Seasonal variability of Baltic sprat fecundity traits

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Abstract

In a number of marine fish species seasonal variability in important spawning traits have been observed. To better understand the reproductive potential of a fish stock, it is thus important to characterise such seasonality. However, the seasonal dynamics in a number of fecundity traits of Baltic sprat (*Sprattus sprattus balticus* S) are not well described yet. In the present study we therefore present data on the seasonal variability in timing of spawning, batch fecundity, number of developing oocytes, oocyte dry weight, spawning fraction, fish condition and atresia in Baltic sprat. Histology techniques in combination with image analyses have been applied to investigate those variables based upon material sampled in 2005 and 2008 in the Bornholm Basin, Baltic Sea. Spawning sprat were detected from January to June. In 2008 signs of ovary maturation were observed also in November. Relative batch fecundity was found to vary two-fold with 85 eggs g⁻¹ ovary free body weight observed in early spawning season (January 2005) and 165 eggs g⁻¹ ovary free body weight in late spawning season (June 2008). However, variability in batch fecundity during peak spawning was rather low. A seasonal decrease in oocyte dry weight was related to an increase in batch fecundity. Spawning fraction varied over the course of spawning period with values ranging from 0.29 in March to 0.18 in June. Stereometric analyses confirmed the indeterminate fecundity of Baltic sprat. Prevalence of atresia was low during peak spawning in April to June (1.0 – 4.0%) but considerably higher during early spawning period in March (16.4%) and highest in November (38.5%). Female sprat condition was low during spawning period and sharply increased after spawning ceased. The results of the present study are important with respect to the better understanding of fecundity and spawning dynamics of Baltic sprat and will serve to better evaluate the reproductive potential of this ecologically and economically important fish species.

Key words: batch fecundity, oocyte, atresia, spawning dynamics, *Sprattus sprattus*

1. Introduction

Seasonality in marine fish spawning activity plays a critical role in reproductive success and varies in terms of its duration, the degree of synchronisation among individual spawning periods, and the season of occurrence (Lowerre-Barbieri et al., 2011). For successful reproduction, it is essential that the produced early life stages are released into an environment where conditions are favouring survival, but a trade-off with other selective forces acting on parental spawning time may exist (Wright and Trippel, 2009). The phenology of environmental features of marine habitats is highly variable, which influences 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 increases the probability of at least some offspring cohorts to find optimum conditions for growth and survival (Sherman et al., 1984; Alheit, 1988), and will therefore increase the probability of successful reproduction.

Multiple-batch spawners often spawn over a prolonged spawning season. In this time frame, changes in environmental conditions can cause variability in the reproductive output in terms of batch fecundity, spawning fraction and frequency, as well as egg quality (Alheit, 1993; Trippel et al., 1997). This seasonal variability may be due to changes in food availability and the resulting scope of individual fish to allocate energy either to reproduction or to somatic growth and maintenance. For a better understanding of these mechanisms which potentially drive the reproductive potential of a stock, it is important to characterise the aforementioned seasonal variability in spawning parameters.

The spawning season of Baltic sprat (*Sprattus sprattus balticus* Schneider, 1908), a multiple batch spawner with indeterminate oocyte recruitment, lasts approximately 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). It has been shown that batch fecundity increases during the spawning season (Heidrich, 1925; Alekseev and Alekseeva, 2005). The only observation of spawning fraction for several consecutive months is given by Kraus and Köster (2004), who reported quite high variability in this parameter over the peak spawning season for the Bornholm Basin. Some observations on the oogenesis in Baltic sprat exist (Haslob et al., 2012; Alekseev and Alekseeva, 2005), but the seasonal dynamics and resulting consequences for spawning fraction and/or batch fecundity are still unknown.

Atresia plays a role in down regulating the realised fecundity in a number of marine fish species when conditions turn to be unfavourable in terms of food availability or hydrographic conditions. 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 energetic reserves stored prior to the spawning season. Hence, potential fecundity is determined before the onset of spawning. In fish species with indeterminate oocyte recruitment, atresia might also play an important role to re-allocate surplus production of oocytes at the end of the spawning season (Murua and Motos, 2006; Korta et al; 2010). For Baltic sprat, no prior studies have investigated atresia; however, its occurrence and seasonality is an important aspect in fecundity studies.

