earlier mentioned. Despite an existence growth discrepancies among depth groups, it is suggested that larger larvae caught in deeper water column might have been migrated from the upper part (see Fig. 10). This is likely since larvae sized ~15 – 16 mm were also found at the edge of upper limit distribution although they were

fewer in number (Fig. 31). With more developed swimming apparatus than smaller individuals, larger larvae are able to move up and down in water column, therefore, they could be found in all depth categories mentioned. With respect to the influence of photoperiod on vertical migration, Last (1980) and Dänhardt (2003) found that the North Sea sprat showed low feeding activity and less movement by which larvae sink to deeper layer during dark. Based on the facts that sprat larvae are phototaxis-positive organisms, therefore, it is unsafe to conclude a higher/lower growth rate for larger larvae according to different depth.

4.2.2 RNA/DNA ratio

Nutritional condition of sprat larvae

RNA/DNA is a useful indicator to determine the nutritional condition in fish larvae. The evidence was derived largely based on laboratory experiments in particular at discriminating the condition between fed and starved larvae (Clemmesen, 1994; Blom et al., 1997; Sato et al., 1998; Fukuda et al., 2001; Johnson et al., 2002; Caldarone et al., 2003; Wongtschowski et al., 2003). From field investigation, to the lesser extent, a close relationship between nutritional condition and to hydrographic condition, food abundance, and primary production has been shown (Shimizu et al., 1989; Rooker et al., 1997; Chícharo et al., 1998; Chiu & Huang, 1998; Fukuda et al., 2001; Ramírez et al., 2001). In the present study, the nutritional condition of the Baltic sprat larvae was assessed both temporally by contrasting the condition between larvae collected during April and May 2001 and vertically from a permanent station in the central Bornholm Basin performed during the latter sampling. The fraction of larvae having RNA/DNA ratio ≤ 1 was approximately 6% and 1% of the total sample from the first and second sampling respectively. Within the vertical column larvae with RNA/DNA ratios of about 1 was less than 2% of total number found at depth between 60-80 m. According to Clemmesen (1994) RNA/DNA ratio approximated 1 is considered to be the critical point for fish larvae regardless of species. Based on these facts, sprat larvae were largely in good condition during spawning season 2001.

The RNA/DNA ratio of larvae collected in May tended to be better than in April. Ferron & Legget (1994) stated that among sources of environmental variability shown to influence condition in fish is temperature. In the Bornholm Basin, the average temperature was higher in May (6.21°C) compared to April (4.32°C), therefore with the approximately 2°C differences can be accounted for better condition in the second survey. Previously, Buckley (1982) found a linear relationship between the ratio and growth rates observed at 5, 7, and 10°C on larval winter flounder. By adding temperature as second independent variable

(Buckley et al., 1984) improved the relationship, from which Buckley (1984) concluded that growth is temperature and food dependent. Bergeron (1997) pointed out that temperature affects the activity of RNA i.e. RNA concentration increased with higher growth rate for all acclimation temperatures. However, for a given growth rate Goolish (1984) *in* Clemmesen (1996) found higher tissue RNA concentration at lower temperature postulating a compensatory mechanisms for lower RNA activity. Furthermore, Høie et al. (1999) suggested that fluctuation in RNA and DNA is closely related to environmental and genetic factors. The influence of temperature and prey availability was also reported from field investigation (e.g. Esteves et al., 2000). One might try to identify which factor is predominant between temperature and food for larvae condition. To the latter McGurk et al., (1992) performed a laboratory experiment with sand lance and herring larvae. They found that the nutritional status of first feeding larvae was not driven solely by prey concentration and temperature, but by an interaction between prey concentration and temperature and the ability of larvae to feed effectively. For the Baltic sprat observed in the current study it is difficult to determine whether each factor plays a more important role than other or combination of all factors.

