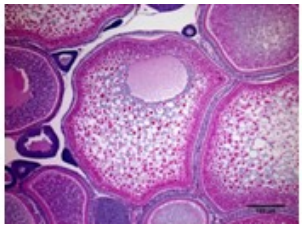
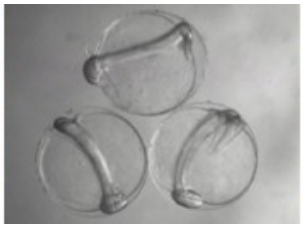
Reproductive ecology of Baltic sprat and its application in stock assessment

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Zusammenfassung

Die Europäische Sprotte (*Sprattus sprattus* L.) ist ein kleiner pelagischer Schwarmfisch, der im Schelfgebiet des Nordostatlantiks weit verbreitet ist. Eine besondere Stellung nimmt diese Art im pelagischen Ökosystem der Ostsee ein, da sie einerseits dem Ostseedorsch als Hauptnahrung dient und andererseits als abundanteste planktivore Fischart in diesem System einen erheblichen Prädationsdruck auf Zooplankton und Ichthyoplankton ausüben kann. In der vorliegenden Arbeit wurden einige wichtige Reproduktionsmerkmale der Sprotte untersucht, die nicht nur für die Bestandskunde, sondern auch für zukünftige Studien zur Populationsdynamik und Ökologie dieser Art wichtig sind. Bei der Sprotte handelt es sich um einen Portionslaicher, der mehrere Eiportionen über eine längere Laichsaison abgibt. Dabei ist der Gesamtumfang der saisonalen Eiproduktion nicht vor der Laichzeit determiniert. Aus einem Reservepool werden ständig neue Oozyten rekrutiert, die heranreifen und schließlich abgelaicht werden. Aus dieser Eigenschaft heraus ergeben sich einige Besonderheiten in Bezug auf Untersuchungen zur Reproduktionsbiologie dieser Art. In der vorliegenden Arbeit konnte sowohl die beobachtete jährliche als auch die saisonale Variabilität in den untersuchten Reproduktionsmerkmalen mit der vorherrschenden Hydrographie und der Größe des Laicherbestandes in Zusammenhang gebracht werden. Die Fruchtbarkeit, d.h. die Anzahl abgegebener Eier pro Laichportion, zeigte sowohl Schwankungen zwischen den untersuchten Jahren als auch zwischen verschiedenen Gebieten in der Ostsee, und ein großer Teil dieser Variabilität konnte mit der vorangegangenen Wintertemperatur und der Gesamtgröße des Laicherbestandes erklärt werden. Das Geschlechterverhältnis und der Anteil an reifen Individuen im Gesamtbestand wurden für verschiedene Gebiete in der südlichen und zentralen Ostsee anhand von logistischen Modellen berechnet. Der Anteil an kleinen Sprotten, die bereits zum Laichgeschehen in einer Laichsaison beitragen, zeigte ebenfalls einen Zusammenhang mit der Wintertemperatur und der Bestandsgröße. Saisonale Schwankungen in der Fruchtbarkeit, dem Anteil laichender Weibchen am Laicherbestand, der Follikelatresie im Ovar sowie dem Eidurchmesser und Eitrockengewicht wurden untersucht. Eine weitere wichtige Beobachtung konnte im Zusammenhang mit der Ovarentwicklung und der Follikelzersetzung nach der Ovulation (postovulatory follicles, POFs) gemacht werden. Zum ersten Mal werden in der vorliegenden Studie histologische Details von POFs im Ovar der Sprotte dargestellt. Dabei konnte gezeigt werden, dass die komplette Zersetzung dieser Strukturen nach der Ovulation etwa solange dauert wie das Intervall zwischen zwei Laichzeitpunkten. Dies sind wichtige Ergebnisse für zukünftige Studien, um die Laichfrequenz der Sprotte abschätzen zu können. Desweiteren wurde anhand experimenteller Daten ein Modell zur Sprotteientwicklung in Abhängigkeit der umgebenden Wassertemperatur entwickelt. Alle Ergebnisse wurden abschließend dazu benutzt, um die Sprottbestandsgröße im Bornholm Becken mittels einer Eiproduktionsmethode, der „Daily Egg Production Method (DEPM)“, abzuschätzen. Die Ergebnisse zeigen, dass dies ein vielversprechender Ansatz zur Bestandsabschätzung dieser Art ist. Die Vorteile dieser Methode liegen in der Unabhängigkeit von Fischereidaten und darin, dass alle wichtigen Eingangsdaten direkt aus Feldbeobachtungen gewonnen werden können. Außerdem bezieht diese Methode die natürliche Variabilität der Reproduktionsmerkmale mit ein, was im Standardassessment nicht der Fall ist. Allerdings wurden in Bezug auf die Laichfrequenz und die tägliche Eiproduktion, zwei essentielle Eingangsparameter der DEPM, verbliebene Unsicherheiten aufgezeigt, die in zukünftigen Studien beseitigt werden sollten. Die in der vorliegenden Arbeit gesammelten Erkenntnisse und Daten können eine Grundlage für weitere Studien zur Verbesserung der Bestandsabschätzung sein und dazu dienen, alternative Indizes für die Bewertung des Reproduktionspotentials des Sprottbestandes in der Ostsee zu entwickeln (z.B. Laicherbestand der Weibchen, oder gesamte Eiproduktion des Bestandes einer Laichsaison), die DEPM weiterzuentwickeln und zu implementieren, und die Populationsdynamik und Ökologie dieser Art weiter zu erforschen.

Summary

The European sprat (*Sprattus sprattus* L.) is a small planktivorous marine pelagic clupeoid species with a wide distribution in shelf areas of the Northeast Atlantic. Sprat is an ecological key species in the Baltic pelagic ecosystem. On the one hand, sprat serves as main prey for the Baltic cod stock and on the other hand, it is the most abundant planktivorous fish species in the Baltic Sea. Thus, sprat has the potential to exert predation pressure on both ichthyoplankton and zooplankton. Sprat is an indeterminate batch spawner, releasing several egg batches over a protracted spawning season. Oocytes recruit from a reserve pool throughout the spawning season. Due to this feature, some peculiarities challenge the investigation of the reproductive biology of this species. In the present study, a number of reproductive traits of Baltic sprat were investigated, all of which are essential with respect to the assessment and further studies of the population dynamics of this species of the Baltic Sea. Interannual, seasonal and spatial variability in the investigated reproductive traits of Baltic sprat was revealed and could partly be attributed to hydrographic conditions and sprat stock size. Absolute and relative batch fecundity was found to differ among areas and between years in the southern-central Baltic. The seasonality of some important spawning traits, *i.e.* batch fecundity, spawning fraction, atresia, oocyte dry weight and oocyte diameter were analysed combining histology and modern image analysis methods. Models of sex ratio and maturity at length were established for different areas in the Baltic. The proportion of small sprat contributing to spawning, and thus forming a part of the spawning stock, was found to be related to ambient winter temperatures and spawning stock size in the Bornholm Basin. In the present investigation histological details of sprat ovary development and postovulatory follicles are presented for the first time. Important results on the ovarian dynamics in relation to postovulatory follicles (POF) were described with the major finding that the degeneration of POF equals the spawning interval. This is an important result for future studies to estimate the spawning frequency of sprat. A temperature dependent model on Baltic sprat egg development was established using an experimental approach. All obtained results and data were finally used to implement the Daily Egg Production Method (DEPM) to the Baltic sprat stock in the Bornholm Basin. Results clearly demonstrated that the DEPM is a promising approach to assess this important pelagic fish stock in the Baltic Sea. In contrast to the standard procedure in sprat stock assessment, this approach takes into account observed variability in sprat reproductive traits. The main advantage of this method is that it is independent from fishery data and all input parameters can be achieved by field observations. However, some uncertainties concerning the spawning frequency and the daily egg production, two crucial input parameters for this method, were identified which require improvement. The knowledge and data obtained by the present work may further serve as basis to (i) enhance existing assessment methods and to test alternative indices for sprat stock reproductive potential (*e.g.* female spawning stock biomass or potential egg production), (ii) implement alternative assessment methods (*e.g.* DEPM) and (iii) further investigate the population dynamics and ecology of Baltic sprat.

Introduction

Baltic Sprat

The European sprat (*Sprattus sprattus* L.) is a small planktivorous pelagic clupeoid species with a wide distribution in shelf areas of the Northeast Atlantic, covering the coasts of Norway, the North Sea, Irish Sea, Bay of Biscay, the western coast of the Iberian peninsula down to Morocco (*Sprattus sprattus sprattus*; Linnaeus, 1758), the northern parts of the Mediterranean, the Black Sea (*Sprattus sprattus phalericus*; Risso, 1826), and the Baltic Sea (*Sprattus sprattus balticus*; Schneider, 1908) (Fig. 1). Sprat is able to tolerate salinities as low as 4 psu and especially juveniles are known to enter estuaries (Whitehead, 1985). In the Baltic Sea, sprat is located at its northern limit of geographic distribution (Muus & Nielsen, 1999). It is distributed throughout the western and eastern parts of the Baltic, up to the Gulf of Finland in the north. Within its range of distribution in this brackish sea, different sprat stock components experience different hydrographic conditions with decreasing water temperatures and salinities from West to East. Morphology, growth rates and other life history traits were reported to differ among different areas in the Baltic (Ojaveer and Kalejs, 2010; Lindquist, 1971). The question if these observed differences justify a separation of the Baltic sprat population into distinct stock units has until today not been answered satisfactorily, which is partly due to mixing of sprat in spawning and wintering areas (Ojaveer and Kalejs, 2010).

Sprat is an ecological key species in the Baltic pelagic ecosystem. On the one hand, sprat serves as main prey for the Baltic cod stock (Rudstaam *et al.*, 1994); on the other hand it is the most abundant planktivorous fish species in the Baltic. By predation on ichthyoplankton, sprat is able to affect the recruitment of cod and through cannibalism also that of sprat (Köster & Möllmann, 2000a; Köster & Möllmann, 2000b; Köster & Schnack, 1994). Via predation on zooplankton it acts as a key player for top down control in the Baltic pelagic ecosystem, with the copepods *Pseudocalanus* sp., *Acartia* spp., and *Temora longicornis* being the main prey organisms (Möllmann *et al*. 2004).

Data on the development of the Baltic sprat stock inhabiting the ICES sub-divisions 22-32 (Baltic proper) is available since 1974 (Fig. 3a). In the 1980s the sprat stock was at low levels, with a minimum of 527.000t in 1980. The sprat stock reached maximum values in the 1990s, with a maximum value of 2.950.000t in 1995. These observed high stock levels can be explained by a combination of declining predation pressure by the collapsed cod stock and some years of strong recruitment. In recent years the sprat stock has decreased again, with a total stock biomass of 1.781.000t in 2009 (ICES, 2010). The estimated spawning stock biomass follows in general the trend of the total stock biomass. Since the year 2000 the sprat spawning stock is fluctuating around 1 mio. t (Fig. 3c).

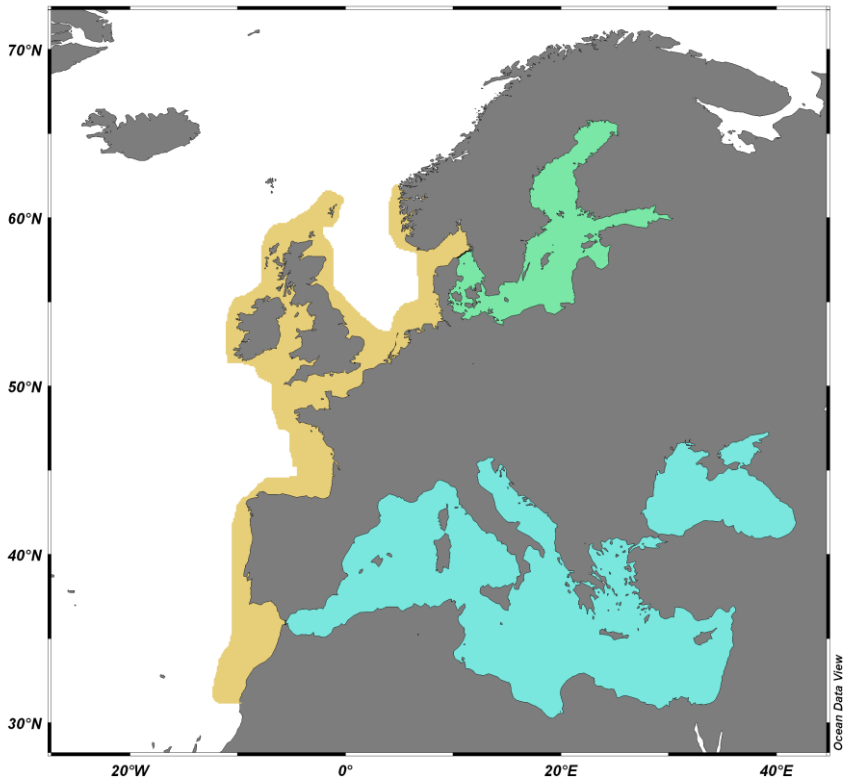

Fig. 1: Distribution of the three different sub-species of European Sprat. Green: *Sprattus sprattus balticus* (Schneider, 1908). Yellow: *Sprattus sprattus sprattus* (Linnaeus, 1758). Blue: *Sprattus sprattus phalericus* (Risso, 1826). Distribution chart redrawn after Whitehead (1985).

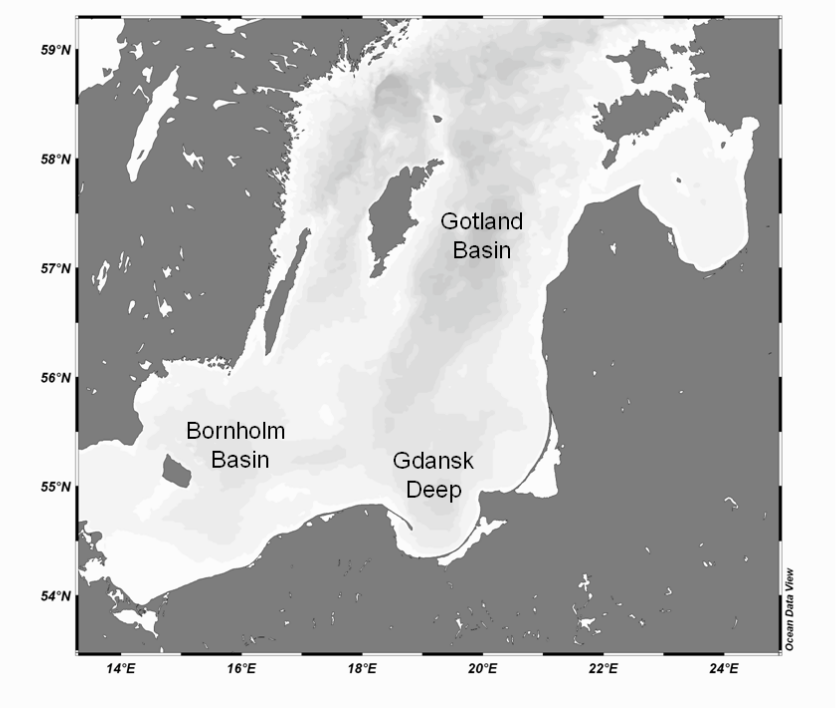

Fig. 2: The central Baltic Sea with the deep Basins serving as major spawning grounds for the Baltic sprat stock.

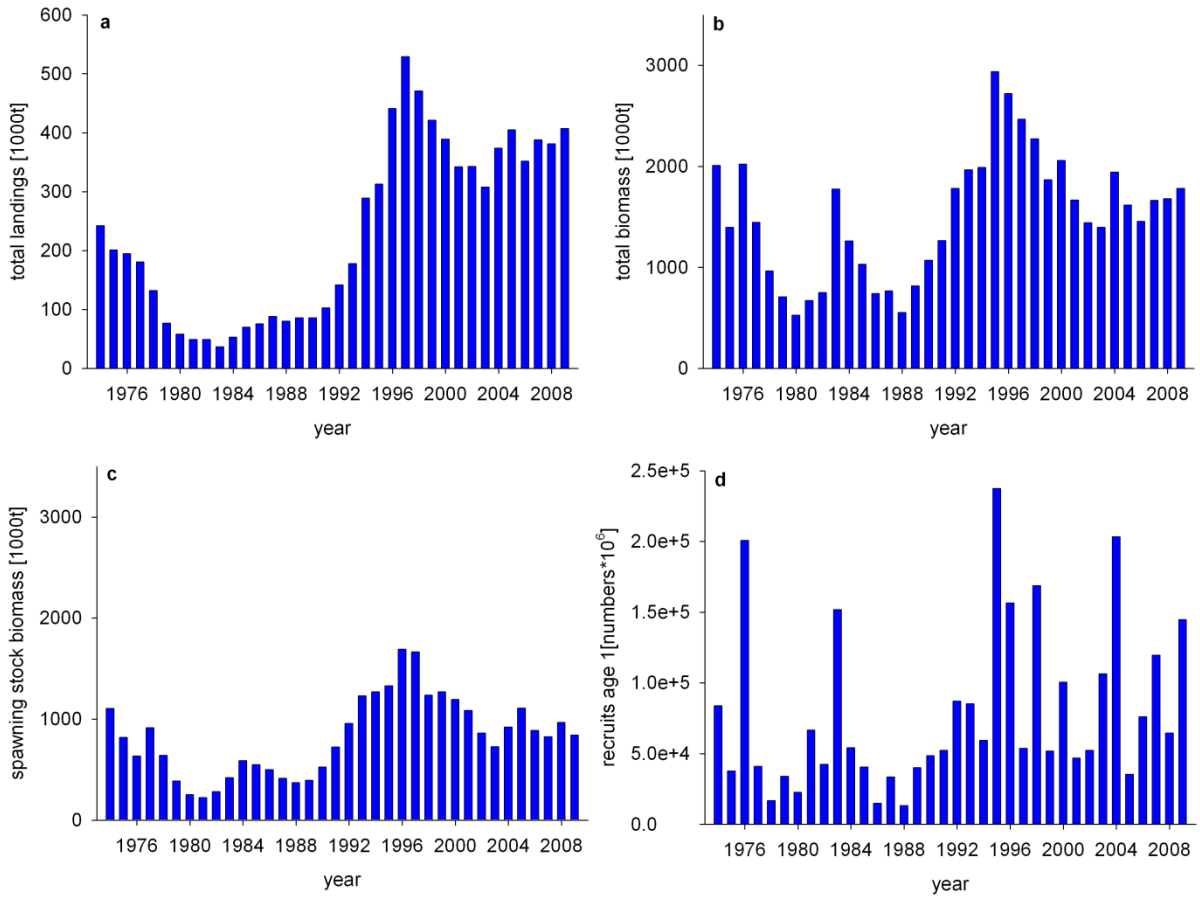

Fig. 3: Baltic sprat XSA output for the Baltic Sea ICES SD 22-32. a) total landings; b) total biomass; c) spawning stock biomass; d) recruits at age 1.

The recruitment of Baltic sprat is highly variable throughout the observed time period, but more years with strong recruitment occurred since the 1990s (Fig. 3d). Processes controlling this variability are not fully understood yet. As one important environmental factor influencing sprat recruitment success, water temperature is discussed in the literature. Nissling (2004) reported a low survival of sprat eggs when ambient temperatures fall below a threshold of 4 °C. Further, low ambient temperatures will slow down the egg development rate and growth rate of larvae (Nissling, 2004; Petereit *et al*., 2008), which may increase the mortality of sprat eggs by predation. Increasing temperatures will in turn accelerate the developmental rates of sprat eggs and larvae. Further, food availability for larval sprat is mainly driven by the abundance of *Acartia* spp., the main food organism of larval sprat, mediated by increasing water temperatures (MacKenzie and Köster, 2004; Dickmann *et al*., 2007). Besides temperature also a number of other climatic processes will affect larval survival and hence might play a role for recruitment processes. The feeding success of sprat larvae was not only found to be linked to prey density, but also to small-scale turbulence rates and light condition (Voss *et al*., 2008). Baumann *et al*. (2006) hypothesized that climate conditions leading to dispersal of sprat larvae out of the central basins to coastal areas may have a negative influence on the recruitment, whereas climatic conditions resulting in retention in the central basins may support a strong recruitment.
Besides its ecological importance, Baltic sprat stock is one of the most important commercial fish species in the Baltic. The highest catches were recorded concurrently with the highest stock level in 1997 with 529.400 t (Fig. 3a). In 2009, sprat catch for the Baltic proper was about 407.100 t, and for the first time the total allowable catch (TAC) was utilised in 100%.

The Assessment of the Baltic Sprat Stock

Today, the Baltic sprat stock is assessed as a single stock unit for the whole Baltic using a virtual population analysis (VPA) with an extended survivors analysis (XSA) (ICES, 2010). This approach is age based, and maturity ogives are kept constant over the whole time period. As tuning fleet, a clupeid targeting international acoustic survey is in use covering the Baltic proper in autumn and spring. As one alternative assessment method, the multi species virtual population analysis (MSVPA; ICES 2006) has been applied, which takes the predation mortality caused by the Baltic cod stock into account. Further, the latter approach resolves the stock biomass of cod, herring, and sprat area disaggregated on ICES sub-division basis. These two assessment methods, the XSA and the MSVPA, are strongly dependent on fishery catch data.

Spawning traits of Baltic Sprat

Important spawning areas of the Baltic sprat stock are located in the three central Basins of the Baltic, namely the Bornholm Basin, the Gdansk Deep and the Gotland Basin (Aro, 1989; Parmanne *et al*., 1994; Fig. 2). In the most northern parts of the Baltic, sprat spawning occurs and sprat eggs can be found in the plankton, but no larvae (Sjöblom and Parmanne, 1980). The main spawning season lasts from March to late June. The spawning stock is migrating into the basins (Aro, 1989), and the largest part spawns within the deep basins (>60 m). It remains to be resolved which factors drive the onset of maturation and spawning in Baltic sprat. It is likely that temperature plays an important role (Karasiova, 2002), but also the availability of suitable prey resources to allow for sufficient energy reserves for gamete production might be of importance. In 2002, a second spawning event was observed in autumn, which was explained by the inflow of unusual warm water masses into the central Baltic (Kraus *et al*., 2003).

In general, most individuals contribute to the spawning stock in their second year of life, but for some years also a considerable high proportion of the one year old sprat has been observed to be mature, thus contributing to spawning (ICES, 2002). Until today, it could not be explained what is driving the high variability in the proportion of mature specimens in age one. The last comprehensive studies on sprat maturation were conducted by the ICES Study Group on Baltic Herring and Sprat Maturity (ICES, 2002). However, the results were not conclusive enough to incorporate results into the standard stock assessment. Due to this lack of knowledge, the ICES Working Group of Baltic Fisheries Assessment (WGBFAS) is using a long term average of the maturity ogive (ICES, 2010a). Given the fact that the young of the year sprat can provide a considerable high proportion of the total stock biomass in years with strong recruitment, the spawning stock biomass estimate might be biased significantly. Hence, the WGBFAS recommends further analysis of this issue (ICES, 2010a).

As many other clupeoid fish, sprat is a species with indeterminate oocyte recruitment, spawning several batches of eggs during a prolonged spawning season (Heidrich, 1925; Alheit, 1988). In such species the amount of oocytes which will be spawned is not determined before the spawning season, and pre-vitellogenic oocytes can develop and be recruited at any time during the spawning season (*de novo* vitellogenesis, Hunter and Goldberg, 1980). Thus, batch fecundity is the only suitable measure of fecundity in indeterminate spawners. Further, in such species the annual fecundity, or potential seasonal egg production, can only be estimated when batch fecundity, the percentage of females spawning per day, and the duration of the spawning season is known.

Fecundity of marine fish may vary for the same species between areas and seasons (Alheit, 1988; Lambert *et al*., 2003) and might be influenced by several biotic and abiotic environmental parameters. Estimates of Baltic sprat batch fecundity are scarce, and often the sample sizes of the investigations were too small to allow conclusive analyses on the dynamics of fecundity. Batch fecundity data for Baltic sprat were published first by Heidrich (1925), but only for the Kiel

Bight area. Petrova (1960) gives some information on batch fecundity of sprat from the eastern parts of the Baltic. Some data on batch fecundity are available for the Gdansk Deep (Alekseev & Alekseeva, 2005). The only data available for the Bornholm Basin were published by Müller *et al*. (1990) and are based on a very small sample size.
For several clupeids it has been shown that spawning frequency is dependent on the age or size of the female fish (Parrish e*t al*., 1986; Claramunt *et al*., 2007). For some species, tank experiments were conducted to investigate spawning frequency, which is probably the best way to obtain a precise estimation of this parameter (Leong, 1971; Ganias *et al.*, 2003). As it is not in all cases feasible to conduct tank experiments, other methods were developed in order to assess the spawning frequency by means of field sampling: (i) the hydrated oocyte method and (ii) the postovulatory follicle method (Hunter and Macewicz, 1985). The first method takes into account all females with hydrated oocytes in their ovaries assuming that these individuals will spawn within the next few hours. The second method makes use of the postovulatory follicles in order to estimate the fraction of spawning females per day. A prerequisite for this approach is the detailed knowledge of the histological features of postovulatory follicles with respect to their deterioration and duration. The postovulatory follicle method is often preferred as hydrated females might be oversampled due to higher vulnerability to the fishing gear in use or due to the forming of spawning aggregations (Alheit, 1985). The spawning frequency of Baltic sprat has never been studied in detail so far. Alekseev & Alekseeva (2005) provided a rough estimate of four days, obtained by the proportion females in spawning condition. The same approach was used by Kraus and Köster (2004) leading to similar results. However, histological features of ovarian maturation or postovulatory follicles have never been published for Baltic sprat so far.
A sound knowledge of the reproduction parameters described above would be valuable to build up models to calculate the total daily or even annual egg production of the spawning stock. This knowledge could be used to enhance existing stock-recruitment models and the stock assessment methods in use. Further, alternative assessment methods, like the Daily Egg Production Method (Parker, 1980; Lasker, 1985; Stratoudakis *et al*., 2006) could be applied.

The Daily Egg Production Method

Several applications have been developed to estimate the size of fish stocks by means of the abundance of their early life stages (Lockwood *et al*., 1981; Parker, 1980), either eggs or larvae. Combining the results of ichthyoplankton surveys with data of the adult stock regarding length frequency, weight at age, sex ratio, maturity and fecundity, enables the estimation of the adult stock size or biomass. The main advantage to standard assessment methods is that these methods are independent from fishery data and theoretically all parameters can be observed in the field, so that the use of uncertain assumptions can be reduced to a minimum.
The choice for an adequate ichthyoplankton method to assess a fish stock is species specific and depends strongly on the species spawning strategy. *E.g.* for Atlantic herring (*Clupea harengus*), which spawns benthic eggs during a single spawning event, a larvae survey has been applied for the North Sea herring stocks to get an additional, fishery independent index of stock development. For species with pelagic eggs it is important whether the annual fecundity of the targeted fish species is determinate or indeterminate.
For species with determinate fecundity the egg production is determined prior to the onset of spawning and the Annual Egg Production Method is used (*e.g.* mackerel, *Scomber scombrus*, Lockwood *et al*., 1981). In indeterminate species the fecundity is not determined prior to spawning. For such species it is difficult, or even impossible, to estimate the annual fecundity and the Daily Egg Production Method (DEPM) has been developed (Parker, 1980).
The DEPM has been applied for several stocks of pelagic fish species in the past worldwide (Fig. 4; Stratoudakis *et al*., 2006). For some stocks it is nowadays used on a routine basis to validate

other assessment methods in use, *e.g.* Northern anchovy (*Engraulis mordax*; Lasker, 1985), Bay of Biscay anchovy (*Engraulis encrasicolus*; ICES, 2009) and European sardine (*Sardina pilchardus*; ICES, 2009). Although originally developed for clupeoid species it has also been successfully applied for other species, such as hake (*Merluccius merluccius*; Murua *et al*., 2010), recently.

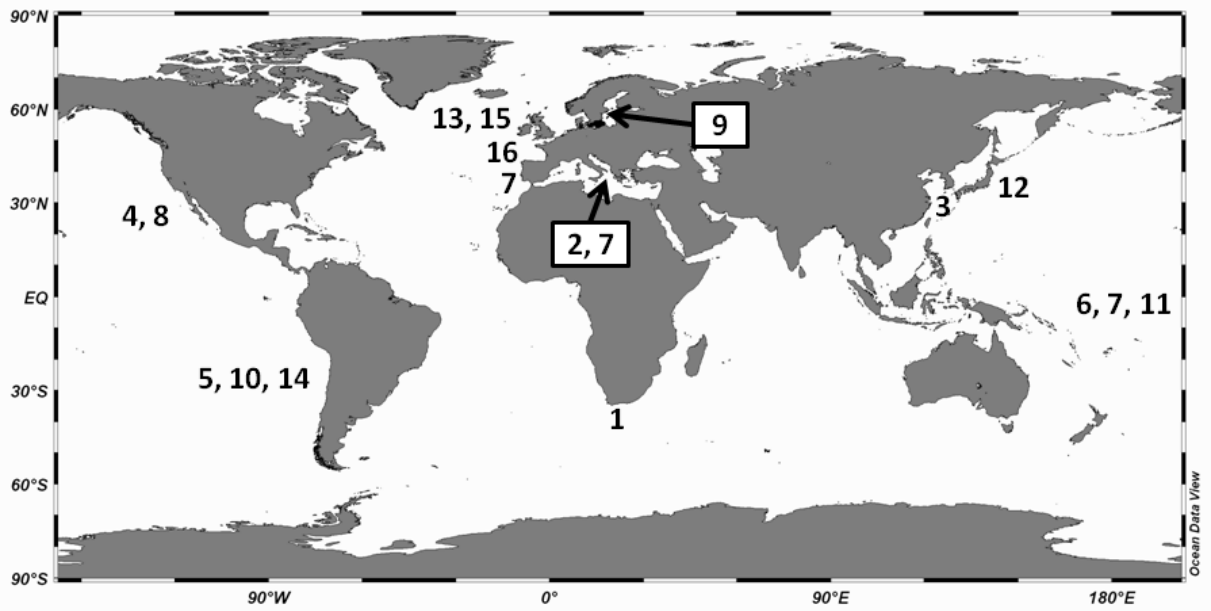

Fig. 4: Worldwide DEPM applications. 1 *Engraulis capensis*, 2 *Engraulis encrasicolus,* 3 *Engraulis japonicus,* 4 *Engraulis mordax*, 5 *Engraulis ringens*, 6 *Encrasicholina sp.*, 7 *Sardina pilchardus*, 8 *Sardinops sagax*, 9 *Sprattus sprattus*, 10 *Strangomera bentincki*, 11 *Pagrus auratus*, 12 *Scomber japonicus*, 13 *Scomber scombrus*, 14 *Trachurus symmetricus*, 15 *Trachurus trachurus*, 16 *Merluccius merluccius.* Redrawn after Stratoudakis *et al*. (2006).

Aim of the present study

The goal of the present study is to enhance the knowledge of reproductive traits of Baltic sprat, which may be utilised in a number of future studies to further enhance the understanding of sprat population dynamics.
Since sprat is a serial batch spawner with indeterminate oocyte recruitment, the DEPM would be the adequate choice as an alternative assessment method for this small clupeoid. The obtained knowledge and data from the present study will form the basis for an application of the DEPM for a wide range of years for which ichthyoplankton and fishery surveys for research purposes were conducted in the Bornholm Basin. For the Baltic sprat stock there are considerable differences in the output from the area disaggregated MSVPA and the acoustic survey (Köster and Möllman, 2000a). Thus, there is a need for a fishery independent assessment tool to validate the results of these methods. The DEPM has been applied to Baltic sprat before (Kraus & Köster, 2004), but not on a regular basis. Essential data were scarce or even not available in the past. In the present study, all important data which are needed to apply the DEPM have been investigated for the Baltic sprat stock. A consecutive series of sprat batch fecundity data covering important sprat spawning areas in the central Baltic Sea has been established (Chapter I). The seasonal variability in batch fecundity and spawning frequency has been studied with modern image analysis methods using stereology (Chapter II). Stock structure parameters needed for the DEPM as length frequency distributions, sex ratios, and maturity ogives have been analysed and updated (Chapter III). A study to assess the degeneration time of postovulatory follicles was conducted

which enables a more precise estimation of the spawning frequency, a crucial parameter for the DEPM (Chapter IV). The egg development of sprat in relation to ambient temperature has been analysed in an experimental approach (Chapter V). The latter data are necessary to correct the field abundance data of the earliest egg stage with respect to egg stage duration and mortality. Finally, the DEPM has been applied to the sprat stock in the Bornholm Basin and results have been compared to other stock assessment methods (Chapter VI). Figure 5 gives a schematic overview of the work steps which were conducted in the present work to apply the DEPM for Baltic sprat. All chapters of the present work relate directly to this scheme with a focus on the spawning traits of Baltic sprat.

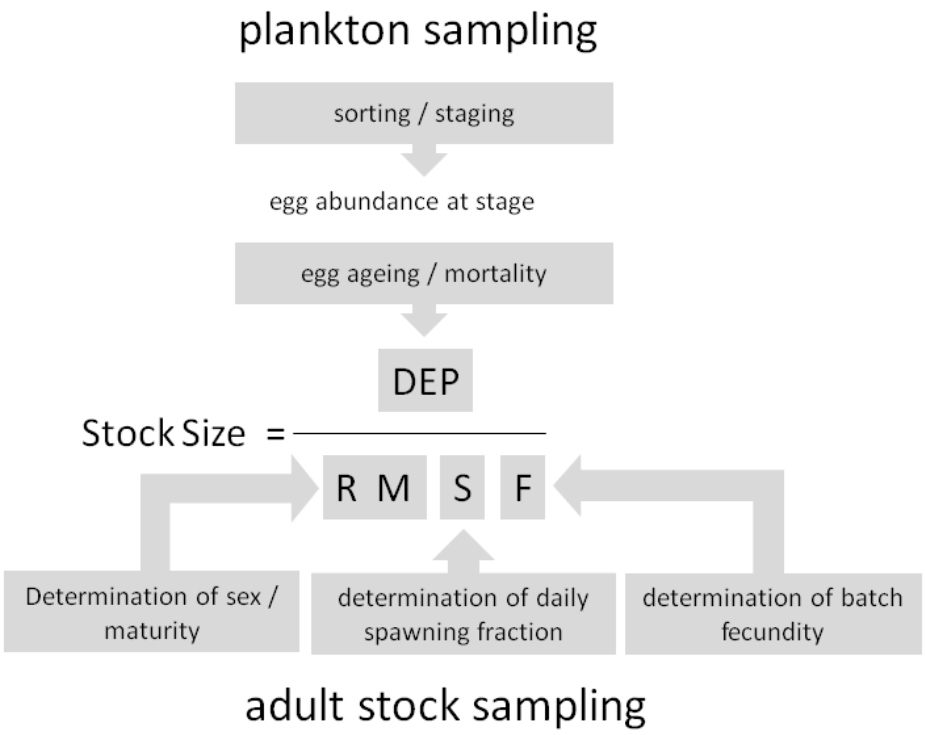

Fig. 5: Scheme of the applied DEPM equation for estimation of the sprat stock size and associated work steps. DEP = total daily egg production for the survey area; R = sex ratio, *i.e.* proportion of females; M = proportion mature females; S = spawning frequency; F = batch fecundity.

Chapter I: Spatial and interannual variability in Baltic sprat batch fecundity

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Abstract

Absolute and relative batch fecundity of Baltic sprat (*Sprattus sprattus balticus*) during peak spawning time was investigated for several years over the last two decades by applying the hydrated oocyte method. Batch fecundity was analysed for three important spawning areas of sprat in the central Baltic Sea, namely the Bornholm Basin, Gdansk Deep and Southern Gotland Basin. Environmental parameters such as hydrography, fish condition and stock density were tested in order to investigate the observed variability in sprat fecundity. Absolute batch fecundity was found to be positively related to fish length and weight. Significant differences in absolute and relative batch fecundity of Baltic sprat among areas and years were detected, and could partly be explained by hydrographic features of the investigated areas. A non-linear multiple regression model taking into account fish length and ambient temperature explained 70% of variability in absolute batch fecundity. Oxygen content and fish condition were not related to sprat batch fecundity. Additionally, a negative effect of stock size on sprat batch fecundity in the Bornholm Basin was revealed. The obtained data and results are important to assess the stock reproductive potential of this important Baltic fish stock.

Key words: batch fecundity, sprat, hydrated oocyte method

I. 1 Introduction

Knowledge on fecundity is essential to estimate the reproductive potential and egg production of a fish stock. Fecundity data allow a fishery independent estimation of the spawning stock size by egg production methods, *e.g.* the Daily Egg Production Method (DEPM; Lasker, 1985; Parker, 1980). However, fecundity in fish is often highly variable and may be influenced by a number of factors such as fish size, nutritional status, food availability, fish density, and other environmental parameters, *e.g.* temperature or salinity (Lambert *et al*., 2003). Thus, fecundity may vary between stocks of the same species, which experience different environmental conditions in their specific habitat (Leal *et al*., 2009). Therefore, spatial and temporal variability in fecundity needs consideration when evaluating the reproductive potential of a stock or assessing the spawning biomass of certain stock components using egg production methods.
In the present study, a time series of Baltic sprat *Sprattus sprattus balticus* (Schneider, 1908) batch fecundity was established applying the hydrated oocyte method (Hunter *et al.* 1985). Baltic sprat is a key species in the pelagic ecosystem of the Baltic Sea (Rudstam *et al*., 1994). It is the

most abundant planktivorous fish species in the Baltic, and the main prey of cod (Bagge *et al.*, 1994) as well as an important predator on early life stages of cod (Köster and Möllmann, 2000; Köster and Schnack, 1994). Consequently, the population dynamics of the Baltic sprat stock has major impact on the stock dynamics of the commercially important cod stock in the Baltic Sea. The Baltic sprat stock itself is heavily exploited with catches of 407.100 t for the year 2009 (ICES, 2010). Therefore, it is relevant to improve the knowledge and understanding of the reproductive biology of this living resource. Main spawning grounds of sprat in the central Baltic Sea are the Bornholm Basin, the Gdansk Deep and the Gotland Basin (Aro, 1989; Köster *et al.*, 2003).

Hydrographic conditions differ substantially among these three spawning areas as a function of the general hydrodynamics of the central Baltic. The salinity and volume of the upper water layers of this semi-enclosed brackish sea are mainly influenced by the amount of precipitation and fresh water river run off, while the renewal of the deep parts of the basins with oxygenated and saline water masses from the adjacent North Sea depends on inflow events. These processes lead to a highly stratified water column with a permanent halocline in the mid-water layer, and oxygen depleted water layers in the deep parts of the basins. The depth of the halocline as well as the oxycline depends on the frequency of inflow events. In general, salinity levels decrease from western to eastern parts of the central Baltic, and also temperatures are lower in the eastern parts.

The Baltic sprat spawning season generally ranges from March to July with a peak in egg production in May/June. However, peak spawning time may be delayed by extremely cold winter temperatures (Karasiova, 2002). Sprat is a species with indeterminate oocyte recruitment releasing a number of successive egg batches over a protracted spawning season (Heidrich, 1925). Consequently, the annual egg production is seasonally indeterminate and batch fecundity is the only appropriate fecundity measurement (Hunter *et al*., 1985; Murua *et al*., 2003). Batch fecundity and spawning frequency show intra- and interannual variability in sprat and differ among areas (*e.g.* Heidrich, 1925 Kiel Bight; Alheit, 1988 North Sea). Data on Baltic sprat batch fecundity are scarce and conclusive investigations on its variability are lacking. The available information on Baltic sprat batch fecundity (Heidrich, 1925; Petrova, 1960; Polivaiko, 1980; Müller *et al*. 1990; Kraus and Köster, 2004; Alekseev and Aleksseva, 2005) is scattered over years and areas. Furthermore, studies are often based on low sample sizes, and thus do not allow for comprehensive spatial and temporal comparisons.

The understanding of the observed variations in fecundity is essential for the establishment of reliable predictions of sprat Stock Reproductive Potential (SRP). In addition, a model on sprat fecundity including historical data on stock structure and egg abundance data would allow reconstruction of the spawning stock biomass of Baltic sprat for a wide range of years using egg production methods.

In the present study, we quantified sprat fecundity on the aforementioned three spawning areas (Bornholm Basin, Gdansk Deep, and Southern Gotland Basin) during the reproductive season over a period of several years and analysed individual batch fecundity. The data obtained were used to build predictive models explaining spatial and temporal variability in sprat batch fecundity in relation to environmental parameters.

I. 2 Methods

Female sprat were caught by trawling during peak spawning time in different years in three areas of the central Baltic Sea: Bornholm Basin (1991, 1995-1996, 1998-2008), Gdansk Deep (2000-2004, 2006, 2008) and the Southern Gotland Basin (2000-2006, 2008) (Fig. 1; Tab. 1). Only females with fully hydrated oocytes were sampled for fecundity analyses. The body cavity was opened, maturity stage determined by macroscopic inspection of the ovaries. In order to exclude actively spawning females from the analyses which might have released part of the egg batch,

running ripe females were not sampled. The entire fish was preserved in a buffered 4% formaldehyde seawater solution for the later fecundity analyses in the laboratory. Obtained data of fish lengths and weights were not corrected for possible preservation effects, since no data were available for sprat.

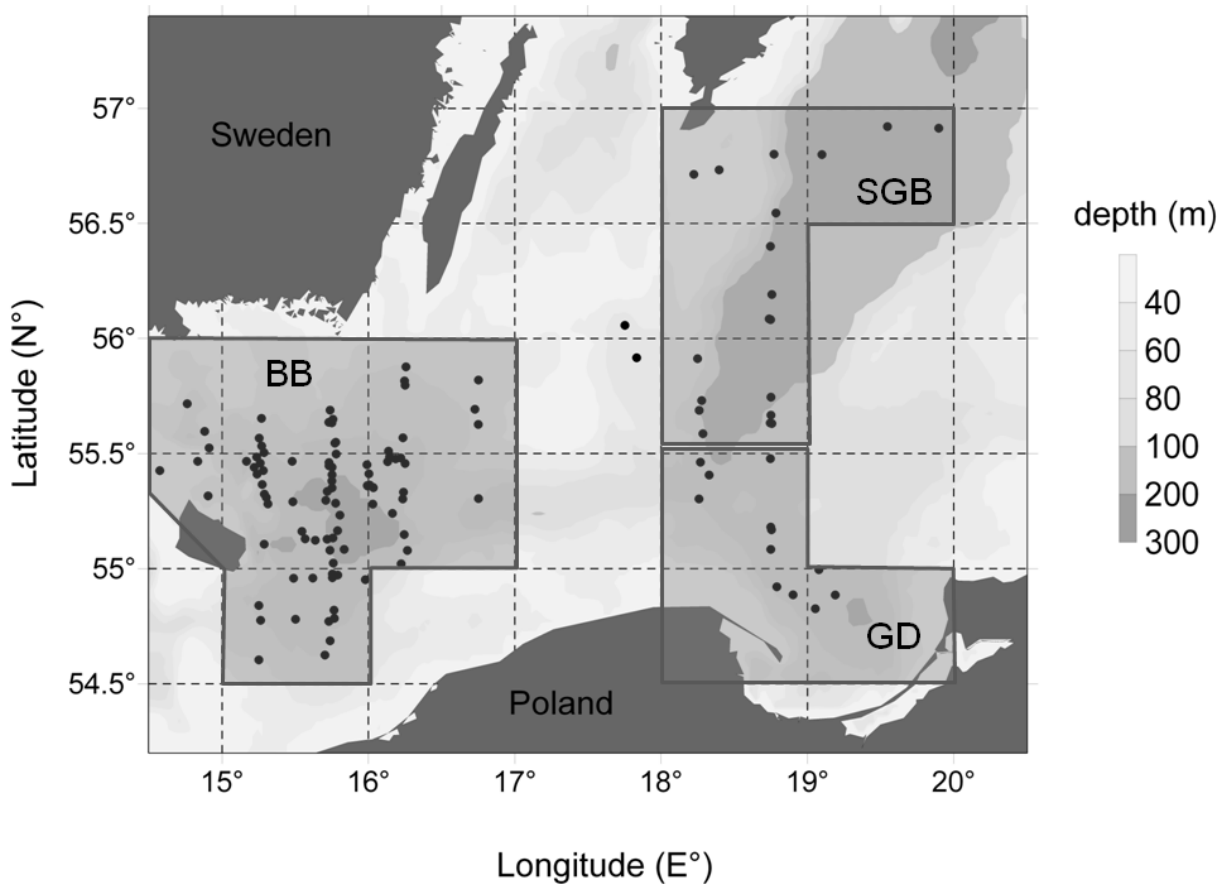

Fig. 1: Sampling locations of sprat in the central Baltic. Dots denote stations sampled within the present study. Shaded areas indicate the three defined sampling areas: BB = Bornholm Basin, GD = Gdansk Deep, SGB = Southern Gotland Basin.

The batch fecundity (*BF*), *i.e.* the number of oocytes released per single spawning event, was estimated gravimetrically applying the hydrated oocyte method (Hunter *et al.*, 1985). For each sampled ovary, the number of eggs per batch (*i.e.* the absolute batch fecundity) was estimated by weighing the entire ovary (*OW*) and by counting the hydrated oocytes (*NOS*) in a subsample of approximately 10% of the ovary (*SW*). The *NOS* was determined using a stereo microscope, and raising the counted numbers to total numbers in the ovary by the weight proportion:

$$BF = \frac{OW}{SW} * NOS \quad (1)$$

In addition, total body weight (*W*), gutted weight (*GW*) and total length (L_T) were recorded for every analysed female sprat. The relative batch fecundity (*RBF*) was calculated for each fish by dividing the absolute batch fecundity by the ovary free body weight (*OFBW*) (Alheit, 1988):

$$RBF = \frac{BF}{OFBW} \quad (2)$$

To obtain a measure of the female sprat condition the Fulton's Condition Index (*K*) was calculated for each fish:

$$K = \frac{GW}{TL^3} * 100 \quad (3)$$

In order to assess variability in absolute batch fecundity, analyses of covariance (ANCOVA) were performed with total fish length as continuous covariable, and year and area as category variables. Cases with no significant linear relationship between length and batch size were excluded from these analyses. All data were square root transformed to meet assumptions of normal distribution and homoscedasticity. K-S tests indicated minor deviations from normal distribution in some cases. However, as ANOVA analyses are quite robust against deviations from normal distribution, deviations from normality were not given much emphasis in the subsequent analyses. The year effect was tested separately for each of the sampling areas. The area effect could only be tested for years, in which two or more significant linear regressions were found. A regression model to predict absolute batch fecundity was established, including fish length and mean temperature as independent variables.
Differences in relative batch fecundity were tested applying a two factorial analysis of variance (ANOVA) with years and areas as independent variables. For this analysis, only years in which the sampling covered all three areas were used in order to avoid gaps in the model design (years included in ANOVA analysis of relative batch fecundity: 2000, 2001, 2002, 2004, 2006, and 2008). In cases were significant differences were detected, a *post hoc* multiple comparison was performed (Tukey's HSD for unequal sample sizes).
Relative fecundity was related to different biotic and abiotic variables in order to identify potential causes of the observed variability. Water temperature, salinity and oxygen content were used as abiotic variables, whereas sprat condition and stock abundance estimates were used as proxies for density dependent processes, *e.g.* trophic interactions. Hydrographic data were obtained from the hydrographic database of ICES (International Council for the Exploration of the Sea). Mean water temperature, salinity, and oxygen content for the analyses were estimated for the first and second quarter of each year for all three areas. These average hydrographic values were calculated for the entire water column, from the surface to depths where oxygen values lower than 1 ml l^{-1} are avoided by sprat (Stepputtis *et al.*, 2011). Data on sprat stock sizes for use in the analyses were obtained from the most recent run of an area disaggregated Multi Species Virtual Population Analyses (MSVPA) conducted by the ICES Study Group of Multi Species Assessment in the Baltic (ICES, 2006). Or as an alternative, stock abundance estimates for ICES sub-division 25, obtained from the international Baltic acoustic survey conducted in May, were applied. Data on fish condition were obtained from the present study.