The seasonal dynamics in a number of fecundity traits of Baltic sprat are not well known yet. Thus, the main objective of this paper was to investigate the seasonal variability in a number of fecundity traits to gain better knowledge of the spawning dynamics of this ecologically and economically important fish species. For this purpose, ovaries were sampled over the whole spawning season and were analysed with histological and image analysis methods. Moreover, a stereological approach was applied to assess the number of developing oocytes and to quantify atretic oocytes. The presented results will enable to better evaluate the reproductive potential of this stock.

2. Methods

Sprat were sampled in 2005 and 2008 during several research cruises conducted in the Bornholm Basin, Baltic Sea (Fig. 1; Table 1). For the year 2005, determination of sex and maturity was exclusively conducted macroscopically immediately after the haul was on board. For this purpose at least 10 individuals per 1cm length class were staged. Additionally, females with hydrated oocytes were sampled and preserved in a buffered 8% formaldehyde solution in January, April and May 2005 for subsequent batch fecundity analyses. To assure a proper fixation, the body cavity of each fish was slit open. Samples for the year 2008 were taken in March, April, May, June, August and November (Table 1). In this year, up to five 2-kg sub-samples of sprat were taken from pelagic fishery hauls and fixed as described above. In the laboratory, at least 20 female sprat were then sampled randomly from the sub-samples for subsequent quantitative determination of ovarian developmental phases by histology. To

increase the sample size for batch fecundity analysis, additional females with hydrated ovaries were collected from the same sub-samples when available. From all sampled females, the ovaries were removed for histological processing. One histological cross-section of one of the ovary lobes (tissue embedded in paraffin; 3µm sections; Hematoxylin staining) was produced from each sampled ovary. The ovary lobe (left or right) was chosen randomly. During the cruise in August 2008, only few sprats 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, including all hydrated ovaries collected for batch fecundity analyses in order to check for post ovulatory follicles (Table 1).

For all females analysed in the laboratory, total weight (0.1 g), gutted weight (0.1 g), total length (*TL*, 0.1 cm), and ovary weight (*OW*, 0.1 mg) and ovary free body weight (*OFBW*, 0.1 g) was determined. Condition index (*K*) was calculated taking into account *TL* and *OFBW*:

$$K = \frac{OFBW}{TL^3} * 100$$

(1)

The gonadosomatic index (*GSI*) was calculated taking into account *OW* and *OFBW*:

$$GSI = \frac{OW}{OFBW} * 100 \quad (2)$$

2.1. Ovarian development phases

All histological sections were read to assess the ovarian development phase based on the presence of the most advanced oocyte developmental stage (Table 2) according to Brown-Peterson et al. (2011). Five oocyte developmental stages were distinguished: (i) primary growth (PG), (ii) cortical alveolar (CA), (iii) vitellogenesis (VIT), (iv) germinal vesicle migration (GVM), and (v) hydrated oocytes (HYD). Moreover, the presence of recent post ovulatory follicles (POFs) was recorded. Recent POFs (<24h) were identified using histological criteria published for Baltic sprat (Haslob et al., 2012). Additionally, each ovary section was checked for the presence of α -atretic oocytes and based on that, the prevalence of atresia was estimated as the proportion of females with oocyte atresia.

2.2. Stereology

The number of developing oocytes (*NDO*) and atretic oocytes were estimated stereometrically for a subsample of ovaries (n=98) covering all months in the year 2008 (Table 3). *NDO* was estimated for spawning capable females using CA, VIT, and GVM oocytes, while atresia was estimated only in α -atresia stage, considering such when a break in the chorion was clearly visible. Histological sections of ovaries were analysed with a stereometric approach following the methodology described in Emerson et al. (1990) and Murua et al. (2003), i.e. a model- or assumption-based stereology. An ImageJ (Rasband, 1997-2009) routine was developed to overlay digital images of histological sections with a Weibel grid (Weibel et al., 1966). The used Weibel grid contained 168 points and had an area of 0.023 cm² (Fig. 2). The distance between points (125 μ m) was smaller than the average of the smallest cortical alveolar. The points touching cross sections of specific oocyte types were then counted and also the total number of cross sections of this oocyte type (see legend of figure 2 for further details). For each ovary, three to four non-overlapped areas of the same histological section were analysed stereometrically and a mean value for the number of oocytes within these areas was calculated for each oocyte type using the modified Weibel formula (Weibel et al., 1966) to calculate the number of developing oocytes (*NDO*) and atretic oocytes:

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

where

- OV* = ovary volume
- C* = size distribution coefficient
- β = shape coefficient
- N_a* = number of oocyte transections per unit area
- V_i* = volume fraction occupied by oocyte type *i* in the histological section