Variation in nutritional condition was also found within vertical water column in this study where better ratios occurred mostly in the upper layer. By grouping water column into depths 05-30 m and 35-80 m, it was shown that smaller larvae up to 12 mm demonstrated variation in nutritional condition, whereas larger individuals showed relatively constant RNA/DNA ratios in both water columns. Moreover, variation in RNA/DNA ratio within each size class plotted against depth confirmed that the nutritional condition of smaller larvae in the upper layer was better although the correlation coefficient were largely weak. The r^2 values were decreasing with size. The explanation accounted for this discrepancies is similar to aforementioned above. With respect to environmental variable, the average temperature at the upper, intermediate, and bottom layers were 7.85, 4.57, and 6,37°C in average (see also Fig. 7), respectively. Vertical variability in condition of sprat larvae in the Bornholm Basin is in agreement with Dänhardt (2003) who reported that overall sprat larvae of 0-10 m depth was significantly better in nutritional condition compare to those caught at depth between 65-75 m investigated from the same area in July 2000.

In April, most of larvae consisted of first feeding individuals (~3.5-6.0 mm) which is in transitional phase of development and physiological change such as mouth opening and esophagus, development of liver, gill bladder and pancreas (all cited in Clemmesen, 1994). Whereas, in May larvae were mostly dominated by length between 8-15 mm of which a

relatively stable condition over increasing size could be observed (Fig. 33). It is suggested that in particular during pre-metamorphosis high variation in nutritional condition in fish larvae would be expected. A stable RNA/DNA ratio characteristics for well nourished larvae independent of length may only be reached when a metabolic balance is achieved (Clemmesen, 1994).

The variability of the RNA/DNA ratio must be ascribed to that of larval RNA content (Clemmesen, 1988; Bergeron, 1997). Whereas, the evidence so far showed a much greater stability in DNA content. Alternatively, Bergeron (1991) in Bergeron (1997) proposed another ratio likely to detect starvation in fish larval by means of the relative DNA content (DNA/dry weight). Regarding this matter, sprat larvae showed differences in DNA/DW at size both horizontally and vertically (Fig. 35 & 44). With an assumption that DNA was at a constant level, larvae with higher dry weight are considered to have larger cell size, therefore, they are considered better in nutritional condition. Therefore, larvae with better condition were found in May than April as well larvae from the upper than the lower layer. With increasing size the rate of DNA/DW decreased slower as larvae become larger. Within the vertical environment, interestingly, the deeper the water the higher the DNA/DW ratio which might be interpreted that in constant DNA and at similar size, larvae in the deeper water were less in weight (hypotrophy) compare to those in the upper water column. By linking to environmental variables with hypotrophy it may be accounted for lower temperature and less food availability and accordingly slower growth rates experienced by larvae in the deeper water.

The existence of diel variation in RNA/DNA ratio in response to different photoperiod was reported on wild-caught *Sardinus pilchardicus* (Chícharo et al., 1998). They reported that larvae collected during the night showed higher RNA/DNA ratio compare to larvae sampled during the day suggesting endogeneous rhythm in the production of RNA. Furthermore, from laboratory-reared *Paralichthys olivaceus* Gwak (2002) found that different period of sampling caused variation in RNA/DNA ratio from which sampling design was suggested to be distinguished between dark and light time. However, in the present study no diel variation in the ratio was found both horizontally and vertically. (Fig. 34, 42, 43). Based on laboratory experiment, the sensitivity of RNA/DNA ratio, accordingly the changes in the ratio, depending on temperature and food ration, would takes about 2-3 days (Clemmesen, 1993; Canino, 1994). In this case, Clemmesen (1996) pointed out that generally the ribosome are able to react to changes in the nutritional condition over a range of hours by decreasing or

increasing their activity. But a reduction in the ribosome number and therefore RNA content only occurs after longer starvation periods over a range of days.

Instantaneous protein growth rates (G_{pi})

The growth patterns of Baltic sprat are not well described. Based on RNA/DNA ratios, the growth of sprat larvae was estimated horizontally from different sampling periods in April-May and vertically in the second survey. There were indications that nutritional condition and growth rates were poorer in the early spawning season (April), when low temperature may have hindered fast growth. Furthermore there was evidence that larvae's vertical distribution was influencing the growth rate. High growth rate was only observed from the upper layer. Three explanation accounts for this phenomenon. First, lower temperature in the deeper water reduces metabolisms and growth. Next, the food availability was found to be higher in the upper layer especially that of microzooplankton as primary food for premetamorphosis stage (Dickmann et al., 2003). Finally, the low oxygen content in the deeper layer may influence the swimming ability of and therefore prey capture success. This was reported from the Baltic cod (Nissling, 1994).