I. 3 Results

Absolute batch fecundity

The absolute batch size of Baltic sprat varied between a minimum of 206 and a maximum of 4244 eggs. The absolute batch size at peak spawning time as the mean of all examined sprat ovaries approximated 1533 (SD±637) eggs (n=1142). The number of eggs per batch increased with total fish length and fish body weight, respectively. Most significant results were obtained from linear regression models with batch size as dependent and total fish length as independent variables (Fig. 2; Tab. 1). In some cases no significant linear relationship could be obtained, probably due to insufficient sample sizes or a low coverage of the fish size spectrum.
As sampling effort was highest in the Bornholm Basin, linear relationships were available for all data sets (Fig. 2). An analysis of covariance for these data showed significant differences in

slopes and intercepts of the regression lines among years ($p<0.05$). The regression lines of the years 1991, 2001, and 2004 were parallel, with a steeper slope compared to all other years. In this group, the intercept of the 1991 regression line was found to be significantly higher than in the years 2001 and 2004 ($p<0.05$). All the remaining regression lines obtained from the Bornholm Basin data were parallel, but also with differing intercepts. In this second group, the year 1995 possessed the highest, and the year 1996 the lowest intercept values. The three linear regressions of the Gdansk Deep fecundity data (2000, 2004, and 2006) showed no significant differences. Similar results applied to the five data sets compared for the Southern Gotland Basin (2001, 2002, 2004, 2005, and 2006). The ANCOVA analyses within years, testing for differences among areas, revealed lower intercept values for the Southern Gotland Basin samples compared to those of the Bornholm Basin for all years included. The regression results of the Gdansk Deep and Southern Gotland Basin showed significant differences in intercepts for 2004 and 2006, whereas no differences in regressions were detected between Bornholm Basin and Gdansk Deep.

Tab. 1: Overview of sampling years, area (BB = Bornholm Basin, GD = Gdansk Deep, SGB = Southern Gotland Basin), research vessel (AL = RV Alkor, WH = FRV W. Herwig III) number of fish analysed (n), relative batch fecundity (RBF±SE), and linear regression coefficients (r^2, r, p-value, y0, a) of batch fecundity and fish length.

year	area	ship	sampling period	*RBF*	n	r^2	r	p	y0	a
1991	BB	AL	29th – 31th May	148.5 (±4.5)	55	0.26	0.51	<0.05	-3171	421
1995	BB	AL	18th May	139.6 (±8.3)	16	0.28	0.53	<0.05	-1402	263
1996	BB	AL	19th May	85.9 (±6.5)	26	0.29	0.54	<0.05	-2948	320
1998	BB	AL	20th – 22th May	129.6 (±4.6)	52	0.15	0.39	<0.05	-1650	259
1999	BB	WH	1st – 7th June	111.6 (±4.8)	48	0.37	0.61	<0.05	-2467	306
2000	BB	AL	25th – 28th May	134.1 (±4.7)	51	0.28	0.53	<0.05	-2971	381
2001	BB	WH	30th May – 6th June	136.2 (±4.2)	62	0.35	0.60	<0.05	-4076	469
2002	BB	WH	9th – 17th May	117.5 (±4.3)	61	0.21	0.45	<0.05	-1583	246
2004	BB	WH	13th – 18th May	128.0 (±4.1)	67	0.40	0.63	<0.05	-5561	581
2005	BB	WH	16th – 20th May	125.6 (±3.3)	102	0.57	0.76	<0.05	-2569	330
2006	BB	WH	24th – 25th May	130.0 (±2.8)	142	0.51	0.72	<0.05	-2566	335
2007	BB	AL	16th – 17th April	137.6 (±8.9)	14	0.36	0.60	<0.05	-1843	277
2008	BB	WH	12th – 17th May	139.9 (±3.8)	78	0.27	0.52	<0.05	-3042	368
2000	GD	AL	2nd June	126.7 (±7.4)	20	0.43	0.65	<0.05	-4704	519
2001	GD	WH	27th – 28th May	110.8 (±8.6)	15	0.02	-0.13	0.63	2495	98
2002	GD	WH	5th – 6th May	85.2 (±6.4)	27	0.04	-0.19	0.35	2969	171
2004	GD	WH	20th – 21th May	118.6 (±10.0)	11	0.45	0.67	<0.05	-2632	331
2006	GD	WH	31th May – 1st June	119.8 (±4.7)	51	0.33	0.57	<0.05	-2207	297
2008	GD	WH	12th – 17th May	149.5 (±6.9)	23	0.06	0.24	0.27	-659	176
2000	SGB	AL	4th June	111.2 (±10.5)	10	0.35	0.59	0.07	-5101	538
2001	SGB	WH	28th May	89.2 (±7.1)	22	0.36	0.60	<0.05	-3513	377
2002	SGB	WH	5th – 6th May	90.4 (±5.0)	44	0.10	0.32	<0.05	-1680	225
2004	SGB	WH	19th May	99.6 (±4.2)	64	0.48	0.69	<0.05	-2191	279
2005	SGB	WH	22th – 23th May	106.9 (±5.3)	39	0.39	0.62	<0.05	-1428	215
2006	SGB	WH	31th May – 1st June	93.8 (±5.8)	33	0.41	0.64	<0.05	-1572	221
2008	SGB	WH	12th – 17th May	102.8 (±11.1)	9	0.19	0.43	0.25	-1162	184

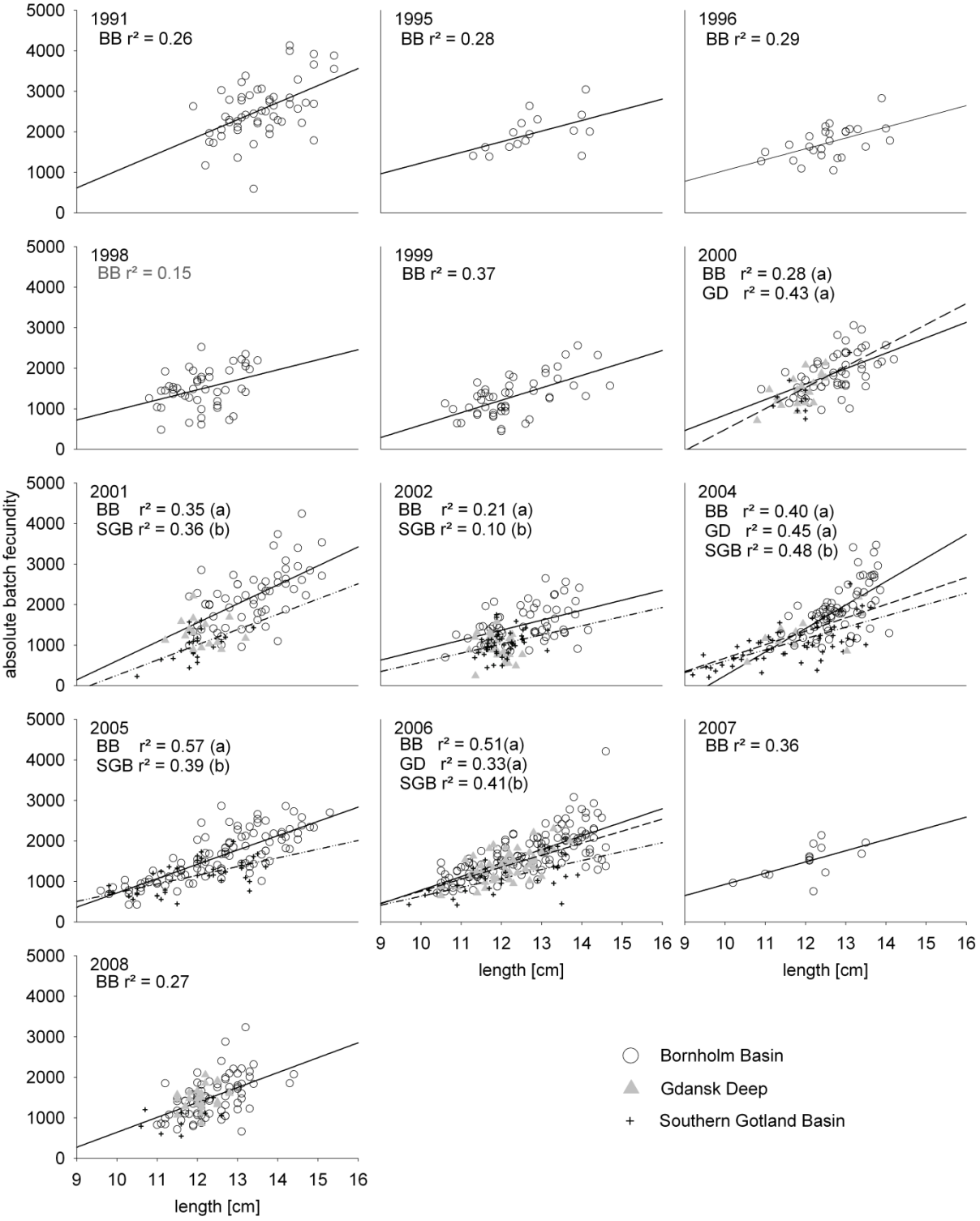

Fig. 2: Relationships between sprat absolute batch fecundity and sprat total length for the Bornholm Basin (BB = white circles; solid lines), Gdansk Deep (GD = grey triangles; dashed lines), and Southern Gotland Basin (SGB = crosshairs; dashed-dotted lines) for different years. Shown are significant linear regressions ($p<0.05$). Characters in parentheses denote significant differences in intercept of regressions within years (ANCOVA, $p<0.05$).

Relative batch fecundity

Data on relative batch fecundity (*RBF*) were analysed by a two factorial analysis of variance (ANOVA) (Fig. 3; Tab. 2) using years and areas as categorical variables. Similar to absolute batch fecundity, the relative batch fecundity showed variability among years and areas. The ANOVA revealed as well a year as an area effect, and an interaction between both (p=0.007). In the Bornholm Basin, mean relative fecundity values (±SE) ranged between 85.9 (±6.6) eggs g^{-1} in 1996 and 148.5 (±4.5) eggs g^{-1} in1991. In most of the observed years relative fecundity was higher in the Bornholm Basin compared to the other two areas although not significant in every case. Comparable low mean values were found for the Southern Gotland Basin, ranging between 89.2 (±7.2) eggs g^{-1} in 2001 and 111.2 (±10.7) eggs g^{-1} in 2000 (Fig. 3), which were always lower than the Bornholm Basin values in the respective years. The relative batch fecundity data for the Gdansk Deep ranged in most cases between the other two areas, with the exception of 2008, where it was slightly higher than in the Bornholm Basin, but not significant (HSD p=0.98). In 2002, it was as low as the Southern Gotland Basin value (HSD p=1.00).

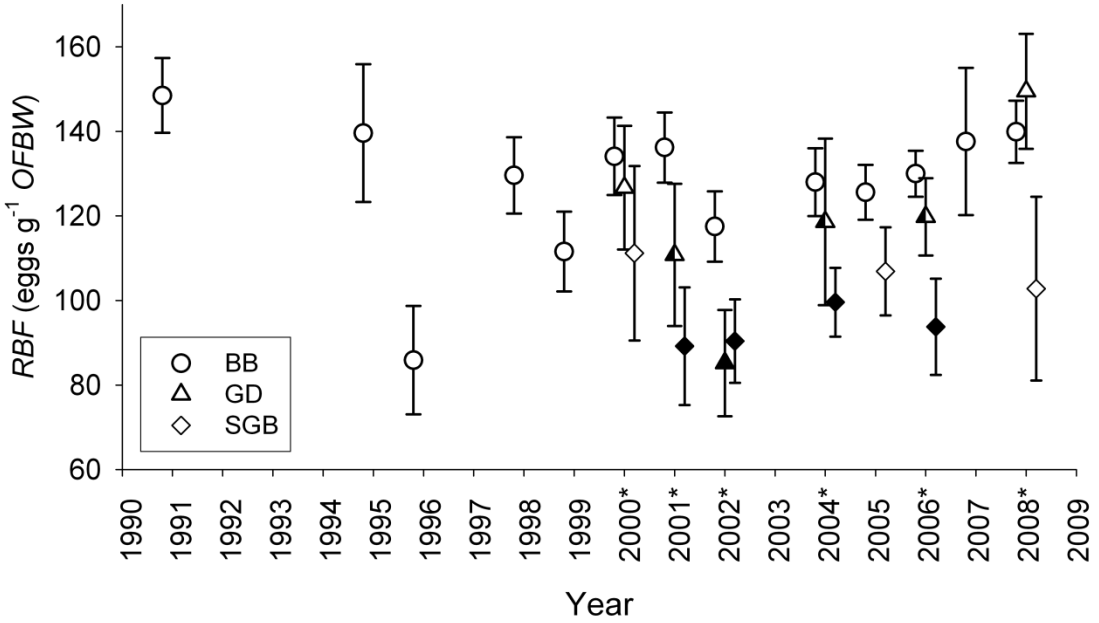

Fig. 3: Mean relative batch fecundity *RBF* (eggs g^{-1} *OFBW*) for different years in the Bornholm Basin (circles), Gdansk Deep (squares), and Southern Gotland Basin (diamonds). Asterisks at the x-axis indicate years included in an ANOVA. Different symbol fill colours denote significant differences ($p<0.05$) for that given year. Vertical bars denote 95% confidence limits.

Tab. 2: ANOVA results testing effects among year and area on relative batch fecundity. Included were the years 2000 - 2002, 2004, 2006, and 2008. All three areas were included.

	SQ	df	MQ	F	p
constant	6029464	1	6029464	5463.65	< 0.0001
year	51890	5	10378	9.40	< 0.0001
area	98766	2	49383	44.75	< 0.0001
year*area	27187	10	2719	2.46	0.007
error	851947	772	1104		

Considering all three areas, the *RBF* showed the strongest relationship with temperature followed by salinity (Fig. 4). Oxygen content revealed no significant relation to *RBF*. Lowest values of mean water temperature and salinity were observed in the Southern Gotland Basin, where in general also the lowest *RBF* values were observed (Fig. 4a and 4d). The lowest *RBF* estimates observed in the Bornholm Basin were associated with the lowest observed mean temperature, but not with low salinity (Fig. 4). Female condition as well as stock size did not result in any significant relationships when considering all areas together. When investigating possible relationships between *RBF* and environmental parameters for each of the three basins separately, only the effect of mean temperature on *RBF* remained for the Bornholm Basin, whereas for the Gdansk Deep and the Gotland Basin no significant relationships were detected at all. This is probably due to the low number of years covered, and the limited observed range of the measured hydrographic variables within these two eastern basins. For the Bornholm Basin separately, using quarter 1 mean temperature as independent variable, resulted in the highest proportion of explained variability (Fig. 5a). Mean salinity showed no relationship, while oxygen content was found to be negatively related to *RBF* (Fig. 5b). From the biotic parameters which were tested *RBF* showed no significant relationship with neither the individual condition index nor MSVPA stock size estimates. In contradiction, the stock size estimate obtained by the acoustic survey revealed a negative relationship with *RBF* explaining as much as 64% of the variability (Fig. 5c). For this last case only 8 years could be included into the regression analysis. Therefore, the significance of this result must not be over-interpreted. However, it might be an indication of density dependent processes affecting sprat fecundity.

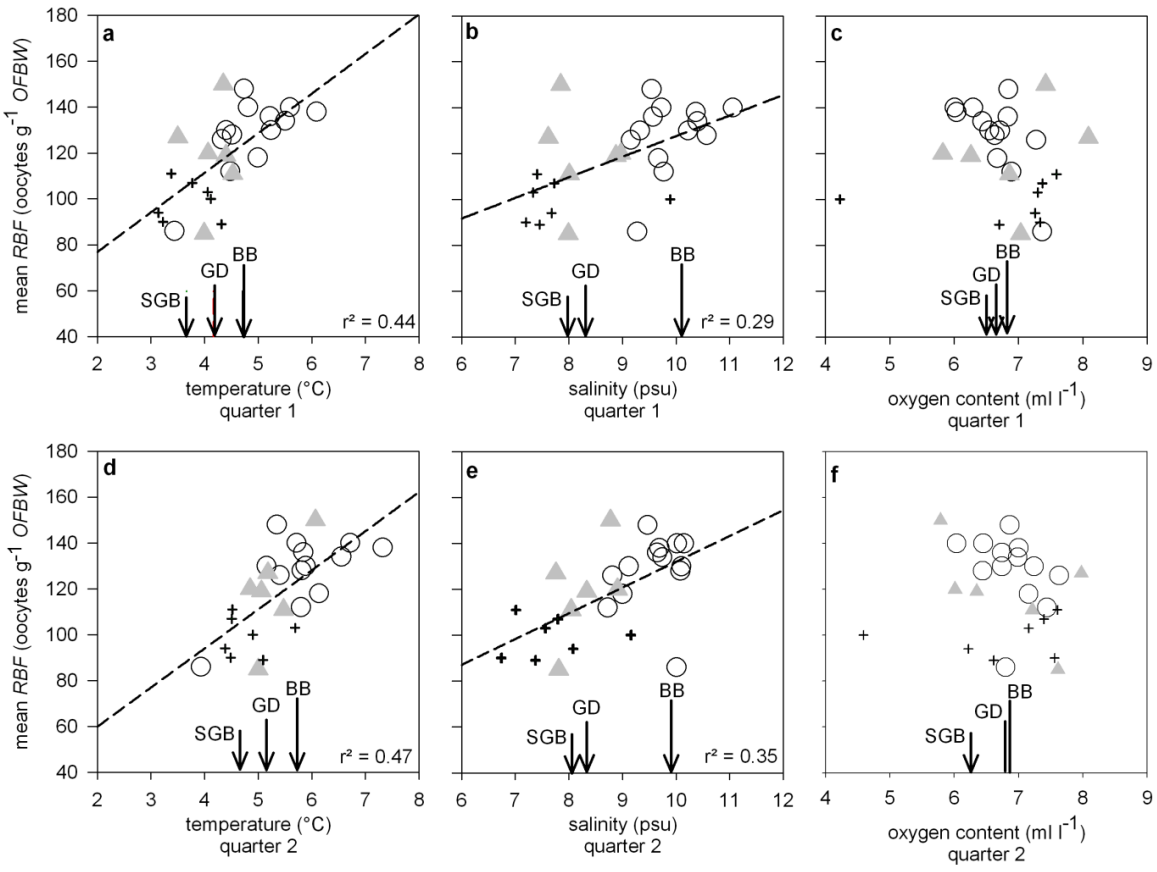

Fig. 4: Relationship between relative batch fecundity (*RBF*) and mean hydrographic parameters measured during the first quarter of the year (a, b, c), and the second quarter (d, e, f). Black dashed lines display significant linear relations. Black arrows indicate the mean of the respective hydrographic parameter observed for the years 1990-2008 within the respective area. Bornholm Basin (BB) = white circles, Gdansk Deep (GD) = grey triangles, Southern Gotland Basin (SGB) = crosshairs.

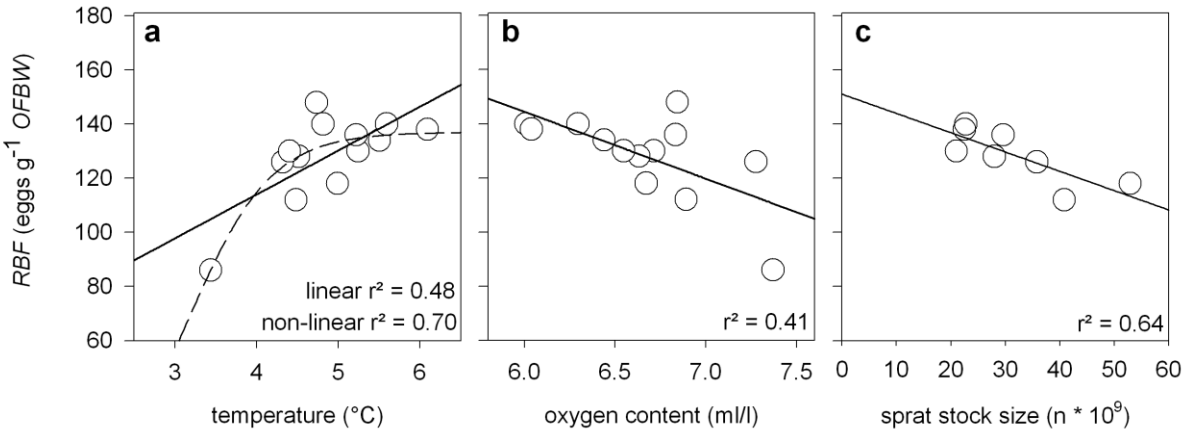

Fig. 5: Significant relationships between relative sprat batch fecundity (*RBF*) and (a) mean winter temperature (solid line linear, dashed line non-linear), (b) mean winter oxygen content, and (c) sprat stock size obtained by an acoustic survey. All panels refer to the Bornholm Basin only.

Non-linear regression models

A multiple non-linear regression with mean values of batch fecundity (*BF*) as dependent variable, fish length class (*TL*) and mean temperature (*T*) as independent variables explained 70% of the variability in *BF* (n=181). The following exponential model was fitted and results are presented in Fig. 6 and Tab. 3:

$$BF = a * TL * exp^{(-0.5*\left(\frac{\ln\left(\frac{T}{Tmax}\right)}{b}\right)^2)} + c \quad (4)$$

where *a*, *b*, *c* and *Tmax* are regression coefficients.

In the case of the relative batch fecundity (*RBF*), it was assumed that fecundity follows rather a flat top curve instead of a continuous linear increase with increasing temperature. Therefore, a model was established describing *RBF* as a sigmoid function of length and temperature (r^2 = 0.70; $p<0.05$):

$$RBF = \frac{a}{1+e^{\frac{-(T-T_0)}{b}}} \quad (5)$$

where *a*, *b* and T_0 are regression coefficients, and *T* is the mean winter temperature (Fig. 5a; Tab. 3).

Tab. 3: Non-linear regression results. All parameter estimations are significant ($p<0.05$). *BF* = absolute batch fecundity, *RBF* = relative batch fecundity, *T* = mean water temperature.

*BF = a * Length * exp (-0.5*(ln(T/Tmax)/b)^2)+c*

parameter	estimate	standard error	t-value (df=10)
a	359.54	23.46	15.33
T_{MAX}	6.97	2.50	2.78
b	1.46	0.55	2.68
c	-2753.16	243.51	-11.31

RBF = a / exp (-1*(T-T_0)/b)

parameter	estimate	standard error	t-value (df=175)
a	137.03	5.74	23.88
T_0	3.17	0.20	2.46
b	0.50	0.19	16.29

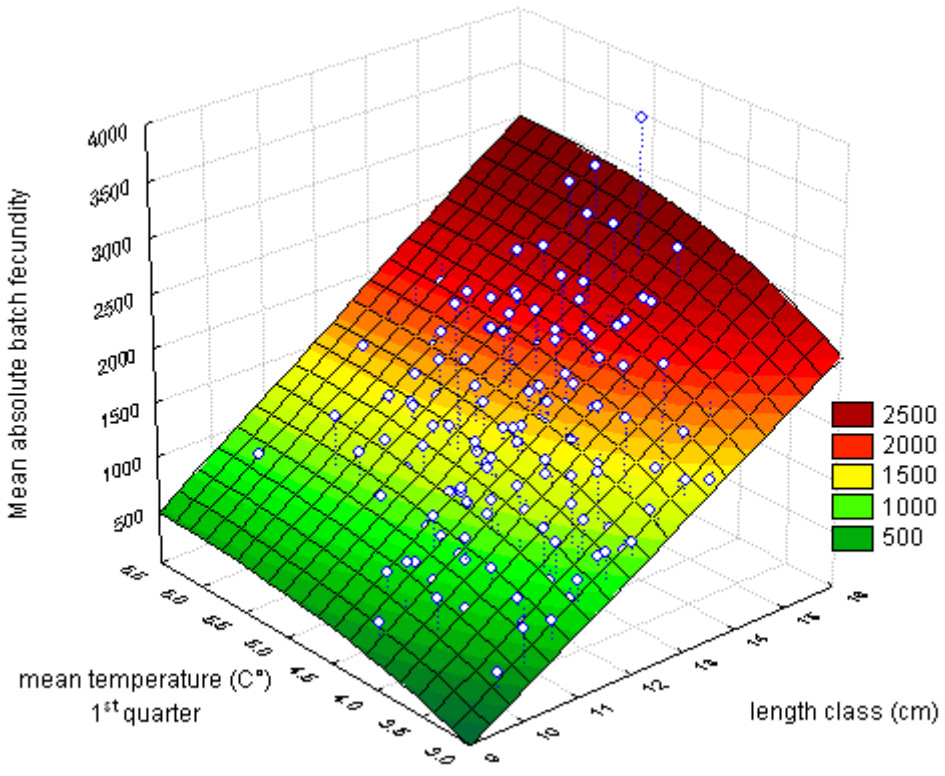

Fig. 6: Non-linear regression model of mean batch fecundity with fish length class and mean temperature as predictor variables. n=179; $r^2 = 0.70$.

I. 4 Discussion

This is the first study to reveal differences in batch fecundity of sprat among areas and years in the Baltic. The deep basins of the central Baltic Sea serve as main spawning area for sprat and are characterised by different hydrographic conditions. The differences found in sprat fecundity

can partly be related to these hydrographic differences among basins, but also to interannual hydrographic variability. Batch size was found to be positively related to mean temperature but also with mean salinity. Larger batch sizes were found in the Bornholm Basin, where the mean water temperature and the mean salinity in the depth range of sprat prevalence is in general higher than in the Gdansk Deep and the Southern Gotland Basin. Sprat in the Baltic lives at the northern boundary of the geographical distribution of this species (Muus & Nielsen, 1999) and is adapted to warmer and more saline waters. Consequently, the observed higher fecundity in years with higher water temperatures may result from better growth, earlier maturation, and enhanced gonadal development leading to a higher egg production (Grauman and Yula, 1989). Low water temperatures reduce and delay the onset of the spring zooplankton production in the Baltic (Dippner *et al*. 2000), which will reduce the availability of prey species, especially of warm adapted copepods as *Acartia spp*. (Möllmann *et al*. 2004), for sprat. This reduction of food availability after a cold winter may negatively affect individual sprat fecundity in the following spawning season. Higher batch fecundity was also associated to higher temperatures in other clupeoids, *e.g.* the engraulids *Encrasicholina heterobola* (Milton *et al.*, 1995) and *Engraulis japonicus* (Funamoto and Aoki, 2002).

However, the highest observed water temperatures did not result in the highest fecundity values. Hence, it was assumed that fecundity is related to temperature in the form of a flat top curve rather than showing a linear relationship. Further, it is reasonable to assume that at some point, when temperatures reach a certain critical level, fecundity will decrease again, following an optimum curve. Optimum or tolerance curves describing physiological or biological processes are a common phenomenon in ecology, especially in relation to temperature effects (Huey and Stevenson, 1979; Pörtner and Peck, 2010). Consequently, fitting relative fecundity as well as absolute fecundity data to models with a temperature dependent plateau resulted in a quite high percentage of explained variability. This might be explained by temperature induced physiological stress, which may affect the egg production negatively when ambient temperatures exceed a critical value. Such an effect was described for Mediterranean sardine (*Sardina pilchardus sardine*), which seems to down regulate batch fecundity by atresia when ambient temperature increases above a certain level (Ganias, 2009).

The linear regressions of sprat length and absolute batch fecundity showed distinct differences in slopes and intercepts between some years and areas. Differences in intercepts can be explained by variability in environmental conditions, affecting the egg production of the population without an interaction with fish size. The observed differences in the slopes of regressions can be explained in two ways: (i) it is an artefact of sampling; (ii) size dependent effects play a role, which affect smaller and larger sprat differently. The first case might be true, as in the three years where the slope of regression was found to be steeper compared to the other years, the small length classes are not well represented in the samples. The second case might be true, if *e.g.* food availability, ambient hydrographic conditions, or conditions during the winter, affect young fish and old fish differently. Young fish may be forced to allocate more energy into maintenance and somatic growth to survive when conditions are suboptimal, instead of investing energy into reproduction.

The only available information on sprat relative fecundity existing so far for the Bornholm Basin is given by Müller *et al*. (1990) with a value of 122 eggs g^{-1} for the year 1988. This value is within the range observed for this area in the present study. However, this estimate is based on the total female weight, and therefore probably underestimates the relative fecundity compared to the findings of the present study. In contrast to the findings of the present study Alekseev & Alekseeva (2005) reported a decreasing relative fecundity with increasing fish length for Baltic sprat from the Gdansk Deep. They observed values ranging from 137 to 163 eggs g^{-1} depending on fish size. They explained their observation due to decreasing growth potential of older fish and the decreasing ability of these old age groups to convert consumed food into biomass. The data obtained within the present study did not reveal a size effect on *RBF*. Hence, the assumption

by Alekseev and Alekseeva (2005) cannot be supported. However, they used gutted weight instead of the ovary free body weight to calculate *RBF* and results of these two studies are therefore not directly comparable.

In comparison to other sprat stocks, the observed values of mean relative batch fecundity for central Baltic sprat are low. For sprat of the southern North Sea and from the Kiel Bight, the mean relative batch fecundity was described with 413 eggs g^{-1} and 232 eggs g^{-1}, respectively (Alheit, 1988). Both areas are characterised by higher salinities than the central Baltic. Egg size of sprat eggs increases from the North Sea towards the Baltic related to decreasing salinity (Russell, 1976). This trend proceeds within the Baltic, as sprat egg size increases from west to east in relation to the decreasing salinity (Nissling *et al*. 2003). As the ovary size is restricted to the fish body size, an increase in oocyte size with decreasing salinity may lead to a decrease in the number of hydrated oocytes per batch, thus explaining the influence of salinity on batch fecundity, especially the decreasing trend from western to eastern areas. From the presented results it can be concluded that in the Bornholm Basin ambient temperature exerts the major effect on sprat batch fecundity. However, in combination with the information on sprat egg size from literature, it seems reasonable that spatial differences on a longitudinal axis from west to east can be explained by differences in salinity.

The history of sprat stock abundance showed highest values during the 90s of the last century, with a record of 2.937.000 t total biomass in 1995 (ICES, 2010). A decrease in weight-at-age was observed during this period of high sprat stock. This was explained by density dependent effects and a change in the abundance of important food organisms due to climatic processes (Cardinale *et al.*, 2002). The present study indicated a density dependent effect on sprat fecundity for the Bornholm Basin, which was negatively related to the acoustic stock size estimated by an acoustic survey. It cannot be ruled out that quality and quantity of food may have a significant effect on sprat growth and, subsequently, on fecundity. A food effect on fecundity has been shown before for other fish species. For example, increased food availability had a positive effect on fecundity in Mediterranean sardine (*Sardina pilchardus*; Ganias, 2009; Somarakis *et al*., 2004). Further, it was demonstrated that the variability in Baltic cod fecundity is related to the availability of prey (Kraus *et al*. 2002). However, as only few fecundity data exist for years in which the sprat stock showed highest abundances, and also zooplankton abundance data were not available for the present study, this issue should be addressed in future studies.

Differences in batch fecundity among years may also be affected by variability in the timing of spawning. An increase in batch size towards peak spawning and a decrease again to the end of the spawning season has been observed in former studies on sprat from Kiel Bight and the German Bight (Heidrich, 1925; Alheit, 1988). Data on batch fecundity presented by Alekseev and Alekseeva (2005) corroborate this trend for sprat in the south-eastern Baltic. However, it seems that batch fecundity during the main spawning season is relatively stable. For example, Kraus and Köster (2004) detected no changes in batch fecundity from March to June in 1999. Karasiova (2002) observed the timing of sprat peak spawning mainly in May/June over a broad range of years in the south eastern Baltic, with an exception for the year 1996, where the peak spawning shifted to July in the Gdansk Deep area. Therefore, we cannot entirely exclude the possibility that the variations we found in batch fecundity of Baltic sprat may at least partly be explained also by variability in the timing of spawning between years and areas.

With a model on Baltic sprat batch fecundity and available time series on egg abundances in the central Baltic, it would be possible to estimate stock sizes of certain sprat stock components for a wide range of years with egg production methods, for which direct information on batch fecundity are lacking. Such fishery independent stock estimates, even if the applicability to the entire stock may be limited, may serve as a tool to validate stock abundance data obtained by other assessment methods, *e.g.* acoustic surveys or virtual population analysis (VPA). This is especially interesting for the Baltic sprat stock, where conflicting results on stock size

estimations from acoustic surveys and the Multi Species VPA approach hampered quantitative studies on recruitment processes of sprat and cod so far, for example the estimation of predation pressure on cod eggs by sprat in the Bornholm Basin (Köster and Möllmann, 2000). The obtained non-linear regression model from the present study, taking into account an interaction between fish length and temperature, explained a quite high percentage of the variability in Baltic sprat batch fecundity. But it should be seen as a first step towards modelling Baltic sprat reproductive potential as further factors potentially impacting sprat fecundity, *e.g.* prey availability and growth, have not been taken into account so far.

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Chapter II: Seasonal variability of sprat spawning traits

Abstract

A number of spawning traits of Baltic sprat (*Sprattus sprattus balticus* S) have been analysed for the years 2005 and 2008 in order to reveal seasonal variability. Timing of spawning, batch fecundity, number of developing oocytes, spawning frequency, fish condition and atresia where included into analyses. Histology techniques in combination with image analyses have been applied to investigate different spawning stages of material sampled in 2008. Spawning sprat were detected from January to June. In November 2008 first signs of ovary maturation were observed. Relative batch fecundity was found to be low early in the year compared to summer with 85 eggs g^{-1} ovary free body weight observed in January 2005 and 165 eggs g^{-1} ovary free body weight observed in June 2008. Variability in batch fecundity during peak spawning was low. A seasonal decrease in oocyte dry weight and diameter was related to an increase in batch fecundity towards the end of spawning season. Spawning frequency was found to be relatively stable over the course of the spawning period with values slightly decreasing from 0.22 in March to 0.18 in June. Stereometric analyses confirmed the indeterminate spawning strategy of Baltic sprat. Prevalence of atresia was low during peak spawning (1 – 3%) but considerably higher during early spawning period (11%). In ripening ovaries from November atresia prevalence was 38%. Female sprat condition was low during spawning period and sharply increased after spawning ceased. The combination of histology and stereometric methods proved to be a valuable tool for analysing maturation, fecundity and atresia in Baltic sprat. The results of the present study are important with respect to the spawning dynamics of Baltic sprat.

Key words: batch fecundity, spawning frequency, developing oocytes, atresia

II. 1 Introduction

Strong seasonality in spawning activity is a common life history trait of temperate marine fish species. For a successful reproduction, it is essential that the produced early life stages are released in an environment where abiotic conditions and food availability are suitable for survival; a mechanism known as the "Match-Mismatch" concept (Cushing, 1975; Cushing, 1990). Consequently, the spawning strategy of many temperate species has evolved to be synchronised with the peak of primary and/or secondary production to match with sufficient food availability for larvae (Sherman *et al*., 1984). Marine habitats show a high variability in their environmental features, which will determine the optimum spawning time. Thus, batch spawning over an extended spawning season has evolved in many marine fish species producing pelagic eggs (Murua and Saborido-Rey, 2003). This spawning strategy will increase the probability of at least some offspring cohorts to find optimum conditions for growth and survival (Alheit, 1988), and will therefore increase the probability of successful reproduction.
In species that exhibit serial spawning, batch fecundity, spawning fraction and frequency, as well as egg quality may change over the course of the spawning season (Alheit, 1993; Trippel et al., 1997). For multiple spawning fish species, *e.g.* Bay of Biscay anchovy (*Engraulis encrasicolus*; Motos, 1996), European sardine (*Sardina pilchardus*; Zwolinski *et al*., 2001), and European hake (*Merluccius merluccius*; Murua *et al*., 2006), batch fecundity has been shown to vary over the spawning season. Spawning frequency might also vary interannually due to the stock age structure, food abundance or other environmental factors (Claramunt *et al*., 2007; Ganias, 2009).

An increasing spawning frequency with progressing spawning season has been described for anchovy (Motos, 1996). In the case of spawning frequency, it has also been stated that it may not vary seasonally when stocks inhabit environmentally stable habitats (McEvoy and McEvoy, 1992; Hunter and Lo, 1997). Fish egg quality, often assessed in terms of egg diameter or egg dry weight, has also been shown to vary over the spawning season (Blaxter and Hempel, 1963; Bagenal, 1971; Riveiro *et al*., 2004).

For a better understanding of the reproductive potential of a stock, it is important to characterise this aforementioned seasonal variability of spawning parameters. This is especially important for studies investigating the spatial and inter-annual variability of spawning traits. For such comparisons, it is a prerequisite to assure that the parameters to be compared are taken from the same phase in the spawning period (Alheit, 1988).

The spawning season of Baltic sprat (*Sprattus sprattus balticus* S.) is prolonged, lasting from February to August in the Baltic proper (Ojaveer and Kalejs, 2010). The onset of spawning may be dependent on temperature conditions, with extremely low winter temperatures causing a delay in the onset of spawning (Karasiova, 2002). For Baltic sprat, a multiple batch spawner with indeterminate oocyte recruitment, it has been shown that batch fecundity increases during the spawning season (Heidrich, 1925; Alekseev and Alekseeva, 2005). The only observation of spawning fraction and an estimate of spawning frequency for several consecutive months are given by Kraus and Köster (2004), who reported quite high variability in these parameters over the peak spawning season for the Bornholm Basin.

Sprat egg diameter and dry weight were found to decrease during the spawning season (Nissling *et al.*, 2003), which may be due to varying hydrographic conditions. How this change in egg size affects batch fecundity has never been investigated for Baltic sprat.

Atresia plays a role in down regulating the realized fecundity in a number of marine fish species. This is especially the case for determinate spawners, *e.g.* cod (*Gadus morhua;* Kraus *et al*. 2008), plaice (*Pleuronectes platessa*; Kennedy *et al*., 2007), and sole (*Solea solea*; Witthames and Greer Walker, 1995). These species are capital breeders (Jönsson, 1997), in which the cost of reproduction is financed by feeding prior to the spawning season. Hence, potential fecundity is determined before the onset of spawning. When conditions turn out to be sub-optimal, due to low food availability or unfavourable hydrographic conditions, ripening oocytes can be reabsorbed, and the gained energy may be used for the maintenance of essential physiological processes. In fish species with indeterminate oocyte recruitment, atresia might not play an important role, as the energy demanding process of oocyte recruitment can be immediately stopped if environmental conditions become sub-optimal. However, for European sardine it has been observed that higher levels of atresia occur when temperature conditions exceed a certain critical threshold (Ganias, 2009). Thus, also in these species atresia seems to be a mechanism to quickly activate energy reserves from ripening oocytes, which had been actually determined for spawning. For Baltic sprat, no prior studies have investigated atresia. Therefore, this aspect was included in the present investigation in order to assess if and to what extent atresia might occur in Baltic sprat ovaries and whether seasonal changes can be detected which might be explained by environmental factors.

In the present study, the seasonal changes of batch fecundity, developing oocytes number, diameter and dry weight as well as the prevalence of atretic oocytes were analysed. For this purpose, ovaries were sampled over the spawning season and analysed with histological methods. For the first time in this species, a stereological approach (Emerson *et al*., 1991; Murua *et al*., 2003) was used to assess the number of developing oocytes.

II. 2 Methods

Sprat were sampled in 2005 and 2008 during several research cruises conducted in the Bornholm Basin (Tab. 1). For the year 2005, determination of maturity was exclusively conducted macroscopically immediately after the haul on board. For this purpose at least 10 individuals per 1cm length class were staged. Additionally, hydrated females were sampled in January, April and May for batch fecundity analyses in 2005. Samples for the year 2008 were taken in March, April, May, June, August and November. In this year, up to five sub-samples of 2 kg sprat were taken from pelagic fishery hauls. Samples were immediately fixed in a buffered 8% formaldehyde solution. To assure a proper fixation, the body cavity of each fish was opened. In the laboratory, the sex and maturity of each fish was determined in a first work step by macroscopic inspection of the ovaries. Then, at least 20 female sprat were then sampled randomly from the sub-samples for subsequent quantitative determination of maturity stages by histology. For analysis of batch fecundity, additional females with hydrated ovaries were collected when present in the fishery hauls. From these females, the ovaries were removed and again fixed in a buffered formaldehyde solution for further processing. Histological sections (tissue embedded in paraffin; 3µm sections; Hematoxilin staining) were produced from each sampled ovary. During the cruise in August 2008, only few sprat were caught, and only five females could be collected for histological analysis. Only one sprat sub-sample was available for November 2008. A total of 471 ovaries were analysed histologically for the year 2008 (Tab. 1).

Tab. 1: Female sprat sampling for the year 2005 and 2008. DA = RV "Dana", AL = RV "Alkor", WH = RV "Walther Herwig III".

cruise	date of sampling	size range	number of females		
			histology	oocyte stage	fecundity
AL251	26th – 28th January 2005	11.4 – 14.0	0	0	25
AL255	20th – 23th April 2005	10.1 – 14.9	0	0	45
WH275	16th – 19th May 2005	9.6 – 15.3	0	0	102
DA 0208	13th - 15th March 2008	9.9 - 14.5	129	110	55
AL318	19th - 21th April 2008	10.5 - 13.4	113	104	44
WH312	12th - 18th May 2008	10.6 - 14.4	128	101	55
AL320	7th - 10th June 2008	11.2 - 14.2	70	60	31
AL324	27th August 2008	13.3 - 13.8	5	5	0
DA 0808	15th November 2008	9.8 - 14.6	26	26	0

For all females analysed in the laboratory, weight, gutted weight, total length, and ovary weight and ovary free body weight (*OFBW*) was determined. Fulton's condition index (*K*) was calculated taking into account total fish length (*L*) and *OFBW*:

$$K = \frac{OFBW}{TL^3} * 100 \qquad (1)$$

Oocyte developmental stages

All histological sections were checked for the developmental stage of oocytes and the presence of recent postovulatory follicles (POF). According to Brown-Peterson *et al*. (2010) five oocyte developmental stages were distinguished: (i) primary growth, (ii) cortical alveoli, (iii) vitellogenesis, (iv) nucleus migratory and (v) hydrated oocytes. Recent POF (<24h) were

identified using histological criteria published for Northern anchovy (*Engraulis mordax*) by Hunter *et al*. (1985). Additionally, each ovary section was checked for the presence of atretic cells.

Number of developing oocytes

The number of specific types of developing oocytes (*NDO*) was estimated for a subsample of ovaries covering all months in the year 2008 for which samples were available. For this purpose, the histological sections of ovaries were analysed with a stereometric method (Emerson *et al*., 1991; Murua *et al*., 2003). Stereology is the tri-dimensional interpretation of bi-dimensional sections of a structure (Weibel *et al*., 1966). In fecundity studies, it allows the estimation of the number of oocytes within an ovary from histological sections of this ovary. This is done by the use of a point grid, the "Weibel Grid", which is overlaid over the histological image of the section to be analysed (Fig. 1). The grid is constructed from hexagonal cells with known size. The distance between each point has to be less than the diameter of the smallest particle to be counted. For this procedure, an ImageJ (Rasband *et al.*, 1997-2009) application was used, allowing an automated process of overlaying digital images of histological sections with the Weibel grid (the ImageJ application has been published on www.fresh-cost.org). The used Weibel grid contains 168 points and has an area of 0.023 cm² (Fig. 1). For each ovary, four randomly defined areas of the histological section were analysed. As shown by Emerson *et al*. (1991), four areas are sufficient for an accurate estimation of the mean. However, in some few cases it was only possible to analyse three areas. First, each point of the grid which is touching the cross section of a developing oocyte is counted to determine the area of the respective oocyte groups. Second, the total number of individual oocyte cross sections within the area of the Weibel Grid is counted. By definition, oocytes touching the right hand and lower border of the Weibel Grid are not counted (Weibel, 1979). Finally, the Weibel formula (Weibel *et al*., 1966) was used to calculate the number of developing oocytes (NDO):

$$NDO = OV * \frac{C}{\beta} * \frac{{N_a}^{3/2}}{{V_i}^{1/2}} \quad (2)$$

where
OV = ovary volume
C = size distribution coefficient
β = shape coefficient
N_a = number of oocyte transections per unit area
V_i = the partial area of oocytes in the histological section

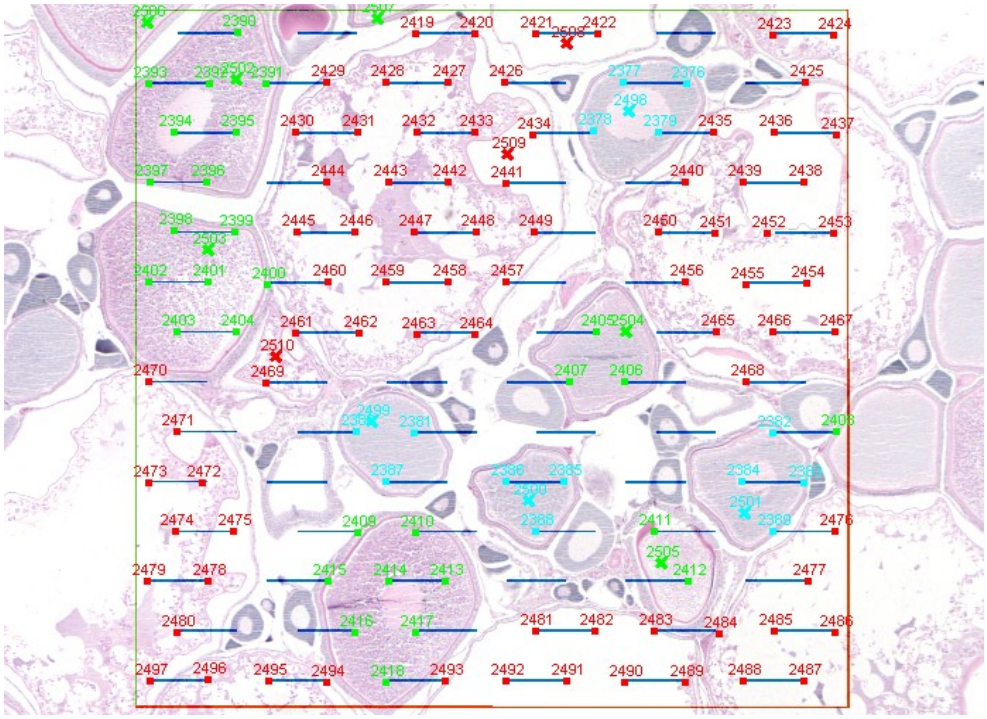

Fig. 1: Histological image of sprat ovary overlaid with the Weibel grid. The grid contains 168 points and has an area of 0.023 cm² (edge length 1516µm). The 84 horizontal bars visible in the grid represent two test points at their ends (n=168). Displayed are the colour coded point counts to estimate the partial area (V_i) of cortical alveoli oocytes (blue points), vitellogenic oocytes (green points), and oocytes with migratory nucleus (red points). Colour coded crosses represent the count of numbers of oocytes in different developmental stages (N_a). Oocytes touching the red borders of the grid are not counted.

Stereology has never been previously applied for Baltic sprat. Thus, ovary volume had to be determined as a function of ovary weight. Further, the correction factors *C* and β had to be estimated.

Based on a subsample of 21 sprat ovaries, covering a broad range of ovary weights, the relationship between ovary weight and ovary volume was estimated following the method introduced by Scherle (1970). Ovary weight (*OW*) was measured to the nearest 0.001 g. A beaker filled with 4% formaldehyde solution was placed on a scale and each ovary was immersed into the fluid. The weight of the displaced fluid was measured. Then the volume of each ovary was calculated by dividing the displaced weight of the solution through its density (4% formaldehyde solution: ρ=1.029 kg m^{-3}). A linear regression model was fitted to the obtained data of *OW* and *OV* which was used to estimate the ovary volume for each analysed ovary in the subsequent analysis.