Ovary volume was determined as a function of ovary weight. This function was estimated for a subsample of 21 sprat ovaries, covering a broad range of ovary weights, following the method introduced by Scherle (1970). The shape coefficient β describes the ratio between the longest and shortest axis of an oocyte sectioned through the nucleus. We consider this coefficient to be species-specific and hence a single value for sprat was estimated and used to

estimate NDO of all stages. The coefficient C was calculated for each analysed ovary with the formula given by Williams (1977):

$$C = \left(\frac{M_3}{M_1} \right)^{\frac{3}{2}} \quad (4)$$

M_1 is the mean oocyte diameter estimated as the mean of the shortest and longest axis (OD):

$$M_1 = \left[\frac{\sum_{i=1}^n OD_n}{n} \right] \quad (5)$$

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

$$M_3 = \left[\frac{\sum_{i=1}^n OD_n^3}{n} \right]^{\frac{1}{3}} \quad (6)$$

For all analysed sections the longest and shortest axis of oocytes sectioned through the nucleus were measured with an image analysis system in order to calculate C and β . The estimation of C assumes that the nucleus diameter is proportional to oocyte diameter over the whole range of measured oocytes, i.e. all oocytes have the same probability of being measured independently of their diameter. For several species it has been shown that this assumption is not correct, and an allometric growth of the nucleus and oocytes has been observed (Murua et al. 2003; Domínguez-Petit, 2007). Therefore, a correction factor D was introduced to estimate C . First, a non-linear regression model was fitted to the dataset of oocyte and nucleus diameters determined from the analysed histological sections. Both diameters were estimated as the mean of the shortest and longest axis. D was then calculated for each size class i of 25 microns, as:

$$D_i = \frac{\overline{LND}}{\overline{ND}_i} \quad (7)$$

Where $\overline{LND}$ is the estimated nucleus diameter from the largest observed oocyte with visible nucleus and $\overline{ND}_i$ is the estimated mean nucleus diameter from the i_{th} oocyte size class. The number of oocytes by size class i used to estimate M_1 and M_3 was then multiplied by D_i . Because the nucleus is only observed in CA, VIT, and GVM oocytes C was only used to estimate NDO of these stages.

2.3. Batch fecundity

Absolute batch fecundity was estimated by two methods: (i) gravimetrically by using females with fully hydrated ovaries, applying the hydrated oocyte method (Hunter et al., 1985), and (ii) using the stereometric method described above. The gravimetric method was used for all hydrated ovary samples. Batch fecundity was estimated by weighing the whole ovary and by counting the hydrated oocytes in a sub-sample of approximately 10% of the ovary. The stereological method was applied to a sub-sample ($n=26$) from the 2008 sampling. By dividing the absolute batch fecundity value by ovary free body weight ($OFBW$), relative batch fecundity was calculated.

2.4. Spawning fraction

The hydrated oocyte and recent POF stage combined were used to estimate a spawning fraction for each month based on the samples taken randomly in 2008. As neither the duration of the hydrated stage nor the most recent POF stage is known for Baltic sprat (Haslob et al., 2012) this approach cannot serve as a direct measure of the daily spawning fraction but gives rather a relative measure to allow a seasonal comparison within the present study. The mean and variance of this spawning fraction was estimated with the following equations given by Piquelle and Stauffer (1985):

$$\bar{S} = \frac{\sum_{i=1}^n m_i \times y_i}{\sum_{i=1}^n m_i} \quad (8)$$

$$Var(\bar{S}) = \frac{\sum_{i=1}^n m_i^2 \times (y_i - \bar{S})}{\left(\sum_{i=1}^n \frac{m_i}{n}\right)^2 \times n(n-1)} \quad (9)$$

where m_i is the number of mature females in the i_{th} haul, and y_i is the proportion of females with hydrated ovaries and females with recent POFs divided by two. For the 2005 samples no histology was conducted. Therefore, only the proportion of females with hydrated ovaries was taken into account to estimate the fraction of actively spawning females.

2.5. Hydrated oocyte dry weight

Hydrated ovaries from female sprat of 2008 samplings were selected to determine the dry weight of hydrated oocytes (n=66). Approximately 50 hydrated oocytes from each selected hydrated ovary were manually separated. To remove formalin, the oocytes were rinsed twice in a sieve with distilled water for 2 min (1 min for each dip). Mean dry weights were determined by drying the oocytes for 24 h at 110 °C and then dividing the weight by the number of oocytes per replicate. Although we are aware that this procedure to determine oocyte dry weight is biased due to losses whilst stored in formaldehyde (Hislop and Bell, 1987), it was considered the best and unique option for ovaries preserved at sea.