Total mortality experienced by a cohort of fish is a function of the daily instantaneous mortality rate and the length of the time interval this mortality operates (Houde, 1987). Transition from one stage to the next, which often is a transition from higher to lower mortality, is determined more by size than by age (Copp & Kovác, 1996). Size is an important factor in the prey-predator interactions, and affects both the larval efficiency as predator, and its vulnerability towards other predation (Houde, 1987). In the Baltic, sprat larvae is estimated to metamorphose at age approximately 30 d (Baumann, et al., 2003). Assuming 3.5 mm hatch size and average somatic growth $0.4 \text{ mm}\cdot\text{d}^{-1}$, larvae reach metamorphosis at $\sim 15 \text{ mm}$ (estimation from length at age based otolith increments is $\sim 20 \text{ mm}$ which is considered to be overestimated). It may be concluded that different growth rates will cause variation in the duration to reach metamorphosis, consequently, the longer the duration the more vulnerable to predation and the weaker to pursue the prey. Therefore, the larvae with high instantaneous growth rate (G_{pi}), probably are larvae with good chances of survival.

4.2.3 Joint analysis

By contrasting between fed and unfed laboratory-reared cod larvae, Clemmesen & Doan (1996) detected a coupled response in RNA/DNA ratio and otolith microstructures to feeding condition, therefore, they concluded that such a coupling analysis make possible to

determine whether the larva's condition is improving or deteriorating. Furthermore, Clemmesen (1996) performed similar analysis on wild-caught *Engraulis anchovieta* of which she revealed a positive relationship between the sum of several last increments with RNA/DNA. However the coefficient of correlation (r^2) values ranged between 0.16-0.27 which was relatively weak. The highest was obtained from the relationship between the sum of the last two increments at nutritional condition. Most recently, Morales-Nin et al. (2002) performed a similar study on the larvae of three species of Antarctic fish. They found a correlation between the two but not significant. The current study presents a similar analysis from wild-caught Baltic sprat larvae comparing between two water column. There were indications that sprat larvae in the upper layer were higher in dry weight at age compare to those in the deeper water (Fig. 46). A correlation between the sum of the last two increments and RNA/DNA ratio showed wider increments were found on larvae from the surface layer indicating a better somatic growth. In relation to nutritional condition, however, the higher the ratio there was a tendency of relatively constant increment widths. The explanation could be the existence of a delay response from otolith development (Fig. 51). The results confirmed the relative constancy in DNA regardless from the water column, whereas RNA showed a higher variation at depth 5-30 than that of 35-80 m (Fig. 50). Moreover, despite no variation could be detected in the lower layer, it is apparently that higher RNA/DNA ratios and wider increments were found in the top layer. A plausible explanation for the superiority of upper layer is mentioned previously.

The joint study of otolith microstructure and nucleic acids in the same larvae can be a very useful tool to study long-term growth rates and recent growth in fish larvae in relation with environmental variables. However great care must be taken when joint studies are made, because otolith extraction can lead to a loss of larval tissue and therefore loss in nucleic acid contents (PARS, 2001). In the present study, RNA/DNA ratios were unaffected by otolith dissection in all but size class 8 mm from top layer and 14 mm from deeper layer. However, this may be associated with very few sample ($n=2$ each). Therefore, it may be concluded that joint study of otolith microstructures and nutritional condition on sprat could be conducted even with early larvae .

4.2.4 Coupled hydrodynamic modeling and otolith microstructure analysis

The use of otolith microstructures data coupled with hydrodynamic model has been able to determine spawning areas and larval advection pathways, e.g. in sillaginids (Fowler et al, 2000; Jenkins et al., 2000) and clupeids (Allain et al., 2001). It is believed that the increment widths

is an expression of growth rate in fish larvae (e.g. Campana & Neilson, 1987), therefore, a combination with circulation model would also be able to investigate, for example, the growth trajectory of larvae.