The coefficient *C* was calculated with the formula given by Williams (1997):

$$C = \left(\frac{M_3}{M_1}\right)^{3/2} \quad (3)$$

M_1 is the mean oocyte diameter:

$$M_1 = [(\sum_{i=1}^{n} D_n)/n] \quad (4)$$

M_3 is the cube root of the third moment about the mean of the oocyte size distribution:

$$M_3 = \left[\frac{\sum_{i=1}^{n} D_n^3}{n}\right]^{1/3} \quad (5)$$

The shape coefficient β describes the ratio between the longest and shortest axis of a transected oocyte. For all analysed sections the longest and shortest axis of transected oocytes were measured with an image analysis system in order to calculate *C* and β. To obtain an unbiased estimate of the diameter, it is important only to measure oocytes which have been transected through their centre. This was achieved by only taking oocytes into account which have been transected through their nucleus. Emerson *et al*. (1991) recommend measuring at least 50 oocytes for the determination of *C*. This estimation of oocyte size distribution assumes that the nucleus diameter is constant over the whole range of measured oocytes. For several species it has been shown that this assumption is not correct (Murua *et al*. 2003; Domínguez Petit, 2006). Therefore, the relationship between oocyte diameter (*OD*) and nucleus diameter (*ND*) was determined from the analysed histological sections and a correction factor *D* was calculated:

$$D_i = \frac{LND}{ND_i} \quad (6)$$

Where *LND* is the *ND* from the largest observed oocyte with visible nucleus and ND_i is the mean nucleus diameter form the i_{th} oocyte size class. *LND* and ND_i were obtained by a non-linear regression model fitted through the observed data of nucleus diameter and oocyte diameter relationship. The obtained size distributions for each ovary where then corrected by multiplying the relative frequency of one oocyte size class by D_i. A total of 98 ovaries was analysed by stereology (Tab. 2).

Tab. 2: Female sprat with ovaries in different development stages analysed with stereology for the spawning season in 2008. CA = only cortical alveoli oocytes, VIT = vitellogenic, NM = nucleus migratory, HYD = hydrated.

month	size range (cm)	number of processed fish				
		CA	VIT	NM	HYD	total
March	9.9 - 14.5	4	14	10	10	38
April	11.1 - 13.1	1	5	8	6	20
May	11.0 - 13.9	2	8	5	5	20
June	11.5 - 14.2	0	8	7	5	20

Spawning frequency

The sum of hydrated oocytes and recent POF divided by two was defined as the proportion of females spawning per day and was used to estimate the spawning frequency. This procedure apparently reduces the error, and it has been demonstrated that spawning frequency estimation is more precise (Korta *et al*., 2010). This method assumes that the hydrated stage and the duration of recent POF lasts approximately one day each. The mean and variance of spawning frequency was estimated with the following equations (Piquelle & Stauffer, 1985):

$$S = \frac{\sum_{i=1}^{n} m_i \times y_i}{\sum_{i=1}^{n} m_i} \quad (7)$$

$$Var(S) = \frac{\sum_{i=1}^{n} m^2 \times (y_1 - \bar{S})}{\left(\sum_{i=1}^{n} m_i / n\right)^2 \times n(n-1)} \quad (8)$$

where m_i is the number of mature females in the i_{th} haul, and y_i is the proportion of hydrated females and females with recent POF divided by two.

Batch fecundity

Batch fecundity was estimated gravimetrically by using females with fully hydrated ovaries, applying the hydrated oocyte method (Hunter *et al.*, 1985). For the year 2008 ovaries with recent POFs were excluded from the analysis, in order to minimise the risk of underestimation of batch fecundity. It might be that some females had already started spawning before the catch, or that some females lost hydrated oocytes due to handling while being sampled. Hydrated oocytes were separated from the ovaries and counted manually under a stereo microscope. For some ovaries, batch fecundity was also estimated with stereology. This method is described in detail below. By dividing the absolute batch fecundity value by the ovary free body weight (*OFBW*) the relative batch fecundity (*RBF*) was calculated for each fish.

Dry weight and diameter of hydrated oocytes

With an image analysis system (Leica QWin), diameters of hydrated oocytes were determined to the nearest µm for the 2008 sampling. For this purpose hydrated oocytes were stained with benguela rosa and photographed under a stereo microscope. In addition the hydrated oocyte dry weight was measured from a subsample of ovaries, for each cruise in 2008 where hydrated females were found. The completed hydration process of ovaries, for which oocyte dry weight and diameter were determined, was confirmed histologically.

II. 3 Results

Oocyte developmental stages

In 2005, female sprats in spawning condition were found already in January samples. Although no histological analysis was performed for samples from this year, incidence of spawning was obvious from macroscopic inspections of sprat ovaries since hydrated females were present in the samples. In 2008, spawning females were found in March, April, May and June samples (Tab. 3). Spawning was fully established during April, May and June, as nearly all of the analysed ovaries contained oocytes in the cortical alveoli and the vitellogenic stage in these months. In March 2008, the share of ovaries with vitellogenic oocytes was slightly lower with 90%, and also the proportion of ovaries containing POF (42%) was not as high as in the following three months. The proportion of ovaries containing oocytes in the nucleus migratory stage and hydrated oocytes varied considerably within the spawning period. While the proportion of ovaries with nucleus migratory oocytes increased from 25% in March to 43% in June, the proportion of females with hydrated oocytes was relatively stable in the range of 33% to 37%. Ovaries containing recent POFs decreased from March to June, but the percentage of ovaries containing POFs, regardless of POF stage, increased up to 100% in June. In August, none of the analysed ovaries showed signs of developing oocytes or recent spawning. In November, 77% of analysed ovaries contained cortical alveoli, and 19% vitellogenic oocytes, but no POF were

detected. The presence of vitellogenic oocytes is indicating that spawning will commence again soon.

Tab. 3: Proportion (%) of different oocyte development stages, post ovulatory follicles (POF), and atretic oocytes. CA = cortical alveoli, VIT = vitellogenic, NM = nucleus migratory, HYD = hydrated.

month	n	oocyte development				POF		atresia
		CA	VIT	NM	HYD	<24h	all	
March	110	99.1	90.1	25.2	33.3	8.1	42.3	10.8
April	104	100.0	99.0	26.9	36.5	5.8	88.5	1.0
May	101	100.0	97.0	29.7	34.7	5.9	76.2	3.0
June	60	100.0	100.0	43.3	35.0	1.7	100.0	0.0
August	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
November	26	76.9	19.2	0.0	0.0	0.0	0.0	38.5

Coefficient estimation for stereology

The relationship between ovary weight and volume could well be defined by a linear regression model with the intercept forced through the origin (Fig. 2; r^2=0.99; $p<0.05$):

$$OV = 0.931\ (\pm 0.018\ SE) * OW \qquad (9)$$

This regression model was used in the stereological analysis to calculate the ovary volume from each analysed ovary.

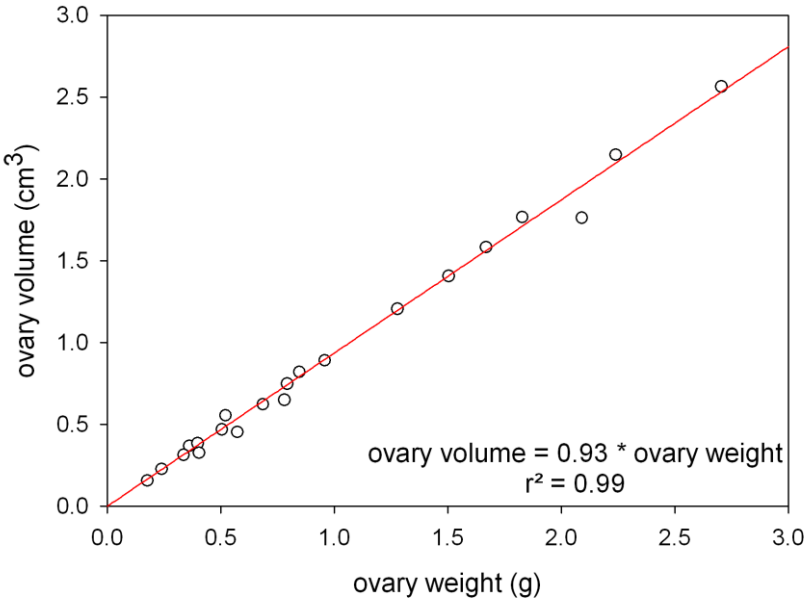

Fig. 2: Relation between sprat ovary weight and ovary volume with linear regression model forced through the origin.

The individual *C* values for the analysed ovaries ranged between 1.02 and 1.46. In some cases it was not possible to measure a sufficient number of oocytes for calculating *C*. In these cases, a mean value from ovaries in the same developmental stage was used (Tab. 4).

Tab. 4: Mean values of the size distribution correction factor *C* obtained for ovaries in different developmental stages. CA = cortical alveoli, VIT = vitellogenic, NM = nucleus migratory, HYD = hydrated; sd = standard deviation.

stage	*C*	sd
CA	1.04	0.02
Vit	1.13	0.04
NM	1.23	0.08
HYD	1.15	0.12

The shape correction factor β was estimated with 0.83 (±0.1.2 SD; n=4237 oocytes measured) for the analyzed ovaries. The relationship between oocyte diameter (*OD*) and nucleus diameter (*ND*) is displayed in Fig. 3a. A power function was fitted to the data (r^2=0.87: $p<0.05$):

$$ND = 8.91(\pm 2.32\ \text{SE}) * OD^{0.46\ (\pm 0.04\ \text{SE})} \quad (10)$$

LND was estimated by this model for the largest observed oocyte with visible nucleus. Then *D* was calculated for each oocyte size group using equation 7 (Fig. 3b).

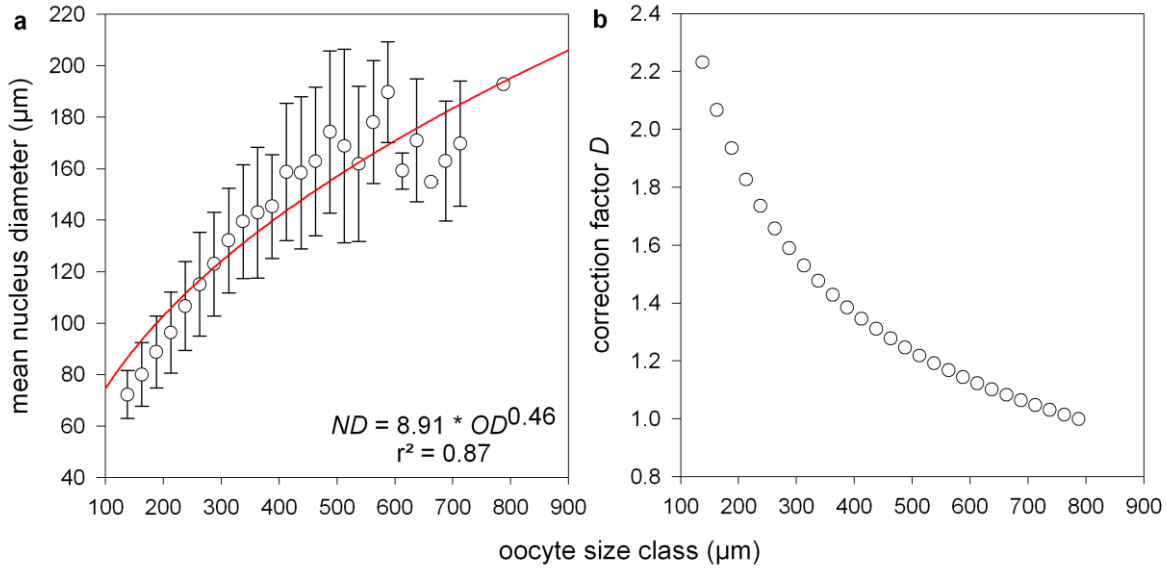

Fig. 3: Relationship between nucleus diameter (±SD) and oocyte size class (a), and the relationship between correction factor *D* and oocyte size class (b).

Number of developing oocytes

The number of oocytes in specific developmental stages increased with fish length (Fig. 4a). The number of the cortical alveoli stage was highest, followed by the vitellogenic oocytes and the nucleus migratory oocytes. Over the spawning season, the relative number of oocytes in the cortical alveoli stage showed an increasing trend from March (1613 ± 562 SD) until June (2342 ± 819 SD) (ANOVA, $p<0.05$; HSD-test, $p<0.05$). Relative numbers of oocytes in the vitellogenic stage showed a slight increase of values from March until May, followed by somewhat lower values for June. However, no significant differences were detected between months in this case (ANOVA, $p>0.05$). Oocytes in the nucleus migratory stage showed lowest values in March, compared to the nearly equal values observed for April, May and June (Fig. 4b). Since no homogeneity of variances could be achieved through transformation, only the extended median test could be performed in this case, which does not reveal significant differences between months. The proportions of cortical alveoli, vitellogenic and nucleus migratory oocytes were the same for April and May 2008, and also similar to March and June. The mean proportions of these three developmental stages were 60% cortical alveoli, 25% vitellogenic and 14% nucleus migratory. Nucleus migratory and hydrated oocytes were never observed parallel in the same ovary, indicating that all nucleus migratory oocytes belong to the developing batch to be spawned. However, comparing the observed numbers of these two developmental stages, this was only supported by the data from March and April, but not for May and June, where the number of hydrated oocytes in the ovaries was approximately the half of nucleus migratory. A comparison between batch fecundity obtained by manual counting and the stereometric method revealed substantial differences (Fig. 5). Batch fecundity estimated with the stereometric method was higher in all cases than the batch fecundity estimated manually by the hydrated oocyte method.

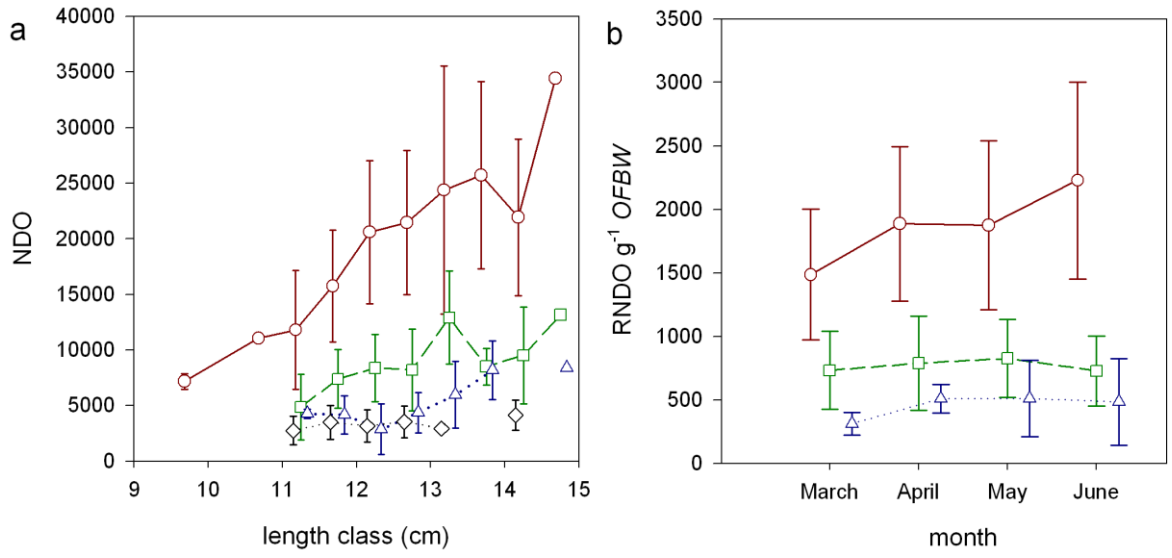

Fig. 4: Stereology results. (a) Relationship of mean *NDO* with fish length, (b) relative *NDO* corrected for ovary free body weight over the spawning season. Red circles = cortical alveoli, green squares = vitellogenic oocytes, blue triangles = nucleus migratory, black diamonds = hydrated oocytes. Error bars denote standard deviations.

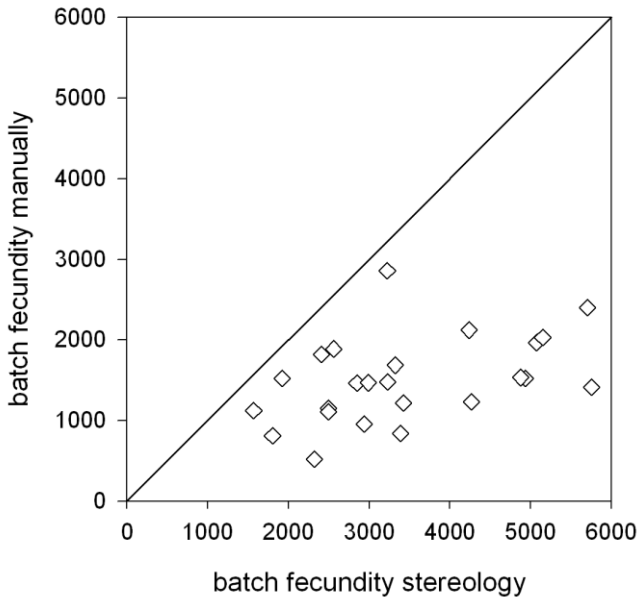

Fig. 5. Comparison between batch fecundity obtained by the hydrated oocyte method (manual counts hydrated oocytes) and the stereometric estimation.

Atresia in Baltic sprat

Atretic oocytes were found in sprat sampled in March, April, May and November 2008. In March 10.8% of female sprat were found to have atretic oocytes, while in April and May the value was considerably lower, with only 1% and 3% respectively. The highest level of atresia was found in November 2008 with 38% of all examined ovaries containing atretic oocytes. In all cases, atresia was associated to ovaries in the vitellogenic or cortical alveoli stage. Stereologic analyses revealed a percentage of 2% to 30% of atretic oocytes in the ovaries for March, with the exception of one fish in which mass atresia was detected. In this special case the number of atretic oocytes exceeded the number of healthy oocytes. In two ovaries analysed stereologically from May, the percentage of atretic cells was 3.6% and 8.2%. Atretic ovaries from November were not analysed stereologically.

Batch fecundity

In 2005, hydrated females were found in January, April and May. In 2008, hydrated females were detected from March to June. In August of both years, no females in spawning condition were detected any more, indicating that the spawning season had ceased already in late summer. In 2005, a total of 172 hydrated ovaries were analysed for batch fecundity. In 2008, a total of 178 ovaries contained hydrated oocytes. From these ovaries, 144 were used for fecundity estimation; the rest was excluded due to the presence of recent POFs detected by histological inspection. However, excluding ovaries with recent POF from fecundity analyses did not lead to significantly different results, probably because of the high variability observed in batch fecundity. Hence, the results of both years are still comparable. Variability in batch fecundity was found to be high in both years. It ranged from 426 to 2865 hydrated oocytes in 2005, and from 333 to 3234 hydrated oocytes in the year 2008. Batch fecundity showed an increasing trend with increasing fish length, and data were fitted to linear regression models with length as continuous co-variable (Fig. 6). Analysis of covariance revealed no statistically significant differences among the slopes of regressions, as well for 2005 as for 2008 (ANCOVA; $p<0.05$).

The differences in the intercepts were found to be statistically significant among months in both years (ANCOVA; $p<0.05$), with lower intercepts for the regressions obtained for January 2005 and March 2008, respectively.

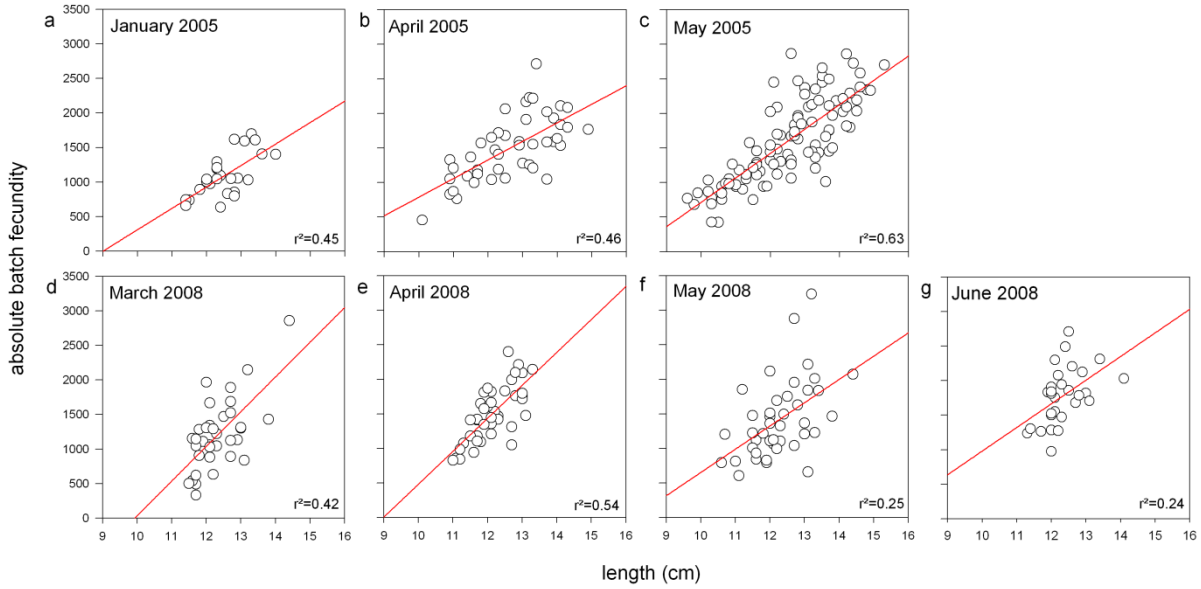

Fig. 6: Relationship between total fish length and batch fecundity in Baltic sprat for different months in 2005 (a, b, c) and in 2008 (d, e, f, g). Red lines show significant linear regressions ($p<0.05$).

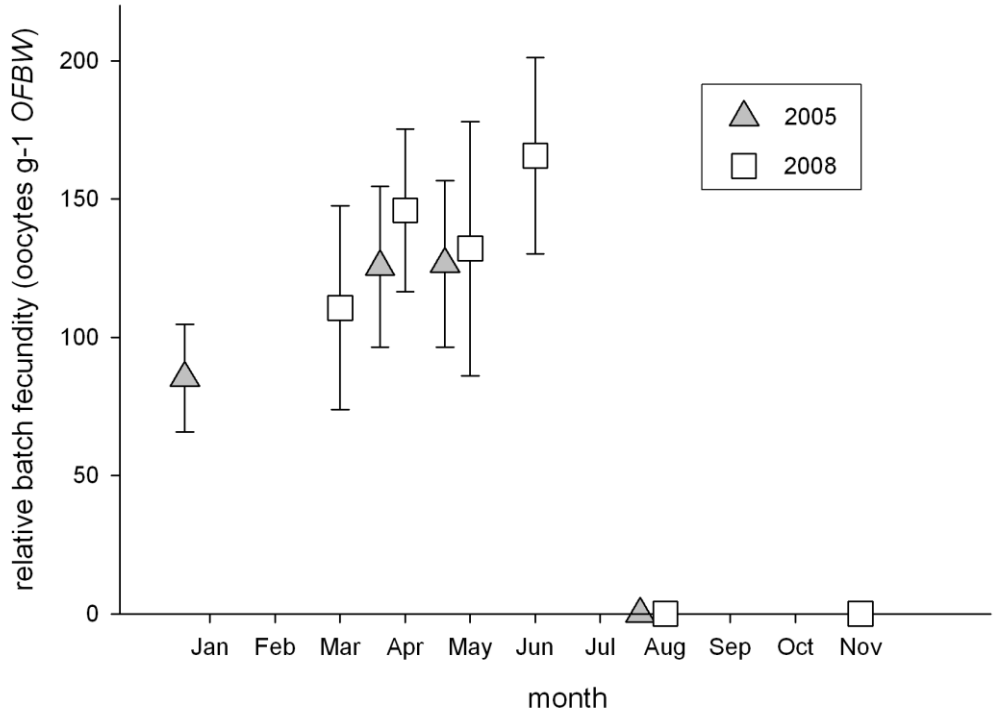

Fig. 3: Seasonal variability in mean relative Baltic sprat batch fecundity for the years 2005 (triangles) and 2008 (squares). Error bars denote the standard deviation of the mean. Numbers of analysed sprat are listed in table 1.

The mean relative batch fecundity of January 2005 was significantly lower compared to April and May 2005 (ANOVA, $p<0.05$; HSD-Test, $p<0.05$), which did not differ significantly from each other. The relative mean batch fecundity in March 2008 was significantly lower than in all other months of that year (ANOVA; HSD-Test, $p<0.05$; Fig. 7). Batch fecundity for April and May 2008 did not differ significantly, whereas batch fecundity from June 2008 was significantly higher than batch fecundity from May 2008. However, June values did not differ significantly from values observed for April 2008. Between 2005 and 2008 no significant differences were found between the months of April and May.
The Fulton's condition index observed in 2008 decreased from March to April, then increasing slightly to May and June, and increased sharply towards August. November values were only slightly lower compared to August values.

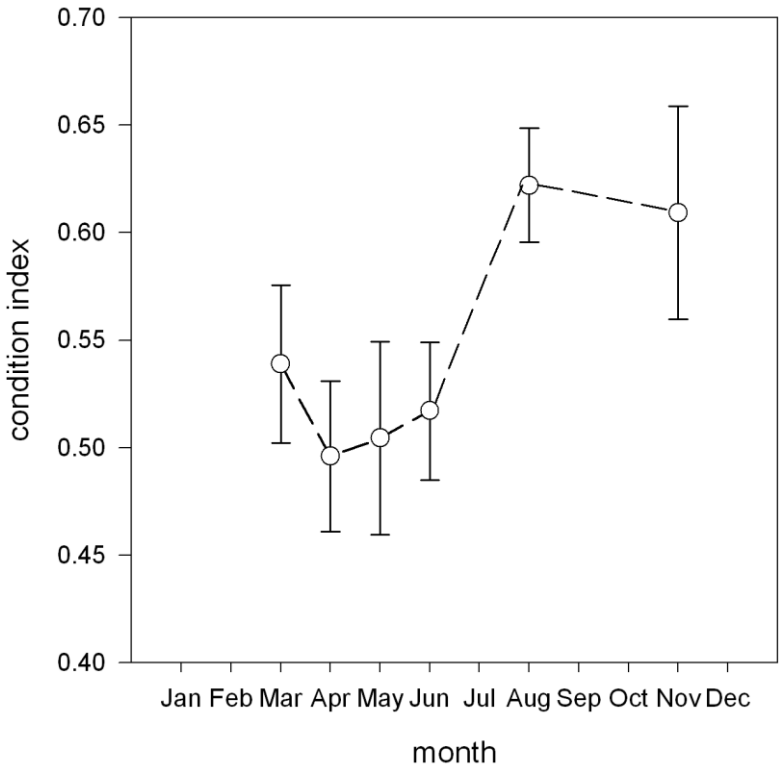

Fig. 4: Change in Fulton's condition index of female Baltic sprat over the course of the spawning season in 2008. Error bars denote the standard deviation. Numbers of analysed sprat are listed in table 3.

Hydrated oocyte dry weight and diameter

The dry weight of hydrated oocytes was not related with fish length or weight in Baltic sprat. Significant differences were found for the mean dry weights of hydrated oocytes among the tested months (ANOVA, $p<0.05$; Fig. 5a). Values of March (0.025 mg ±0.008 SD) and April (0.0029 mg ±0.008 SD) differed significantly from May (0.017 mg ±0.005 SD) and June (0.015 mg ±0.004 SD) values (HSD, $p<0.05$).
The mean diameter of hydrated oocytes was not related with fish weight. Also no relation was found with fish length for March, April and May. Only for June a weak positive relation was found with length ($r^2=0.17$; $p=0.04$). Therefore, in subsequent analyses length was not included as a covariate and the seasonal effect on oocyte diameter was tested with an ANOVA. The mean

oocyte diameter was found to decrease with progressing spawning season (ANOVA, $p<0.05$; Fig. 5b). The observed values of March (1232 µm ± 122 SD), April (1230 µm ± 109 SD) and May (1203 µm ± 119 SD) differed not significantly, but mean oocyte diameter of June (1138 µm ± 82 SD) was significantly lower compared to March and April values (HSD, $p<0.05$). This trend is similar to the decreasing trend in oocyte dry weight.

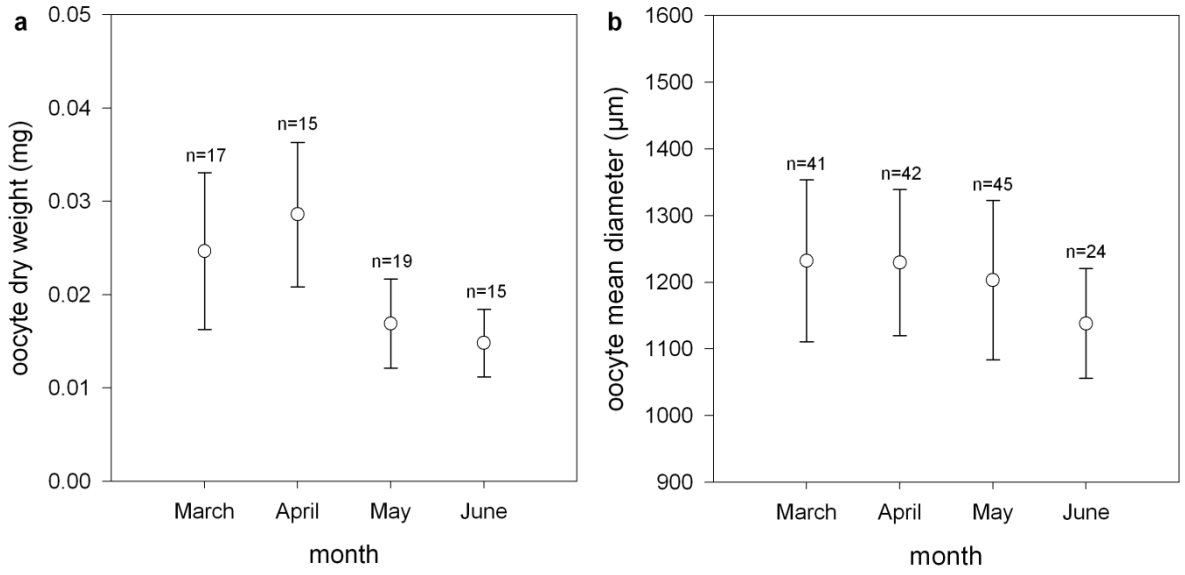

Fig. 5: Seasonal variability in hydrated oocyte mean dry weight ±SD (a), and mean oocyte diameter ±SD (b) of Baltic sprat.

Spawning frequency

Spawning frequency decreased during the spawning season with highest values in March (0.22; CV=1.11), slightly lower values in April (0.21; CV=0.65) and May (0.21; CV=0.44), and the lowest observed value in June (0.18; CV=0.19).

II. 4 Discussion

Timing of spawning

Baltic sprat spawning activity was already detected for January in the year 2005, albeit at relatively low levels. This is earlier than mentioned in most reports of sprat spawning for the central Baltic and demonstrates that the perception of the spawning dynamics of this species is not very clear yet. This is certainly due to the lack of continuous data sampling over the whole year in this area, which would enable a more precise resolution of the spawning dynamics in relation to environmental factors. For Baltic sprat, a prolonged spawning season has been reported with the peak of spawning activity from spring to midsummer. Kraus *et al*. (2003) reported a second spawning peak in autumn 2002, which was explained by an exceptional warm water inflow into the central Baltic basins in summer. The data of the present study show that sprat ovaries sampled in November 2008 already contained oocytes in the vitellogenic stage. This indicates that spawning probably commenced some days later. Either this was due to the occurrence of a second spawning peak as observed for 2002 (Kraus *et al.*, 2003), or the next regular spawning season after the resting phase in summer already begun. As no data were

available for winter and the early period of the following year, this question cannot be answered. However, if female sprat allocated energy to oocyte development during winter time on a regular basis, either during a second spawning peak in autumn or due to an early beginning of spawning activities, this must have consequences for growth and eventually survival through the winter. If such second spawning peak will result in viable offspring, which eventually survives and recruits to the population is questionable.

Number of developing oocytes

The increasing number of cortical alveoli oocytes from March until June clearly confirms the indeterminate oocyte recruitment in Baltic sprat, which has been described before (Heidrich, 1925; Polivaiko, 1980; Alheit, 1993). New oocytes recruited from the reserve pool throughout the spawning season. The mean number of oocytes in the cortical alveoli stage was always higher compared to the number of vitellogenic oocytes, which was, in turn, higher than that of oocytes in the nucleus migratory stage in all four months, although differences were not significant for April, May and June between the latter two stages. If it is assumed that the recruiting oocytes continuously develop to the next developmental stage until final hydration is reached, than the observed differences in numbers between the stages gives evidence for stage-specific development durations. Thus, cortical alveoli would last more than twice as long as the vitellogenic and nucleus migratory stages, which show a similar stage duration because quite similar numbers of both were found in the ovaries.
In the present study, the results of batch fecundity estimation with the stereometric method clearly indicated that the number of hydrated oocytes is overestimated in all cases compared to the gravimetric method and manual counting. This is in contrast to findings for hake (*Merluccius merluccius*; Domínguez-Petit, 2006) and also Brazilian sardine (*Sardinella brasiliensis*; Isaac-Nahum *et al.*, 1988), for which good agreement has been reported between the stereologic approach and conventional methods. However, the stereometric method makes some assumptions about the three dimensional nature of the oocytes within the ovary, which are not necessarily true. In addition, some bias is always introduced through the histological processing, which may cause some deformation of the ovary tissue, and especially the shrinkage of large oocytes. To overcome these simplifications and potential sources of error, correction factors on shape and the form of the size distribution of oocytes are included into Weibel's formula. In theory, the stereometric approach would best work for perfect spheres. Hence, bias increases with progressive developmental stages of the oocytes. Therefore, the estimation of batch fecundity must have the greatest bias, as the hydrated oocytes are most vulnerable to deformation due to the histological processing and their shape differs most from being spherical in the histological sections. It might be concluded that also the numbers of migratory nucleus oocytes are biased, which can explain the difference observed between numbers of vitellogenic and nucleus migratory. It is also possible that this observation represents the asynchronous oocyte recruitment in Baltic sprat. Nevertheless, the stereometric approach proved to be a valuable, time-saving tool to estimate the number of developing oocytes, especially the cortical alveoli and vitellogenic stage in a number of sprat ovaries. A further advantage is that the oocytes cannot only be counted, but the exact developmental stage of oocytes can be determined using histology. In addition, this method enables quantitative analyses of atresia in fish ovaries.

Atresia

Atresia was detected in Baltic sprat ovaries, with lowest values during the peak spawning period in April, May and June, and highest values in November and March, when the development of oocytes started again. This seasonal pattern of atresia could be due to sub-optimal conditions for spawning during the onset and towards the end of the spawning period. Ganias (2009) reported

that the prevalence of atresia in European sardine in the eastern Mediterranean was related to temperature. Therefore, it is possible that ambient temperature and/or food availability were not in the range required by sprat for maximum reproductive output, and less energy could therefore be allocated to reproduction. Atresia might be a mechanism for indeterminate spawners to react quickly to short-term changes in environmental conditions, especially a sudden shortage of food supply. However, batch fecundity was already lower in March compared to the rest of the peak spawning period, and only in 10% of female sprat atresia was detected. Hence, it seems that the direct regulation of fecundity in advance of oocyte recruitment is more important, and better reflects the adjustment of reproductive output to the prevailing environmental conditions on a population level. The high levels of atresia found in November can be interpreted similarly. As described by Kraus *et al*. (2003) for 2002, this observed oocyte recruitment may be a second spawning peak, triggered by exceptionally favourable environmental conditions late in the year. If conditions changed, parts, or all of the already recruited oocytes, could have been reabsorbed in November 2008. This cannot be solved here, because further observations on adult sprat or ichthyoplankton samplings covering the following months are lacking.

Seasonality in batch fecundity

Variability in fecundity has been described before for Baltic sprat (Heidrich, 1925; Alekseev & Alekseeva, 2005). The peak spawning might be delayed by extremely cold temperatures in spring, following a hard winter (Karasiova, 2002). Thus, it cannot be ruled out that inter annual comparisons of batch fecundity might be biased by this seasonality (Alheit, 1988) if the shape of the fecundity curve over the whole spawning season is unknown. In the present study, batch fecundity was found to be lower in the beginning of the spawning season and increased towards its end. It also becomes clear from the presented data that fecundity values during the main spawning season do not vary significantly. Highest batch fecundity was found in June, whereas in August the spawning activity has completely ceased already, thus the value of batch fecundity in this month was theoretically assumed to be zero. This means that the fecundity decreases faster towards the end of the spawning season than it is increasing at the beginning. It also appears possible that the recruitment of new oocytes is stopped immediately towards the end of the spawning season and the organic substance of already developed oocytes is reabsorbed via atresia. The presented observations on batch fecundity make it reasonable to assume that the fecundity curve increases at the beginning of the spawning season, reaches a plateau at peak spawning, and decreases rapidly towards the end of the spawning season. Consequently, when comparing Baltic sprat batch fecundity during peak spawning between years, the probability of bias due to seasonality is low, as Baltic sprat fecundity values will be rather stable over several months during spring and early summer.

Sprat condition during spawning season

Fish with a capital breeding strategy show a distinct reduction in condition over the course of the spawning season (Ganias, 2009; Henderson *et al*., 1996). The condition data obtained during the present study for the year 2008 suggest that sprat condition was relatively low from March to April, and then slightly increased until June (i. e. the end of the spawning season). Thus, it is likely that in March, sprat recruit oocytes at the expense of remaining energy reserves from overwintering. In April, when more prey organisms for sprat are available, incoming energy might be transferred directly into egg production. Thus, the condition increases in parallel to batch fecundity. When spawning has ceased in midsummer, energy from food uptake can entirely be transferred into somatic growth and condition increases again sharply. This implies that winter condition affects the initial batch fecundity of the following spawning season in Baltic sprat. The worst scenario would be a mild winter with low prey abundance. But also an

extremely cold winter with low food abundance and a delayed zooplankton production in spring (Dippner *et al*., 1997) will affect sprat egg production negatively.

Oocyte diameter and dry weight

The dry weight as well as the diameter of hydrated oocytes decreased during the course of the peak spawning. This is well in line with the increasing trend of batch fecundity. It is discussed in the literature that there is a trade-off between egg size and fecundity in fish, and that egg size is a maternal response to the variability in environmental conditions (Castro *et al*., 2009). Larger eggs often seem to be of better quality, and larval size is directly correlated to egg size. Hence, producing larger eggs may increase larval survival probability. It has been hypothesised that the production of larger eggs at the beginning of the spawning season is related to colder temperatures in multiple batch spawners, *e.g.* in *Engraulis ringens* and *Sardina pilchardus* (Castro *et al*., 2009; Riveiro *et al.*, 2004). This might also be the case in Baltic sprat. Eggs are heavier and larger at the beginning of the spawning season, when temperatures are colder. These eggs contain probably more lipids and proteins. Low temperatures will decelerate the development and growth, and consequently the need of exogenous feeding for larvae will also be delayed. Given that the yolk-sac larvae in a cold environment are provided with a sufficient amount of nutrients, this deceleration of development might be of advantage to match with suitable food availability, which may not be fully achieved early in the spawning season. In turn, oocytes with a smaller amount of nutrients will also result in successful embryonic and larval development in warmer ambient water temperatures towards the end of spawning season despite of their shorter development time, since first feeding larvae are at once in an environment where sufficient prey organisms are available. Thus, the female fish can then produce more eggs to be spawned to further increase the probability of successful reproduction.
A change in weight and size of pelagic fish eggs has also consequences for their specific gravity. In the strongly stratified Baltic Sea, this determines their vertical distribution in the water column (Nissling *et al.*, 2003). Neutral buoyancy is a prerequisite for the successful development of most pelagic fish eggs, especially in the central Baltic basins, where anoxic water layers occur in deeper water layers (Wieland *et al*., 2000). Therefore, the change in sprat egg size over the spawning season may also be in response to changes in physical factors of the ambient environment, in order to maintain neutral buoyancy.

Spawning frequency

Baltic sprat spawning frequency showed only minor changes over the course of peak spawning in 2008, which is in contrast to previous findings for the Bornholm Basin. In the year 1999, Kraus and Köster (2004) observed a very high spawning fraction for April (63-93%), and an average value of 27% for May, June and July, with somewhat lower values towards the end of spawning season. The exceptionally high values in April, which would translate into a rather unrealistic spawning frequency of approximately one day, was explained as being an early phase of spawning, with no spawning pattern yet established in the stock and ongoing migration of actively spawning females into the investigation area. The values observed for May to July by Kraus and Köster (2004) come close to the values of the present study, and also the slight decrease towards the end of spawning activity is in line with the present findings. Possibly, the spawning activity in 1999 was delayed compared to the year 2008, where spawning was already fully established in March, thus this estimate of spawning frequency was not biased due to ongoing migration processes of actively spawning females. Another source of uncertainty is the difference in methods used to determine the spawning frequency. The previous estimation of spawning frequency by Kraus and Köster (2004) was solely based on macroscopic inspection of sprat ovaries applying the hydrated oocyte method (Hunter and Macewicz, 1985), whereas the

present findings also take the presence of postovulatory follicles (POF) into account. Macroscopic determination of the maturity stage is certainly not as precise as histological maturity classification. Further, it has been shown that spawning clupeoids form spawning aggregations and are probably more vulnerable to the fishing gear, which might lead to an overestimation of the proportion of hydrated females (Goldberg and Hunter, 1984; Alheit, 1985). Therefore, the postovulatory follicle method was proposed to estimate more precisely the spawning frequency from field samplings (Hunter *et al.*, 1985; Ganias *et al*., 2003). Both methods still are dependent on the assumption that the fraction of sampled fish showing a certain maturity criterion, only hydrated or the average of hydrated and females with recent POF, represents the daily spawning fraction of the female stock. If the duration of recent POF lasts longer in Baltic sprat due to low ambient temperatures, compared to other clupeid species, spawning frequency would be overestimated. For other pelagic fish species the use of hydrated females and recent POF were shown to be a good estimate of spawning frequency (Isaac-Nahum *et al*., 1988; Korta *et al*., 2010; Domínguez-Petit, 2006). However, neither the exact duration of the hydrated oocyte stage nor the POF stage duration is known for Baltic sprat. Additionally, there exists contradictory information about the diel spawning behaviour of Baltic sprat in literature (Balzar, 1994; Alekseev and Alekseeva, 2005). But the observed spawning stages in the present study can be used to compare the relative rate of spawning during spawning season.

The observed inter-annual and intra-annual consistency in spawning frequency, with the exception of April 1999 values (Kraus and Köster, 2004), is in line with observations in other clupeoid batch spawners. For Northern anchovy (*Engraulis mordax*), low variability in spawning frequency has been reported as long as the environmental conditions are relatively stable (Hunter and Lo, 1997). Ganias *et al*. (2003) found low variability in Mediterranean sardine (*Sardina pilchardus*) spawning frequency, which was explained by stable environmental conditions in addition with equally sized females, as spawning frequency has also been shown to be dependent on fish size (Parrish *et al.*, 1989; Claramunt *et al.*, 2007; Ganias *et al.*, 2003).

Spawning frequency is an essential input parameter for the DEPM (Parker, 1985), but also the most biased parameter in the majority of DEPM applications (Stratoudakis *et al*., 2006). It has to be stated here, that the results of the present study are based on comparatively low sample sizes and further investigations to clarify the recent findings on sprat spawning frequency would be desirable. However, the low variability in spawning frequency and batch fecundity, as observed for Baltic sprat in the present study, would translate into a continuous daily egg production over the course of peak spawning, which meets the underlying assumption to estimate the spawning stock biomass with the DEPM (Alheit, 1993). Hence, this method seems adequate to alternatively estimate Baltic sprat stock, independent of fishery data and on a regional scale, for years in which ichthyoplankton data are available.

Conclusion

The results of the present study clearly demonstrate that sampling for studies on fecundity and maturation dynamics should be extended to time periods beyond the peak spawning. This may especially become relevant when a possible shift to warmer ambient temperatures, due to climate change, affects the pelagic environment, and will cause changes in the life history traits of organisms assigned to all trophic levels of the ecosystem. Only a sampling coverage over the whole year, and that for several years with contrasting environmental conditions, will give the possibility to explore in detail mechanisms of spawning dynamics of marine fish species in relation to environmental variability in the field. The combination of histology and stereometric methods is recommended to differentially uncover oocyte development, fecundity and atresia in further studies on sprat maturation dynamics.

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Chapter III: Observations on sex ratio and maturity ogives of Baltic sprat

Abstract

Data on length frequency distributions, growth, sex ratios and maturity ogives of Baltic sprat were collected from 1999 to 2009 in different ICES sub-divisions of the south-central Baltic Sea. Regression models were constructed to predict sex ratio and proportion mature sprat in relation to fish length. Additionally, a data set obtained from the former Study Group on Baltic Herring and Sprat Maturity (SGBHSM) was included into the analyses in order to investigate the variability in size at maturity. A shift in the most frequent length class was observed from West to East with a higher proportion of larger sprat in ICES sub-division 24. The pattern in sex ratios was found to be similar for each ICES sub-division with a higher proportion of females in the larger size classes. Spatial variability in size at which sprat mature was not found to be statistically significant. Von Bertalanffy Growth Functions were fitted to age-at-length data to analyse sprat growth in relation to sex. In all areas, female grew larger and mature at a larger size compared to males. Size at maturity could be related to winter temperature and stock size for sprat in ICES sub-division 25. The results of the present study are relevant to estimate the proportion of sprat stock contributing to spawning and form the basis to develop alternative indices to assess the stock reproductive potential of this stock in the Baltic.

Key words: maturity ogive, sex ratio, growth, Baltic sprat

III. 1 Introduction

Maturity of fish at a given size or age is an important parameter to calculate the spawning stock biomass of a fish stock. However, standard assessment methods often do not account for variability in this reproductive trait and constant maturity ogives are generally used for spawning stock biomass (SSB) estimation. This is also the case for Baltic sprat. The standard stock assessment used for Baltic sprat is a virtual population analysis (Parmanne, 1994) with an extended survivors analysis (XSA; Shepard, 1999; ICES, 2010a). In this assessment, maturity ogives are kept constant, disregarding variability among areas and years. Further, the estimation of the spawning stock biomass does not account for variability in sex ratios. The same is true for an alternative assessment method, the multi species virtual population analysis (MSVPA), which accounts for natural mortality caused by predation, but not for variability in maturity or sex ratios to estimate the SSB. These standard assessment procedures are in contrast to the observation that males often mature at smaller sizes than females, causing a skewed sex ratio in the spawning stock due to male dominance of the younger size or age groups as it was observed for Baltic sprat (Grygiel and Wyszyński, 2003). The size or age at which fish mature is linked to fish growth and, thus, may vary in relation to variability in environmental conditions (Stearns and Crandell, 1984). Growth during early life may be affected by ambient temperature, food availability or food quality. Thus, interannual changes in climatic conditions (*e.g.* winter temperature), spatial differences in the hydrography of the various spawning areas and density dependent effects caused by fluctuations in population sizes may be important drivers for observed variability in both maturity and sex ratio (Blaxter and Hunter, 1982). Moreover, it was demonstrated that fishing pressure may act as selective force causing a decrease in size at first maturity (Heino *et al*., 2002). A decrease in size or age at first maturity was attributed to heavy

exploitation in a number of fish stocks, *e.g.* pilchard (Armstrong *et al*., 1989) and plaice (Nash *et al*., 2000).
Hence, observations and studies to reveal causes and consequences of such variability in these reproductive traits are necessary. The last effort to analyse the variability in Baltic sprat maturity was performed by the Study Group on Baltic Herring and Sprat Maturity (SGBHSM; ICES, 2002) in a time series from 1976 to 1987 and 1995 to 1996, revealing a high variability in the proportion of mature fish in age group one and, to a certain extent, age group two. However, it was not possible to explain the observed variability in maturity in the two youngest age groups by means of environmental conditions or changes in the stock structure. Therefore, the procedure of constant maturity ogives was maintained in sprat assessment and no new data or analyses on sprat maturity were provided since then (ICES, 2010a).
It remains essential to report observations on these reproductive traits and to establish time series. Only such continuous data sets will reveal plausible relations between the observed traits and environmental conditions and will form the basis to develop process models which might be incorporated into assessment methods to enhance the SSB estimation.
In the present study, observations on Baltic sprat length frequencies, growth, sex ratios, and maturity are reported for different years and areas in the Baltic Sea. Fish length dependent regression models on these parameters were established and the relationship between the length of first maturity (L_{50}), winter temperature and SSB was explored.