3. Results

3.1. Ovarian development phases

In 2005, actively spawning sprat were found in January, April, and May but not in August. Although no histological analysis was performed in this year, incidence of spawning was obvious from macroscopic inspections of sprat ovaries, especially as hydrated females were present in the samples. In 2008, actively spawning females were found in March, April, May, and June samples (Table 4). Spawning was fully established during April, May and June, as a large proportion of the analysed ovaries were in spawning capable phase showing GVM or HYD oocytes or contained POFs. In March 2008, the share of ovaries containing POF (52.7%) was not as high as in the following three months. The proportion of ovaries containing oocytes in the germinal vesicle migration stage was around 25% in March, April, and May but increased to 38.3% in June (Table 4). Ovaries containing hydrated oocytes varied slightly within the spawning period (33.6 – 36.5%). The proportion of 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, 57.7% of analysed ovaries were in early development phase, and 19% contained vitellogenic oocytes, but no POF were detected.

3.2. Coefficient estimation for stereology

The relationship between ovary weight (OW) and ovary volume (OV) could well be defined by a linear regression model with the intercept forced through the origin ($r^2=0.99$; $p<0.05$; fig. 3):

$$OV = 0.931 (\pm 0.018 \text{ SE}) * OW \quad (10)$$

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

The individual C values for the analysed ovaries ($n=34$ for which it was possible to measure at least 50 oocytes per ovary section) ranged between 1.02 and 1.46. It was not possible to measure a sufficient number of oocytes for every analysed ovary to estimate C . Therefore, a mean C value was estimated for each specific ovarian developmental phase and was applied for those ovaries for which it was not possible to estimate a specific C value directly (Table 5). The shape correction factor β was estimated with 0.83 (± 0.12 SD; $n=4237$ oocytes measured) for the analysed ovaries.

A power function was fitted to describe the relationship between oocyte diameter size class (OD_i) and nucleus diameter (ND) ($r^2=0.87$; $p<0.05$; Fig. 4a):

$$ND = 8.91 (\pm 2.32 \text{ SE}) * OD_i^{0.46 (\pm 0.04 \text{ SE})} \quad (11)$$

LND was estimated by this model for the largest observed oocyte with visible nucleus. Then D_i was calculated for each oocyte size group using equation 7 resulting in the following function (Fig. 4b):

$$D_i = 21.49 * OD_i^{-0.46} \quad (12)$$

3.3. Number of developing oocytes

The number of oocytes in specific developmental stages increased significantly with fish length in spawning capable females (regression analysis, $p < 0.05$). The number of cortical alveolar oocytes was highest, followed by the vitellogenic oocytes and the oocytes in the germinal vesicle migration stage (Fig. 5). Over the spawning season, the relative number of oocytes in the cortical alveolar stage showed an increasing trend from March (1524 ± 541 SD) until June (2232 ± 839 SD) (Fig. 6), but without statistical significance (ANOVA, $p = 0.06$). Relative numbers of oocytes in the vitellogenic stage showed a slight increase of values from March (693 ± 298 SD) until May (827 ± 320 SD), followed by somewhat lower values for June (693 ± 329 SD). However, also in this case no significant differences were detected between months (ANOVA, $p = 0.66$). Oocytes in the germinal vesicle migration stage showed lower values in March (385 ± 183 SD), compared to April (445 ± 134 SD), May (507 ± 266 SD) and June (458 ± 308 SD). Since no homogeneity of variances could be achieved in the latter case, only the extended median test could be performed, which did not reveal significant differences between months.

The proportions of cortical alveolar, vitellogenic and germinal vesicle migration oocytes were very similar for all months. The mean proportions of these three oocyte stages over all ovaries were 61% (± 0.04 SD) cortical alveolar, 24% (± 0.03 SD) vitellogenic, and 15% (± 0.01 SD) germinal vesicle migration. Germinal vesicle migration oocytes and hydrated oocytes were never observed parallel in the same ovary, indicating that all germinal vesicle migration oocytes belong to the developing batch to be spawned.