Recently, a simulation which combining 3D hydrodynamic Baltic Sea model and biological information consisting of feeding environment and somatic growth estimated by otolith microstructure has been able to simulate the growth probability and survival of Baltic cod larvae. The results revealed that retention and dispersal from the main spawning ground is the key process influencing larval survival which is associated to the presence or absence of the copepod *Pseudocalanus elongatus* as a main food for this species (Hinrichsen et al., 2002). Application of such a biophysical model for sprat larvae is promising because sprat has the same spawning ground with cod in the Bornholm Basin (STORE, 1999). It is also supported by a well-documented hydrographic condition in the Baltic Sea from which a highly accurate model has been developed and verified (Lehmann, 1995; Hinrichsen et al., 1997; Voss et al., 1999).

In the absence of the growth formula required by the model, alternatively, sprat larval growth has been estimated based on normalisation of the total increment widths with temperature during spring and early summer 2001. In this case the short-term somatic growth has been considered to be influenced predominantly by temperature. The combination between “reverse” hydrodynamic modelling and otolith microstructure presented in Fig. 55 shows a slightly higher gain in otolith growth per 1°C of sprat larvae found in April compared to May. This was due to the mean differences in increment widths between both months were relatively small (0.9 and 1.1 µm), whereas the temperature differences during larval trajectory was much higher 0.3 °C (13 d) and 4 °C (18 d) in April and May, respectively.

The results presented may be influenced by the unresolved vertical distribution of sprat larvae. The model assumes that larval distribution is 10 m (Voss, 2001), whereas the otolith data used in this analysis were derived from bongo sample which were collected from various depth. Estimated from percentiles analyses between age and length (increment-based radius) in the vertical sampling, it was shown that larvae from the deeper layer tend to be lower in growth rate compare to the those in the upper part. Therefore, it is crucial to resolve the vertical distribution pattern of sprat larvae in the future corresponding to their environmental condition.

In the future, application of the coupled model should be based on more biological data. Temperature alone may not be enough to estimate growth probability of sprat larvae. In this case, depending on the goal, the studies which deal with the interaction processes

between an individual and its environment requires more biological information in addition to otolith microstructures (e.g. Hinckley, 2001; Dower et al., 2002; Hinrichsen et al., 2002).

Validation on growth of sprat larvae revealed a relatively high agreement between the model and mean distribution of RNA/DNA over the Bornholm Basin. Both illustrations show that the areas where higher growth coincides in the coastal area. This is in agreement with Voss et al. (2003) who concluded that the coastal area in the Bornholm Basin is the nursery ground for sprat larvae in the following spawning season. Despite using a relatively complex and different model simulation and different species (the Baltic cod), Hinrichsen et al. (2002) confirmed a relatively similar conditions as they found a higher survival probability for cod in the coastal region, in particular in the north off Bornholm Island, followed by the north-eastern and to a lesser extent in the south-eastern basin.

Chapter 5. General discussion

5.1 Growth and nutritional condition: importance for larval survival

Variability in fish recruitment has been attributed to either variations in the size of reproductive stocks or changes in the environment (Planque & Frédou, 1999). This implies that the relationship between spawner abundance (any of the following metrics of the size of the spawning stock: spawning stock biomass, the number of spawners, the number of eggs, or some index of spawner abundance) and subsequent recruitment is one of the fundamental issue in fishery management (Cushing, 1971; Myers & Barrowman, 1996). However, many researchers believe that there is no relevant relationship between species abundance and recruitment (reviewed by Wooster & Bailey, 1989; Koslow et al., 1987). In fact, Myers & Barrowman (1996) recently confirmed that recruitment fluctuation depends on spawner abundance. By analysing 364 spawner-recruitment time series data (derived from virtual population analysis (VPA) and cohort analysis which taken into account data series of minimum 5 years), they determined the highest and the lowest recruitment ($S_{R_{max}}$ and $S_{R_{min}}$, respectively) from which they calculated the relative rank (r) in order to compare ranks across population. The r values were then plotted against the $S_{R_{max}}/S_{R_{min}}$ ratio. From their compilation they found that maximum recruitment (R) occurs when spawner abundance is at its highest level and *vice versa* as well as that the greater recruitment occurs as spawner abundance above the median. Importantly, from their calculation they were able to convince that the relationship between the two was completely determined by the environment instead of either autocorrelation presents in spawner-recruitment recruitment series or as the product artifact of a necessary relationship between recruitment and subsequent spawner abundances.