III. 2 Methods

Sprat were sampled on several research cruises for the years 1999 to 2009 within the south central Baltic (Fig. 1; Tab. 1). Samples were taken within the Arkona Basin (ICES sub-division 24), the Bornholm Basin (ICES sub-division 25), the Gdansk Deep (ICES sub-division 26), between Sweden and Gotland (ICES sub-division 27) and the Southern Gotland Basin (ICES sub-division 28). In total 39284 sprats were analysed. Data for length frequency distributions were obtained by measuring the total length to the nearest cm of subsamples of at least 200 specimens of each haul. Data on sex and maturity were determined by macroscopic inspection of at least 10 individuals per length class from each haul. All specimens showing evidence of ripening gonads and all those with post-spawning characteristics were classified as mature, *i.e.* contributing to the SSB. Two different maturity keys were used. For the years 1999 to 2003, the maturity key of Alekseev and Alekseeva (1996) was applied. Thereafter (2004 to 2009) a standard maturity key, which is also used for the German standard surveys in the Baltic, was applied. Data obtained by means of the former maturity key were converted to the latter key in order to achieve comparable data (see Annex I). Since samplings were either based on 1 or 0.5 cm length classes, all collected data were standardised to 1cm length classes. Length frequency distributions, sex ratios and maturity ogives were calculated by pooling all data from the second quarter of the year in order to obtain an estimate for the main spawning season of sprat in these areas (Ojaveer *et al*., 2010).

The sex ratio was modelled as proportion females in a given length class L (PF_L) with a logistic equation:

$$PF_L = P_{min} + \frac{a}{1+\exp\left(-\left(\frac{L-PF_{50}}{b}\right)\right)}$$

where P_{min} is the observed minimum proportion of females which serves as a start point for the logistic regression. L is the fish length class and PF_{50}, a and b are regression coefficients. PF_{50}, the inflection point of the logistic curve, describes the length where 50% of individuals are

females. This regression is only valid for length classes larger than or equal to the length class at which P_{min} was observed.

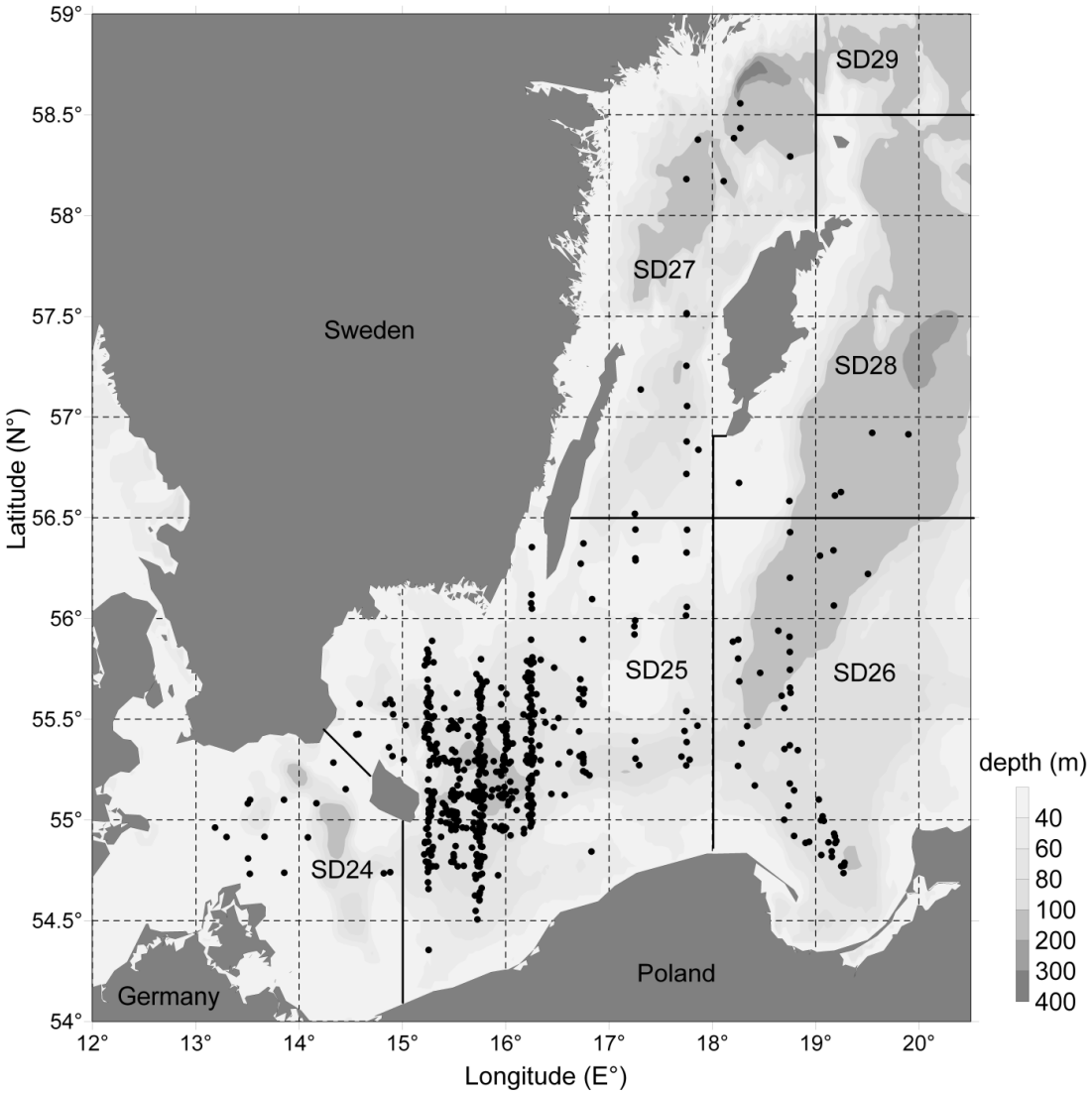

Fig. 1: Sampling stations within ICES sub-divisions (SD) in the Southern- and Central Baltic Sea. Each dot represents one fishery haul. For details on cruises and sampling see table 1.

Tab. 1: Sampling of Baltic sprat in different ICES sub-divisions (SD). Cruise (AL = RV"Alkor", HE = RV"Heincke", WH = RV "Walther Herwig III"), month, year, number of fish analysed for maturity and number of hauls per cruise.

ICES sub-division	cruise	month	year	number of sprat	number of hauls
SD24	WH206	June	1999	977	9
	WH228	June	2001	97	4
	WH239	May	2002	123	5
	WH251	May	2003	133	5
	WH263	May	2004	246	12
	WH275	May	2005	117	2
	WH288	May	2006	714	4
			Total	**2407**	**41**
SD25	AL141	April	1999	1178	13
	AL143	May	1999	1738	14
	WH206	June	1999	2053	25
	HE131	April	2000	983	12
	AL161	May	2000	831	15
	AL182	June	2001	1706	20
	WH228	June	2001	510	14
	AL200	April	2002	1074	17
	WH239	May	2002	249	15
	HE168	May	2002	770	14
	AL205	June	2002	572	13
	AL219	April	2003	878	16
	WH251	May	2003	124	11
	AL220	May	2003	127	9
	WH263	May	2004	422	25
	AL238	June	2004	564	11
	AL255	April	2005	888	17
	WH275	May	2005	754	9
	AL258	May	2005	534	11
	AL276	April	2006	2069	31
	AL277	April	2006	783	14
	WH288	May	2006	2683	22
	AL279	June	2006	464	10
	AL297	April	2007	1884	22
	WH299	May	2007	2119	15
	AL299	May	2007	617	11
	AL318	April	2008	3187	15
	AL320	June	2008	470	12
	AL335	April	2009	1503	16
	AL338	May	2009	124	2
			Total	**31858**	**451**

Tab. 1: continued.

ICES sub-division	cruise	month	year	number of sprat	number of hauls
SD26	WH206	May	1999	258	6
	AL161	June	2000	275	4
	WH228	May	2001	97	3
	WH239	May	2002	70	4
	WH251	May	2003	142	5
	WH263	May	2004	73	7
	AL238	June	2004	399	8
	AL255	April	2005	241	5
	WH275	May	2005	318	4
	AL277	April	2006	297	5
	WH288	May	2006	856	7
			Total	**3026**	**58**
SD27	WH206	May	1999	50	1
	WH228	May	2001	18	1
	WH239	May	2002	34	2
	WH263	May	2004	126	4
	WH288	June	2006	659	8
	WH299	May	2007	656	6
			Total	**1543**	**22**
SD28	WH206	May	1999	148	3
	AL161	June	2000	129	2
	WH228	May	2001	95	2
	WH263	May	2004	78	3
			Total	**450**	**10**

The maturity ogives were modelled with a logistic equation as proportion mature individuals, mature females and mature males, respectively:

$$P_m = \frac{a}{1+\exp\left(-\left(\frac{L-L_{50}}{b}\right)\right)}$$

where P_m is the predicted probability of maturity, L the fish length class, the regression coefficient L_{50} is the length class where 50% of individuals are mature or the length at first maturity, a and b are regression coefficients. To detect possible differences in mean values of L_{50} between sexes, different years and areas, analyses of variance were performed, respectively.
Length based maturity data from former studies of the Study Group of Baltic Herring and Sprat Maturity (ICES, 2002) were analysed. These data were sex combined data only. L_{50} values were calculated for ICES SD25 applying the same method as described above. A time series was constructed combining these data with new results obtained by the present study. Regression analyses were performed to investigate whether the observed variability in this data set could be explained by a possible effect of stock size or hydrographic conditions. To explore density effects, the total and spawning stock size obtained by the standard sprat assessment were used (ICES, 2010a). To explore the effect of environmental conditions, data from the ICES hydrograhic database were utilised. Mean values of salinity, oxygen content and temperature

were calculated for year quarters over the water column where oxygen content exceeded 1ml L^{-1}, since sprat were assumed to avoid lower oxygen concentrations (Stepputtis *et al.*, 2011).
To investigate sprat growth, Von Bertalanffy Growth Functions (VBGF) were fitted to sex separated length-at-age data which were obtained from acoustic surveys for the years 2001 – 2006:

$$L_t = L_{inf} * (1 - e^{(-k*(t-t_0))})$$

where L_t is the length at age t, and L_{inf}, k and t_0 are regression coefficients.

III. 3 Results

Size frequency distributions revealed differences among ICES sub-divisions (SD). The proportions of sprat >13.5 cm length was higher in SD24 compared to the other areas (Fig. 2). The proportion of 12.5 cm and 13.5 cm length classes is nearly equal in SD24. In SD25 the 12.5 cm is the predominant length class, whereas in SD26, SD27 and SD28 the highest proportion lies in the 11.5 cm length class. Pooled over all years, the distributions are bimodal in SD24, SD27 and SD28, but unimodal in SD25 and SD26. However, in some years size frequency distributions in SD25 also revealed a pronounced bimodality with relatively high proportions of small individuals, which is not visible in the overall mean distribution.
The coefficients of determination obtained for the growth models were in the range of 0.68 to 0.84 for females and 0.53 to 0.72 for males, respectively (Tab. 2). For female sprat higher values of L_{inf} and k were found compared to males, for every ICES sub-division apart from SD28. Thus, female sprats appear to grow faster to their theoretical maximum length, which is in addition larger than the maximum length of males (Fig. 3).
The observed trends in sex ratio were similar for all analysed areas in the Baltic. The smaller size groups <10.5cm were male dominated, whereas the larger size groups were female dominated (Fig. 4; Tab. 3). In most of the cases P_{min} was observed in the 10.5 cm length class, with the exception of SD24, where this value was observed at 12.5 cm. In the latter SD, the proportion females ranged more or less around 50% in size classes <12.5 cm. The PF_{50} values obtained by the regression models ranged between 12.93 cm (SD 26) to 13.62 cm (SD 24).
Interannually variability in proportion mature was highest in the small length classes <11.5 cm for all areas (Fig. 5). The mean (±SE) L_{50} value for both sexes combined was highest in SD24 with 10.41 (±0.21) cm, the lowest value was observed in SD25 with 9.37 cm (Fig. 6; Tab. 4). ANOVA did not reveal any statistically significant difference in sex combined L_{50} values among different areas (p=0.17).
For all analysed areas and years, female sprat matured at a larger size than male sprat (Tab. 5; Tab. 6; Fig 7 - 10). In ICES sub-division 25 the mean (±SD) female L_{50} value was 10.17 (±0.4) cm, which was significantly higher than the mean L_{50} value observed for males of 8.97 (±0.5) cm (t-test; p<0.001; n=11). A significant difference between female and male mean (±SD) L_{50} values was also found for SD26, with 9.86 (±0.37) cm and 8.91 (±0.62) cm, respectively (t-test; p=0.02; n=5). For the other areas, low sample sizes prevented meaningful sex separated regression analyses for most of years so that in these cases only the sex combined mean L_{50} values were computed (Fig 6a).
The L_{50} values obtained from SGBHSM data for the years 1976 – 1987 of ICES sub-division 25 were in general higher than the values obtained from the present study. The combined data set was plotted against ambient winter temperature and spawning stock biomass (Fig. 11). Linear regressions revealed a negative relationship between the L_{50} value and both ambient temperature during the first quarter (r^2=0.53; Fig. 12a) and spawning stock biomass (r^2=0.38; Fig. 12b). Ambient temperature and SSB were not correlated and data were normally distributed. Thus,

assumptions to perform a multiple linear regression model were met. The following model explained 65% of the observed variability:

$$L_{50} = -0.61(\pm 0.13SE) * T - 0.07(\pm 0.03SE) * SSB + 12.35(\pm 0.49SE) \quad n=22;\ r^2=0.65$$

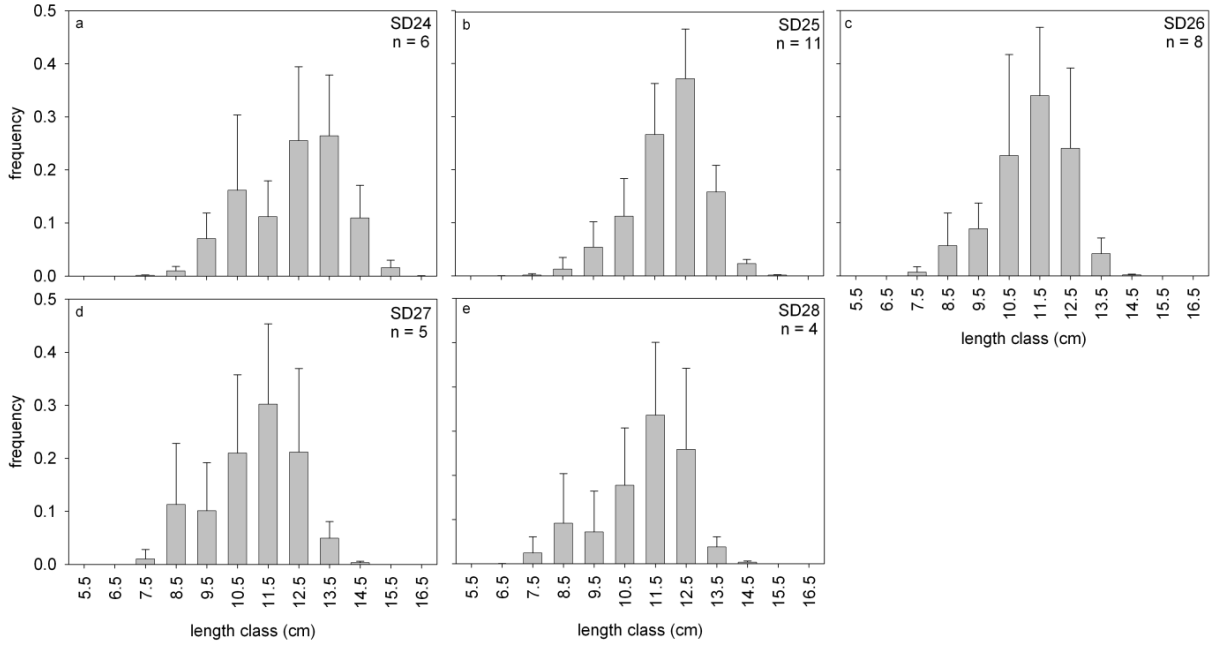

Fig. 2: Length frequency distributions as unweighted means from several years (n) observed for different ICES sub-divisions for the second quarter of the year. Error bars denote standard deviations among years (n) which were included in the analysis.

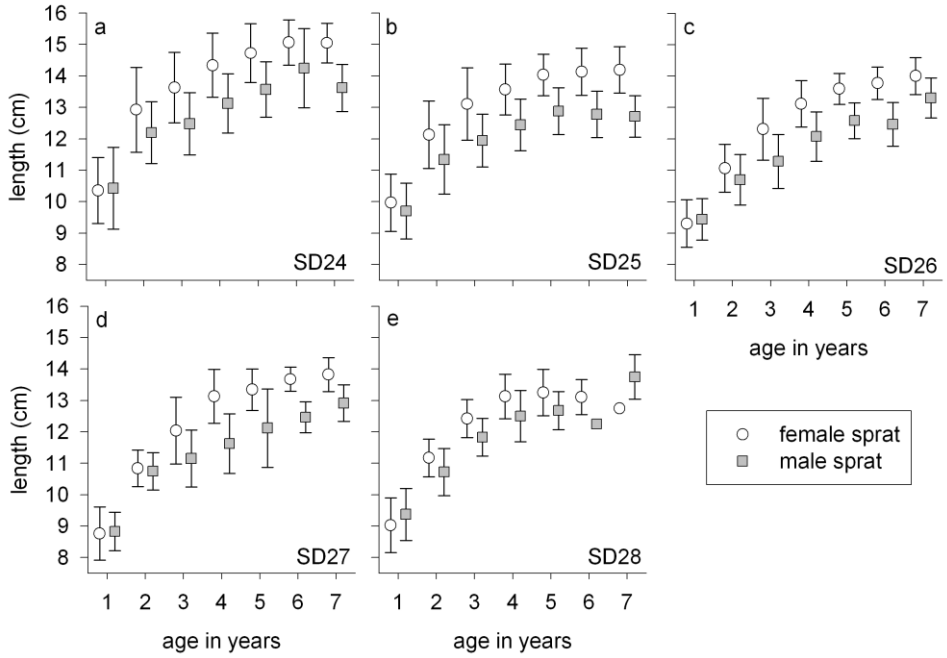

Fig. 3: Length at age data for different ICES sub-divisions (SD) for several years combined. Error bars denote the standard deviation of the mean. Symbols are slightly offset on the x-axis for better visibility.

Tab. 2: Parameters of the Von Bertalanffy Growth Function $L_t = L_{inf} * (1\text{-}exp^{-k*(t-t0)})$. Presented are the results for different ICES sub-divisions (SD) separated for female and male sprat, years included in the analysis, number of sprat (n), coefficients of determination, regression parameters, standard error, t and p values.

female sprat	years included	n	r^2	coefficient		standard error	t	p
SD24	2001-2006	546	0.72	L_{inf}	14.98	0.15	101.00	0.00
				k	0.69	0.07	10.61	0.00
				t_0	-0.71	0.15	-4.91	0.00
SD25	2001-2006	836	0.68	L_{inf}	14.25	0.09	156.70	0.00
				k	0.66	0.04	14.72	0.00
				t_0	-0.84	0.12	-6.89	0.00
SD26	2001-2006	324	0.82	L_{inf}	14.43	0.22	65.29	0.00
				k	0.45	0.04	10.16	0.00
				t_0	-1.32	0.19	-6.85	0.00
SD27	2001-2002, 2004-2006	186	0.84	L_{inf}	14.13	0.20	70.23	0.00
				k	0.51	0.05	9.73	0.00
				t_0	-0.89	0.18	-4.88	0.00
SD28	2001, 2004	121	0.84	L_{inf}	13.52	0.18	73.17	0.00
				k	0.70	0.09	7.92	0.00
				t_0	-0.57	0.19	-3.00	0.00
male sprat	years included	n	r^2	coefficient		standard error	t	p
SD24	2001-2006	311	0.53	L_{inf}	13.72	0.30	46.15	0.00
				k	0.62	0.13	4.74	0.00
				t_0	-1.32	0.41	-3.23	0.00
SD25	2001-2006	670	0.59	L_{inf}	12.91	0.13	101.64	0.00
				k	0.65	0.06	10.16	0.00
				t_0	-1.15	0.19	-6.03	0.00
SD26	2001-2006	295	0.6	L_{inf}	13.89	0.53	25.98	0.00
				k	0.29	0.06	4.68	0.00
				t_0	-3.02	0.65	-4.68	0.00
SD27	2001-2002, 2004-2006	124	0.67	L_{inf}	12.40	0.28	44.88	0.00
				k	0.60	0.12	5.05	0.00
				t_0	-1.14	0.38	-2.97	0.00
SD28	2001, 2004	98	0.72	L_{inf}	13.84	0.62	22.43	0.00
				k	0.38	0.10	3.85	0.00
				t_0	-1.96	0.60	-3.25	0.00

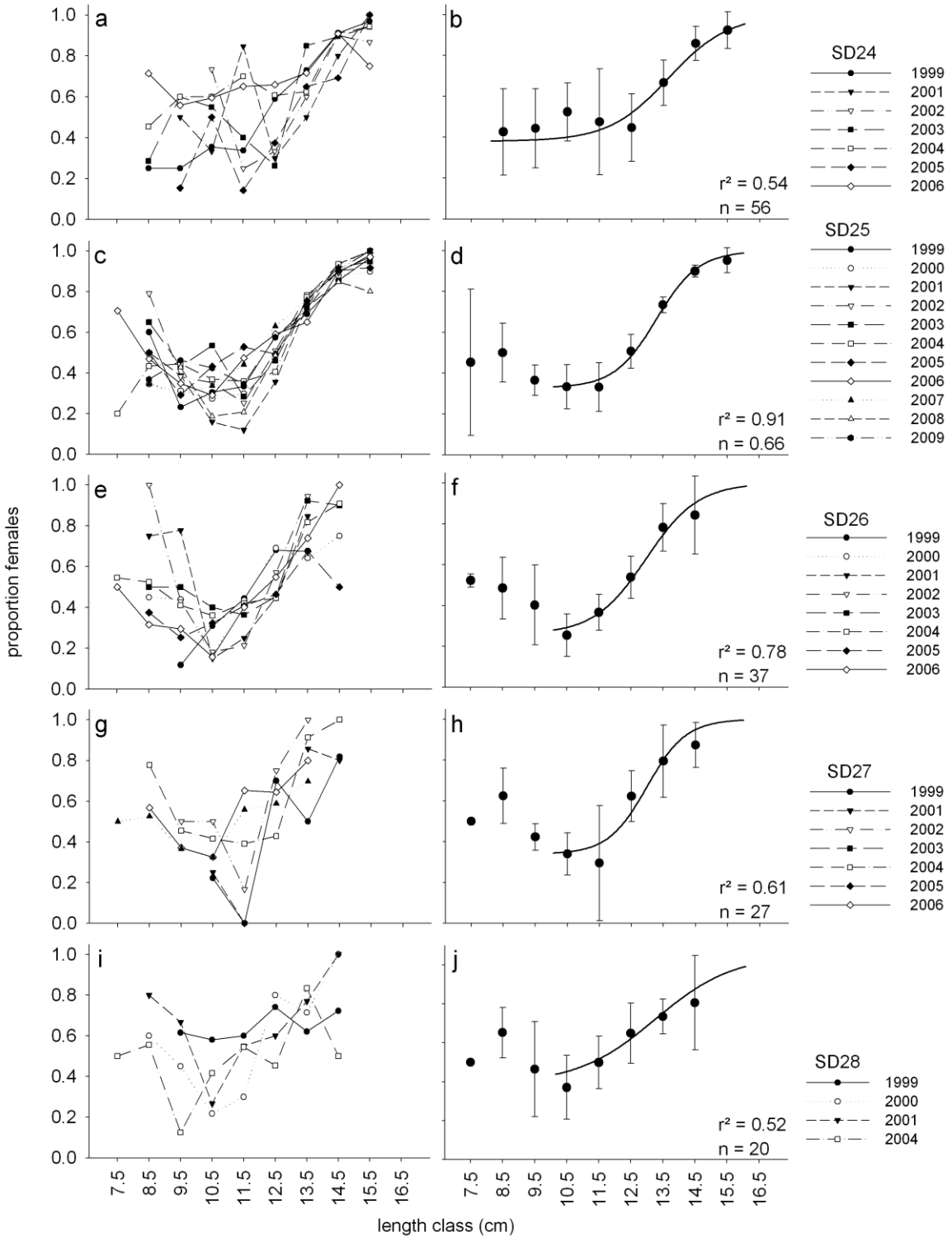

Fig. 4: Observed Baltic sprat sex ratios (left panels) during the second quarter of the year with corresponding regression models including means ± standard deviations (right panels) for different ICES sub-divisions: SD24 (a, b), SD25 (c, d), SD 26 (e, f), SD 27(g, h) and SD28 (i, j).

Tab. 3: Parameters of logistic regression models of Baltic sprat sex ratios as proportion of females (*PF*) in a given length class (*L*): $PF_L = P_{min} + (a / (1+\exp^{-1*(L-PF50)/b}))$. Presented are the results for different ICES sub-divisions (SD) for all years combined, coefficients of determination (r^2), number of sprat (n), standard error, t and p values.

ICES sub-division		coefficient	standard error	t	p
SD24	$r^2 = 0.54$	$P_{min} = 0.38$			
		$a = 0.62$	0.16	3.96	<0.001
	n = 56	$b = 0.94$	0.40	2.36	0.02
		$PF_{50} = 13.62$	0.68	20.15	<0.0001
SD25	$r^2 = 0.91$	$P_{min} = 0.33$			
		$a = 0.67$	0.03	21.15	<0.0001
	n = 66	$b = 0.66$	0.09	7.33	<0.0001
		$PF_{50} = 13.26$	0.12	113.63	<0.0001
SD26	$r^2 = 0.78$	$P_{min} = 0.26$			
		$a = 0.74$	0.13	5.73	<0.0001
	n = 37	$b = 0.82$	0.23	3.59	<0.01
		$PF_{50} = 12.93$	0.39	32.80	<0.0001
SD27	$r^2 = 0.61$	$P_{min} = 0.34$			
		$a = 0.66$	0.19	3.49	<0.01
	n = 27	$b = 0.61$	0.32	1.91	0.07
		$PF_{50} = 12.96$	0.52	24.97	<0.0001
SD28	$r^2 = 0.52$	$P_{min} = 0.37$			
		$a = 0.63$	0.48	1.31	0.21
	n = 20	$b = 1.23$	1.00	1.23	0.24
		$PF_{50} = 13.17$	2.24	5.87	<0.0001

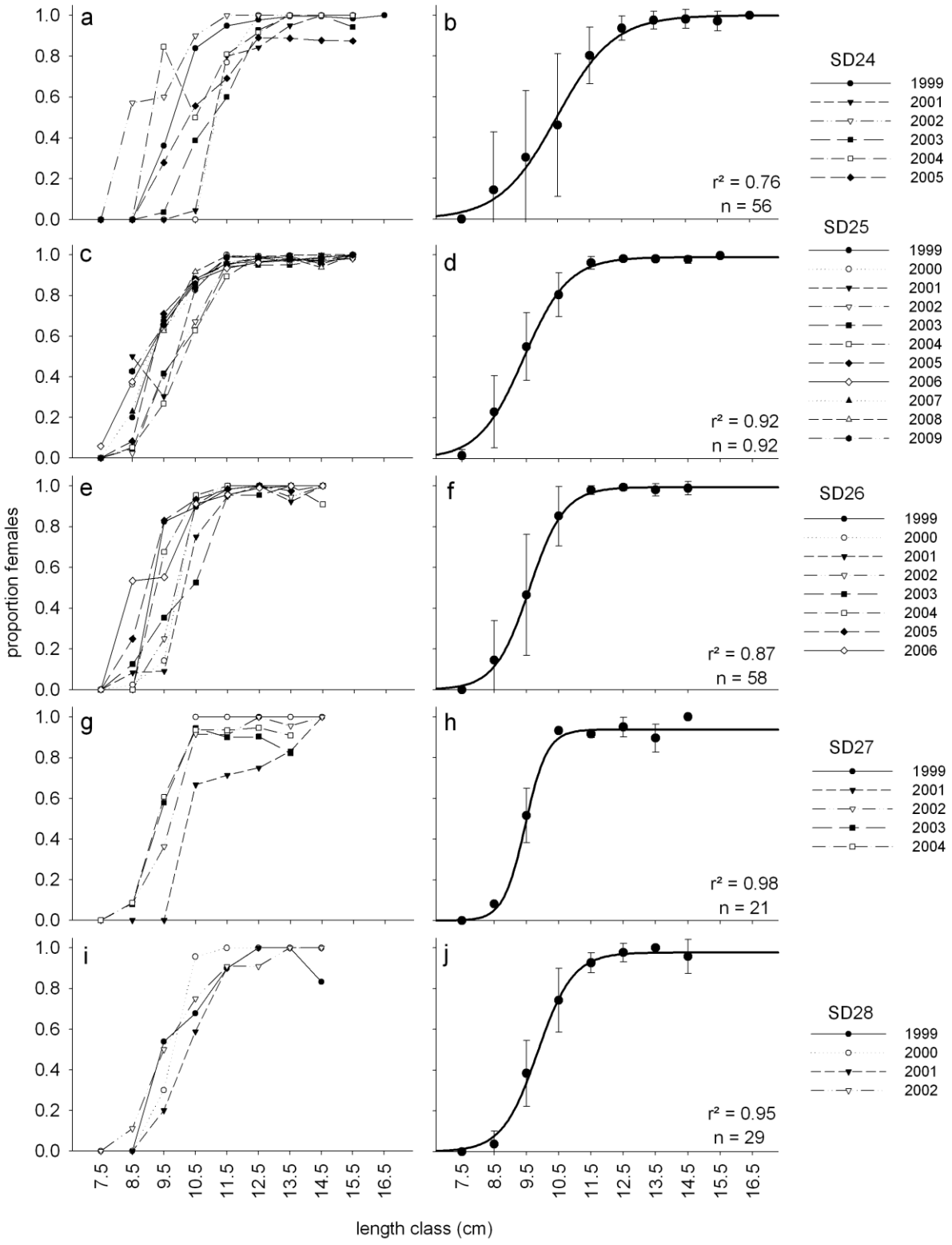

Fig. 5: Observed length based Baltic sprat maturity ogives (left panels) during the second quarter of the year with corresponding regression models including mean values ± standard deviation (right panels) for different ICES sub-divisions: SD24 (a, b), SD25 (c, d), SD 26 (e, f), SD 27(g, h) and SD28 (i, j).

Tab. 4: Parameters of length based regression models of Baltic sprat maturity ogives as proportion mature individuals: $P_m = a \,/\, (1+\exp^{-1*(L-L50)/b})$. Presented are results for both sexes combined for different ICES sub-divisions pooled for all years.

ICES sub-division		coefficient	standard error	t	p
SD24	$r^2 = 0.77$ n = 56	$a = 1.00$	0.05	21.33	<0.0001
		$b = 0.89$	0.19	4.70	<0.0001
		$L_{50} = 10.41$	0.21	50.55	<0.0001
SD25	$r^2 = 0.92$ n = 92	$a = 0.99$	0.01	73.04	<0.0001
		$b = 0.70$	0.06	11.71	<0.0001
		$L_{50} = 9.37$	0.06	147.22	<0.0001
SD26	$r^2 = 0.87$ n = 58	$a = 0.99$	0.03	38.14	<0.0001
		$b = 0.55$	0.09	6.01	<0.0001
		$L_{50} = 9.55$	0.10	98.11	<0.0001
SD27	$r^2 = 0.90$ n = 37	$a = 0.94$	0.03	36.17	<0.0001
		$b = 0.30$	0.11	2.81	0.0082
		$L_{50} = 9.59$	0.08	114.93	<0.0001
SD28	$r^2 = 0.95$ n = 29	$a = 0.98$	0.03	38.40	<0.0001
		$b = 0.56$	0.09	6.54	<0.0001
		$L_{50} = 9.80$	0.10	103.03	<0.0001

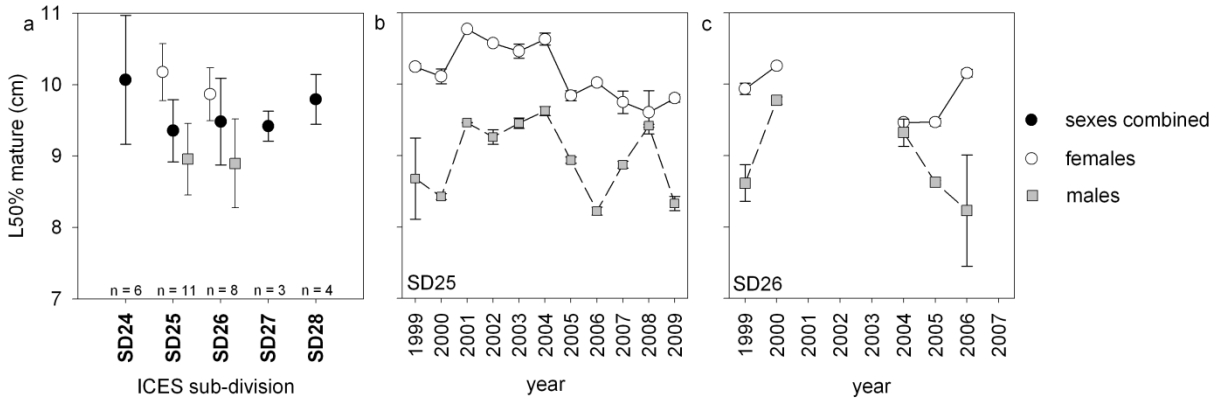

Fig. 6: Length at 50% maturity (L_{50}) as regression coefficient values for sprat. (a) mean values for different ICES sub-divisions, error bars denote standard deviation. (b) female and male sprat observed for ICES sub-division 25 and (c) female and male sprat observed for ICES sub-division 26. Error bars denote the standard error of the regression coefficient.

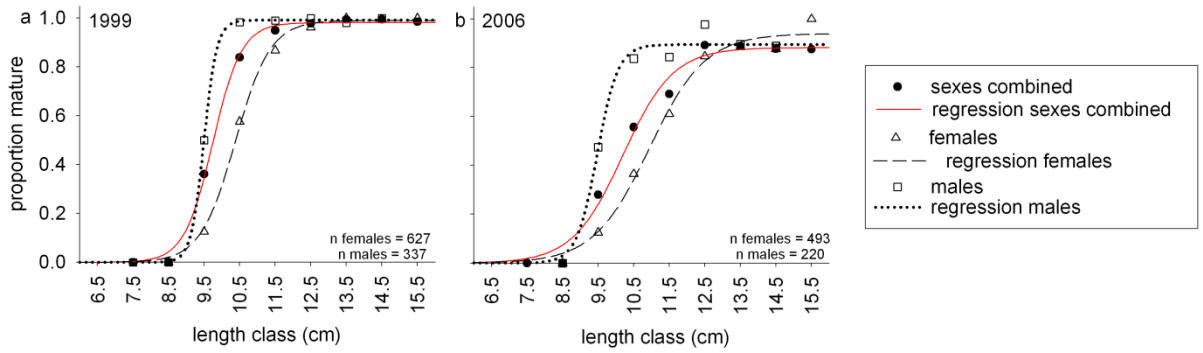

Fig. 7: Length based sprat maturity ogives for both sexes combined and sex separated observed for ICES sub-division SD24 in the second quarter of the year.

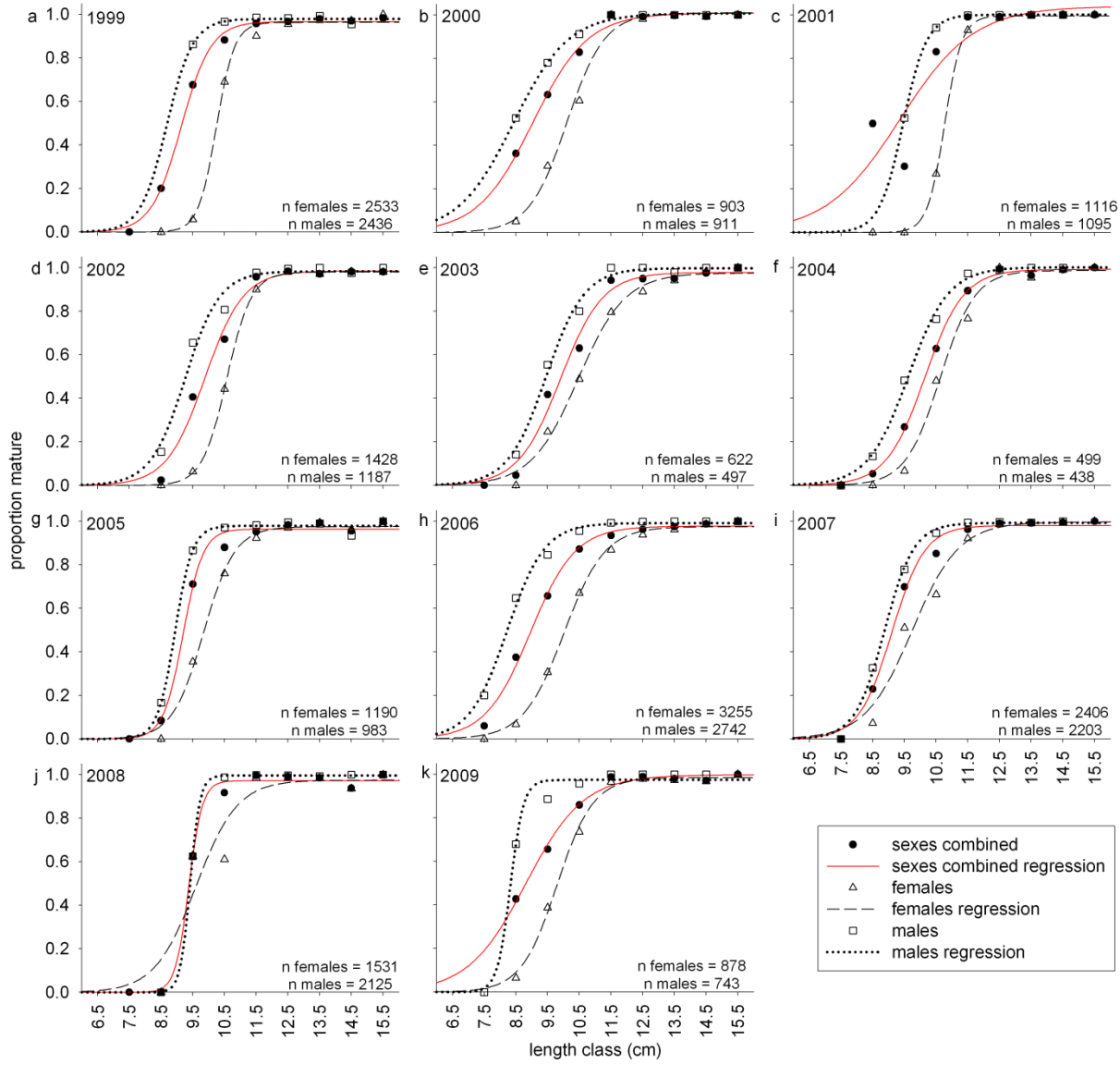

Fig. 8: Length based sprat maturity ogives for both sexes combined and sexes separated observed for ICES sub-division 25 in the second quarter of the year.

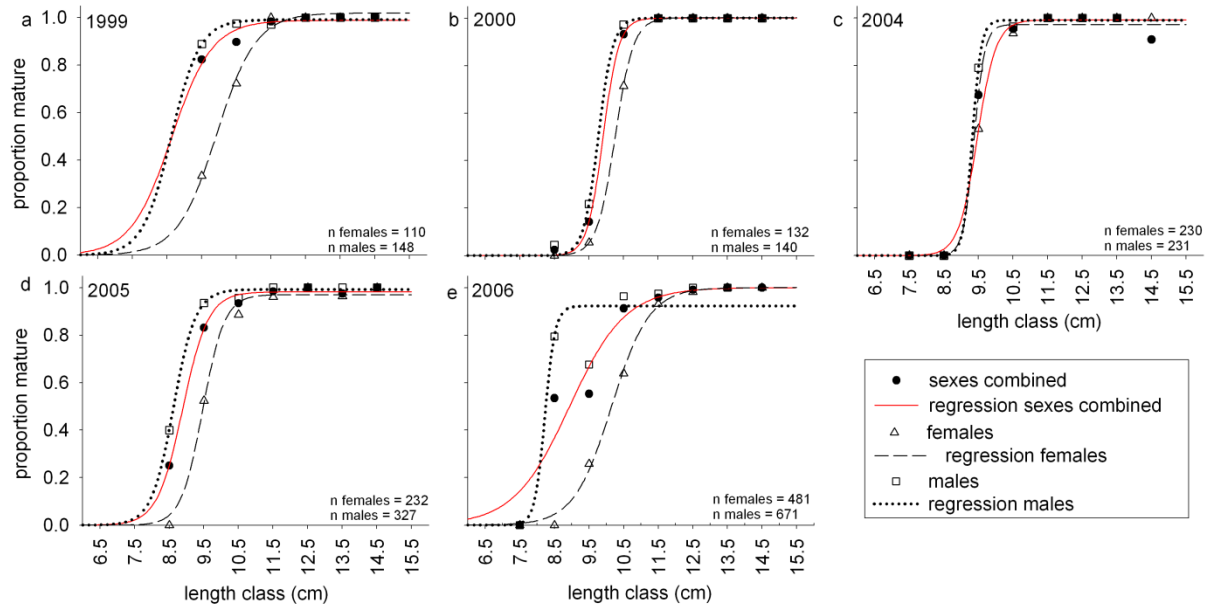

Fig. 9: Length based sprat maturity ogives for both sexes combined and sex separated observed for ICES sub-division SD26 in the second quarter of the year.

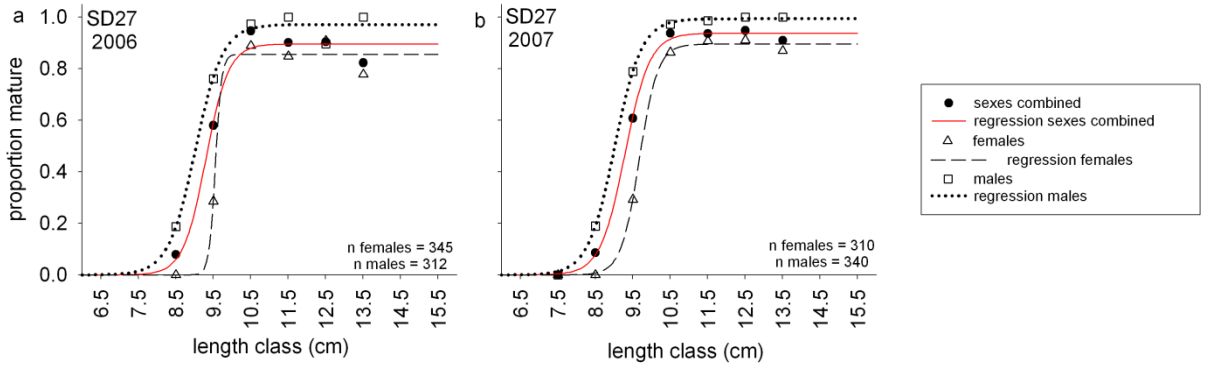

Fig. 10: Length based sprat maturity ogives for both sexes combined and sex separated observed for ICES sub-division SD27 in the second quarter of the year.

Tab. 5: Parameters of length based regression models of Baltic sprat maturity ogives as proportion mature individuals: $P_m = a / (1+\exp^{-1*(L-L50)/b})$. Presented are results for female sprat for different ICES sub-divisions pooled for all years.

ICES sub-division		coefficient	standard error	t	p
SD24	$r^2 = 0.79$ n = 56	$a = 1.01$	0.05	20.93	<0.0001
		$b = 0.86$	0.17	4.96	<0.0001
		$L_{50} = 10.92$	0.20	55.07	<0.0001
SD25	$r^2 = 0.96$ n = 89	$a = 0.99$	0.01	70.27	<0.0001
		$b = 0.61$	0.05	12.33	<0.0001
		$L_{50} = 10.21$	0.06	177.68	<0.0001
SD26	$r^2 = 0.72$ n = 59	$a = 0.92$	0.04	20.91	<0.0001
		$b = 0.46$	0.13	3.53	0.0009
		$L_{50} = 10.01$	0.17	60.57	<0.0001
SD27	$r^2 = 0.98$ n = 21	$a = 0.91$	0.02	51.59	<0.0001
		$b = 0.29$	0.06	4.89	0.0001
		$L_{50} = 9.67$	0.06	174.26	<0.0001
SD28	$r^2 = 0.95$ n = 29	$a = 0.97$	0.03	35.73	<0.0001
		$b = 0.45$	0.08	5.62	<0.0001
		$L_{50} = 10.31$	0.09	117.83	<0.0001

Tab. 6: Parameters of length based regression models of Baltic sprat maturity ogives as proportion mature individuals: $P_m = a / (1+\exp^{-1*(L-L50)/b})$. Presented are results for male sprat for different ICES sub-divisions pooled for all years.

ICES sub-division		coefficient	standard error	t	p
SD24	$r^2 = 0.62$ n = 52	$a = 0.96$	0.05	18.63	<0.0001
		$b = 0.77$	0.23	3.35	0.0016
		$L_{50} = 9.90$	0.23	42.36	<0.0001
SD25	$r^2 = 0.67$ n = 87	$a = 1.00$	0.03	39.73	<0.0001
		$b = 0.87$	0.15	5.81	<0.0001
		$L_{50} = 8.65$	0.14	62.73	<0.0001
SD26	$r^2 = 0.62$ n = 53	$a = 0.97$	0.04	24.55	<0.0001
		$b = 0.64$	0.18	3.58	0.0008
		$L_{50} = 8.88$	0.18	49.24	<0.0001
SD27	$r^2 = 0.89$ n = 19	$a = 1.00$	0.04	24.04	<0.0001
		$b = 0.57$	0.13	4.26	0.0006
		$L_{50} = 9.09$	0.15	61.73	<0.0001
SD28	$r^2 = 0.93$ n = 26	$a = 0.97$	0.02	40.66	<0.0001
		$b = 0.39$	0.08	4.84	<0.0001
		$L_{50} = 9.31$	0.08	116.23	<0.0001

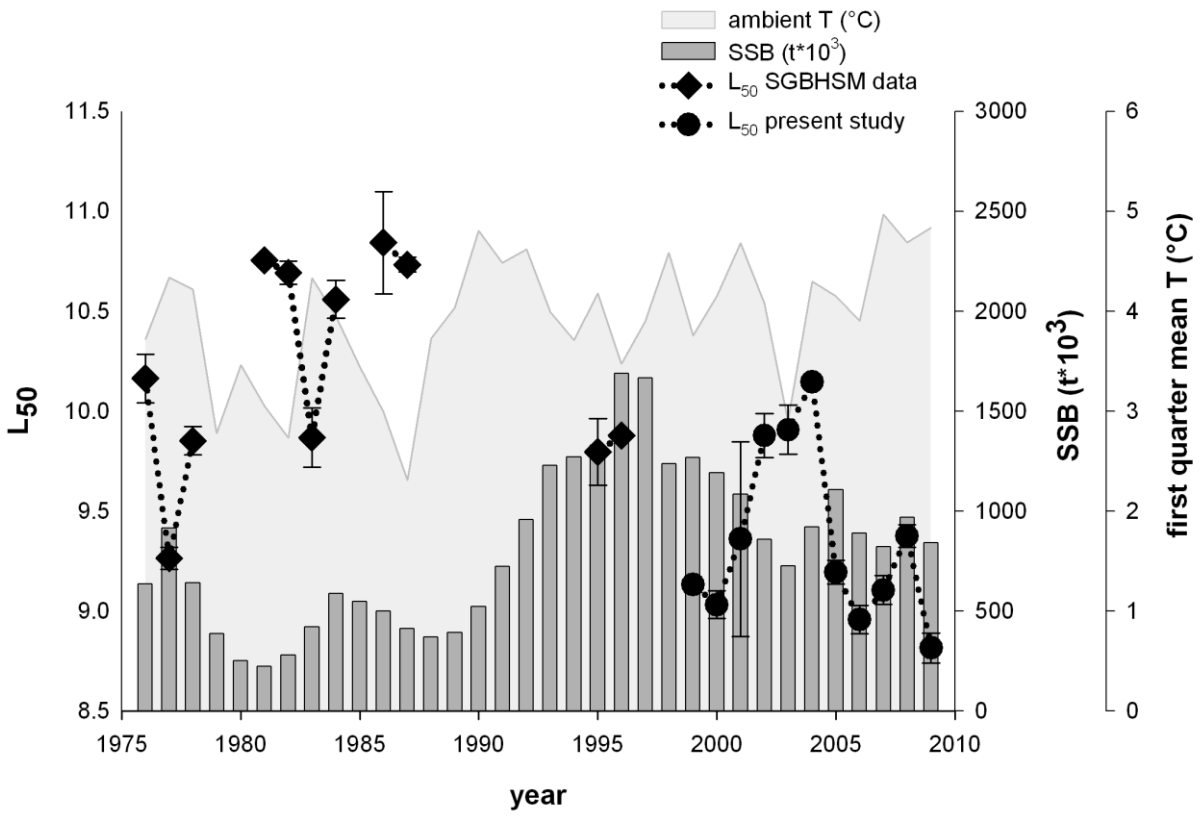

Fig. 11: Time series of L_{50} values estimated for the ICES SD25 from SGBHSM data (black diamonds) and the present study (black circles) in relation to SSB (grey bars) and ambient winter temperature (grey area).

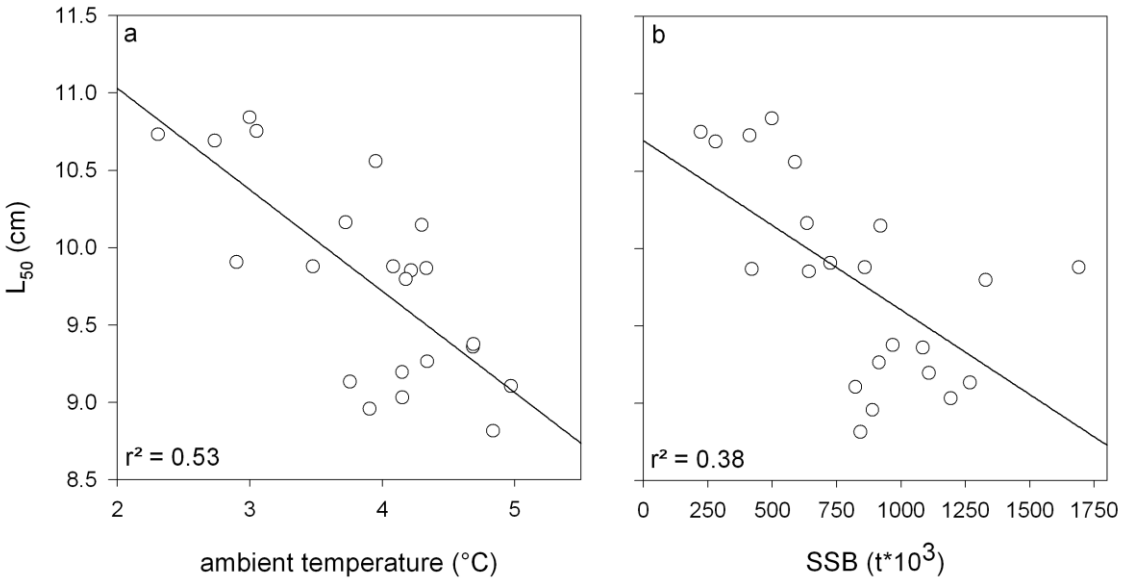

Fig. 12: Relationship of sex combined L_{50} values for ICES SD25 with (a) ambient temperature and (b) spawning stock biomass (SSB).

III. 4 Discussion

The observed skewed sex ratios can be explained by the sex specific differences in growth rate. This pattern has been observed before also for other marine fish species, *e.g.* cod (*Gadus morhua*; Nash *et al*., 2010) or hake (*Merluccius merluccius*; Murua *et al*., 2010).
Spatial differences in size at maturity have been observed for other clupeid stocks, *e.g.* sardine (*Sardina pilchardus*; Silva *et al*., 2006). The present study did not reveal statistically significant differences in the L_{50} values among areas. However, it is possible that such spatial differences might also be detected for Baltic sprat if more years can be included into future analyses. Similar to the sex ratio, the size at maturity is also dependent on growth. Hence, the observed differences in L_{50} values between female and male sprat may also be explained by sex specific growth rates. Larger L_{50} values for female sprat compared to male sprat have been reported for the time period 1980-2000 for the Bornholm Basin and the Gdansk Deep, respectively (Grygiel and Wyszyński, 2003). The sex separated L_{50} values from this former study were well in the range of the results from the present study.
For sprat from the north-eastern parts of the Baltic, an increase of mature individuals in age group one and two has been reported for the 1990s compared to the 1980s (Kaljuste and Raid, 2002) which was explained by an increase of ambient temperature. Similar results were reported by Grygiel and Wyszyński (2003) for Baltic sprat from south-eastern parts of the Baltic. Reglero and Mosegaard (2006) found a relationship between otolith size and onset of maturation in Baltic sprat and concluded that fish size at the end of the first growing season determines whether sprat matures at age one or age two. Results of the present study support the hypothesis that temperature conditions may influence the maturation of sprat since lower L_{50} values were related to warmer ambient winter temperatures. This finding indicates that a higher proportion of small sprat contribute to spawning after a mild winter. The results of the present study are solely length based, thus, it is not possible to relate the results to maturity at age directly. However, the decrease in L_{50} might be a combination of high growth rates and energy storage during the first growing season followed by a mild winter, provided sufficient prey availability. If in addition the spring zooplankton production starts earlier due to warmer water temperatures and earlier stratification, small sprat may already be able to allocate energy to reproduction and thus contribute to spawning. However, due to the lack of age-based maturity for most of the presented data it remains unclear whether the observed decrease in L_{50} with increasing stock size is due to decreased growth of age group two, which could then be explained by food limitation, or if also younger individuals contribute to spawning already.
The obtained models on sex ratios and maturity ogives, sex combined as well as sex separated, can directly be used in a number of applications to study the Baltic sprat stock. Both reproductive traits are important parameters needed for the daily egg production method to assess sprat stock size from fishery independent information. In combination with knowledge of batch fecundity, spawning frequency and the duration of the spawning season, these data enable the estimation of the female spawning stock biomass (FSSB) or the potential egg production of sprat. Further, these data are especially relevant for the implementation of stage based matrix models, which have proven to be a valuable tool to assess the population dynamics of exploited fish stocks (*e.g.* Mantzouni *et al*., 2007; Pertierra *et al*., 1997; Butler *et al*., 1993), and for which the reproductive output of the adult population is a prerequisite (Caswell, 2001). The potential egg production of the stock could also be used in future studies to test if the SSB, in the way it is estimated today in the standard procedure of sprat assessment, is robust to the simplification of ignoring variability in maturity ogives and sex ratios. Kraus *et al*. (2002) demonstrated that the use of potential egg production as alternative measure of stock reproductive potential improved the stock-recruitment relationship for Baltic cod (*Gadus morhua*) by 10%, compared to the use of SSB. On the contrary, it was suggested that for hake (*Merluccius merluccius*), another gadoid species, the implementation of alternative indices of reproductive potential did not improve the

assessment (Murua *et al*., 2010). For the clupeoid sprat, future studies should test a possible effect of changing assessment procedures towards the utilisation of more realistic data with respect to the reproductive potential of the stock. More effort is needed to (i) build up conclusive models to explain variability in reproductive traits in order to model reliable data to fill the gaps in existing time series and (ii) to recover suitable historical data which may exist but have not been used for such purposes so far. The latter would give the opportunity to investigate longer time periods with contrasting environmental conditions.

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Chapter IV: The dynamics of postovulatory follicle degeneration and oocyte growth in Baltic sprat

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Abstract

Ovaries of Baltic sprat (*Sprattus sprattus balticus* S.) were analysed histologically to identify stages of postovulatory follicles (POF) and to assess the oocyte development pattern. Samples were taken every 3 h during a 24 h trawl survey conducted in the Bornholm Basin in April 2007. Gonad histology revealed spawning of sprat throughout the day which hampered the exact aging of POFs. However, it was possible to define four stages of POFs, according to their histological features. The occurrence of these POF stages (I to IV) corresponded clearly to the development of the leading oocyte cohort. Further, the oocyte recruitment pattern revealed that the spawning batch can be identified prior to hydration. The POF stages I and II were present almost exclusively in vitellogenic ovaries, POF III were found in ovaries in the germinal vesicle stage, and POF stage IV was found exclusively in actively spawning fish with hydrated ovaries. Since only in very few ovaries POF were absent (5%), and in each ovary only one POF stage was present, the duration of POF degeneration approximately equals the average batch interval, *i.e.* the time lag between subsequent spawning events. Thus, aging of POFs can be realised when assuming 24 h duration of the hydrated stage and by combining the histological maturation stages of oocytes, the defined POF stages, and the evolution of the diameter of oocytes in the most advanced mode. A spawning interval of approximately 4.5 days was estimated for Baltic sprat using hydrated females and females with the most recent POF stage as spawning markers.

Keywords: oocyte recruitment; post ovulatory follicle; spawning fraction; *Sprattus* sprattus

IV. 1 Introduction

Like many other small pelagic clupeids, Baltic sprat release several batches of eggs over a protracted spawning season and show indeterminate oocyte recruitment (Alheit, 1988; Heidrich, 1925), *i.e.* the number of oocytes that will potentially be spawned during the breeding season is not determined prior to spawning. This means that individual potential fecundity cannot be estimated. However, the spawning stock biomass of these species can be determined from egg production methods, *e.g.* the Daily Egg Production Method, DEPM (Hunter, 1985) if information on a number of reproductive parameters is available. The use of DEPM requires the estimation of i) mean daily egg production from ichthyoplankton surveys and ii) the average daily individual fecundity. The latter involves the estimation of spawning frequency, batch fecundity, female weight and maturity status as well as sex ratio. Uncertainty in daily fecundity estimation of clupeids mainly resides in a lack of precision when estimating spawning fraction, either by the hydrated oocyte method or the postovulatory follicle (POF) method (Stratoudakis *et al*., 2006). The latter method requires histological preparation of ovaries to classify POF into daily cohorts.

Although the Daily Egg Production Method is a valuable, fisheries independent tool to estimate the spawning stock biomass of small pelagic fishes, such as anchovy and sardines (Stratoudakis *et al*., 2006), it has never been applied to the Baltic sprat stock on a regular basis. Thereby, a DEPM application would be especially interesting for the Baltic sprat assessment, where conflicting results on stock size estimations from acoustic surveys and a multi species virtual population analysis (MSVPA) approach hampered quantitative studies on recruitment processes of sprat and cod so far, for example the estimation of predation pressure on cod eggs by sprat in the Bornholm Basin (Köster and Möllmann, 2000). The Bornholm Basin serves sprat and cod as an important spawning ground (Aro, 1989). Therefore, this area is surveyed since years by means of ichthyoplankton and fishery surveys and data needed to implement the DEPM are available already (ICES 2002; Köster *et al*., 2005; Haslob *et al*., 2005). Thus, besides a future application even a retrospective stock assessment with the DEPM in order to validate former assessments is possible. However, the lack of adequate estimations of spawning frequency hampered the implementation of the DEPM for this pelagic species in former studies (Kraus and Köster, 2004). Neither have detailed histological investigations of Baltic sprat ovaries been published before nor was it attempted to age POF of sprat.

The best way to estimate the duration of POF is by laboratory tank experiments, where fish spawn under controlled conditions and adult fish are sampled at a specified time after spawning (Macewicz *et al*., 1996). As this is often not practicable, an alternative method was developed to estimate the spawning fraction from a series of samples collected over a 24 h period in the field (Alheit *et al*., 1984; Goldberg *et al*., 1984). On the basis of such samples distinct histological stages of POF have to be defined in relation to the daily peak spawning time. Therefore, this method is strictly speaking only suitable for fish species with a daily synchronous spawning behaviour, *e.g.* the Northern Anchovy (*Engraulis mordax*; Hunter *et al*., 1985) or the Mediterranean Sardine (*Sardina pilchardus sardina;* Ganias *et al*., 2003). For sprat, contradictory observations on the diel spawning behaviour are reported in literature. Simpson (1971) reported the diel spawning of Sprat from the Irish Sea between 22:00 p.m. and 06:00 a.m., with a peak between midnight and 04:00 a.m. For Baltic sprat, an asynchronous diel spawning pattern was observed (Alekseev and Alekseeva, 2005). The results of the present study also suggest a rather asynchronous spawning pattern, and direct aging of POF with respect to a diel peak in spawning was not possible.

In the present paper, we introduce a method for staging POF according to (i) their histological features of degeneration, and (ii) the dynamics of the oocyte growth of the most advanced oocyte cohort. Further, we propose the use of females with hydrated oocytes and recent POF to determine the spawning frequency. This study is the first to analyse the histological features of ovarian maturation stages and POF of Baltic sprat in detail.

IV. 2 Methods

Baltic sprat were sampled with a pelagic trawl during a research cruise with RV “Alkor” in the Bornholm Basin during the main spawning season in April 2007 (Fig. 1). Pelagic trawls were carried out in the central basin defined by the 80 m depth line every 3 h over a 24 h period (n=9; Tab. 1). From each haul a 2 kg sub-sample (n≈200) of sprat was preserved in a buffered 10% formaldehyde solution. To assure proper fixation of the samples, the body cavity of each fish was slit open prior to fixation.

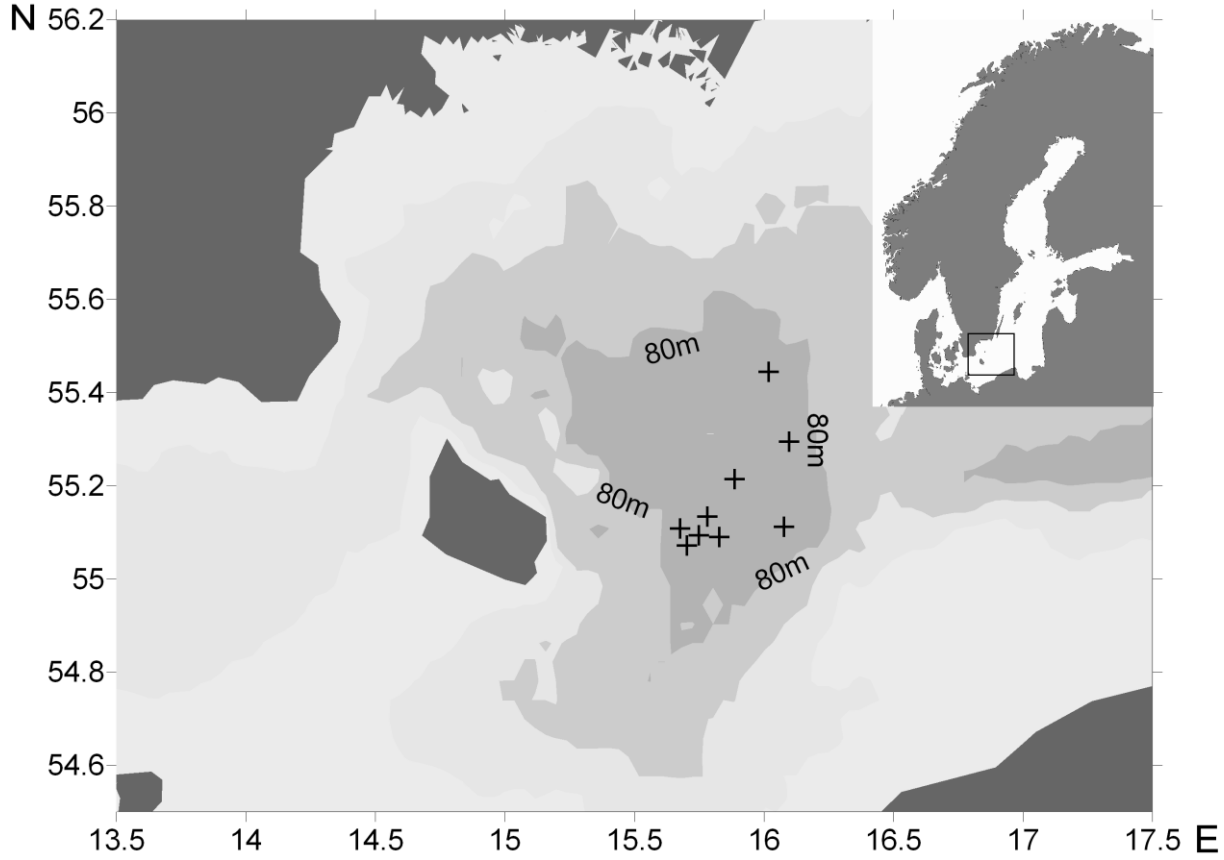

Fig. 1: Map of the sampling area in the Bornholm Basin (ICES Sub-Division 25). Fishery hauls 1 – 9 (crosses) were conducted within the 80 m isobath.

Histological processing

In the laboratory, at least 20 females were taken randomly from each sample for histological analysis of the ovaries. From these specimens, the total body weight (nearest 0.1 g), ovary free body weight (nearest 0.1 g), and total length (nearest 0.1 cm) were measured. The ovaries were removed, weighed to the nearest 0.001 g, and fixed again in a 4%-formaldehyde solution. A tissue sub-sample from the middle part of each ovary was embedded in paraffin. Histological sections of 3 µm were cut and stained, using the Hematoxilin-Eosin method. All histological sections of ovaries were analysed for the developmental stage of oocytes and the presence of postovulatory follicles (POF). Five oocyte developmental stages were distinguished (Brown-Peterson *et al*., 2010): primary growth, cortical alveoli, vitellogenesis, germinal vesicle migration (GVM) and hydrated oocytes (Fig. 2) forming the basis to classify the sampled females into three distinct phases of the reproductive cycle (Brown-Peterson *et al*., 2010): i) developing, ovaries containing only vitellogenic oocytes, ii) spawning capable, ovaries with oocytes undergoing GVM, and iii) actively spawning, hydration is present in the ovary.
According to their histological features, POFs have been assigned into different stages of degeneration (Fig. 3). Referring to observations from other clupeid fishes, the shape, the size of lumen and the state of the granulosa layer were mainly used for that purpose (Ganias, 2003; Hunter and Macewicz, 1985).

Oocyte recruitment related to POF stage durations

To assess the oocyte development pattern, 40 ovaries from two consecutive hauls (haul 6 and 7) were processed with the whole-mount method as described below. In addition, tissue of the same ovaries was analysed histologically. Thereby it was possible to relate the oocyte developmental pattern directly to histological results. For the whole mount method at least 50 mg of ovary tissue was removed from the sample. The oocytes were then separated into different size classes with 600 µm, 300 µm and 150 µm sieves. After separation oocytes were photographed, automatically measured to the nearest µm (diameter) and counted with image analysis software (*Leica Qwin Software*). The separation with sieves into different size groups facilitates the image analysis, as no large oocytes will cover smaller ones. As all oocytes were measured, no bias in size frequency was introduced by sieving the material. However, for subsequent analysis only oocytes >200 µm diameter were taken into account in order to exclude primary growth oocytes. The oocyte diameters were grouped in 25µm intervals and length frequency distributions were established for each analysed ovary. Cohorts were identified in each oocyte size frequency distribution and for each cohort the median oocyte diameter was calculated. The median was chosen as some frequency distributions of the more advanced oocyte cohorts showed significant deviations from normal distribution (K-S-Test; $p<0.05$). By sorting the analysed ovaries according to their median oocyte diameter of the leading cohort, the oocyte growth dynamics was analysed with respect to the according POF stage and ovarian maturation stage of each analysed ovary. In order to estimate the relative duration of POF stages, those ovaries for which oocyte diameters were measured and which contained POFs were sorted by the median diameter of the most advanced oocyte cohort to display the whole spawning interval. Further, the POF stage and gonadal development stage were assigned to each ovary.

Estimation of spawning frequency

The mean spawning frequency (*S*), the proportion of females spawning per day, and its variance (*Var(S)*), were estimated with the following equations (Piquelle & Stauffer, 1985):

$$S = \frac{\sum_{i=1}^{n} m_i \times y_i}{\sum_{i=1}^{n} m_i} \quad (1)$$

$$Var(S) = \frac{\sum_{i=1}^{n} {m_i}^2 \times (y_i - \bar{S})^2}{\left(\sum_{i=1}^{n} m_i/n\right)^2 \times n(n-1)} \quad (2)$$

where m_i is the number of mature females in the i_{th} haul and y_i the proportion of spawning females. Each haul was used in this calculation. Prevalence of actively spawning females can be estimated from the proportion of imminent spawning females with hydrated oocytes (or less often germinal vesicle migration stage), or recent spawners, *i.e.* with presence of POFs. However, the proportion of females with hydrated oocytes or recent POF was highly variable between hauls. To reduce variability, the sum of females with hydrated oocytes and females with recent POF divided by two was used to estimate the prevalence of spawning females (Korta *et al*., 2010b). To estimate the spawning interval in days the duration and the peak spawning time during the day has to be known. In the case of Baltic sprat it was assumed that the duration of the hydrated stage and POF I is approximately 24 h each, and thus represents the daily spawning fraction of the population (Murua *et al*., 1998; Korta *et al*., 2010a).

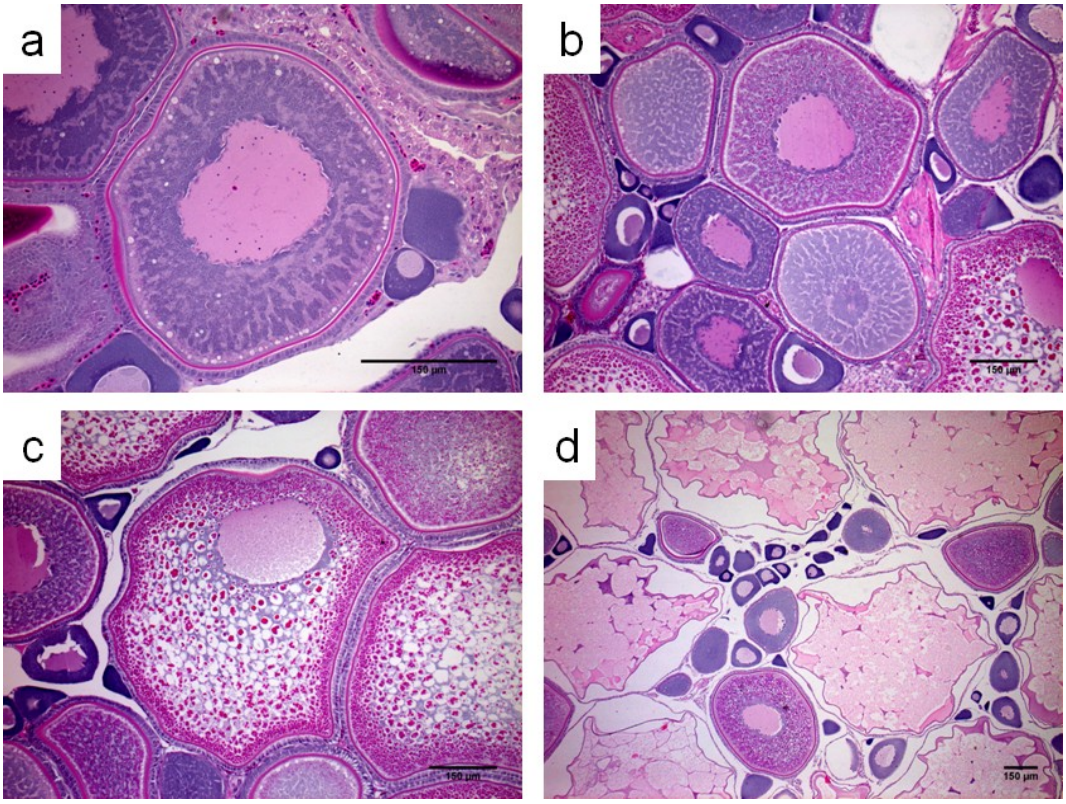

Fig. 2: Stages of oocyte development. (a) cortical alveoli, (b) vitellogenesis, (c) germinal vesicle migration and (d) hydrated.

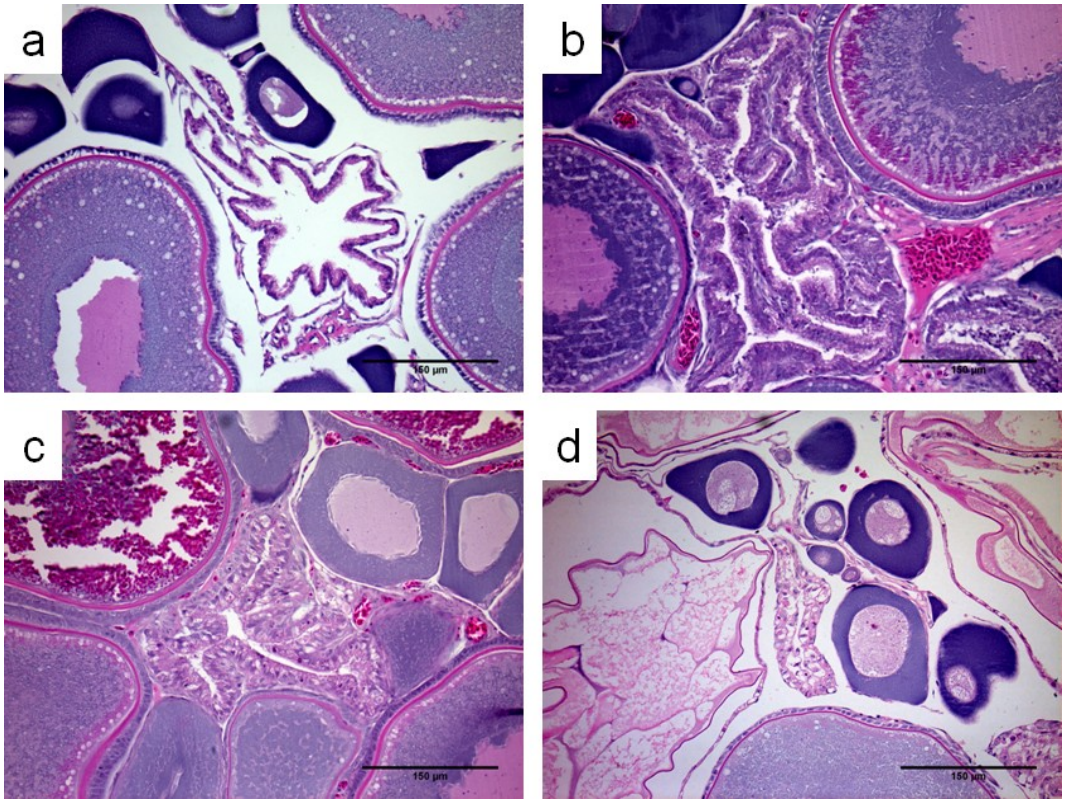

Fig. 3: Stages of postovulatory follicles of Baltic sprat. (a) POF I < 24h, (b) POF II, (c) POF III and (d) POF IV > 72h.

IV. 3 Results

The histological classification of all analysed ovaries revealed 23% (±15% SD) females with hydrated oocytes, 41% (±19% SD) in the vitellogenic and 36% (±20% SD) in the germinal vesicle migration stage (Tab. 1).

Tab. 1: Date and time of sampling, n = number of sub-sampled females for histologic analysis; sex ratio as proportion of females from at least 200 specimens; fraction of maturity stages and POF stages derived from histological analysis.

haul	date	time	n	sex ratio	Fraction of:							
					vitellogenesis	GVM	hydrated	POF I	POF II	POF III	POF IV	No POF
1	16 April 2007	06:18	21	0.41	0.19	0.57	0.24	0.00	0.10	0.38	0.43	0.10
2	16 April 2007	09:00	23	0.48	0.30	0.57	0.13	0.09	0.17	0.35	0.30	0.09
3	16 April 2007	12:00	23	0.22	0.86	0.14	0.00	0.65	0.22	0.09	0.00	0.04
4	16 April 2007	15:00	20	0.22	0.50	0.10	0.40	0.25	0.25	0.05	0.40	0.05
5	16 April 2007	18:00	20	0.31	0.35	0.15	0.50	0.20	0.10	0.15	0.55	0.00
6	16 April 2007	21:00	20	0.58	0.50	0.25	0.25	0.10	0.25	0.35	0.20	0.10
7	17 April 2007	00:00	21	0.54	0.38	0.38	0.24	0.14	0.19	0.38	0.24	0.05
8	17 April 2007	03:00	21	0.30	0.38	0.48	0.14	0.29	0.10	0.38	0.19	0.05
9	17 April 2007	06:00	21	na	0.24	0.57	0.19	0.10	0.10	0.52	0.29	0.00

Spawning activity showed an asynchronous diel pattern, as females with hydrated ovaries were found throughout the day. However, the proportion of females with hydrated ovaries varied largely between hauls over the entire 24 h period. The highest proportion of hydrated females was found at 06:00 p.m. with nearly 50% of females containing ovaries with hydrated oocytes. The lowest value was found at noon with no females having hydrated oocytes (Fig. 4a).
In 95% of the analysed ovaries POFs were detected. POFs were classified into four different stages based on their histomorphology:

I. The POF has a clear convoluted shape and a rather big lumen. It consists of two cell layers, theca and granulosa with clearly visible nuclei and relatively thin width with similar morphology to the follicle wall in advanced vitellogenic oocytes. The cross sectional area is larger compared to that of other identified POF stages. This type of POF occurred exclusively in ovaries where vitellogenesis was the most advance stage of oocyte development (Fig. 3a).

II. The POF has still a distinct convoluted shape but the lumen has become smaller. The two cell layers are still distinguishable but first signs of degeneration are visible: Vacuoles become visible and nuclear pycnosis occurs. The thickness of the two cell layers has clearly increased and granulosa cells are more rectangular. Again, this type of POF occurred exclusively in ovaries where oocytes have not developed further than vitellogenic stage (Fig. 3b).

III. The convoluted shape begins to disappear, as the follicle is being resorbed. The POF lumen shrinks and is no longer clearly distinct. It is not possible to distinguish between the two cell layers. The cross sectional area has clearly decreased compared to the two first POF stages. The thickness of the two cell layers has decreased again due to degeneration. This POF type occurred in ovaries with early and advanced germinal vesicle migration stage or in ovaries with early hydrated oocytes (Fig. 3c).

IV. The convoluted shape and lumen are not visible anymore. The follicle has shrunk considerably, becoming more compact and the cross sectional area is very small compared to the other stages. This POF type occurred in ovaries with advanced germinal vesicle migration and hydrated oocytes (Fig. 3d).

Also the occurrence of different POF stages showed an asynchronous pattern throughout the day (Tab. 1). The variability of POF stage proportions between the sub-samples was high for every POF stage. The highest range was detected for POF I with 0% at 06:00 a.m. and 65% at noon (Fig. 4b).

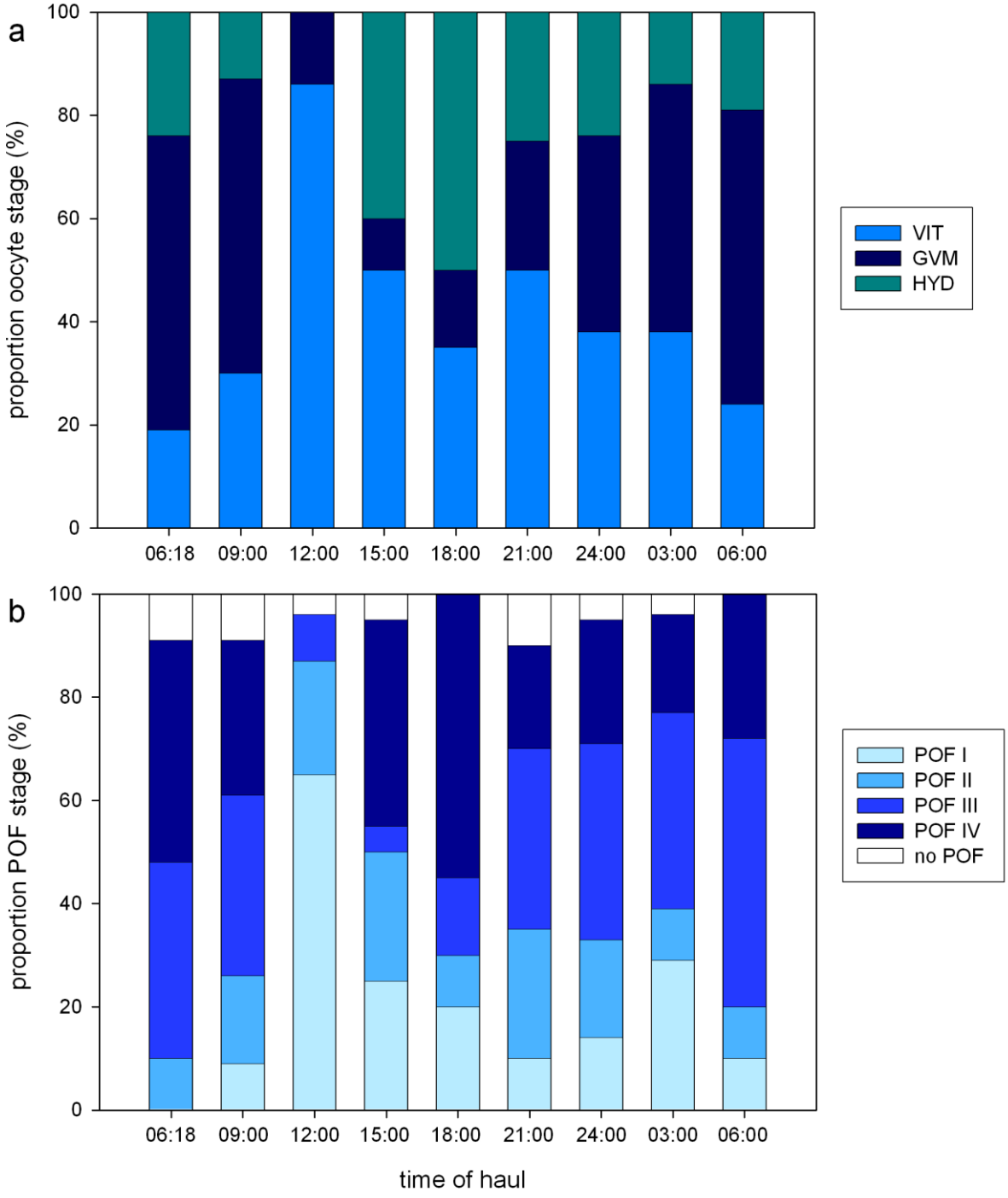

Fig. 4: Share of different oocyte stages (a) and POF stages (b) in fishery hauls conducted over the 24 h period.

The analysis of oocyte size frequency revealed the presence of two clearly distinct cohorts of secondary growth stage oocytes: from the pool of early vitellogenic oocytes, subsequent cohorts develop one by one with increasing oocyte diameter until spawning (Fig. 5).

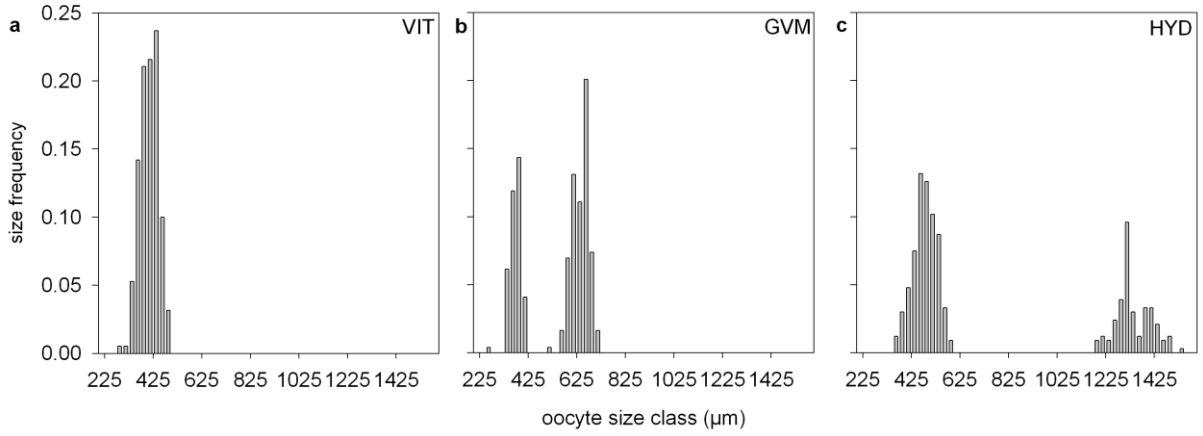

Fig. 5: Typical oocyte size frequencies observed for specific ovarian maturation stages. (a) vitellogenesis (VIT), (b) germinal vesicle migration (GVM), (c) hydrated.

The median oocyte diameter in ovaries with only vitellogenic oocytes ranged from 410 µm to 558 µm, the median of oocytes in the germinal vesicle migration stage ranged from 568 µm to 813 µm (Fig. 6), and the hydrated oocytes ranged from 877 µm to 1388 µm in diameter. While the size increase was clearly visible for the leading cohort, the median diameter of the second cohort remained constant around 400 µm. Although the median values between two cohorts become distinct in early development (Fig. 6), there was an overlap in oocyte size distributions during vitellogenesis, becoming completely separated when the leading cohort enters the germinal vesicle migration stage.

The occurrence of POF stages (I to IV) corresponded clearly to the development of the leading oocyte cohort. The POF stages I and II were present almost exclusively in developing ovaries, and similarly POF III were found in spawning capable females, and POF stage IV was found exclusively in actively spawning fish with hydrated oocytes (Fig. 6). Considering that in very few ovaries POF were absent (5%), and in each ovary only one POF stage was present, the duration of POF degeneration equals the average batch interval, *i.e.* the time lag between subsequent spawning events.

Using formula (1) with the average proportion of hydrated and POF I for the 24 h sampling, as daily spawning marker, resulted in a spawning frequency of 0.22 (CV=0.10).

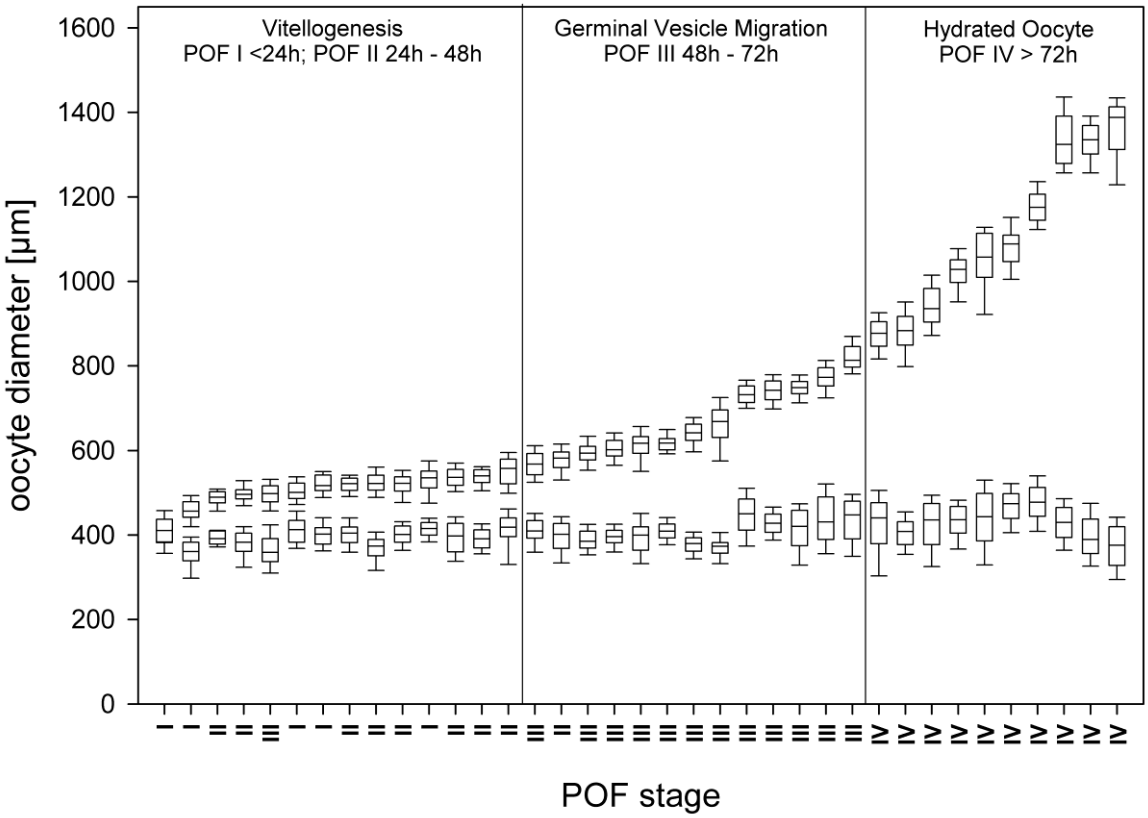

Fig. 6: Development of median diameters of the reserve stock of oocytes (lower row) and developing oocytes (upper row) with corresponding POF stages. Box displays 25th and 75th percentiles, vertical lines display 10th and 90th percentiles. In the upper part of the figure estimated durations of POF stages are denoted.

IV. 4 Discussion

The investigated oocyte development pattern of sprat shows a distinct hiatus between oocytes in the germinal vesicle migratory stage prior to hydration and the remaining reserve oocytes. A similar pattern has been observed for the anglerfish *Lophius litulon* (Yoneda *et al*., 2001) and for *Siganus canaliculatus* (Hoque *et al*., 1999). One example for a clupeid fish with this type of oocyte development pattern is the Mediterranean sardine (*Sardina pilchardus sardina*) as described by Ganias *et al*. (2004). For the latter species it was possible to estimate batch fecundity by using not fully hydrated females. Our results demonstrate that it also could be possible in the case of Baltic sprat to use females in the germinal vesicle migratory stage to estimate batch fecundity. This will be of advantage for the sampling procedure as it is sometimes difficult to obtain enough hydrated females from one haul, since their proportion was shown to be highly variable. Moreover, the risk of including partly ovulated ovaries into the batch fecundity analysis would be minimized, without checking ovaries histologically for POFs. The auto-diametric method to estimate fecundity (Thorsen and Kjesbu, 2001) can be easily applied. Hence, increasing number of samples will enhance fecundity estimation accuracy.
Unlike other clupeid fish species, Baltic sprat does not show a clear diel synchronous spawning behaviour (Alekseev and Alekseeva, 2005). Findings of the present study confirmed this hypothesis as females with hydrated oocytes and recent POF were found nearly throughout the day. Although there was a concentration of hydrated females found around 06:00 p.m. it is doubtful that this is a synchronised diel peak spawning, because (i) the occurrence of recent POF does not confirm this pattern, as no recent POFs occur during the early morning hours, and (ii) if

peak spawning occurred in the afternoon and at dusk, the elevated proportion of hydrated females should be found prior to the peak spawning for the duration of the hydrated stage. For the observed pattern that would translate into approximately 6 h duration for the hydrated stage from noon until the observed peak at 06:00 p.m.. Given the mean percentage of 23% hydrated females during the sampling this would result in more than one spawning per day and female, which seems quite unrealistic. The lack of diel spawning synchronicity is in contrast to many other clupeid species, *e.g.* for *Sardina* spp. or *Sardinops* spp. (Ganias *et al*. 2003; Macewicz *et al*. 1996), which synchronise their spawning during few hours at night time. For these species a reference time exists for the estimation of POF stages by means of field samplings. Alday *et al*. (2008) used an alternative approach to determine the POF duration in anchovy (*Engraulis encrasicolus*). They first defined different stages of POF solely based on histological features of degeneration, and then aged these defined stages in a second step by using information from tank experiments. Since no information from tank experiments with sprat was available for this study and no clear spawning peak during the day was found to serve as reference time, the exact estimation of POF duration was not possible in the present study. Nevertheless, it was possible to define four different stages of POF with respect to their histological features and their state of deterioration. Further, since nearly all analysed ovaries contained POF and in each ovary only one POF stage was present, it can be concluded that the duration of complete POF resorption equals the average batch or spawning interval. The estimated spawning frequency of 0.22 would translate into an average spawning interval of 4.5 days for individual female sprat, meaning that it takes 24h * 4.5 = 108 h from the earliest vitellogenesis stage to the fully hydrated stage. The estimated spawning interval is well in line with estimates in sprat literature, *e.g.*, from the Bornholm Basin (Kraus and Köster, 2004), and from the Gdansk Deep (Alekseev and Alekseeva, 2005). However, these studies also assumed duration of 24h of the hydrated stage, which lacks any evidence from field or tank experiment data. If we assume this spawning interval to be a realistic estimate for Baltic sprat it would be possible to estimate the duration of each ovarian developmental stage by converting their proportions into time. It would also be possible to give a rough estimation of the duration of POF stages III and IV, which are very closely related to the GVM and the hydrated oocyte stage, respectively. This approach would result in approximately 29 h, 38 h and 41 h duration for the hydrated ovaries/POF IV, germinal vesicle migration/POF III, and vitellogenesis stage, respectively. Aging POF I and POF II is more complicated as both are related to the vitellogenic stage. However, the duration of POF I seems to be shorter than that of POF II, because there were less POF I stages recorded. Converting the proportions of POF I and POF II to time results in 15 h duration for POF I and 26 h duration for POF II. The duration of POF I is shorter than 24 h, while the duration of hydration might last longer than exactly one day. However, the total duration of hydration and POF I together was 44 h, *i.e.* ca. 48 h, as assumed to calculate the spawning frequency.

Although the spawning interval estimated in the present study was well in line with literature values, our results differ considerably from historical observations by Heidrich (1925), who reported a spawning frequency of 7 to 9 days from the Kiel Bight area. This difference might be due to different environmental conditions between the Western and Central Baltic Sea, but it was also criticized that Heidrich might have underestimated the spawning frequency because of methodical shortcomings (Alekseev and Alekseeva, 2005; Kraus and Köster, 2004).