An examination on spawner-recruitment relationship of the Baltic sprat has been conducted by Köster et al. (2003) based on multispecies population virtual analysis (MSVPA) for the eastern Baltic Sea (Sub-division 26 and 28) during 1977-1996. They sequentially analysed the relationship between one stage to another from eggs, early larvae, late larvae, and 0-group of this species that constitute the year class strength. The investigators found a positive relationship in any two successive stages but between larval abundance and year class strength. The investigators concluded that the period between the late larval and early juvenile stage appeared to be critical for sprat recruitment. It was suggested that ambient temperature and wind stress would be potential variable to be accounted for this phenomenon. In the present study, it was demonstrated that variation in temperature due to different sampling period and different water column was reflected in different growth and condition of sprat

larvae inferred from otolith microstructure analysis and RNA/DNA ratio. It was also shown that larvae encountered outside the central basin or those found in the coastal regions exhibited relatively higher growth rates as well as nutritional condition suggesting the influence of wind-induced larval transport. Although, the evidence reported in the present study derive from pre-metamorphosis individuals, the method may also be applied for other stages of larval sprat in an attempt elucidating the environmental impact on recruitment variability of this species. The analysis would be better to be incorporated with a well managed sampling period, i.e sampling period is intended to cover until juvenile stage for the whole spawning period and so that possible to estimate the abundance of egg, earla larvae, late larvae and juvenile stages, successively.

Utilising otolith microstructures data resulting from a well-planned field sampling is of importance in order to produce a powerful ageing data set used to observe, e.g. seasonal variation of the 0-group population. Methot (1983) reported that information from otoliths could be used as survival index on *Engraulis mordax*. He calculated a relative survivorship for this species based on the ratio of the fraction larval's birth date to the fraction of annual larvae production per 30 d during 1978-1979. Based on these facts, one could see that seasonal fluctuation in number of juveniles of this species depends on larval abundance. Furthermore, it could also be estimated at which time from a certain spawning season considered which affect considerably to recruitment variability. This was possible to be done by comparing the abundance data of first feeding larvae (max 5mm) with later results from a juvenile survey within the corresponding year, i.e all individuals being analysed were from same 0-group. In this case the author was in favor to Cushing's hypothesis that the age distribution of juveniles –the survivors of the larval stage- is a function of the seasonal distribution of spawning and seasonal changes in larval survival. Unfortunately he did not estimate the growth of the larvae using increment widths, therefore the analysis could not be extended to determine whether fluctuation in numbers of larvae and juveniles was governed by larval growth in association with environmental factors. In another study it was reported an evidence that increment widths could be used as a measure of survival. Based on mesocosms experiments on herring Folkvord et al. (1997) proved that survivors derived from well-fed individuals collected at the end of the experiment (60 d) demonstrated wider increments compared to individuals that were collected within two week intervals.

Pepin et al. (1999) hypothesised that survival probability (or fitness) is persistent, i.e. if it varies less during the larval life of one individual than it does among individuals, then in a population of larvae that starts with a distribution of fitnesses, individual with high fitness will

form an ever larger fraction as the time goes by. This implies an initial high variation in growth and condition distribution will diminish with size or age. All larvae initially have relatively good growth and condition (fuelled by their yolk-sac), but those that find themselves ill-matched to their environment suffer a decrease in condition as they start to feed. Therefore weaker individuals are less likely to survive from one stage to another. Based on 6 species observed, the authors showed that the distribution of RNA/DNA ratios of fish larvae contracts towards its upper tail as size (estimated with DNA) increases, i.e. at the end of size range interest only those from upper distribution limit will survive. In this case they suggest that high mortality during larval period is highly selective mortality. The intensity of removal individuals from a population is directly related to estimated mortality rates. In the current investigation it is not possible to estimate survival in a quantitative way as the larvae available were that of pre-metamorphosis individuals and sampling did not cover the whole time of spawning season from March/April to July/August.