Spawning frequency in the present study was estimated applying the average proportion of hydrated females and females containing POF I in their ovaries. This is in contrast to previous reports on sprat spawning frequency, where only the incidence of hydrated females was used as spawning marker (Kraus and Köster, 2004; Alekseev and Alekseeva, 2005). Although using exclusively hydrated females to calculate this parameter did not result in substantially different results, it is of advantage to include histological analyses in order to enhance the accuracy of estimations, because it was found that the hydrated oocytes might last for more than one day in the ovary, and the POF I duration was shorter. Thus, using only hydrated females may lead to an

overestimation of the spawning frequency, while using only POF I may lead to an underestimation. Hence the approach to combine both stages should give a more balanced estimation of spawning frequency, because the sum of both is closer to 48 h.
Generally, the proportion of hydrated females exceeded the proportions of females with recent POF. This might not only be due to the longer duration of the hydrated stage, but can be due to the specific spawning behaviour of small pelagic fish, forming dense and female dominated spawning aggregations, which were reported to be more vulnerable to the sampling gear. Thus, in previous studies on spawning frequency, it was recommended not to use hydrated females for analysis, because they might be oversampled (Alheit, 1993; Alheit *et al*., 1984), and instead use the postovulatory follicle method. Our results showed that the hydrated oocyte method is appropriate to estimate spawning frequency in the case of Baltic sprat and might also be used to estimate the spawning frequency from historical sprat maturity data sets without parallel histological investigations.
Duration of less than 24 h for the first POF stage, as we propose also for sprat, was reported for several clupeid species such as *Sardina pilchardus pilchardus* off Portugal (ICES, 2000), *Sardinops melanostictus* off Japan (Aoki and Murayama, 1993), and *Sardinops sagax musica* off Chile (Claramunt and Herrera, 1994). In contrast to sprat, for these species only three POF stages have been described. Similar to our observations on Baltic sprat the complete POF duration of northern anchovy lasted also longer than 72 h (Hunter and Macewicz, 1985). For the Mediterranean sardine (*Sardina pilchardus sardina*), the first POF stage was reported to last 10 h (Ganias *et al*., 2003), a comparably shorter duration than for sprat and most other clupeid species. These differences might be related to different temperatures prevailing in the investigated areas, as the resorption of POF is known to be strongly dependent on the ambient temperature (Fitzhugh and Hettler, 1995), and also the growth rates of oocytes have been reported to increase with increasing temperatures (Kurita *et al.*, 2011). Therefore, the POF stage duration and the duration of ovarian developmental stages of Baltic sprat might vary over the prolonged spawning season due to an increase in ambient temperatures over the spawning season lasting approximately from March to June. However, this issue should be addressed in future investigations to further enhance the understanding of POF degeneration and thus spawning frequency of Baltic sprat. The methodology described in the present study, combining histology and image analyses, will clearly improve this type of analyses.
From the oocyte size distribution it can be seen that right after releasing the spawning batch the next cohort of oocytes begins to increase in size to form a new batch. Our results indicate that the batch development cycle lasts approximately 4.5 days. Compared to other species this is rather quick. For Mediterranean sardine a spawning interval of 10 to 11 days was reported (Ganias *et al*., 2003), a value considerably lower than found for other sardine stocks from the Atlantic coast off Spain and Portugal (Ganias *et al*., 2003 and references therein). This was explained by the oligotrophic characteristic of the Mediterranean Sea, as the egg production and spawning frequency might be influenced by food availability and thus condition of the fish. The comparably fast oocyte development of Baltic sprat might therefore be due to favourable feeding conditions during the peak spawning period, which is synchronised with the peak production period of calanoid copepods in the central Baltic, the major prey items for sprat (Möllmann *et al*., 2004; Möllmann *et al*., 2003). Petrova (1960) reported an accelerated oocyte development of sprat which was explained by optimum food availability and also favourable temperature conditions. Hence, it seems to be a common pattern in Baltic sprat, that degenerated POFs and hydrated eggs can be found together in the same ovary. This can be explained by the comparatively slow POF degeneration, due to low ambient temperatures, and relatively fast oocyte development, due to favourable feeding conditions, compared to other clupeid fish where these features have been studied. Thus, the association of the respective POF stages to oocyte recruitment might differ with environmental conditions. It was not possible to investigate the

relative effect of temperature and prey availability on POF duration and oocyte recruitment in the present study, as only samples from one cruise were analysed.
The results of the present study provide an excellent basis for future studies on sprat spawning dynamics, in order to compare reproductive parameters between different stocks, years and seasons. It might also be possible to extend our approach to study the dynamics of gonadal development in other fish species having a similar oocyte recruitment and daily spawning pattern to Baltic sprat, *e.g.* hake (*Merluccius merluccius*, Murua *et al*., 1998). However, so far all estimates of sprat spawning frequency are based on assumptions about the duration of used spawning markers and not on direct observations or experiment results. Therefore, it is strongly recommended to further investigate this important parameter of sprat which would allow a validation of our results and that of other authors. This study can be seen as a first approach to tackle the problem of POF stage duration and to get a more reliable estimate of spawning frequency for Baltic sprat.

Acknowledgements

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Chapter V: Temperature-dependent egg development of Baltic sprat

Abstract

An experimental approach was used to determine the egg development time in relation to temperature in a range from 1.8°C to 16°C. The relationship between incubation temperature and the time to the end of each of four egg stages is presented. For all egg stages, time to complete the respective stage decreased exponentially with increasing ambient water temperature. A multiple regression model on temperature-dependent egg stage development time was established. At the lowest temperature only very few eggs developed successfully, at the highest temperature no successful egg development was observed. The presented results are relevant for the aging of field caught sprat eggs which enables to estimate mortality rates and the daily egg production.

Key words: egg development, egg aging, *Sprattus sprattus*

V. 1 Introduction

In marine fishes, recruitment success is largely relying on the success of their early life stages as these are most susceptible to mortality (*e.g.* Hjort, 1914; Rothschild, 2000; Chambers and Trippel, 1997; Houde, 2002). The exact timing of critical transitions during early life history of temperate species is crucial for survival. Especially pelagic fish eggs are strongly influenced by abiotic factors such as temperature, salinity, oxygen saturation or wind forcing (Grauman and Yula, 1989; Blaxter, 1992; Köster *et al,*. 2003). Temperature plays a central role due to its importance in pacing physiological processes (Blaxter, 1992; Fuiman, 2002). Hence, knowledge about the duration and timing of early life stages is a prerequisite for understanding and interpreting match and mismatch situations between larval predators and their prey (Cushing, 1972).

Several applications have been developed to estimate the size of fish stocks by means of the abundance of their early life stages in the field (Lockwood *et al.*, 1981; Parker, 1980). If the temperature-dependent development is known, observed abundance at specific egg stages can be converted into abundance at age in order to correct for instantaneous mortality and the daily egg production can be estimated from ichthyoplankton samples (Lo, 1985). Fox *et al.* (2003) found faster egg development rates under similar temperature conditions in Irish Sea plaice (*Pleuronectes platessa* L.) populations compared to eggs from North Sea plaice. They suggested genetic differences between the two stocks which could lead to inter-stock differences in egg development rates. Since the application of incorrect egg development rates clearly has the potential to bias the assessment of spawning stock biomass (SSB) using egg production methods, Fox *et al.* (2003) recommend that egg development relationships should be evaluated for each stock separately.

Although sprat is an abundant and important species in the Baltic Sea ecosystem, little is known about temperature effects on their reproduction traits and early life stages. Sprat are indeterminate multiple batch spawners releasing several thousand pelagic eggs per spawning season, which may last from February to July in the Baltic (Ojaveer and Kalejs, 2010). Due to the brackish environment of the Baltic, egg buoyancy is restricted to the more saline, deeper water layers, *i.e.* 40-65 m in the Bornholm Basin, one important spawning area in the Baltic. As spawning season proceeds the vertical distribution of sprat eggs changes towards shallower water layers due to seasonal changes in hydrography (Nissling *et al*., 2003). Historic observations of egg and larval development are limited to few temperature regimes and the

majority of studies use field caught sprat eggs and larvae which causes variation due to the difference in age (*e.g.* Ehrenbaum, 1936; Morawa, 1953). Experimental data on sprat egg development from the English Channel were provided by Thompson *et al.* (1981). For Baltic sprat, Nissling (2004) provides information on temperature dependent egg developmental times in the Gotland Basin and the Gdansk Deep. However, this study does not provide information on the temperature dependent duration of all four egg stages, which is necessary to estimate mortality in the field. The aim of the present study was to establish a temperature-dependent model of Baltic sprat egg development that can be applied to estimate mortality and daily egg production in the field. This is a prerequisite for the application of the Daily Egg Production Method to Baltic sprat.

V. 2 Methods

Sprat eggs were obtained from the Bornholm Basin during a cruise with RV Alkor in March/April 2004 from stripping running, fully hydrated female sprat caught with a pelagic trawl.
Eggs were obtained from a single female. Eggs were fertilised with a mixture of sperm from six males for 30 minutes in unfiltered surface seawater at ambient salinity of 7.1 psu. Subsequently, eggs (n=2906) were transferred into a 500 ml plastic box containing 1.0 µm filtered Baltic seawater with a salinity of 18 psu, which keeps fertilised eggs floating (Nissling *et al.,* 2003; Nissling, 2004). Unfertilised eggs were negatively buoyant. Thus, eggs which had sunk to the bottom were removed (Nissling, 2004). Subsamples of the remaining floating eggs were checked under a stereo microscope after 12 h to ensure fertilisation success. The fertilised eggs were stored in darkness in a temperature controlled room at 6 °C (±0.5 °C) on board. All eggs were transported to the laboratory in Kiel and upon arrival separated into 150 ml beakers filled with 6 °C and 5 µm filtered water with a salinity of 14.8 psu. The time from fertilization to the start of the experiment was 38 h. The salinity of 14.8 psu kept the eggs at all temperatures floating. Each beaker, containing 30 to 156 eggs, was placed into a temperature gradient table (Thomas *et al.*, 1963) in a temperature-controlled room with a light regime of 12 h light and 12 h darkness. The incubation table is an aluminium block, which is heated on one side and cooled on the other side to create a stable temperature gradient (Fig. 1). It contains 6 x 10 holes in which the beakers can be placed. Ten different temperatures were set in three to four beakers (1.8 °C, 3.4 °C, 5.2 °C, 6.8 °C, 8.4 °C, 10.0 °C, 11.6 °C, 13.1 °C, 14.7 °C, and 16.0 °C). The single temperatures were stable with minimum and maximum fluctuations of 0.08 and 0.17 °C, respectively. After the beakers were placed into the table, they were gently acclimated to the chosen temperatures with approximately 1 °C per hour. Before incubation, sub-samples of eggs were checked under a stereo microscope to determine the developmental stage at the start of the incubation. Eggs were assigned to development stages according to Thompson *et al.* (1981). Due to the time lag between fertilisation and incubation all eggs had reached already development stage IB at the start of the incubation. Therefore, the first egg stages IA and IB had to be combined to a single stage I in the subsequent analyses since the end of stage IA was not recorded. Dead eggs were removed every day from the beakers to remain good water quality. Every 24 hours, digital images of randomly chosen sub-samples (1-2 eggs per beaker) of eggs were recorded with a camera system under a stereo microscope. Due to the low numbers, photographed eggs were immediately replaced back into the respective beaker. Afterwards egg developmental stages were determined from digital images (Fig. 2). The duration of the egg stages I to IV was defined as days until the last egg of a temperature group reached the next stage.

Exponential decay models were fitted to the data of different egg stages:

$$td_i = a * T^{-b} \quad (1)$$

where td_i is the duration of the i_{th} egg stage in days, T the ambient temperature (°C) and a and b regression coefficients. To obtain a single model to predict egg duration in relation to ambient seawater temperature a multiple non-linear regression analysis was performed using the following equation:

$$td_i = a * e^{-(b*T)} * S_i^c \quad (2)$$

where td_i is the duration of the i_{th} egg stage in days, T the ambient temperature (°C), S_i the i_{th} egg stage and a, b and c regression coefficients.

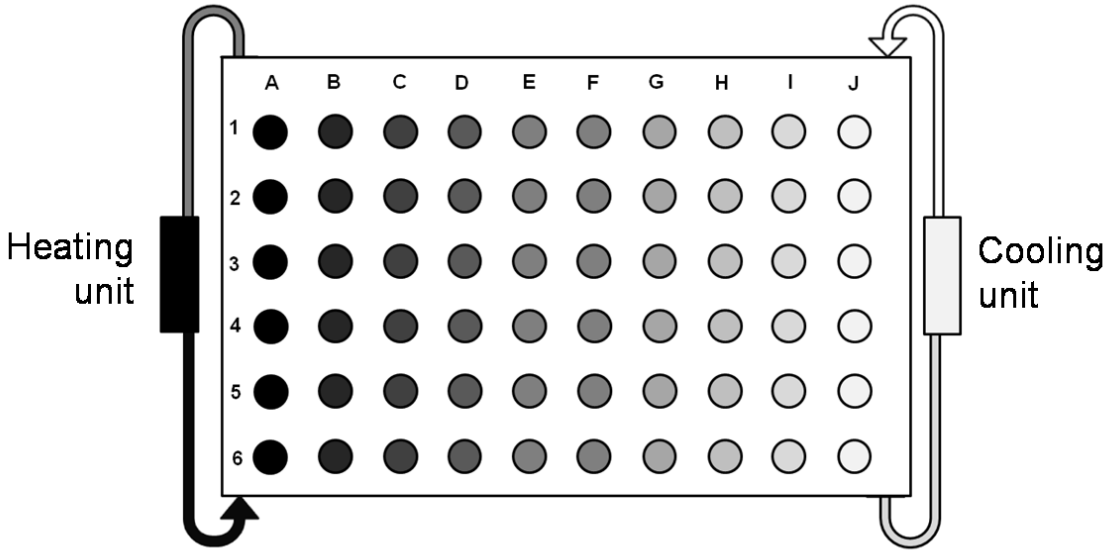

Fig. 1: Schematic of the temperature gradient table used in the egg incubation experiments with 10 temperatures (columns A-J) and six replicates (beakers, row 1-6).

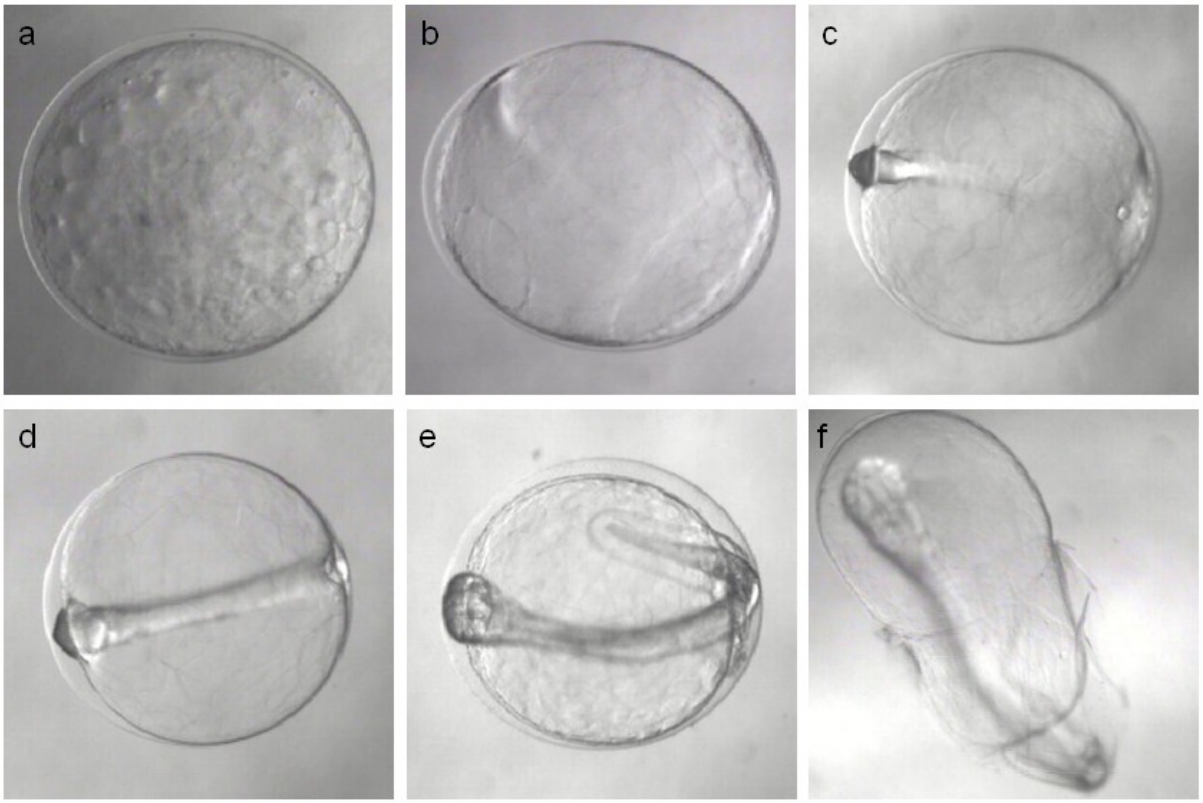

Fig. 2: Sprat egg development stages. (a) stage IB, (b) early stage II, (c) late stage II, (d) stage III, (e) stage IV and (d) hatching sprat larva. Photographs taken by Holger Haslob, IFM-GEOMAR.

V. 3 Results

Sprat egg development was temperature dependent. The duration of each developmental stage (Fig. 2) exponentially decreased with increasing temperature (Fig. 3; Tab. 1). A multiple regression can successfully describe the relationship between stage duration and temperature for the four differentiated stages (r^2=0.97, Tab. 1). For the first egg stage, the duration time was 5 days at the lowest temperature (1.8 °C) and two days for temperatures above 10 °C. The observed time from fertilisation to hatch was 17 days at the coldest temperature and 6 days at 14.7 °C, the highest temperature at which successful egg development was observed. Above 14.7 °C, no successful egg development was observed. At the lowest temperature (1.8 °C), only two eggs survived, one larva having a malformed yolk sac and the other hatching successfully.

Tab. 1: Regression coefficients of exponential models on egg stage duration for specific egg stages in relation to ambient temperature (equation 1) and for all stages combined in a two factor exponential regression model (equation 2).

egg stage		regression coefficients	standard error	r^2	p
I	a	5.57	0.38	0.93	<0.0001
	b	0.08	0.01		0.0002
II	a	8.06	0.35	0.98	0.0019
	b	0.08	0.01		0.0131
III	a	13.61	1.24	0.89	<0.0001
	b	0.11	0.01		0.0002
IV	a	20.33	1.43	0.95	<0.0001
	b	0.13	0.01		<0.0001
stages combined	a	5.58	0.55	0.97	<0.0001
	b	0.12	0.01		<0.0001
	c	0.87	0.08		<0.0001

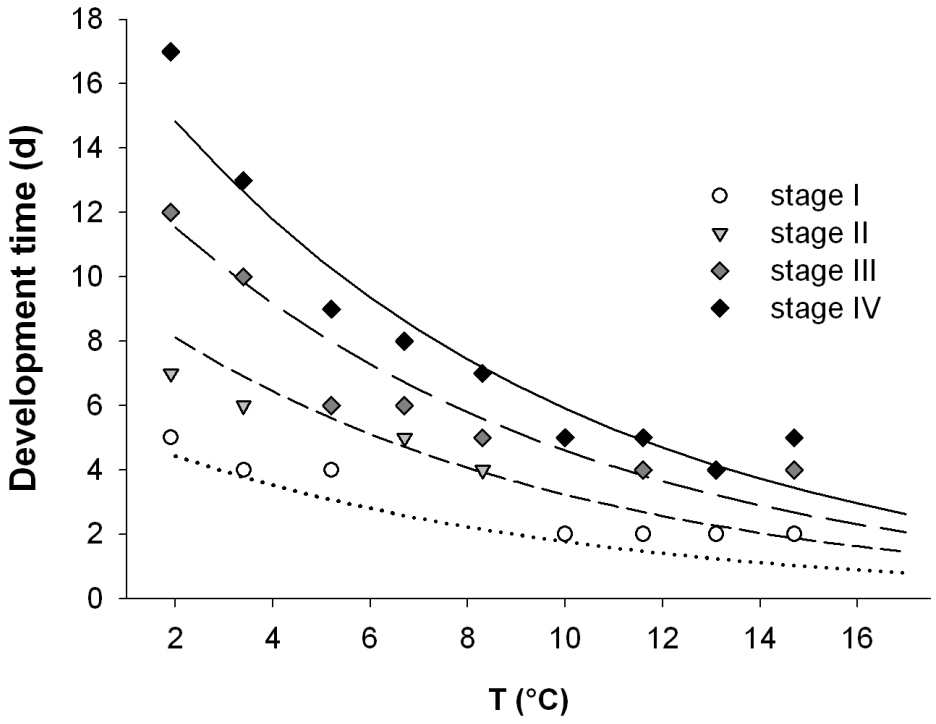

Fig. 3: Development time of stages I-IV of sprat eggs at the different experimental temperatures. Data were fitted to a multiple regression model. Lines represent the model prediction for the respective stage. Parameter estimates (±SE) are given in table 1.

V. 4 Discussion

The obtained egg stage duration times are generally in line with observations by Thompson *et al.* (1981) who performed experiments on the development of sprat eggs from the English Channel (North Sea) using 19 different temperatures from 4.5-20 °C. Compared to the data provided by Nissling (2004) the observed time from fertilisation to hatch was approximately one day longer for the whole temperature range. The deviations in the egg stage durations may be explained by the time lag between fertilisation and incubation in our study. All eggs experienced 6 °C temperature conditions during the transport which might have caused faster development of the eggs in the lower temperatures and a somewhat slower development in the higher temperature treatments.

Furthermore, colder temperatures appeared to increase the variability within experimental groups, as some embryos were not able to hatch while other larvae already had hatched at the coldest temperatures. This was probably due to reduced embryonic activity which may hamper the hatching success in colder temperatures.

No successful egg development was observed at the lower and upper end of the tested temperature range. The lack of successful hatching at the lower temperature extreme agreed with results from experiments conducted by Nissling (2004) who reported higher mortalities for temperatures below 4 °C. Thompson *et al.* (1981) found successful development over the full temperature range, although from 17.4-20 °C hatching occurred prematurely before many eggs reached stage IV. The authors doubted the larvae were sufficiently well developed to survive. Egg survival until hatch between 6 °C and 18.5 °C ranged from 36% to 67%, with higher mortality at the extremes of the experimental temperature range. These findings of successful egg development above 14.7 °C are in contrast to the findings of the present study and may be related to possible genetic adaptations or a difference in incubation salinity between the two experiments.

Results obtained by the present study enable to assess the impact of interannual changes in ambient temperatures on Baltic sprat egg stage duration in the field. Low temperatures prolong the development time of sprat eggs, thus increasing the susceptibility to predation (Nissling, 2004). In the context of a match-mismatch hypothesis (Cushing, 1972), interannual changes in ambient temperatures have consequences for the successful survival of larvae as the time of hatch will be affected, and thus their ability to find sufficient food for first feeding. On shorter time scales, temperatures in the upper halocline, *i.e.* the water layer where sprat eggs occur (Nissling *et al.*, 2003), may be affected even stronger due to the inflow of warm summer surface waters from the Kattegatt (Mohrholz *et al.,* 2006). Further, the effects of temperature elevation due to climate change (IPCC 2002) can be investigated. Alheit *et al.* (2005) compared temperature time-series for the Bornholm Basin from 1970-1987 and 1988-2003 and found an increase in the spring and autumn surface mixed layer temperature by about 1.5 °C. Baltic sprat eggs are supposed to take advantage of a predicted elevation of ambient temperatures (MacKenzie *et al,.* 2007; Nissling, 2004) because present average ambient conditions in the water layer where sprat eggs occur (45 to 65 m; ~4 °C) range below the optimum survival temperature described for sprat eggs (Petereit *et al*., 2008; Nissling, 2004).

The acquired temperature-dependent egg development model can now be applied to sprat egg stage abundance data in order to estimate daily mortality and egg production in the field. This allows the investigation of factors influencing egg survival on spatial and temporal scales as well as the application of the Daily Egg Production Method as a fishery independent stock assessment tool.

Chapter VI: Application of the Daily Egg Production Method to Baltic Sprat

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Abstract

Baltic sprat (*Sprattus sprattus balticus,* Schneider 1908) is a key species in the Baltic Sea, as it is the most abundant planktivorous fish species in this pelagic ecosystem. In the present study the Daily Egg Production Method was applied to Baltic sprat in the Bornholm Basin, an important spawning ground for sprat and cod, for the years 1999 to 2008. Results were compared with stock size estimations obtained by a multi species virtual population analysis and results obtained by an acoustic survey. In general, the results obtained by the egg production method were in the same order of magnitude compared to the other methods, being closer to the acoustic estimate. However, results also revealed pronounced differences between compared methods. Since the accurate determination of the daily spawning fraction bears major uncertainties different scenarios were tested for this parameter. Least deviation to the other assessment methods was obtained when using a daily spawning fraction of 24%, which corresponds well to values described in literature. The applicability of the Daily Egg Production Method to Baltic sprat was clearly demonstrated and it can serve as valuable tool for the estimation of Baltic sprat stock sizes independent from fishery data as well as for spatial sub-areas, *i.e.* distinct spawning areas.

Key words: DEPM, spawning frequency, *Sprattus sprattus*

VI. 1 Introduction

The Daily Egg Production Method (DEPM) was demonstrated to be an adequate tool to estimate the spawning stock biomass of pelagic fish species with indeterminate oocyte recruitment and multiple batch spawning (Parker, 1980; Lasker, 1985). Since then, the DEPM has been applied for many stocks around the world (Stratoudakis *et al*., 2006; Alheit, 1993). One advantage of egg production methods is their independence of catch data from commercial fisheries, which is often biased due to: (i) misreporting of catches, (ii) discards, or (iii) specific fishing patterns. Further, only a single survey during the peak spawning period of the species under investigation is necessary to assess the spawning stock biomass. In addition to the stock size estimate, the DEPM provides valuable biological data of the stock reproductive potential, which is often not taken into account in standard assessment methods, *e.g.* the fecundity and spawning frequency of fish as well as the distribution, abundance and survival of the early life stages. Therefore, the DEPM is a cost and time effective alternative assessment method that combines processes acting on biological traits from the oocyte development to the egg phase in the open sea (Stratoudakis *et al*. 2006) with the potential to provide new insights into the reproductive dynamics of the assessed fish species (Somarakis *et al*., 2004) and its interaction with the environment. One

disadvantage is that the DEPM cannot be applied beyond spawning season. Also, two major methodological challenges remain: first, in order to assess the whole population it has to be assured that the complete spawning area is covered by the ichthyoplankton survey and spatial patchiness in egg distribution is resolved sufficiently. Second, a crucial source of uncertainty in applying the DEPM has been identified in the determination of the spawning frequency (Stratoudakis *et al*., 2006).

Baltic sprat is assessed by the International Council of Exploration of the Sea (ICES) as a single stock unit in the entire Baltic (ICES Sub-Divisions 22-32) using a virtual population analysis with an extended survivor analysis (XSA) based on catch data, which is tuned by an acoustic survey conducted in autumn and spring (ICES, 2010a). The calculation of the spawning stock biomass (SSB) with this standard method does not take into account observed spatial and temporal variability of important reproductive parameters. As comprehensive and coherent investigations on the variability of Baltic sprat maturity are lacking so far (ICES, 2010a), the maturity ogive is kept constant over the whole time series to calculate SSB. In general, more than 90% of the sprat stock older than 2 years is mature. However, there is a considerably variability in the proportion mature in age groups one and two. This may lead to a significant underestimation of sprat SSB in years with a high proportion of young sprat being mature or vice versa. Further, the spawning stock biomass is calculated without taking sex ratios into account. The latter approach is in contrast to observations which show that the sex ratio is skewed towards a higher proportion of females with increasing size or age, respectively (Grygiel and Wyszyński, 2003). Another shortcoming of this assessment is that the stock abundance estimates are not provided for stock components inhabiting different areas of the Baltic Sea. As sprat is known to be an important predator on eggs of eastern Baltic cod (Köster and Schnack, 1994), which has its main spawning ground in the Bornholm Basin, it is desirable to estimate the stock size in this particular area.

Several attempts have been made in the past to estimate sprat stock sizes in the Baltic Sea by egg production methods. Grauman and Krenkel (1986) estimated the sprat stock covering extensive areas from the Arkona Basin up to the central Gotland Basin. Macarchouk (2001; 2007) estimated the sprat stock for the Gotland Basin applying the Hensen-Apstein method. However, these authors made many assumptions and simplifications concerning sprat stock structure, *e.g.* fecundity and spawning fraction, particularly crucial parameters to assess a fish stock with egg production methods. Kraus and Köster (2004) applied for the first time the DEPM to estimate sprat stock abundance for Baltic sprat. They modified the original DEPM model to calculate the stock size based on fish length classes. However, their study was restricted to one year only, but showed that this method might be applied successfully to Baltic sprat.

In the present study, detailed observations on reproduction parameters of Baltic sprat, *i.e.* sex ratios, maturity ogives, spawning fraction, and batch fecundity were combined with total sprat egg production from ichthyoplankton surveys, in order to assess the stock size of Baltic sprat with the DEPM. Stock size estimates were thus achieved for the Bornholm Basin area (ICES Sub-division 25) as a continuous time-series for the years 1999 to 2008. The obtained results were compared with sprat abundance data from acoustic surveys and from an area disaggregated multi-species virtual population analysis (MSVPA).

VI. 2 Materials and Methods

Daily egg production from ichthyoplankton surveys

The abundance of sprat eggs was obtained from ichthyoplankton surveys covering the Bornholm Basin (Tab. 1) on a 45 stations grid (10.0 * 8.5 nm miles; Fig. 1). Double oblique hauls with a Bongo net (Ø=60 cm; 335 µm and 500 µm mesh size) were conducted on each station. In some

years, not all stations were sampled due to gear failure in some cases or bad weather conditions, but a sufficient coverage of the basin was achieved in all years. Samples were preserved in a 4% buffered formaldehyde solution immediately after sampling. Samples obtained by the 335 µm mesh nets were sorted in the laboratory and eggs were assigned to five egg stages (Ia, Ib, II, III, IV according to Thompson *et al*., 1981). The egg abundance was calculated as eggs m^{-2} on each station. Because of uncertainties concerning the identification of egg stage Ib, the first two stages were grouped to a single stage I. The total abundance data where fitted to normal distribution curves to visualise the seasonal course of egg production in the investigated area. It was assumed that the highest observed value is an indicator for the peak spawning and was therefore chosen for the DEPM calculation procedure.

Tab. 1: Ichthyoplankton sampling and daily egg production results used for spawning stock size estimations: cruise (AL = RV"Alkor"), month and year of sampling, number of stations, total egg abundance, mean ambient temperature (°C) integrated over water layers characterised by 8 – 12 psu, stage duration of egg stage I in days, mortality rate, and daily egg production.

cruise	month	year	n stations	total abundance egg stage I	mean ambient temperature	duration egg stage I	daily mortality *z*	daily egg production
AL143	June	1999	42	$5.62*10^{12}$	5.04	3.1	0.46	$3.46*10^{12}$
AL161	May	2000	41	$1.61*10^{12}$	4.57	3.3	0.14	$0.62*10^{12}$
AL182	May	2001	45	$3.72*10^{12}$	4.36	3.4	0.20	$1.53*10^{12}$
AL200	April	2002	29	$3.46*10^{12}$	5.23	3.1	0.86	$2.58*10^{12}$
AL217	March	2003	45	$1.88*10^{12}$	3.47	3.7	0.34	$0.89*10^{12}$
AL238	June	2004	45	$1.55*10^{12}$	4.56	3.3	0.25	$0.69*10^{12}$
AL258	May	2005	42	$1.14*10^{12}$	4.18	3.4	0.20	$0.47*10^{12}$
AL279	June	2006	41	$2.61*10^{12}$	3.15	3.9	0.27	$1.09*10^{12}$
AL299	May	2007	38	$4.36*10^{12}$	5.04	3.1	0.18	$1.86*10^{12}$
AL318	April	2008	45	$3.97*10^{12}$	5.84	3.1	0.20	$1.75*10^{12}$

Total egg abundance in the area of each egg stage was estimated with an objective analysis (Bretherton *et al*., 1976) which interpolates over stations where no data are available. This approach is based on the Gauss-Markov theorem, which gives an expression for the linear least-square error estimate of the variables. The analysis uses a spatial covariance function of measurements and assumptions concerning the measurement noise and small-scale errors inferred from the observed egg abundance data on each single station. In general, sprat eggs were present on all stations within the surveyed area during the peak spawning period. Thus, the whole area of the surveyed station grid was included for every year in the abundance estimate. Taking into account noise levels of 0 to 15% in the egg data resulted in an underestimation of abundances. In the worst case this method underestimates the egg abundances up to 30%. To account for such uncertainties, a confidence interval based on these values was constructed.

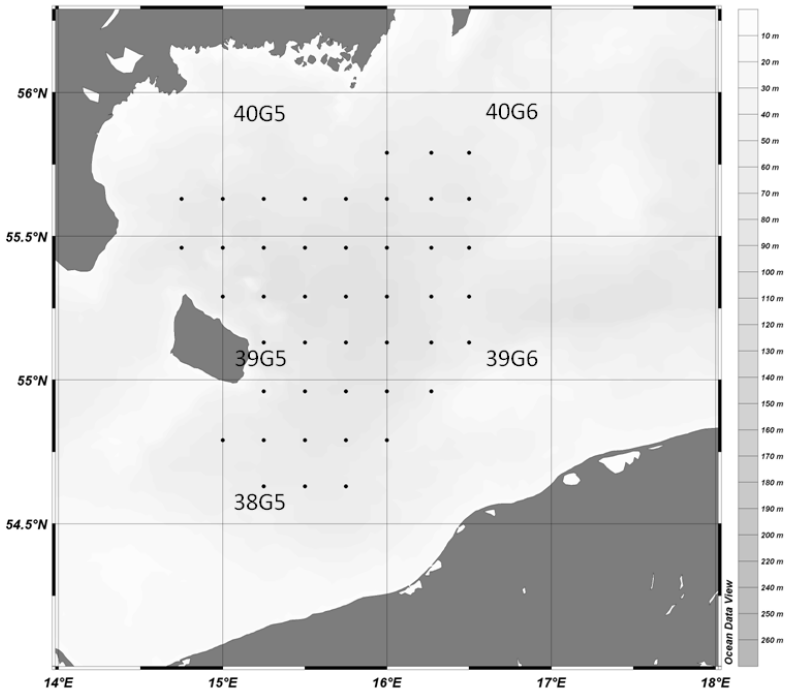

Fig. 1: Investigation area in the south-central Baltic. Dots show position of stations covered by the ichthyoplankton surveys. ICES rectangles used for stock abundance calculation from acoustic survey data, and for downscaling MSVPA abundance data are labelled with their code.

To obtain an estimation of the total daily egg production (*DEP*), a temperature-dependent stage model is required to calculate the duration of egg stages. A model derived from experimental data (Petereit *et al.,* 2008; chapter V) was applied:

$$td_i = 5.58(\pm 0.54\mathrm{SE}) * \exp^{(-1*(0.012(\pm 0.008\mathrm{SE})*T)} * S^{0.87(\pm 0.08)} \qquad (1)$$

where td_i is the endpoint of the i_{th} egg stage, T is the ambient temperature (°C), and S is the specific egg developmental stage. It has been shown that sprat eggs in the Bornholm Basin generally occur in water layers characterised by salinities of 8 – 12 psu (Nissling *et al.*, 2003). Therefore, the mean temperature of these water layers was used. In order to assess the possible bias from estimating the ambient temperatures, the 95% confidence intervals of this parameter were calculated and applied to the *DEP* calculation. All hydrographic data were derived from the ICES hydrographic data base.

Different to the standard procedure in the DEPM (Lo, 1985), it was not possible to classify the sprat egg abundance data directly to daily cohorts, because neither the egg data nor the adult stock data gave evidence for a synchronised spawning of sprat during a certain time of the day. Hence, it was assumed that sprat show no synchronised spawning pattern over the day, a view which is also supported by other authors (Alekseev and Alekseeva, 2005). Further, the duration of the youngest egg stage lasts more than one day for Baltic sprat at the observed ambient temperatures. Therefore, it was not possible to calculate the *DEP* directly from fitting the observed egg abundance data to the exponential decay mortality model (Lo *et al.*, 1985), as this will (i) overestimate the *DEP* by neglecting the mortality acting immediately on the eggs from the time of spawning until the time of sampling, and (ii) would only be an adequate estimation for a synchronised spawning event. Thus, a different approach was chosen to estimate the *DEP*

assuming a continuous egg production over the day and taking into account natural mortality. The general formulation of the exponential decay model reads:

$$AB_{t1} = EP_{t0} * e^{(-z*(t1-t0))} \quad (2)$$

where AB_{t1} is the abundance of a given egg batch at an observed time point t_1 which results of a specific egg production *EP* at t_0, with mortality z acting over a period of time t_1-t_0.
Since this holds true for every egg spawned prior to sampling for the total duration of all four defined egg stages, we may set up an integrated model to predict abundance for each egg stage as follows:

$$AB_i = \int_{td_{(i-1)}}^{td_i} EP_{t0} * e^{(-z*(t-t0))} * dt \quad (3)$$

where the total abundance of all eggs assigned to the i_{th} stage is given by the area under the curve of the exponential decay model between the endpoint of the preceding stage $td_{(i-1)}$ and the endpoint of the i_{th} stage td_i. This area can be approximated by summing the abundance of eggs spawned during certain intervals of time; an hourly egg production *HEP* was chosen as this seems to be sufficiently accurate for the applied temperature-development model. It can thus be written:

$$AB_i = \sum_{t=td_{(i-1)}+1}^{td_i} HEP * e^{(-z_h*(t-t0))} \quad (4)$$

where instantaneous mortality z_h is given for hourly death rates. Predicted abundance AB_i was set as a dummy parameter, and the squared error calculated as the squared difference to the observed abundance of the respective stage. By minimizing the sum of squared errors (SSE) of all available egg stages, equation (4) was solved for *HEP* and z_h. Hereby, both differing stage durations and constant mortality acting upon continuously produced eggs were taken into account. *HEP* and z_h were then multiplied by 24 to yield the *DEP* and the daily mortality coefficient z.
In general, the DEPM survey should be conducted during the peak spawning period, because then the largest part of the stock is present on the spawning ground. For most of the analysed years several ichthyoplankton surveys have been conducted. Thus, in order to match peak spawning, the highest observed egg abundance was chosen to calculate the spawning stock biomass with the DEPM.

Stock structure and spawning fraction

Sampling of adult sprat in the investigation area was carried out either on the same cruises on which the ichthyoplankton survey was conducted or on parallel acoustic surveys covering the Bornholm Basin during peak spawning time of sprat each year (Tab. 2). The length frequency distribution was calculated by measuring subsamples of at least 200 specimens to the nearest cm of each haul. Average length distributions were calculated by weighting the station specific length frequency distributions by the corresponding catch rates. Sex and maturity were determined by macroscopic inspection of at least 10 individuals per 1 cm length class from each haul. To estimate the spawning fraction, the hydrated oocyte method was applied assuming that the hydrated oocyte stage lasts 24 h (Hunter and Macewicz, 1985). This method makes solely use of the incidence of females with hydrated oocytes and assumes that the proportion of females with hydrated oocytes from all females in spawning condition represents the proportion of females spawning per day. However, although it is generally straightforward to identify advanced hydrated oocytes by macroscopic inspection of fish ovaries (Hunter at al., 1985;

DeMartini and Fountain, 1981), this method bears considerable uncertainties. The duration of the hydrated stage has to be known, which is often assumed to last approximately 24 h. Further, the results may be biased since clupeid fish form female dominated spawning aggregations, and thus females with hydrated ovaries may be oversampled in some fishery hauls (Alheit, 1985). To account for these uncertainties, different spawning frequencies were tested. In a first approach the actually observed values per length class were utilised and contrasted to results based on an overall mean of the observed spawning frequency values. This was done to test for the impact of a possible length class dependency of this parameter. In an alternative approach three scenarios were calculated, to investigate in which amount the stock sizes obtained by the DEPM change compared to stock sizes obtained by the acoustic survey and the MSVPA:

Scenario I: a mean spawning frequency of *SF*=0.24 was utilised which was obtained by averaging values from recent literature (Chapter IV *SF*=0.22; Kraus and Köster, 2004 *SF*=0.27; Alekseev and Alekseeva, 2005 *SF*=0.23).

Scenario II: the mean value was halved to *SF*=0.12. This scenario takes into account a report on spawning frequency of sprat from the Western Baltic (SF≈0.11; Heidrich, 1925), and a possible oversampling of hydrated females.

Scenario III: the mean value was doubled to *SF*=0.48 in order to test an extreme underestimation of spawning frequency.

Batch fecundity

For fecundity analyses female sprat with fully hydrated ovaries were collected. Running ripe females were excluded from analyses. Whole fishes were conserved in a buffered 4% formaldehyde seawater solution. In order to assure a proper fixation the body cavity was slit open. Batch fecundity was estimated gravimetrically in the laboratory by counting the hydrated oocytes from an ovarian sub-sample (Hunter *et al*., 1985). Linear regression models with fish total length as predictor, and absolute batch fecundity as response variable were established for each year. For the year 2003 no fecundity data existed. It has been shown that a model of batch fecundity taking into account fish length and ambient temperature explained the largest proportion of variability (see Chapter I). Therefore, this model was used to estimate batch fecundity for each length class for the year 2003:

$$BF_{2003} = a * L * exp^{\left(-0.5*\left(\frac{\ln\left(\frac{T}{Tmax}\right)}{b}\right)^2\right)} + c \qquad (5)$$

where *BF* is the batch fecundity (number oocytes spawned per batch), *L* the fish length class (0.5cm), *T* (3.47°C) the observed mean temperature integrated over the water column for the specific date (March 2003), from surface to depths where low oxygen levels prevent the occurrence of sprat (< 1ml l^{-1}), *Tmax* (6.97°C±2.50SE), *a* (359.54±23.46SE), *b* (1.46±0.55SE), and *c* (-2753.16±243.51SE) regression coefficients (see Chapter I). Temperature data for this model were obtained from the ICES hydrographic data base.

Tab. 2: Adult stock sampling: cruise (AL = RV"Alkor"; WH = RV"Walther Herwig III"), year and month of sampling, numbers of analysed sprat for establishing length class specific sex ratios, maturity ogives, and fecundity analyses, linear regression models on batch fecundity. *BF* = batch fecundity, *L* = total fish length.

cruise	month	year	number of sprat analysed		fecundity models
			sex ratio, maturity	fecundity	linear regressions
AL143	June	1999	1738		
WH206	June	1999		48	$BF = -2467 + L * 306$
AL161	May	2000	831	51	$BF = -2971 + L * 381$
AL182	May	2001	1706		
WH228	June	2001		62	$BF = -4076 + L * 469$
AL200	April	2002	1074		
WH239	May	2002		61	$BF = -1583 + L * 246$
AL217	March	2003	979		
WH263	May	2004		67	$BF = -5561 + L * 581$
AL238	June	2004	564		
WH275	May	2005	618	102	$BF = -2569 + L * 330$
WH288	May	2006	1803	142	$BF = -2566 + L * 335$
AL297	April	2007		14	$BF = -1843 + L * 277$
AL299	May	2007	617		
AL318	April	2008	3187	41	$BF = -4284 + L * 477$

Estimation of stock size

The stock size in numbers at sampling date t (N_t) was estimated by applying a modified formula of the daily egg production method introduced by Kraus and Köster (2004):

$$N_t = \frac{P_0}{\sum_{8cm}^{16cm} L_{t,l} * S_{t,l} * M_{t,l} * SF_{t,l} * BF_{t,l}} \quad (6)$$

where P_0 is the total daily egg production obtained from ichthyoplankton surveys in the field, $L_{t,l}$ is the relative frequency of length class l at date t, $S_{t,l}$ is the sex ratio, $M_{t,l}$ the proportion mature females, $SF_{t,l}$ the fraction of females spawning per day, and $BF_{t,l}$ the batch fecundity.
Stock size estimations obtained with the daily egg production method for the Bornholm Basin were compared with stock size estimations from (i) an acoustic survey targeting sprat population in the central Baltic during peak spawning period, and (ii) an area disaggregated multi species virtual population analysis (MSVPA; ICES, 2006). The abundance data from the acoustic survey are based on ICES rectangles. To obtain comparable stock abundance values, the abundance values from these surveys were summed up over the ICES rectangles covering the Bornholm Basin for each year (38G5, 39G5, 40G5, 39G6, 40G6; Fig. 1). The area disaggregated MSVPA stock abundance estimates are based on an ICES sub-division scale. Thus, they were down-scaled to the area of the Bornholm Basin by the use of distribution patterns obtained from the acoustic survey (Köster, 1994).

VI. 3 Results

Egg abundance and daily egg production

Egg abundance as well as egg distribution patterns showed distinct variability over the observed time period. The horizontal distribution of sprat eggs observed at peak spawning revealed that sprat eggs occurred on nearly each sampled station of the survey grid (Fig. 2). The margins of the basin showed in general lower egg abundances compared to the centre. However, in some years with high egg abundances (*e.g.* 1999), also high values were found at the margins, indicating that not the whole spawning area was covered in some years. The maximum abundance ranged from $5.62*10^{12}$ eggs in June 1999 to $1.14*10^{12}$ eggs in May 2005 (Fig. 3; Tab. 1). Peak egg abundances were mostly observed in May/June. In the year 2003 maximum egg abundance was observed in March, in 2002 and 2008 the maximum egg stage I abundance was observed in April. No egg abundance curves could be fitted in 2002 and 2001, due to limited data points in these years. Especially in the case of 2000 it is therefore not fully assured that the stock size estimation reflects the situation during peak spawning. In May/June 1999 the egg stage I abundance nearly tripled within two weeks from $1.9 * 10^{12}$ to $5.62 * 10^{12}$ (Fig. 3a).
The estimation of daily egg mortality resulted in mortality coefficients ranging from 0.14 to 0.86. Highest daily egg production was observed for June 1999, the lowest value was found in May 2000.

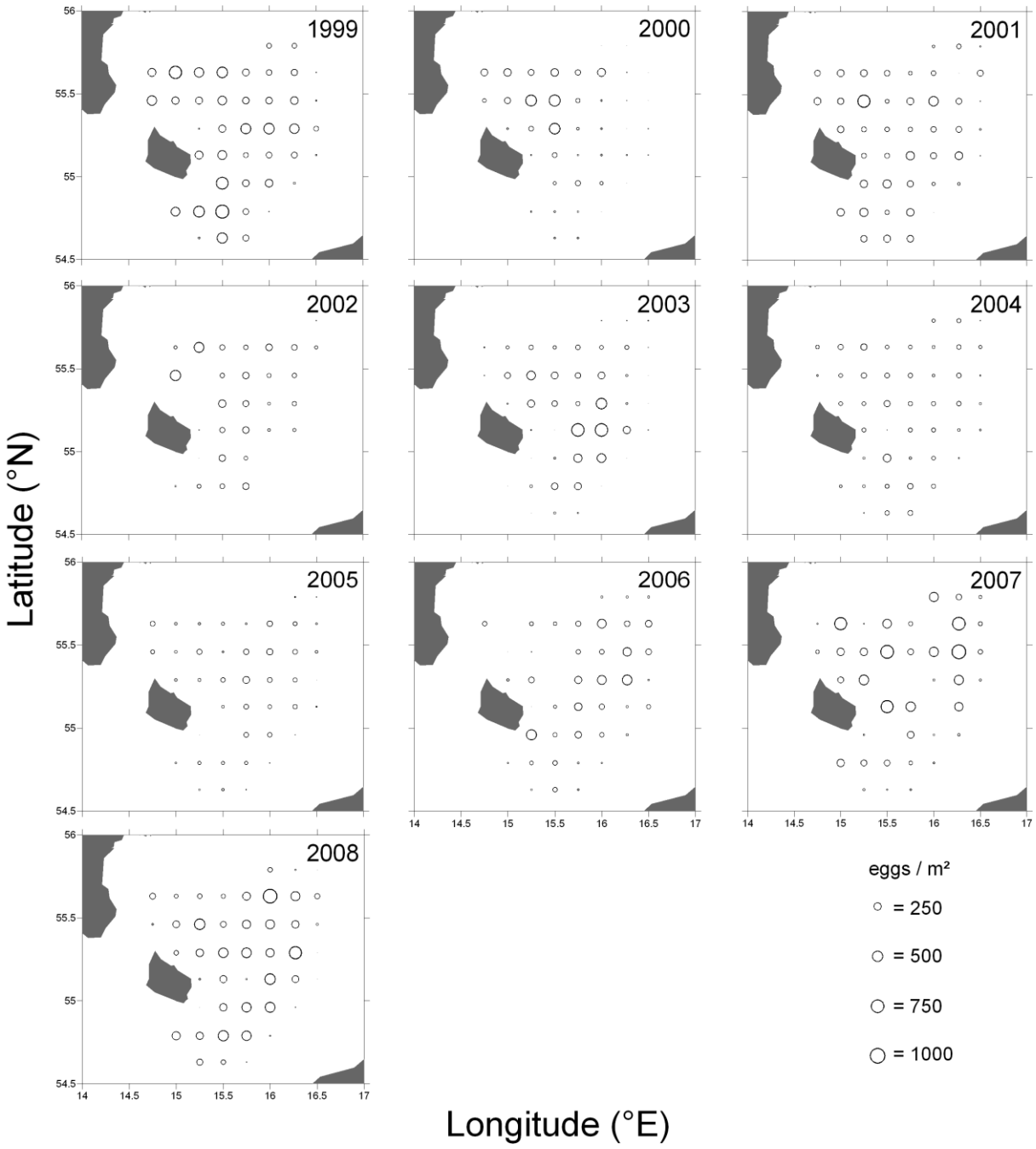

Fig. 2: Horizontal distribution of sprat eggs in the Bornholm Basin during peak spawning time 1999 - 2008.

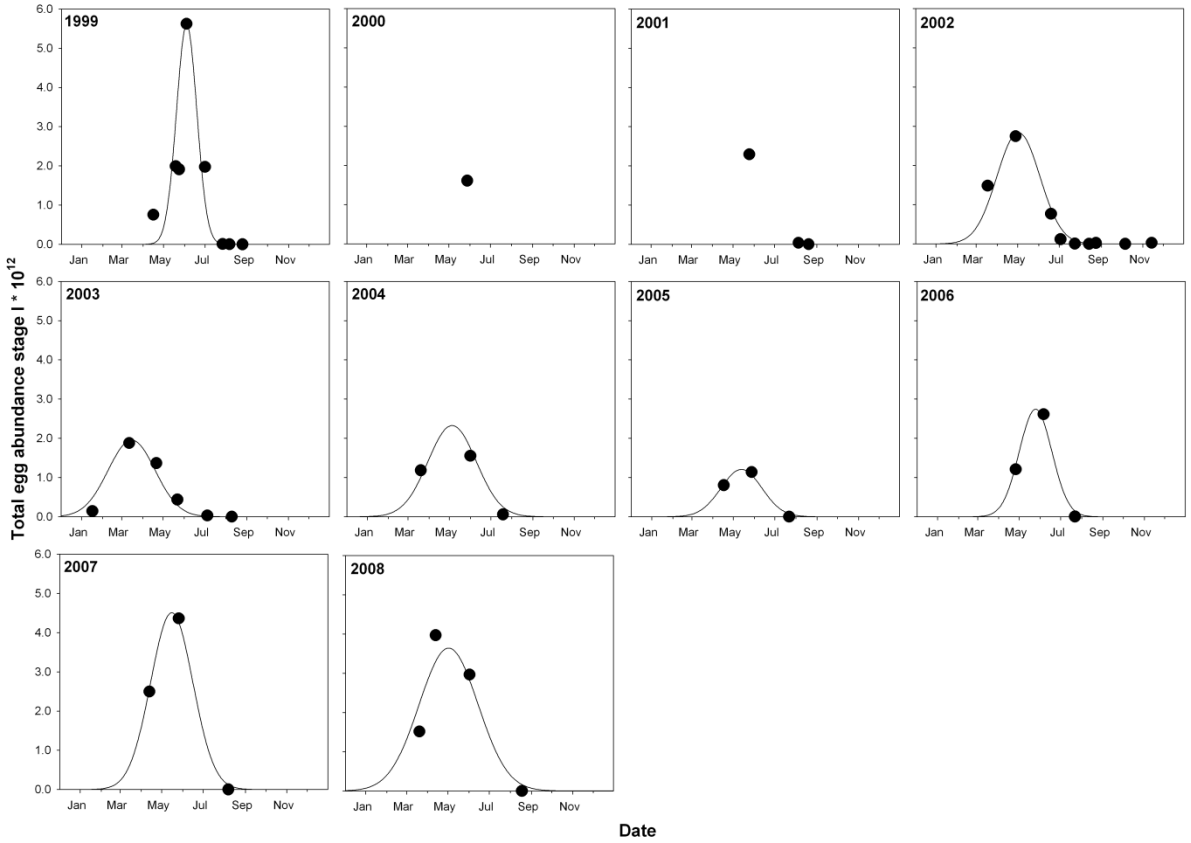

Fig. 3: Total seasonal egg stage I abundance in the Bornholm Basin for the years 1999 to 2008.

Adult stock parameters

Length frequency distributions of the sampled sprat stock showed a similar picture for all observed years (Fig. 4). The majority of fish ranged from > 11 cm to < 13 cm. In 2002, 2003, and 2004, a bimodal distribution was observed caused by a remarkably high proportion of smaller individuals. In 2004, the proportion of length classes was more evenly distributed in comparison to all other years.

Sex ratios showed an increasing trend in the proportion of females with increasing fish size for all observed years (Fig. 4). Variability was higher in the smaller length classes, probably due to uncertainties in macroscopic identification of sexes in immature, small fish < 10 cm. The proportion of mature females showed in some years (*e.g.* 2000, 2001) a remarkably sharp increase with size, changing from around 20% up to 100% from one cm-length class to the next. In general there was a more or less moderate increase in the proportion mature females with L_{50} values ranging between 9.5 cm and 10.5 cm length. In almost all cases the female sprat > 12.5 cm were 100% mature.

The daily spawning fraction of the female stock mainly ranged between 20 and 30% (Fig. 4). The overall mean spawning fraction was 24%. However, considerably higher values for single size classes were observed in some cases, *e.g.* in the year 2004 up to 50% in length class 12-13 cm. Further, in 2003 a remarkably low proportion of females with hydrated oocytes was detected which resulted in very low spawning fractions. A clear trend of spawning frequency with fish size was not obvious. In some years an increase with length could be shown, in some other years even a decrease with fish length was observed. In almost all years no spawning frequency could be estimated for fish < 9.5 cm, although some of these females were classified as mature, but no hydrated females were detected.

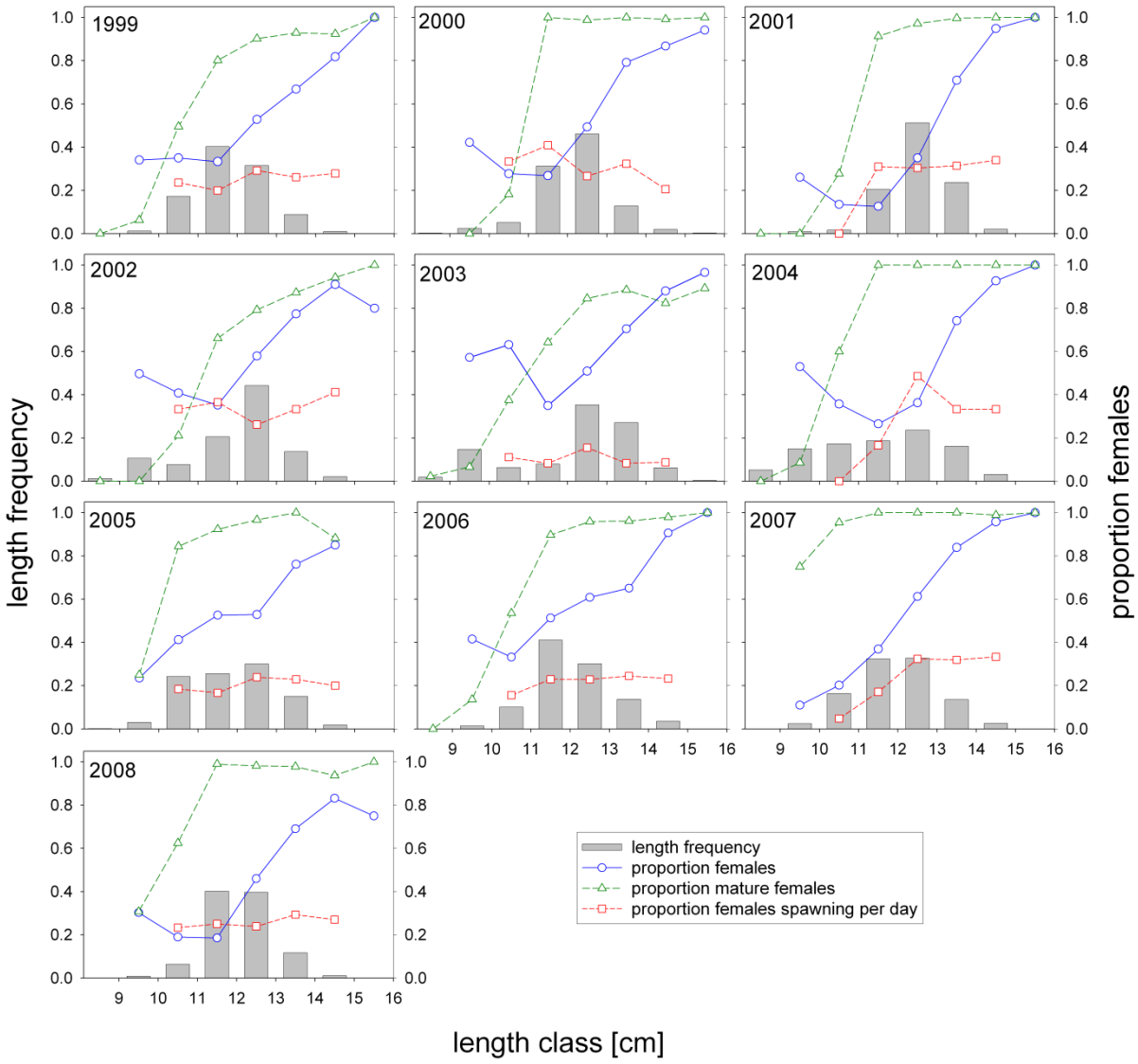

Fig. 4: Adult stock parameters of sprat for the investigated years during the peak of spawning.

Batch fecundity

For all observed years batch fecundity was positively related to fish length (Tab. 2). Linear regression models were all statistically significant ($p<0.05$). Slopes as well as intercepts showed differences between some years (ANCOVA; $p<0.05$). The steepest slopes were found for the years 2001, 2004, and 2008, which were also the years with the lowest intercept. All other regression curves were rather similar with only minor differences. The estimated batch fecundity values for the year 2003 resulted in the following model: $BF = -2753 + L * 321$, where BF is the batch fecundity (oocytes spawned per batch) and L the total fish length (cm), which is in good accordance to the observed values for all the other years.

Stock size

Considering the 95% confidence intervals for mean ambient temperature values, leads to an uncertainty in DEPM based stock size estimates in the range of 6% to 16%. Correcting the calculated egg abundances for the maximum underestimation due to the objective analysis

resulted in 23% higher stock estimates. These results were used to create a confidence interval for the DEPM estimates.

The two approaches using either observed spawning frequency values per length class or observed mean values, revealed a similar trend in the estimated DEPM stock sizes with high interannual variability (Fig. 5). The utilisation of both methods resulted only in a substantial difference for the 2003 stock size estimate (Fig. 5a, b). This can clearly be explained by the lowest observed spawning frequencies in this year. Stock sizes obtained by the DEPM were highest in the year 1999, followed by the lowest stock size in 2000. For the year 2002, a second peak in sprat abundance was detected. The DEPM then revealed a steady decrease until the year 2005 and a continuous increase in stock size for the last three years of the time series.

Generally, variability among the different years was even higher in the acoustic survey stock estimate. Similar to the DEPM, the acoustic survey detected a peak in sprat abundance in 2002, although the acoustic estimate is more than twice as high as the DEPM estimate. The acoustic stock estimate for the years 2004 and 2005 show considerable higher values compared to the DEPM with an opposite trend for these two years. Only in 1999, the acoustic stock size was considerably lower than the DEPM estimate. The acoustic survey revealed that between 31% and 67% of the total stock in ICES sub-division 25 were detected within the Bornholm Basin during the survey. Accordingly, the highest absolute stock sizes in the Bornholm Basin were observed in those years where proportions of fish acoustically detected within the basin were also high. The stock estimates of the MSVPA where down-scaled to the Bornholm Basin using the distributions obtained from the acoustic results (Tab. 3).

The MSVPA stock estimates are generally less variable among years compared to the other two methods. Only for two years (2001 and 2002), it shows a good accordance to the DEPM, when the mean observed spawning frequency was applied (Fig. 5b). For most years, the MSVPA stock size is higher compared to the DEPM, apart from the year 1999. Compared to the acoustic estimate the MSVPA is also higher for most of the years, but is in good accordance for the years 2001 and 2005. Generally the trend of the MSVPA values was similar to that of the acoustic survey, which is due to the use of acoustic distribution patterns to down-scale MSVPA results.

Tab. 3: Baltic sprat stock sizes (numbers * 10^9) obtained by the acoustic survey and the MSVPA for the whole ICES sub-division 25 and the Bornholm Basin. MSVPA stock sizes where down-scaled with the share of the stock in the Bornholm Basin obtained by the acoustic survey.

	quarter 2 acoustic survey			MSVPA	
year	ICES SD25	Bornholm Basin	Share of stock in the Bornholm Basin (%)	ICES SD25	Bornholm Basin
1999	40.81	16.63	40.76	52.76	21.51
2000	n.a.	n.a.	n.a.	44.22	22.68
2001	29.58	9.21	31.14	32.50	10.12
2002	52.91	35.59	67.26	29.40	19.77
2003	11.40	6.47	56.74	28.01	15.90
2004	28.02	14.55	51.92	43.96	22.83
2005	35.79	23.45	65.51	34.05	22.31
2006	21.00	11.50	54.77	n.a.	n.a.
2007	22.46	11.88	52.88	n.a.	n.a.
2008	22.74	10.65	46.83	n.a.	n.a.

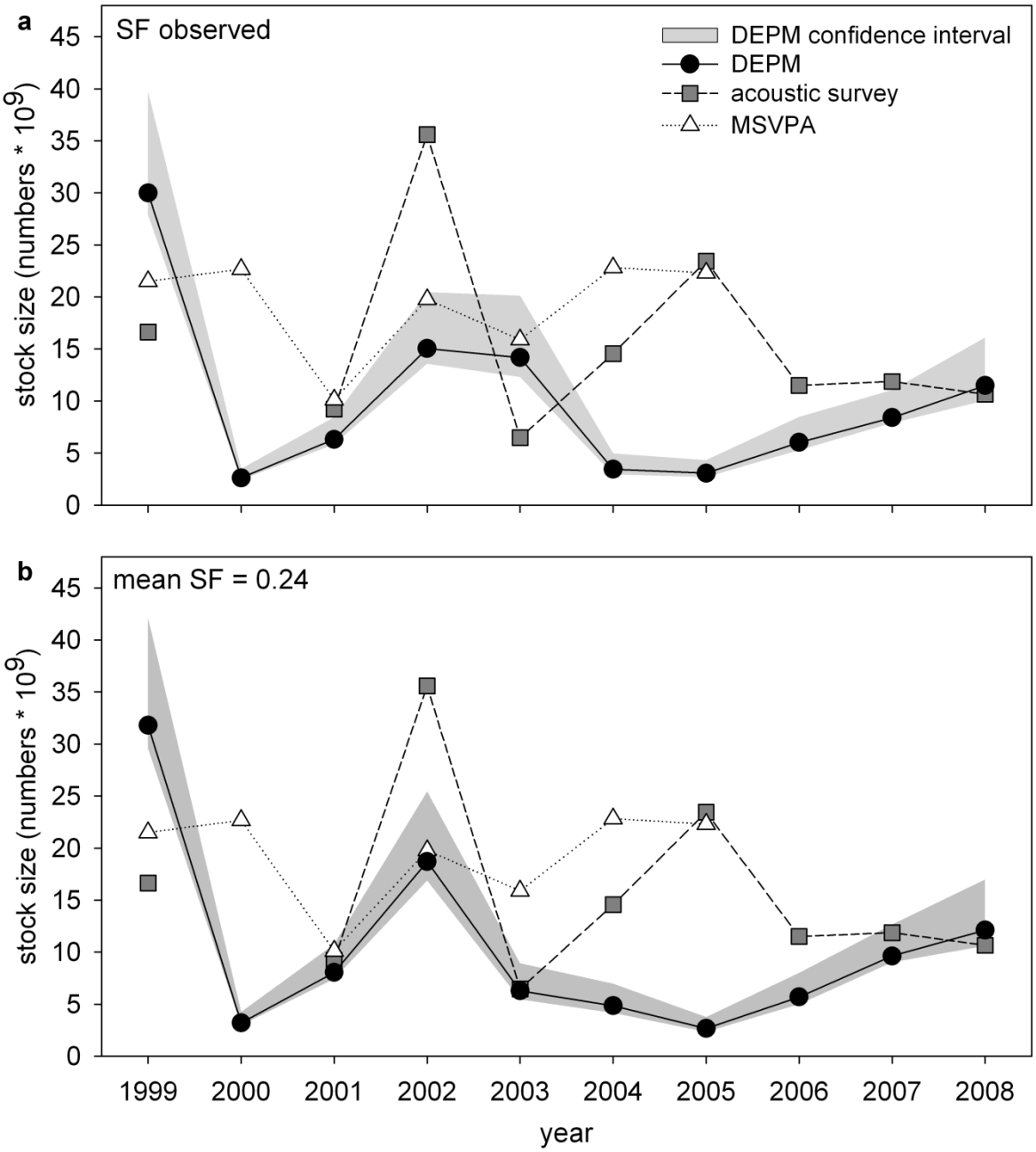

Fig. 5: Stock abundance data obtained from the DEPM (black circles, solid line), the acoustic survey (grey squares, dashed line), and the MSVPA (white triangles, dotted line). The DEPM was calculated for different spawning frequencies: (a) length class specific observed spawning frequency (*SF*) obtained by the present study; (b) overall mean spawning frequency of observed values from the present study. The confidence interval of the DEPM is based on the uncertainty in egg abundance calculation, and the estimation of mean ambient temperature for the egg stage duration model.

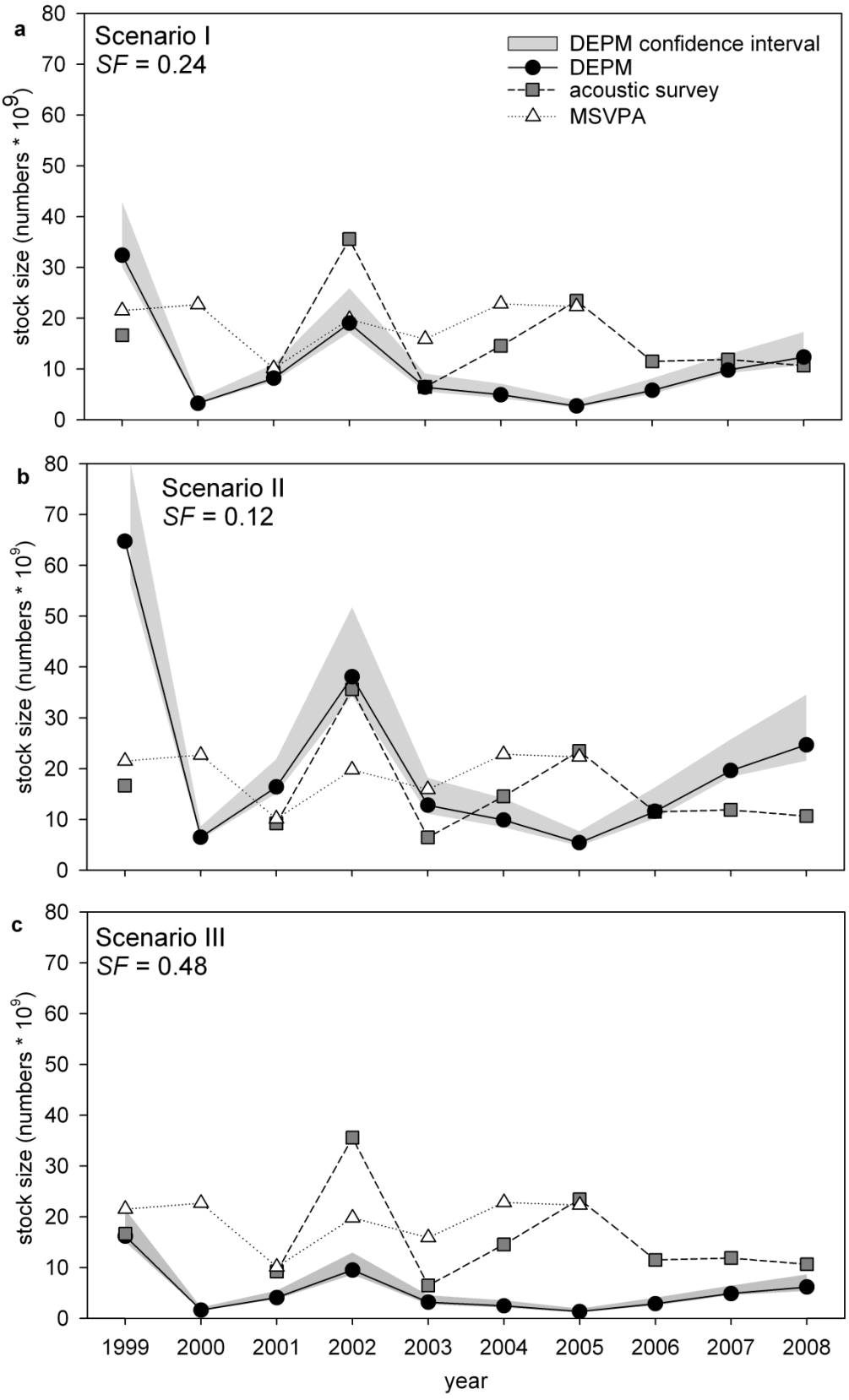

Fig. 6 (a) DEPM scenario I with average spawning frequency (*SF*) obtained by literature values, (b) scenario II with halved *SF*, (c) scenario III with doubled *SF*. The confidence interval of the DEPM is based on the uncertainty in egg abundance calculation, and the estimation of mean ambient temperature for the egg stage duration model.

When comparing the DEPM results obtained by the different scenarios with changes in spawning frequency by ±50% (Fig. 6), the best accordance was achieved by scenario I, *i.e.* when using literature values (Fig. 6a). In this scenario five years fit well to the acoustic estimate (2001, 2003, 2006, 2007, and 2008), with values of the acoustic estimate still lower than the DEPM results, but within the DEPM confidence intervals. This is also a better accordance compared to the scenarios where the observed values of spawning frequency were used. In scenario I most of the MSVPA stock sizes are still considerably higher than the DEPM values, apart from 1999 which is lower. For 2001 and 2002 MSVPA estimates are well within the DEPM confidence interval. In scenario II the DEPM stock sizes doubled and were now higher in a number of years compared to the acoustic and MSVPA estimates, even exceeding both methods more than three times in 1999. An overlap with the DEPM confidence interval was only achieved for 2002 and 2003, for the acoustic survey and the MSVPA respectively. By increasing the spawning frequency by 50% in scenario III the DEPM stock estimates were halved. In this scenario the DEPM estimates, and even the upper limit of its confidence intervals, are lower compared to all other estimates, apart from the year 1999 where it nearly equals the acoustic estimate.

VI. 4 Discussion

The application of the daily egg production method to Baltic sprat inhabiting the Bornholm Basin is a challenging exercise as this stock shows some peculiarities compared to other clupeid stocks for which this method has been applied: (i) the Bornholm Basin is a brackish water habitat with a unique stratified hydrography, (ii) in contrast to other clupeid species, sprat do not show a synchronised diel spawning pattern, (iii) uncertainties reside in the limited amount of information on spawning frequency of Baltic sprat.

The first two points have consequences for the estimation of the daily egg production (*DEP*), which is one of the most influential input parameter for the DEPM. *DEP* is influenced by the temperature dependent egg stage duration, which is essential to estimate the daily mortality rate. Hydrography and egg buoyancy determine the vertical distribution of pelagic fish eggs (Wieland *et al.*, 1994). Thus, the estimation of ambient temperature is not trivial and may be biased, because hydrography and buoyancy may change during the spawning season affecting the vertical distribution of sprat eggs (Nissling *et al*., 2003). An operational model on the vertical distribution of sprat eggs related to hydrographic features is still lacking. Consequently, the vertical distribution of sprat eggs was assumed to be confined to water layers characterised by 8 – 12 psu (Kraus and Köster, 2004), based on observations made by Nissling *et al*. (2003). The results of the present study showed that the stock size estimations derived by the DEPM might change up to 16% due to the range in ambient temperature within the 95% confidence intervals, which corresponds to a possible deviation of 0.8 to 2.2 °C of the mean value. Thus, a biased estimate of the ambient mean temperature due to uncertainties in the vertical distribution of sprat eggs will affect the DEPM estimations.

The lack of a synchronised diel spawning pattern in combination with stage durations of the identifiable egg stages of more than one day were impeding the classification of eggs into distinct daily cohorts (Lo, 1985). Therefore it was not possible to estimate daily egg production and mortality by directly fitting a model of exponential decay through observed abundance values. Since stage I eggs were already experiencing up to three days of unknown mortality, it is impossible to estimate *DEP* from this stage only. Thus, a rather continuous egg production over the day was assumed and daily mortality rates as well as daily egg production were modelled on the basis of the observed abundance data of all four stages. In the case of Baltic sprat, this method will result in a more precise estimation of mortality and daily egg production as the egg stage specific, temperature dependent development duration is taken into account. However, the diel spawning pattern of sprat, and possibly seasonal variation of this parameter, has to be

explored further since contradicting information exists on that issue in literature for Baltic sprat (Kraus and Köster, 2004; Alekseev and Alekseeva, 2005; Balzar, 1994). This would either justify the introduction of the alternative method to estimate the daily egg production from ichthyoplankton field data as proposed in the present study, or would demonstrate the need of an improvement of ichthyoplankton sampling and analyses, as it is not possible to classify the sampled sprat eggs into daily cohorts based on the available data. To overcome the latter problem a finer resolution of sprat egg development would be necessary with clearly identifiable stages lasting less than one day, for example by microscopic identification of cleavages in the youngest egg stages (Simpson, 1971).

Spawning frequency is one of the parameters with the highest uncertainty in the DEPM (Stratoudakis *et al.*, 2006). Usually this parameter can be determined from field data by the postovulatory follicle (POF) method or by the hydrated oocyte method (Hunter and Macewicz, 1985). For both methods the duration of either the POF stages or the hydrated stage has to be known. This can be achieved either by tank experiments or by field observations when the diel spawning is synchronised. For sprat it was assumed that no clear diel synchronicity of spawning is the case, so the POF method cannot be applied without prior tank experiments to determine exact POF durations. Consequently, laboratory experiments on sprat to determine the spawning frequency would be the only way to get an accurate measure of this parameter. However, such laboratory experiments are lacking so far because it was not feasible to keep Baltic sprat in tanks for such experiments. Hence, all recent available information on spawning frequency of Baltic sprat (Kraus and Köster, 2004; Alekseev and Alekseeva, 2005; see Chapter IV) is based on the hydrated oocyte method (Hunter and Macewicz, 1985) assuming a duration of the hydrated stage of approximately 24 h, based upon observations in other clupeid species (Hunter and Macewicz, 1985). Furthermore, this method may be biased through oversampling of spawning females (Alheit, 1985) or uncertainties in the exact classification of maturity stages in cases where no histology was applied. However, results of the present study confirm literature values for this species and provide additional evidence that a spawning interval of 4.5 to 4 days (SF = 0.22 – 0.25) might be realistic for Baltic sprat. The use of these values in the present DEPM application is justified because only the scenario using a spawning frequency in this range resulted in a rather good accordance between stock sizes obtained by the acoustic survey and the DEPM, two totally independent assessment methods, for a number of years. Spawning frequencies in the range of approximately four days have also been described for other clupeid fish, *e.g.* sardine (*Sardina pilchardus*) off Spain (East Cantabria) with a spawning frequency ranging between 0.21 and 0.23 (see review in Ganias *et al*., 2003). Somewhat higher values were reported for Bay of Biscay anchovy (*Engraulis encrasicolus*), ranging between 0.26 and 0.32 (Motos, 1996), which is also in the range of the observed values for Baltic sprat. Compared to most other estimates of spawning frequency for clupeid fish species, this seems to be rather high, and was explained by relatively high temperatures prevailing in this area during the peak spawning of anchovy. Spawning frequencies in the range used for scenario III or even higher have been reported only for tropical species (*e.g. Sardinella brasiliensis*, Isaac-Nahum *et al*., 1988; *Encrasicholina purpurea*, Clarke, 1987). As temperatures in the Baltic are lower during sprat peak spawning compared to the aforementioned examples, we argue that spawning frequency is similar to or less than the observed values. Thus, it is highly recommended that spawning frequency of Baltic sprat is further assessed, and former estimations re-evaluated. This would also be valuable information for a number of other applications, *e.g.* individual based modelling or a matrix population model approach, where the seasonal egg production is important.

No clear size effect of spawning frequency could be shown for sprat in the present study as it has been reported for other clupeid species (Claramunt *et al*., 2007; Ganias *et al*., 2003; Parrish *et al*., 1986). However, Claramunt *et al*. (2007) showed that spawning frequency in dependence of body size is best described by a logistic curve. If this is true also for Baltic sprat, and some of the presented data support this hypothesis, this size dependency would have a considerable influence

on the results of the DEPM in cases where smaller individuals are more abundant compared to larger individuals. The length frequency distributions showed that this is usually not the case. And even if this scenario would occur, the much lower proportion mature and the lower fecundity in the smaller length classes would further dampen the effect of high abundance. For the same reason, the use of fixed spawning frequencies for each length class did not result in pronounced differences compared to the use of length class specific spawning frequencies as in the length classes that most contribute to spawning, the spawning frequency is relatively stable.

Uncertainties in the other adult stock parameters may hold sources of error, but not with the same impact as the discussed uncertainty in the estimation of spawning frequency. Variation introduced by batch fecundity values by sampling or measurement error will certainly not reach the level of impact on DEPM estimates compared to spawning frequency. High variability was observed in sex ratios and proportion mature fish, and especially for the smallest length classes values might be uncertain. This can be explained by (i) uncertainties of sex and maturity classification in these small fish by macroscopic inspection of ovaries, (ii) sample sizes of small length classes may be inadequate in some cases. The determination of relative length frequency distribution is in general straightforward and sample sizes were in all cases sufficient to get a representative estimation. Thus, the error introduced by these other input parameters generally was considerably lower than observed for spawning frequency and thus will not result in a comparable impact on DEPM estimates in the case of Baltic sprat.

We assumed that all oocytes were spawned and have been fertilised successfully. However, fertilisation rates may be dependent on hydrographic parameters, *e.g.* temperature or salinity. Therefore, it is likely that a proportion of oocytes might not be fertilised under certain circumstances (Markle and Waiwood, 1985; Hempel, 1979). Unfertilised eggs rapidly sink down to the bottom and were thus under estimated in our egg production estimates. To further enhance the accuracy of the DEPM, fertilisation success in relation to hydrography should be included in future studies.

In general, the DEPM application for Baltic sprat was in the same order of magnitude as results from the acoustic survey and the area disaggregated MSVPA, showing that its utilisation as alternative, fishery data independent stock estimate is justified. However, for some years considerable deviations between the results of the different approaches were found. This was to some extent to be expected as the results were not strictly comparable, especially when comparing the DEPM with the MSVPA. The DEPM solely assesses the sprat stock in the area covered by the ichthyoplankton survey, whereas the MSVPA uses a completely different approach for down-scaling the assessment to ICES SD 25, *i.e.* using the distribution patterns from the acoustic survey. Further, MSVPA does not account for the female proportion and uses constant maturity ogives, obviously an unrealistic scenario. Another source of uncertainty is the use of commercial catch data in the MSVPA, which might also be biased. Pronounced differences existed in some years between the acoustic stock size estimate and the DEPM. These differences might also be due to uncertainties in the acoustic methodology. Sprat occurs together with herring and cod in the investigated area, thus a separation of species is needed. However, the separation of species by means of their acoustic back scatter signal remains uncertain. Thus, fishery hauls have to be conducted in parallel to crosscheck species composition. In addition, age and weight structure has to be obtained by fishery hauls in order to calculate total abundance and biomass for each species. In general only two fishery hauls per ICES rectangle are conducted within the acoustic survey for this purpose (ICES, 2010b), which might not be sufficiently to represent the investigated area. Further, the spatial resolution of the acoustic survey is comparably low, with only two transects crossing one ICES rectangle (ICES, 2010b). Therefore, extrapolating the acoustic abundance estimate to large areas might introduce a certain error to the estimation of stock size.

Sprat are migrating into the deep basins of the Baltic for spawning, afterwards they again migrate out of the basin for summer feeding, and they return not before late autumn to

overwinter in the warmer water layers below the halocline (Aro, 1989). Deviation between results obtained by the DEPM and the acoustic survey might therefore partly be explained by a different timing of the two applied surveys for some years. However, this can only result in higher stock sizes obtained by the DEPM in the present study. The DEPM was always applied for the time of peak abundance of sprat eggs, assuming that the largest proportion of the stock was located in the Basin whereas the acoustic survey was in some cases not matching the peak of egg abundance. It might be possible that parts of the spawning stock had already migrated out of the basin again while the acoustic survey took place. Thus, the large deviation for the year 2002, where the acoustic estimate was exceeding most of the results obtained by the DEPM scenarios, cannot be explained by a possible migration effect alone. But this might be the case for the comparable high DEPM estimate for 2003 using the observed spawning fraction, which is based on data from March, while the acoustic survey was conducted later in May. However, the high DEPM stock estimate for 2003 is solely due to the low observed spawning fraction. Using the observed mean spawning fraction resulted in nearly equal stock sizes for both methods.

Refinement of methods, especially sampling and survey design to better meet requirements for the DEPM application, will increase the accuracy of the spawning stock estimates. The present application was, to a certain extent, an opportunistic approach as some of the data were obtained from different research cruises. Therefore, sampling of all necessary data within one survey or at least during the same time period is highly recommended to avoid the time lag between *e.g.* ichthyoplankton survey and adult stock sampling (Smith and Hewitt, 1985). Further, histological analyses of sprat ovaries could improve the determination of maturity, batch fecundity and spawning frequency (see Chapter II and IV). The diel spawning pattern of sprat, and possibly seasonal variation of this parameter, have to be explored more extensively, as contradicting information exists on that issue in the literature (Kraus and Köster, 2004; Alekseev and Alekseeva, 2005; Balzar, 1994). This would either justify the introduction of an hourly egg production approach, or would clearly show the need of an improvement in ichthyoplankton sampling and analyses, as it is not possible to classify the sampled sprat eggs into distinct daily cohorts with the available data.

Quantitative studies on recruitment processes of sprat and cod, for example the estimation of predation pressure on cod early life stages by sprat (Köster and Möllmann, 2000) were so far hampered by conflicting results on Baltic sprat stock size estimations from acoustic surveys and the MSVPA approach. Retrospective stock assessments can be conducted by the DEPM using historical egg abundance data. If these data originate from ichthyoplankton surveys where cod egg and larval abundance data were obtained in parallel, this would enable a meso-scale spatial resolution to evaluate the impact of sprat predation. This method would also be suited to extend area specific assessments to eastern Baltic basins, *e.g.* the Gotland Basin, and the Gdansk Deep. Further, the stock can be assessed retrospectively for time periods and areas where no regular trawl survey monitoring is available, but still a considerable proportion of the sprat stock is supposed to be spawning.

In conclusion, the present study revealed the applicability of the DEPM to assess the Baltic sprat stock, but some problems could not finally be resolved. Improvements have to be made in future applications to minimise the remaining uncertainties, especially with regards to spawning frequency and the diel spawning pattern. If these uncertainties can be ruled out, the DEPM is a promising option as fishery independent method to assess the Baltic sprat stock size.

Conclusion and Outlook

In the present study, a number of reproductive traits of Baltic sprat were investigated, all of which are essential with respect to the assessment and further studies of the population dynamics of this key ecological species of the Baltic Sea. Interannual, seasonal and spatial variability in the investigated reproductive traits of Baltic sprat was revealed and could partly be attributed to hydrographic conditions and sprat stock size.

Absolute and relative batch fecundity was found to differ among areas and between years in the southern-central Baltic, where important spawning areas of this species are located and was related to temperature, salinity and stock size (Chapter I). Batch fecundity decreased from west to east which can be explained by a decrease in salinity. The interannual variability of batch fecundity observed within the Bornholm Basin can be best explained by an effect of ambient temperature. However, time series data on growth and zooplankton abundance were not available for the present study. As a relation of relative sprat batch fecundity to stock size was found for the Bornholm Basin, it is likely that fecundity is density dependent and related to food availability, which should be taken into account in future studies in order to improve the models presented in this study to predict sprat batch fecundity. The seasonal variability for Baltic sprat (Chapter II) in batch fecundity was confirmed (Heidrich, 1925; Alheit, 1988). However, it was also found that the batch fecundity is rather stable during the peak spawning period. A slight increase towards the end of the spawning season could be related to a decrease in oocyte dry weight and diameter, which was described already for other clupeid species (Blaxter and Hunter, 1982). This may be in response to changes in hydrographic conditions over the course of the spawning season to (i) maintain neutral buoyancy of eggs which is a prerequisite for survival and (ii) to provide the embryo with larger amounts of nutrients to increase survival probability of yolk sac larvae when ambient temperatures are still low at the beginning of the spawning season.

The present study revealed that the onset of spawning may already start in January (Chapter II), which is earlier than what has been reported before for Baltic sprat (Ojaveer and Kalejs, 2010). Further, evidence was found that a second spawning peak occurred in autumn. Future studies should address whether this is a regular pattern in timing of sprat spawning or whether it is an exceptional spawning event due to environmental factors, as reported for autumn spawning in 2002 (Kraus et al., 2003). This unusual egg production may be a waste of energy reserves and may therefore (i) negatively influence the probability of sprat surviving through the winter period, which might have consequences for year class strength, and (ii) reduce the fecundity of the following regular spawning season due to an early depletion of energy reserves.

A second reproductive trait for which temperature dependence was demonstrated is the length at first maturity (Chapter III). Our results suggest that the proportion of small sprat contributing to spawning, and thus forming a part of the spawning stock, is related to ambient winter temperatures in the Bornholm Basin. This can be explained by the positive effect of warmer temperature on growth, given that sufficient prey is available. Our results suggest that, after comparatively warm winters, a large proportion of small (probably age class one) sprat already migrate into the basins and contribute to spawning. However, maturity data used for these analyses were all length based. Age based maturity ogives should be analysed additionally to confirm this hypothesis and clarify whether the observed shift in L_{50} translates directly into a shift in age at maturity. Obtaining a conclusive model to explain the variability in the proportion mature in the first two age classes would be of importance for the assessment. This would allow to recalculate the SSB for the assessed period and to evaluate the impact of variability in maturity ogives on the sprat stock assessment.

Ojaveer *et al*. (2010) suggested a critical evaluation of the present approach of assessing the sprat stock of the Baltic proper as one single stock unit, because spatial differences in life history traits, *e.g.* morphometrics and growth, have been reported (*e.g.* Lindquist, 1971). The obvious

variability among the areas investigated in the present study, which may be due to spatial differences in growth, support this statement.

Results on the dynamics of oocyte recruitment (Chapter IV) in Baltic sprat proved that the spawning batch is clearly identifiable prior to hydration, although the general indeterminate oocyte recruitment pattern was confirmed. This allows estimating batch fecundity from females in both ovarian developmental stages, *i.e.* the germinal vesicle migration stage and the hydrated stage. The advantage is that (i) sufficient material for batch fecundity analyses can be collected more easily, and (ii) it may be feasible to use image analysis systems to multiply sample sizes and to improve accuracy. The auto-diametric method could be applied to sprat in order to yield theoretical values of potential fecundity but also batch fecundity may be assessed. The auto-diametric method is based on the relationship between mean oocyte diameters and oocyte density in the ovary (Thorsen and Kjesbu, 2001). Once a calibration curve of this relationship has been established fecundity can be estimated by the measurement of oocytes from a sub-sample of ovary tissue. In comparison to the conventional gravimetrical method where oocytes have to be counted manually, large numbers of samples can be processed rapidly applying a computer-aided image analysis system. However, the precision of the auto-diametric method should be tested statistically prior to a possible application to Baltic sprat. Witthames *et al*. (2009) reported that this method was less accurate when applied to the indeterminate spawner hake (*Merluccius merluccius*), compared to species with determined oocyte recruitment as cod (*Gadus morhua*) or plaice (*Pleuronectes platessa*). Results of the present study (Chapter IV) suggest that the reported spawning frequencies of approximately four days might be realistic for Baltic sprat. However, all available information on this reproductive trait are solely based on assumptions so far, including results of the present study, and a validation is a prerequisite for further effort to implement the DEPM and population dynamic models which take into account the daily or seasonal egg production of this stock. The approach of the present study to use histology and modern image analysis to analyse the ovarian cycle of sprat over a 24 h period is in principle adequate. However, sample sizes were limited and only one single 24 h fishery sampling for one time point of the year could be realised. Therefore, this study has to be seen as a pilot study. Important results on the ovarian dynamics in relation to postovulatory follicles (POF) were described in the present study with the major finding that the degeneration of POF equals the spawning interval. More effort in this direction would help to clarify the picture. Several 24 h fishery samplings should be carried out to get a more reliable result on the diel spawning pattern. Vertically resolved ichthyoplankton samplings over more than one 24 h sampling are required to elucidate the spawning behaviour of Baltic sprat. The latter approach was used by Balzar (1994) who found evidence for Baltic sprat spawning at night near the surface. However, this study was restricted to one sampling period in July, *i.e*. at the end of sprat spawning season. It has been reported before that sprat spawning shifts from below the halocline at the beginning of the spawning period to surface water layers towards the end of the spawning season (Alekseev and Alekseeva, 2005). This shift in spawning depth might also affect the diel spawning behaviour. Simpson (1971) was able to determine the diel peak spawning of sprat from ichthyoplankton samples by identification of the earliest cleavage egg stages. This would also be a possible approach to unequivocally determine the diel spawning behaviour of Baltic sprat. Otherwise, tank experiments could be used to investigate spawning frequency in detail (Leong, 1971). The latter approach would have the advantage that the effect of temperature on spawning frequency, duration of ovarian developmental stages and the duration of POF could be investigated in parallel, which is desirable and has been shown to be possible for other species (Kurita *et al*., 2010). However, it is questionable if the latter approach is feasible for Baltic sprat. Sprat is a vulnerable fish which is difficult to handle and to keep in tanks in sufficient numbers and for a sufficient time to perform such experiments successfully. Further, artificial condition in tanks may also affect the behaviour of fish and does not necessarily reflect natural behaviour.

The obtained data were used to implement the Daily Egg Production Method to the Baltic sprat stock in the Bornholm Basin (Chapter VI). Results clearly demonstrated that the DEPM is a promising approach to assess this stock. In contrast to the standard procedure in sprat stock assessment, this approach takes into account observed variability in sprat reproductive traits. However, some uncertainties concerning the input parameters were identified which require improvement. First, it remains difficult to identify distinct daily cohorts of spawned eggs from the ichthyoplankton field samples with the existing classification of sprat egg development stages and the resulting temperature dependent development model (Chapter V). This is due to the relative long duration (more than one day) of the first identifiable egg stage for the observed range in ambient temperature. It may be useful to investigate whether it is possible to define more egg stages and to establish a temperature-dependent development model with a higher resolution (Smith and Hewitt, 1985). Secondly, it was shown that the diel spawning pattern of Baltic sprat is not as synchronous as in many other clupeoid species, a fact which further hampered the precise identification of daily cohorts of spawned eggs and the determination of spawning frequency from histological analyses of sprat ovaries. Improvements are especially necessary in the estimation of spawning frequency of Baltic sprat as discussed above.
A number of important findings and data have been obtained by the present study concerning the reproductive traits of Baltic sprat. This new knowledge may serve as basis to (i) enhance existing assessment methods and to test alternative indices for sprat stock reproductive potential (*e.g.* female spawning stock biomass or potential egg production), (ii) implement alternative assessment methods (*e.g.* DEPM) and (iii) further investigate the population dynamics and the ecology of Baltic sprat. The latter may be accomplished by means of modelling, either individual based models (IBMs) or population based models, for which obtained data of the present work will serve as important input parameters. Various interesting questions concerning the reproductive biology of sprat still remain to be solved. Thus, further investigations are needed that combine sophisticated field sampling, laboratory experiments and modelling activities to complete our understanding of Baltic sprat reproductive biology and its potential application in assessment methods.

A stage-based matrix model to resolve critical life stages of Baltic sprat population in relation to temperature

Baltic Sea sprat recruitment is highly variable which can partly be explained by atmospheric forcing and changes in ambient temperature (Baumann et al., 2006a; MacKenzie and Köster, 2004; Köster et al., 2003). Several important life history parameters of sprat are directly affected by changes in environmental conditions. For instance, the development and survival of early life stages as well as the maturation process and batch fecundity of adults are related to ambient temperature. However, the relative impact of processes acting on individual life stages from eggs to the spawning stock, finally resulting in recruitment, are still poorly understood. To resolve this, it is essential to identify critical life stages with respect to various environmental factors. Because all life stages of sprat are likely to be differentially affected by a possible global climate change, investigating the cumulative effects of a possible increase in ambient temperature on the sprat population dynamics is particularly needed. A promising approach for such a study would be a stage based population model. The basic principle of such models was described by Leslie (1945). Lefkovitch (1965) introduced a stage-based extension of this model. Since then, stage based matrix models have been used successfully in order to investigate the population dynamics of small pelagics (*e.g.* Mantzouni *et al*., 2007; Pertierra *et al*., 1997; Butler *et al*., 1993). This approach accounts for changes in the vital rates of each specific life stage and thus enables the identification of the most critical life stages in terms of population response to environmental forcing (Caswell, 2001).

Three important stage specific life history traits are usually included in such models: (i) the duration of a specific life stage, (ii) the stage specific mortality or probability of survival and (iii) stage specific reproduction output. The life table matrix describes the transition of a population from time *t* to time *t*+1 in terms of vital rates, *i.e.* the probabilities of surviving and staying in stage *i*, the probability of surviving and growing into stage *i*+1, and the reproductive rate per stage and unit time.