Differences in growth and condition has a major impact on recruitment (Houde, 1989). Based on temporal variability in growth rate, larvae showed a higher growth rate and better condition found in the latter spawning season. Likewise, larvae from the upper water column demonstrated superiority in growth rates to those found in the deeper layer. The superiority in growth and condition will increase larval survival which will probably significantly contribute to the year class strength.

5.2 Temperature and wind effects on sprat recruitment in the Baltic Sea

Schnack et al. (2002) pointed out that the recruitment of the Baltic sprat is more dependent on the availability of suitable zooplankton prey, which is strongly related to spring temperature. Variable prey concentrations may affect nutritional conditions, growth rates and subsequently survival of larvae. Concurrently temperature determine the growth rate of fish larvae through which the metabolism rate is being regulated. The larvae that encounter temperature condition which are close to its optimum value for growth and development will be characterised by a higher growth rates and a shorter larval stage to achieved a certain growth stanza.

Based on a differentiation from logistic formulation, temperature mediation in fish larval growth (r) is formulated by Bartsch (2002) as follows:

$$r = r_{opt} - d(T_{opt} - T)^2$$

This is to show that the realised-growth rate is a function of temperature condition as r_{opt} and T_{opt} determined, whereas d is a constant value. Accordingly information on growth as well as

temperature of optimum level is of importance. The use of this formula does not necessarily change the maximum attainable size S for growth stanza S_{∞} . This is in support to the role of temperature on growth and condition of sprat larvae mentioned above.

However, not only a temporal match between larvae and suitable prey, but also transport to favourable nursery areas may be of importance for larval and early juvenile survival. Hydrodynamic modelling of flow fields within the Bornholm Basin has identified two contrasting scenarios. Firstly, low wind speed in variable direction results in a retention within the spawning area and secondly, relatively high wind forcing of westerly or easterly direction results in rapid transport towards different shallow coastal environments offering improved feeding condition (Schnack et al., 2002; STORE 2001). In the absence of major inflow occasion, the easterly and northerly winds regulate the minor water exchange between the North Sea and the Baltic (Krauss & Brügge, 1996), therefore, by incorporating this phenomenon with wind-regulated transport of sprat larvae may cause a variability in survival characteristic encountered by the larvae. In case of a major inflow, favourable conditions for the reproductive success of the Baltic cod are created (e.g. Plikhs et al., 1993). Since cod is the principal predator to sprat, changes in hydrographic condition following the major inflow will affect to the recruitment level of this species.

5.3 Future direction

Clemmesen et al. (2004) suggested that the future work for sprat larval studies in the Baltic is to quantify the age structure of the sprat larvae so that cohorts can be tracked and patterns analysed based on larval age. Therefore, in the present study it would have been more appropriate to cover the whole spawning period and early life stages in spawning season 2001. The changes in growth and condition along with the fluctuation of environmental conditions would resolve the causes of the changes in abundance. Sampling designed must be improved in order to make it possible to estimate the number of earlier stage which are loss from the population based on the survey on the latter stage, i.e. by estimating the instantaneous mortality rate of each stage.

Based on the results, it is shown that coupled analysis of otolith microstructure analysis and RNA/DNA ratio had no effect on larval condition. This gives rise to assume that the observed changes in growth and condition with age in relation to environmental conditions are reliable. The joint analysis is appropriate to be applied consistently in the attempt to elucidate the interaction processes between the larvae and surrounding environment. Additionally, a coupled IBM models based on hydrodynamic and biological

models is of great importance since growth variability as well as environmental condition could be simulated at a time. In the future, it might be possible to conduct such simulations for a vertical environment with a prerequisite an established data from vertical distribution of sprat larvae. More information is required for a successful simulation in particular with regard to biological information such as the minimum and maximum widths in each increment width which express the growth range at a certain time, the length and weight at age, and the feeding habits.

Regarding with otolith microstructure analysis, the validation on the daily nature of increment formation is mandatory to obtain reliable estimates in larval ageing.

Chapter 6. References

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Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertation selbständig angefertigt habe und dabei als Hilfsmittel nur die genannten Quellen benutzt habe. Des weiteren versichere ich, dass die vorliegende Dissertation weder ganz, noch zum Teil bei einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt wurde.

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