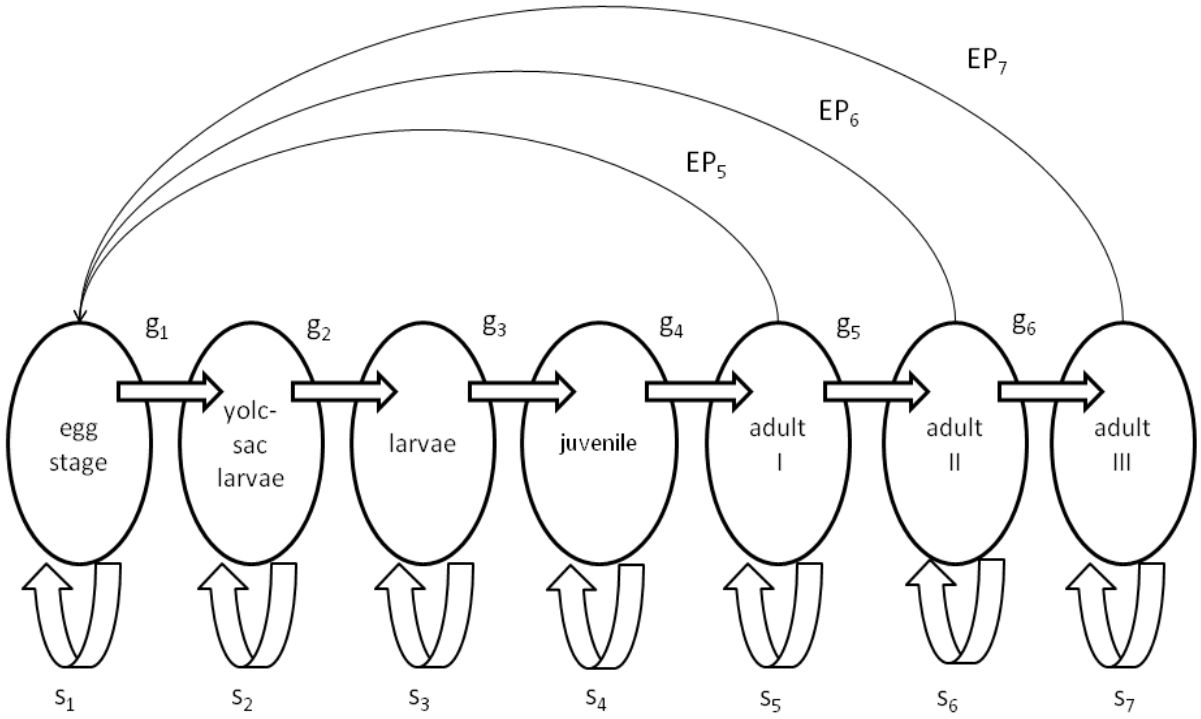

Fig. 1: Conceptual model of a Baltic sprat population matrix. Defined are seven different stages: eggs, yolk-sac larvae, feeding larvae (larvae), juveniles, and three adult stages (I to III). g_i = growth of the i_{th} stage for i = 1 - 6, s_{i} = survival probability of the i_{th} stage for i = 1 - 7, EP_i = egg production of the i_{th} stage for i = 5 - 7.

For Baltic sprat, it would be possible to construct such a model with seven different life stages (Fig. 1). Many of the parameters which are needed to run such a model have been obtained by the present work and can be estimated as a function of temperature: the duration of egg development, growth of yolk-sac larvae (Petereit *et al*., 2008), size at maturity and batch fecundity. However, due to the indeterminate spawning strategy of sprat some assumptions still have to be made in order to calculate the total egg production of the defined adult stages. Spawning frequency and therefore the number of batches spawned over the whole season is still a source of uncertainty, but results of the present study gave some evidence that a spawning interval of approximately four days could be used as initial input parameter. Some input parameters which were not a subject of matter in the present work are available from literature, *e.g.* juvenile and feeding larvae growth rates in relation to temperature (Baumann *et al*., 2008; Baumann *et al*., 2006b; Clemmesen, personal communication). Some parameters have still to be investigated from survey data, *e.g.* juvenile mortality, adult growth and mortality. All these data combined in the described matrix model may allow to model sprat population dynamics in relation to changes in ambient temperature. Predicted elevation in seawater temperature is supposed to result in more favourable conditions for Baltic sprat recruitment as many of sprat life history traits seem to be positively related to temperature (MacKenzie and Köster, 2004;

Nissling, 2004), and the Baltic sprat population resides in the lower temperature range of the species distribution. However, Baltic sprat is only one part of a complex pelagic ecosystem. A change in temperature may also result in a shift in marine production cycles and consequently may lead to mismatch situations with negative impact on sprat recruitment. A temperature elevation in the Baltic may also have the potential to alter the species composition and may favour invasive species, *e.g. Mnemiopsis leidyi*, which potentially is a threat for pelagic fish eggs and larvae via predation (Haslob *et al*., 2007) or via competition for food (Schaber *et al*., 2011). While the matrix model approach may allow the identification of critical life stages with respect to the population growth rate, it is an inadequate tool to quantitatively predict how changes in phenology of the respective sprat life stages and their prey and predators will act on sprat recruitment. To tackle the latter problem, it might be useful to couple results from ongoing process oriented IBM modelling activities and the matrix model approach in future studies.

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Annex I: Maturity Keys

A) Maturity stages for the Baltic sprat (used in LATFRI and ATLANTNIRO)

(From: Alekseejev F.E. & Alekseejeva E.I., 1996. Assessment of gonads maturity stages and study of sex cycles, fecundity, eggs production and maturation rate of marine commercial fishes. Methodical manual. Kaliningrad, AtlantNIRO)

Stage	State	Females	Males
I	Juvenile	Gonads are thin, thread-like, colourless and transparent. Sex of fish is not distinguishable by naked eye.	
II	Immature (non-mature)	Ovaries are small, tubular, slender, and **yellow-orange**. Oocytes are **not visible by eye**. If the colour is dark violet: stage **VI-II**!!!	Testes are thin, flatten, half-transparent and greyish.
III	Ripening	Ovaries increasing the size and at the end of the stage **occupy up to 2/3 of body cavity**. In the beginning of stage oocytes are half-transparent, but at the end of stage non-transparent, yellow. Only at the caudal end (near the ass) there might be some reddish area. Non-transparent and yellow oocytes are **seen by naked eye** through the membrane of ovaries. Diameter of oocytes is 0.2 mm in the beginning of stage and 0.5 mm – at the end of stage. Sub-stages of stage III (differentiation of the ripening females from those who are close to the mature stage) are no longer separated. To distinguish between stage III and IV use the **size** of the oocytes. Oocytes in stage IV are slightly bigger! III is separated from VI-III with the help of the **colour** of the ovary (VI-III: reddish violet).	Size of testes has increased and at the end of stage III they occupy most of body cavity. Testes are elastic on touch. At the end of stage, they are white (though real white ones are hardly available). To distinguish from stage IV, a **cross-section** of testes can be done. **Overrun' (overflow) and exude of milt should not occur, the form should be maintained.** III has also a different shape of the testes with a **triangular form**.
IV	Mature (ripe)	Ovaries occupy all empty space in body cavity. Non-transparent oocyte diameter is 0.5-0.6 mm, so oocytes are **slightly bigger than in III**. The colour of the ovary is **light yellow**, not reddish!	Testes occupy all body cavity, are elastic and **white**. In cross-section, they '**overrun**' (overflow) exuding thick milk.
IV-V	Pre-spawning	Ovaries occupy all empty space in ventral cavity firmly pressing on all other organs. Through the membrane of ovaries are seen **large** (diameter 0.7-1.0 mm) and transparent (**hydrated) oocytes**. In ovary they are evenly distributed between different size ripening and non-transparent oocytes. There are no eggs available in ovaries cavity. It is **very important** to distinguish between IV-V and **VI-IVh** (a new	

		invention!). IV-V is mostly yellow in contrast to VI-IV which is red-violet (but both are of course a bit transparent).	
V (VI-V)	Spawning	Slight pressure upon the belly extrudes eggs from the genital opening (press before cutting the fish!). **Running** during the day is really seldom!	Slight pressure upon the belly **extrudes thin milk** from the genital opening, so press **before cutting** the fish!!!
VI-III	Partly spawned - ripening	Ovaries are similar to the stage III, but have **reddish-violet** colour. Ovaries are soft on touch. In cross-section of ovaries in ovaries cavity are seen separate, not spent eggs. Use the **size** of oocytes to differentiate between VI-III and VI-IV	
VI-IV	Partly spawned - mature	Ovaries are similar to stage IV but reddish-violet. In ovaries cavity can be seen separate, not spent eggs. Do not judge by size of ovary but again by size of oocytes!	Testes are similar to stages III and IV, but **smaller**, soft and **unevenly coloured**: Milk is remaining in the upper part (**white areas**), reddish or brownish spots occur.
VI-IVh	**NEW!**	**New invention** to separate between first time spawners (IV-V) and those which are entering from VI-IV: Similar to IV-V (see above) but the colour is **red-violet**!!!	
VI	Spent (Spawned)	Like an early stage III, but Ovaries are **very flabby** (and small), mainly **dark red**, half-transparent. **Small number** of remaining yellow oocytes can be seen through membrane of ovaries. Cross-section of ovaries cavity has large opening in which can be seen separate, not spent or remaining eggs.	Testes are **small**, soft and **flabby**. Colours of testes are red-brawn frequently with white spots. In cross-section, they not 'overrun' (overflow) but small amount of remaining milk is exuded.

B) Maturity key used by the Heinrich von Thünen Institute for Baltic Fisheries, Rostock, Germany (vTI-OSF)*

code		**Females**	**Males**
0	juvenile	No sex differentiation by the naked eye possible	
1	juvenile	immature, ovary thin, tubular and transparent ovary Ø > 1 -1.5 mm, oocytes not visible	testes thin, tubular greyish Ø > 1 - 1.5 mm
2	resting	Ovary grey-white to yellow-pink, hyaline ovary Ø > 1,5 -3 mm, oocytes not visible	testes grey, compact Ø > 1,5 -3 mm
3	ripening	Ovary yellow-orange, well supplied with blood, hyaline, fills up to 1/2 of body cavity ovary Ø >2 -3,5 mm	testes grey with beginning elucidation, compact, fills up to ½ of body cavity, Ø >2 -3,5 mm
4	ripening	Ovary yellow to light red, opaque to semi-transparent, fills up to 2/3 of body cavity, opaque yellow oocytes (0.2 -0.5 mm) are visible	testes light grey to whitish, fills ½ to 2/3 of body cavity, Ø >2 -3,5 mm
5	ripe	Ovary tight, fills more than 2/3 of body cavity, yellow-reddish, numerous opaque oocytes (0.5 - 0.6 mm)	testes light grey to whitish, at the edges soft, fills 2/3 of the body cavity, releases white milt under pressure
6	spawning	Abdominal cavity swollen, ovary tight, reddish, besides ripening opaque oocytes, numerous transparent oocytes (0.7 -1 mm) visible	testes light grey to white, releases white and runny milt under pressure
7	ripening again	partly spent, ovary red to grey-red, purple, smaller, some hydrated oocytes left, otherwise similar to stage 5	testes coloured irregular, reddish or brownish blots, grey at the lower edge, partly whitish remains of milt
8	spent	spent, ovary grey-red and hyaline, flabby, ovary Ø 2 - 3 mm	testes grey to brownish, small, flabby, reduced in size, Ø 2-3 mm

* translated into English by Holger Haslob, IFM-GEOMAR

Annex II: Histological features of sprat ovaries

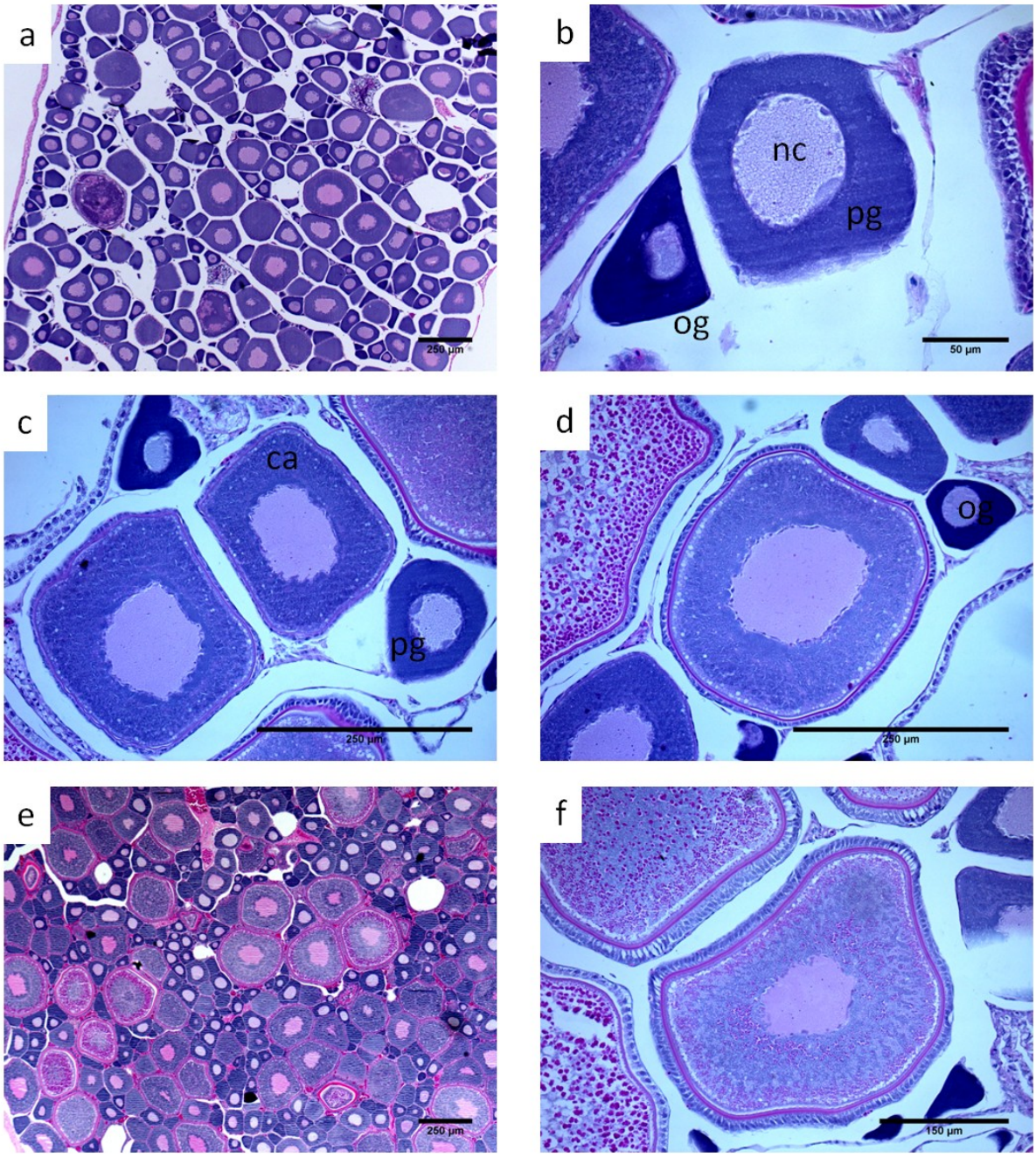

Fig. 1: Histological sections of ovaries from immature/resting until early vitellogenesis. (a) Resting or immature. Only oogonia and primary growth oocytes with large nuclei are visible; (b) oogonia (og) and primary growth oocyte (pg), nc = nucleus. (c) Beginning maturation with secondary growth oocytes in the cortical alveoli stage. The oocyte size has increased and cortical alveoli are visible at the cell periphery. (d) Oocyte with cortical alveoli at the periphery. (e) Early maturation, besides oogonia and primary growth oocytes first signs of vitellogenesis becomes visible. (f) Secondary growth oocyte with first signs of vitellogenesis; yolk granules become visible. Photographs taken by Holger Haslob, IFM-GEOMAR.

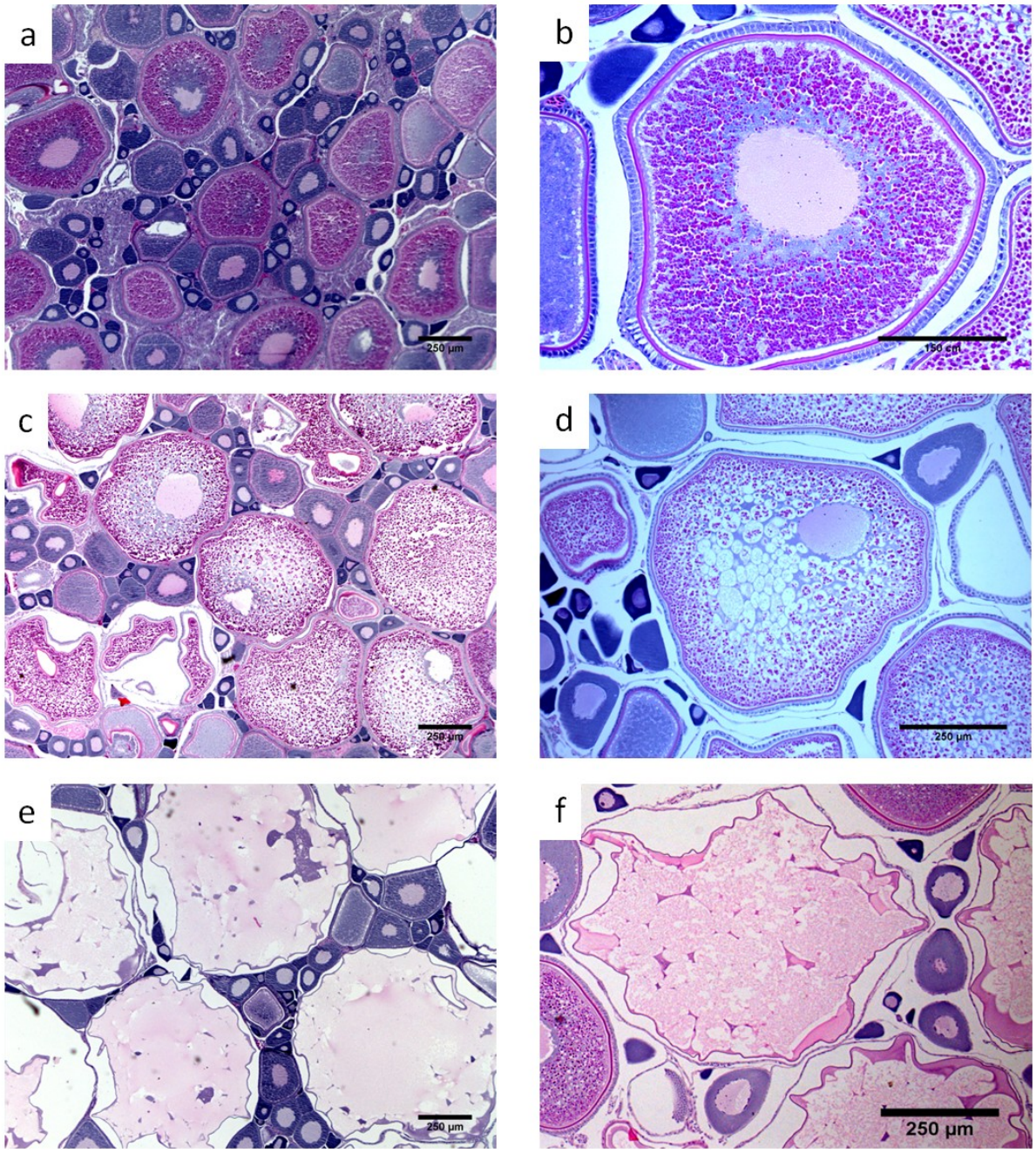

Fig. 2: Histological sections of ovaries from vitellogenesis until full hydration. (a) Oocytes in the vitellogenesis stage besides primary growth and oogonia. Oocytes are completely filled with yolk. (b) Oocyte in advanced vitellogenesis stage filled with yolk. (c) Nucleus migratory stage. The nucleus migrates towards the micropyle and yolk granules begin to hydrolyse. (d) Oocyte in nucleus migratory stage. Yolk in the center of the cell hydrolyses, nucleus migrates toward micropyle. (e) Hydrated stage with fully hydrated oocytes; (f) Fully hydrated oocyte. The yolk has completely hydrolysed and the nucleus has disintegrated. Within a short time these oocytes will be ovulated and spawned. Photographs taken by Holger Haslob, IFM-GEOMAR.

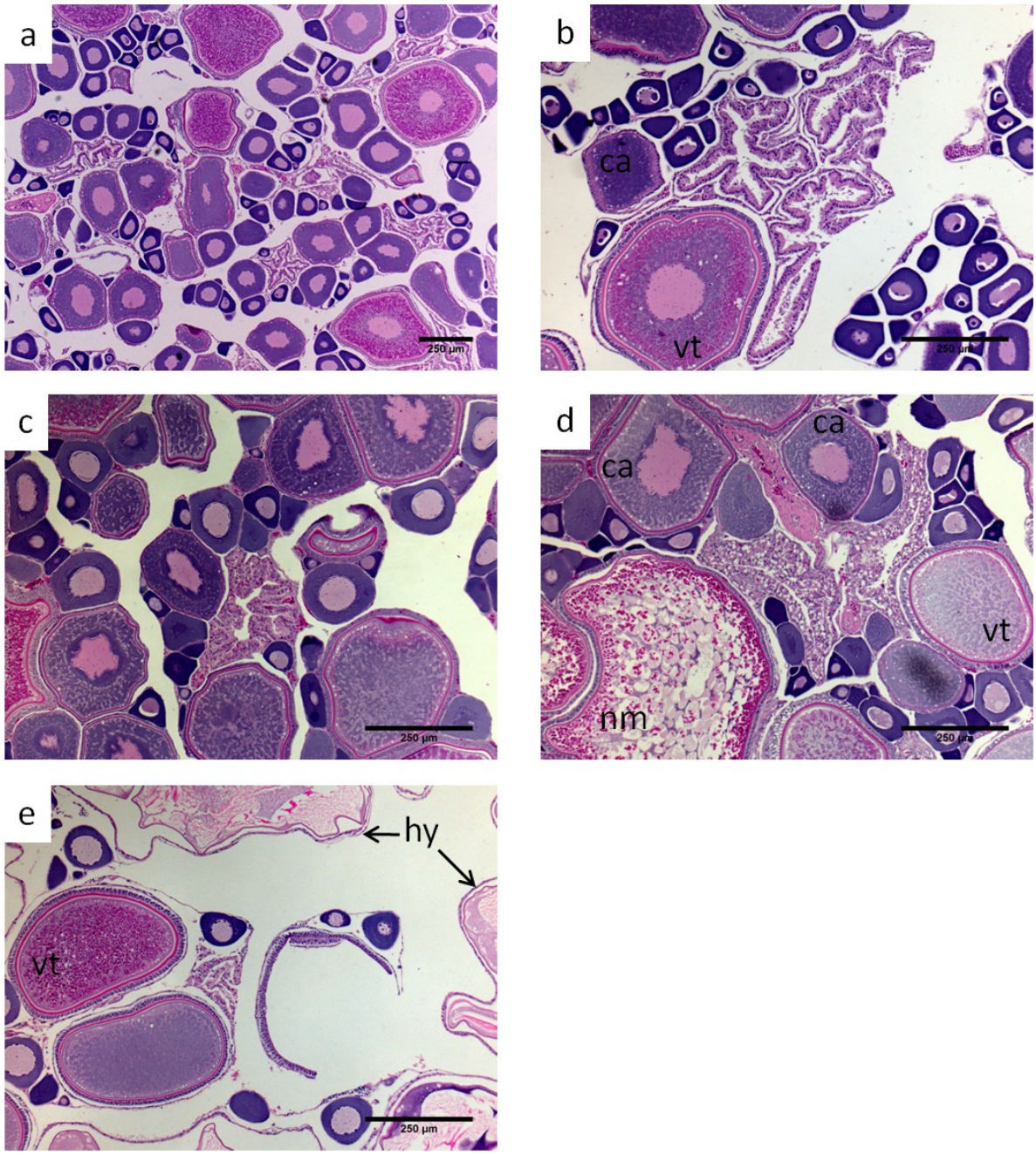

Fig. 3: Deterioration stages of postovulatory follicles. (a) Ovary recently after spawning with numerous fresh postovulatory follicles; (b) recent postovulatory follicles; (c) advanced postovulatory follicle; (d) postovulatory follicles with beginning signs of pycnosis; (e) remains of a postovulatory follicle in the center of the section. ca = cortical alveoli, vt = vitellogenesis, nm = nucleus migratory, hy = hydrated. Photographs taken by Holger Haslob, IFM-GEOMAR.

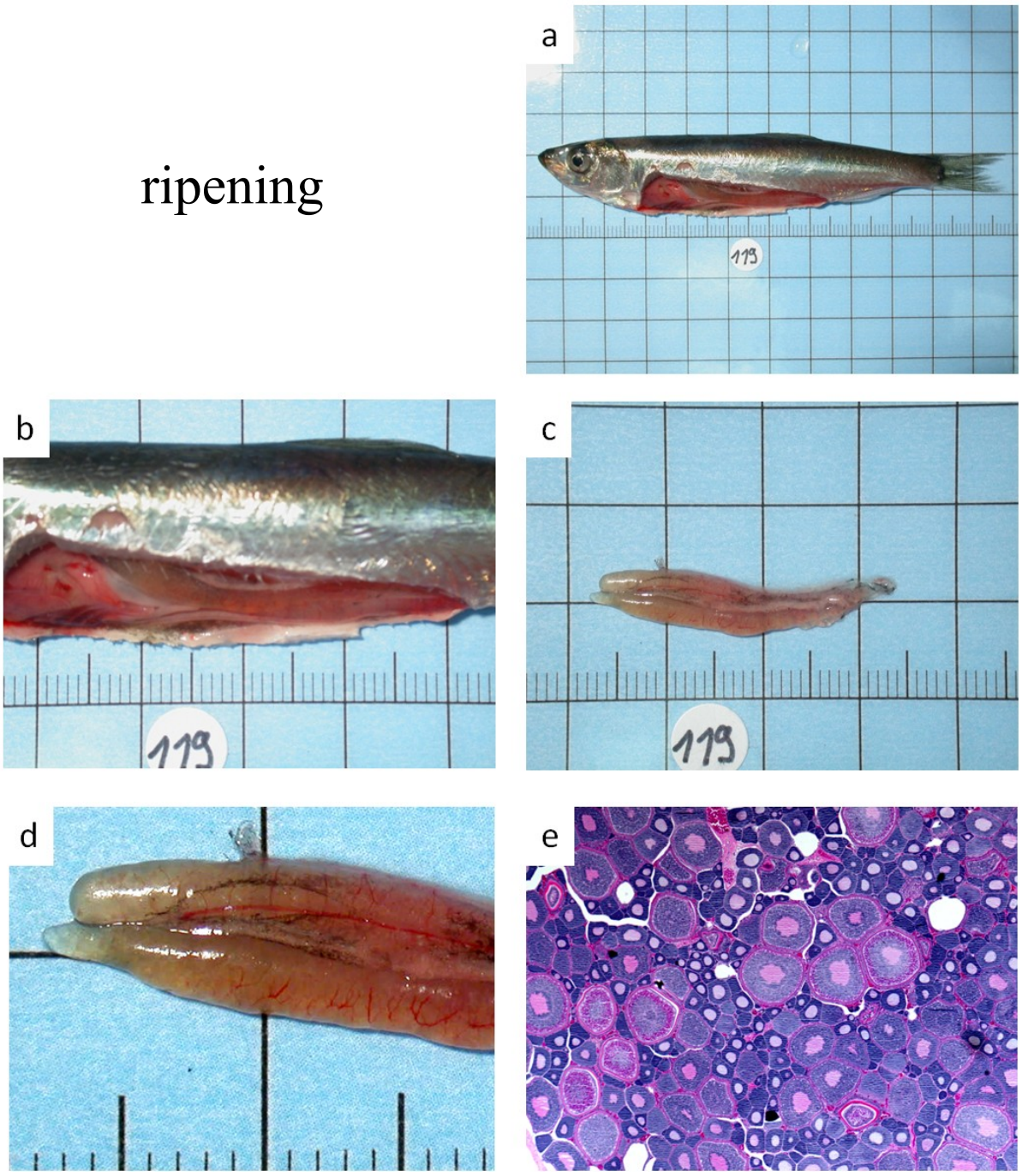

Fig. 4: Macroscopic and histological appearance of female sprat in an early maturation stage. (a) whole fish: total length = 11.4 cm, total weight = 8 g; (b) body cavity slit open; (c) dissected ovary; (d) enlarged ovary with oocytes becoming visible in the anterior part; (e) histological section with visible oogonia, primary growth oocytes and oocytes with beginning vitellogenesis. Edge lengths of squares in panels a – d correspond to 1cm. Photographs taken by Holger Haslob, IFM-GEOMAR.

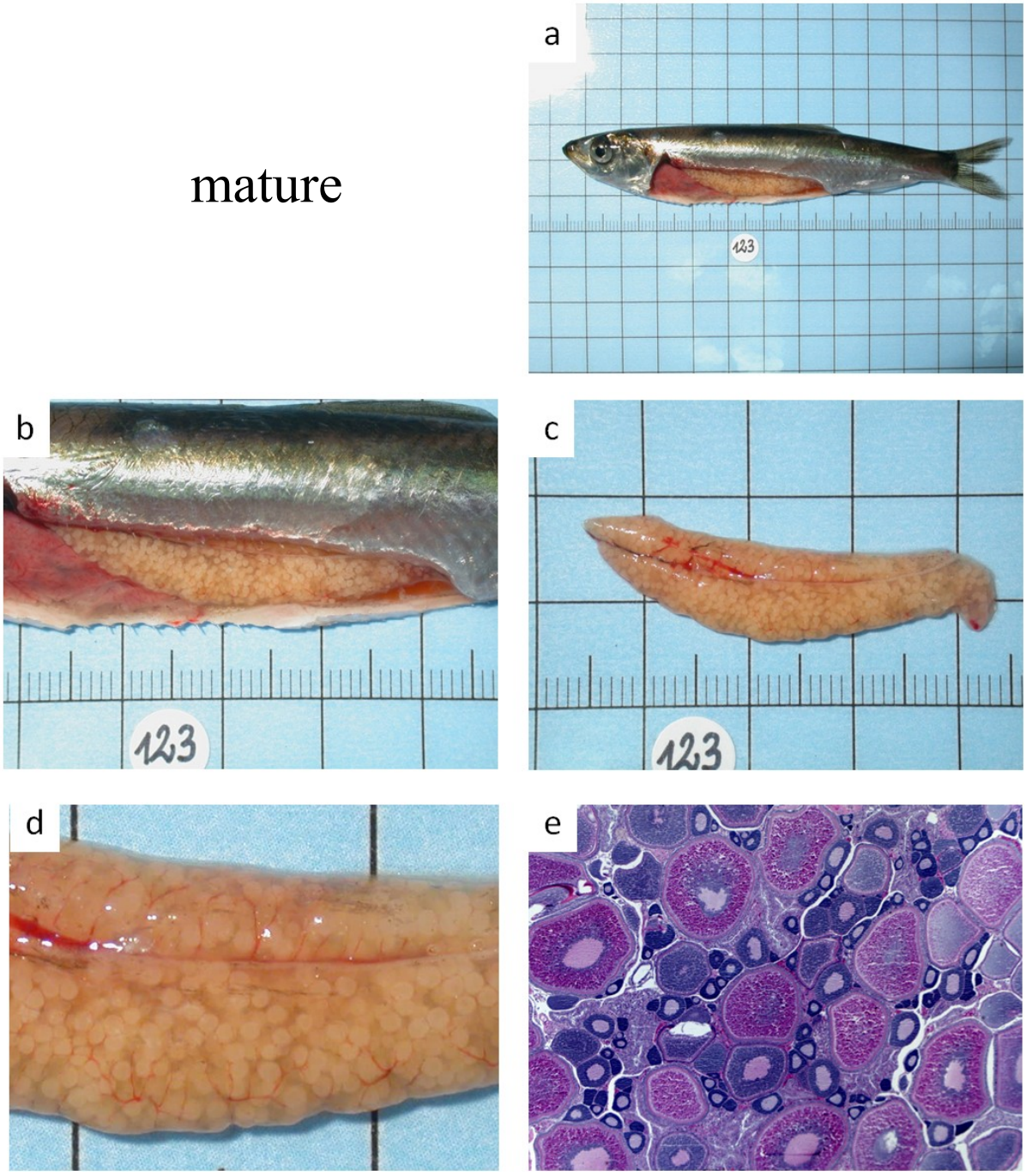

Fig. 5: Macroscopic and histological appearance of mature female sprat. (a) whole fish, total length = 12.7 cm, total weight = 11 g; (b) body cavity slit open; (c) dissected ovary; (d) enlarged ovary with clearly visible, opaque oocytes; (e) histological section corresponding to the macroscopic stage. Besides oogonia and primary growth oocytes several oocytes in advanced vitellogenesis are visible. Also visible are some postovulatory follicles indicating repeated spawning. Photographs taken by Holger Haslob, IFM-GEOMAR.

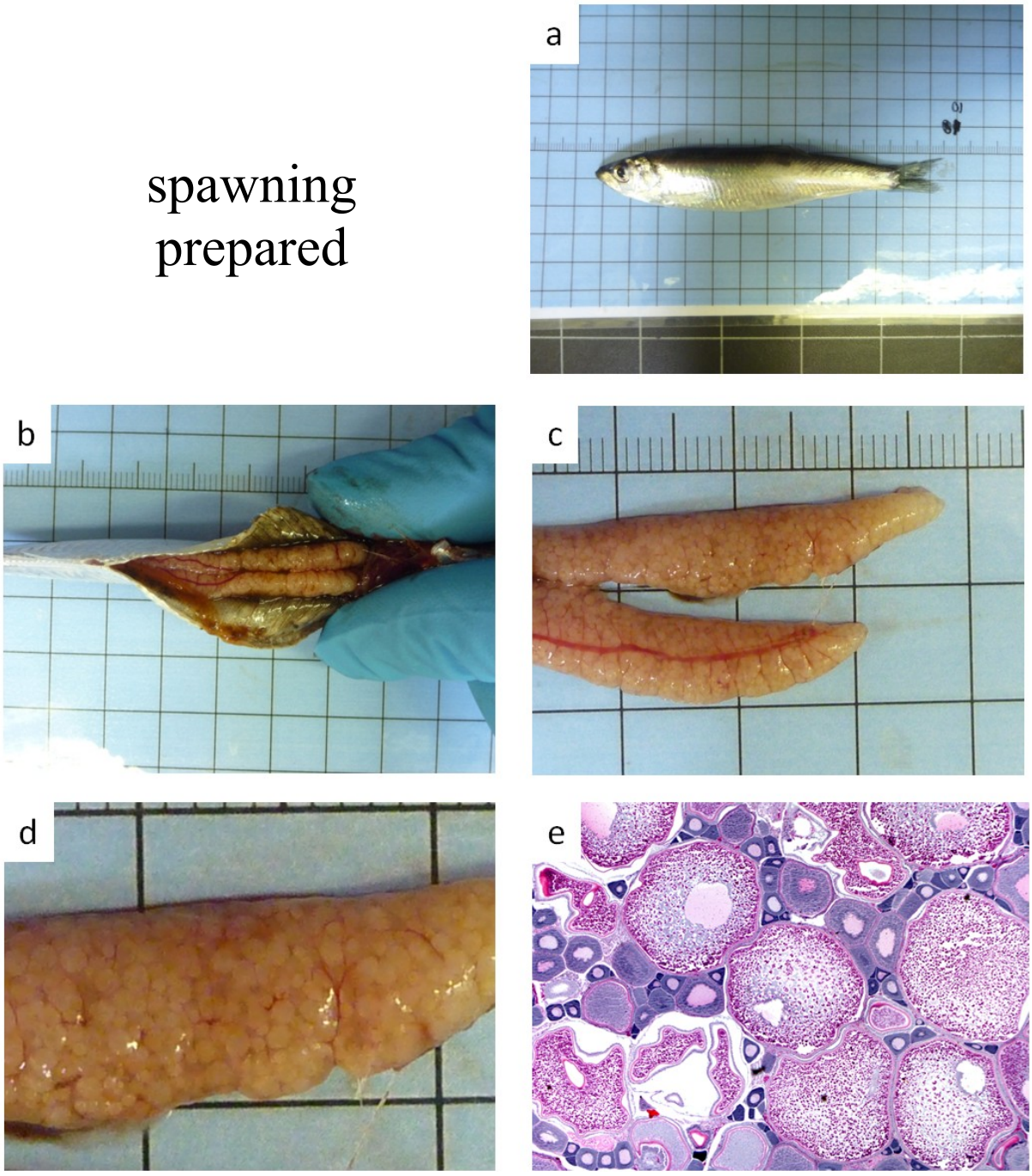

Fig. 6: Macroscopic and histological appearance of female sprat preparing spawning. (a) whole fish, total length = 13.1 cm, total weight not available; (b) body cavity slit open; (c) dissected ovary; (d) enlarged ovary. Compared to stage 4 in this stage the oocytes have increased slightly in size and begin to become transparent in the center; (e) histological section corresponding to this stage. Besides oogonia and primary growth oocytes several oocytes in the migratory nucleus stage are visible. Photographs a-d taken by Dr. J. Schmidt, CAU-Kiel. Photograph e taken by H. Haslob, IFM-GEOMAR.

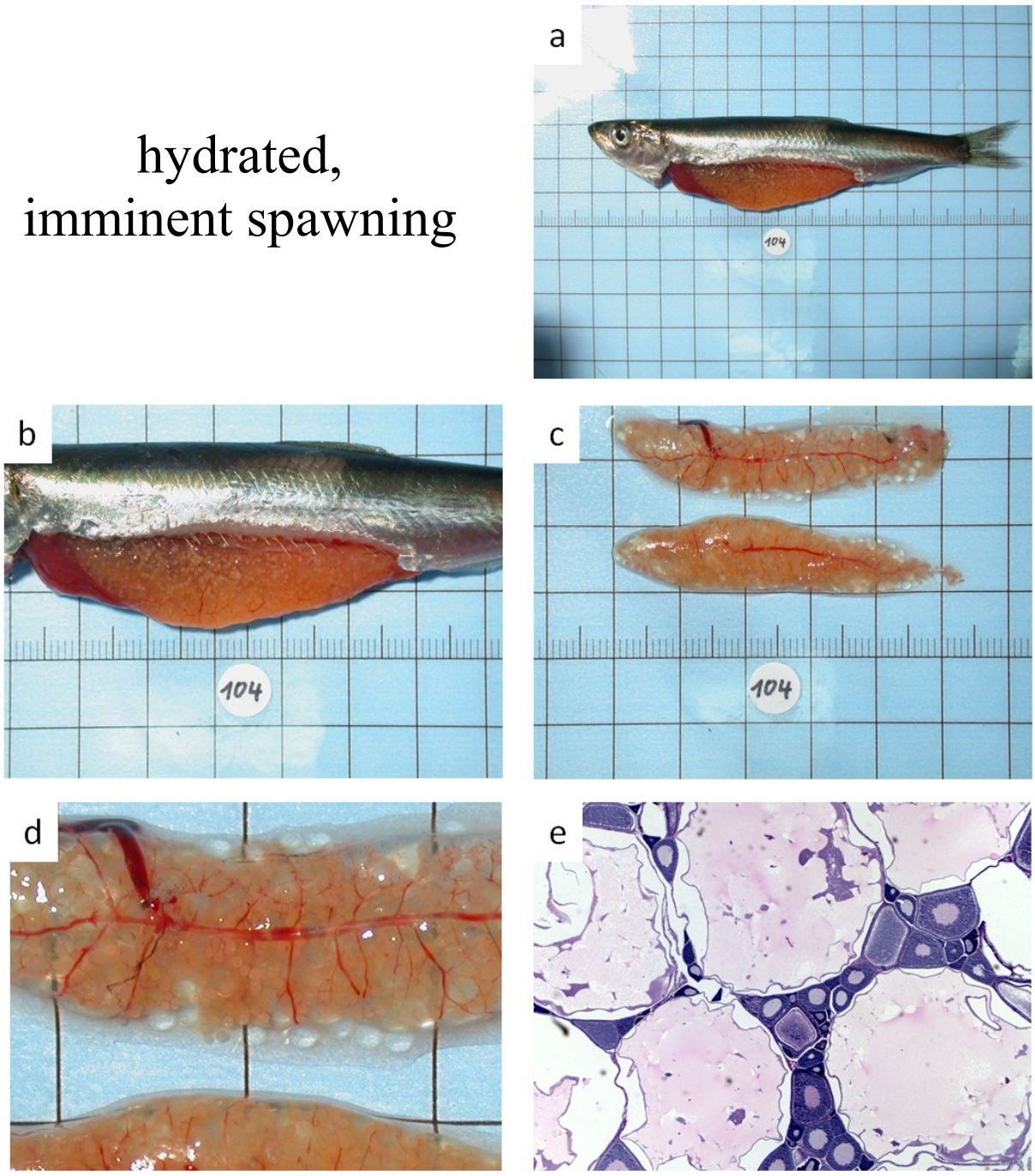

Fig. 7: Macroscopic and histological appearance of female sprat with fully hydrated oocytes. (a) whole fish, total length = 13.0 cm, total weight = 16 g; (b) body cavity slit open; (c) dissected ovary; (d) enlarged ovary with clearly visible and transparent oocytes; (e) histological section of an ovary with hydrated oocytes. Photographs taken by Holger Haslob, IFM-GEOMAR.

Annex III: Histology protocols

Ovaries for histological analyses were fixed in a buffered 4% formaldehyde solution. From each ovary a sub-sample was cut and embedded in paraffin. Prior to embedding the tissue was dehydrated using a semi-enclosed benchtop tissue processor (Leica TP1020) following the work steps listed in table 1. The embedding process was done by using an embedding station (Leica EG1160). The produced paraffin blocks were stored in a refrigerator for at least 24 h prior to sectioning. Shortly before the preparation of sections the paraffin blocks were once more cooled down in an ice box. The rapid cooling process of the blocks enhances the quality of the histology sections and facilitates the sectioning with the microtome. Sections of 3 µm were produced. The sections were transferred onto microscope slides and stored for 24 h in an oven at 48 °C prior to staining. Hematoxilin-Eosin was chosen as stain. An automated staining machine was used (Leica ST5010). The staining process followed the steps listed in table 2.

Tab. 1: Tissue processing protocol

Step	Reagent	Time
1	Ethanol 70%	45'
2	Ethanol 96%	1h 30'
3	Ethanol 96%	1h 30'
4	Ethanol 100%	1h 30'
5	Ethanol 100%	1h 30'
6	1:1 Ethanol:Toluene (v:v)	2h15'
7	Toluene	1h 30'
8	Toluene	1h 30'
9	1/2 Toluene 1/2 Paraffin	2h15'
10	Paraffin	3h

Tab. 2: Section staining protocol

Step	Reagent	Time (min:sec)
1	Xylene	10:00
2	Ethanol 100%	4:00
3	Ethanol 80%	3:00
4	H_2O	2:00
5	Hematoxylin	4:00
6	H_2O	2:00
7	Acid alcohol	0:10
8	H_2O	3:00
9	Litium Carbonate	0:10
10	H_2O	1:00
11	Ethanol 70%	1:00
12	Eosine-Floxine	2:00
13	Ethanol 96%	2:00
14	Ethanol 100%	2:00
15	Xylene	5:00
16	Xylene	3:00

Contributions of authors

Chapter I
This chapter has been accepted for publication in the Journal "Fisheries Research". All analyses, graphics and text writing were done by Holger Haslob. Dr. Jonna Tomkiewicz critically reviewed the manuscript and gave valuable comments. Dipl.-Phys. Hans-Harald Hinrichsen provided all hydrographic data and also reviewed the manuscript. Dr. Gerd Kraus was involved in elaborating the concept of the study and gave valuable advice on the practical work.

Chapter II
All analyses, graphics and text writing were done by Holger Haslob.

Chapter III
All analyses, graphics and text writing were done by Holger Haslob. Dr. Uwe Böttcher provided age based single fish data obtained on the May acoustic surveys on RV Walther Herwig III. Dr. G. Kraus provided data from the ICES Study Group on Baltic Herring and Sprat Maturity (SGBHSM).

Chapter IV
This chapter has been submitted to the "Journal of Sea Research" as a manuscript. All analyses, graphics and text writing were done by Holger Haslob. Dr. Fran Saborido-Rey supervised the histological analysis and critically reviewed the manuscript. Dr. Gerd Kraus critically reviewed and commented the manuscript.

Chapter V
Some results of this chapter already have been published in: Petereit, C., Haslob, H., Kraus, G., Clemmesen, C., 2008. The influence of temperature on the development of Baltic Sea sprat (*Sprattus sprattus*) eggs and yolk sac larvae. Marine Biology 154, 295-306. Dr. C. Petereit performed artificial fertilisation of sprat eggs on board of RV Alkor. The subsequent experiments, observations and data analysis to establish the stage combined temperature-dependent model on sprat egg development were performed by Holger Haslob. Dr. C. Clemmesen and Dr. G. Kraus were involved in elaborating the concept of the study and supported the work with valuable suggestions.

Chapter IV
This chapter is prepared for submission to the "ICES Journal of Marine Science". Most of the analyses, all graphics and text writing were done by Holger Haslob. Dipl.-Phys. H.H. Hinrichsen performed the objective analyses to estimate total egg abundance and provided all hydrographic data. H. Hauss assisted in analyses of daily egg production and egg mortality. Dr. Voss provided ichthyoplankton data for the years 1999-2003 and critically reviewed the manuscript. Dr. Uwe Böttcher crtitically reviewed and commented the manuscript. Dr. G. Kraus was involved in elaborating the concept of the study and critically reviewed the manuscript.

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Curriculum Vitae

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Publikationen

H. Haslob, J. Tomkiewicz, H.H. Hinrichsen, G. Kraus (2011): Spatial and interannual variability in Baltic sprat batch fecundity. Fisheries Research (accepted) doi:10.1016/j.fishres.2011.04.018

M. Schaber, **H. Haslob**, B. Huwer, A. Harjes, H.H. Hinrichsen, M. Storr-Paulsen, J.O. Schmidt, R. Voss, V. Neumann, F.W. Köster (2011): Spatio-temporal overlap of the alien invasive predatory ctenophore Mnemiopsis leidyi and ichthyoplankton in the Bornholm Basin (Baltic Sea). Biological Invasions doi: 10.1007/s10530-011-9936-7

M. Schaber, **H. Haslob**, B. Huwer, A. Harjes, H.H. Hinrichsen, M. Storr-Paulsen, J.O. Schmidt, R. Voss, F.W. Köster (2011): The invasive ctenophore *Mnemiopsis leidyi* in the central Baltic Sea - Seasonal phenology and hydrographic influence on spatio-temporal distribution patterns. Journal of Plankton research doi: 10.1093/plankt/fbq167

H. Haslob, N. Rohlf, D. Schnack (2009): Small scale distribution patterns and vertical migration of North Sea herring larvae (*Clupea harengus*, Teleostei: Clupeidae) in relation to abiotic and biotic factors. Sci. Mar. 73S1: 13-22.

C. Petereit, **H. Haslob**, C. Clemmesen, G. Kraus (2008): The influence of temperature on the development of Baltic Sea sprat (*Sprattus sprattus*) eggs and yolk sac larvae. Marine Biology 154(2): 295-306.

B. Huwer, M. Storr-Paulsen, H.U. Riisgård, **H. Haslob** (2008): Abundance, horizontal and vertical distribution of the invasive ctenophore *Mnemiopsis leidyi* in the central Baltic Sea, November 2007. Aquatic Invasions Vol. 3, Issue 2.

H. Haslob, Clemmesen, C., Schaber, M., Hinrichsen, H., Schmidt, J.O., Voss, R., Kraus, G., Köster, F.W. (2007): Invading *Mnemiopsis leidyi* as a potential threat to Baltic fish. Mar. Ecol. Prog. Ser. 349: 303-306.

C. Petereit, **H. Haslob**, G. Kraus, C. Clemmesen-Bockelmann (2006): The influence of temperature on the development of Baltic and North Sea sprat. GLOBEC International Newsletter 12(1): 295-306.

H. Haslob, J. Tomkiewicz, H.H. Hinrichsen (2005): Spatial and temporal variability in Baltic sprat (*Sprattus sprattus balticus* S.) batch fecundity. ICES CM 2005/Q: 08, 13pp.

Eidesstattliche Erklärung

Hiermit erkläre ich, dass die vorliegende Promotionsarbeit selbständig von mir unter Einhaltung der Regeln guter wissenschaftlicher Praxis der DFG angefertigt wurde. Die Dissertation ist in Form und Inhalt meine eigene Arbeit und es wurden keine anderen als die angegebenen Hilfsmittel verwendet. Diese Arbeit wurde weder ganz noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegt. Teile der Arbeit wurden zur Begutachtung in Fachzeitschriften eingereicht wie in „Contributions of Authors“ dargelegt. Dies ist mein einziges und bisher erstes Promotionsverfahren. Die Promotion soll im Fach Fischereibiologie erfolgen. Des Weiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

Kiel, den

Holger Haslob