3.4. Batch fecundity

A total of 172 ovaries was analysed for batch fecundity in 2005. In 2008, a total of 184 ovaries contained hydrated oocytes from which 144 were used for batch fecundity estimation (Table 1); the rest was excluded due to the presence of recent POFs. Variability in batch fecundity was 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 was significantly related to fish length in all months (Fig. 7). Analysis of covariance revealed no statistically significant differences among slopes of regressions between months of each year (ANCOVA; $p < 0.05$). But, differences between 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. For the year 2005 it was not possible to exclude ovaries containing POF from fecundity analysis because no histology was performed for this year samples. However, repeating the regression analysis for the year 2008 including ovaries with POFs, did not reveal any significant different results (ANCOVA, $p > 0.05$). This is probably due to the high natural variability observed in sprat batch fecundity.

Mean relative batch fecundity was significantly lower in January 2005 compared to April and May 2005 (ANOVA, $p < 0.05$; HSD-Test, $p < 0.05$), which did not differ significantly from each other (Fig. 8). Similarly, the relative mean batch fecundity in March 2008 was significantly lower than in all other months of that year (ANOVA; HSD-Test, $p < 0.05$). Relative 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. Neither in 2005 nor in 2008 significant differences were found in batch fecundity between April and May (Fig. 8).

A comparison between batch fecundity obtained by the gravimetric method and the stereometric method revealed substantial differences (Fig. 9). Batch fecundity estimated with the stereometric method was higher in all cases than the batch fecundity estimated by the gravimetric method.

3.5. Spawning fraction

Based on the proportion females with hydrated oocytes spawning fraction in January 2005 was 0.01 (CV=0.04). It increased to 0.20 (CV=0.83) in April and to 0.21 (CV=2.11) in May. The estimated spawning fraction during the spawning season 2008 decreased, with highest values in March (0.29; CV=2.00), lower values in April (0.22; CV=0.49) and May (0.24; CV=0.61), and the lowest observed value in June (0.18; CV=0.19).

3.6. Atresia in Baltic sprat

Atretic oocytes were found in sprat samples from March, April, May and November 2008 (Table 4). In March, 16.4% of female sprat were found to have atretic oocytes, while in April and May the value was considerably lower, with only 1% and 4% respectively. The highest prevalence of atresia was found in November 2008 with 38% of all examined ovaries

containing atretic oocytes. In all cases, atresia was associated to developing ovaries with oocytes in the vitellogenic or cortical alveolar stage. Stereologic analyses revealed an intensity 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 latter case the number of atretic oocytes exceeded the number of normal developing oocytes. In the two ovaries with atretic oocytes in May, the intensity of atresia was 3.6% and 8.2%. Atretic ovaries from April and November were not analysed stereometrically.

3.7. Condition factor and gonadosomatic index

The condition index observed in 2008 decreased from March to April, then increasing slightly to May and June, and increased sharply towards August. The November value was only slightly lower as the August value (Fig. 10). The gonadosomatic index (GSI) showed the opposite trend to the condition index (Fig. 10).

3.8. Hydrated oocyte dry weight

The dry weight of hydrated oocytes was not related to 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. 11). Values of March ($0.025 \text{ mg} \pm 0.008\text{sd}$) and April ($0.0029 \text{ mg} \pm 0.008\text{sd}$) differed significantly from May ($0.017 \text{ mg} \pm 0.005\text{sd}$) and June ($0.015 \text{ mg} \pm 0.004\text{sd}$) values (HSD, $p < 0.05$).

4. Discussion

4.1. Timing of spawning

Baltic sprat spawning activity was already detected for late January in the year 2005, albeit at relatively low levels, indicated by the low proportion of actively spawning females, i.e. with hydrated oocytes. This is earlier than the previous reported onset of sprat spawning for the central Baltic (Ojaveer and Kaljes, 2011) 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. In the present study sprat sampled in November 2008 were in developing phase containing ovaries with oocytes in the vitellogenic stage. Either this was due to the occurrence of a second spawning peak in autumn as observed for 2002 (Kraus et al., 2003), or the next regular spawning season after the resting phase in summer already begun. However, in sprat the oocyte development seems to be a rapid process (Haslob et al., 2012), as typical in income breeders, so it is very unlikely that oocyte recruitment takes place well before spawning. Nevertheless, as no data were available for winter and the early period of the following year, this question cannot be answered. However, if a considerable proportion of the female sprat stock allocates energy to oocyte development already during autumn/winter time in some years, either due to a second spawning peak in autumn or due to an early start of spawning activities, this might have negative consequences for somatic 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.

4.2. Number of developing oocytes

The constant proportion of cortical alveolar oocytes in relation to further developed oocytes in spawning capable females from March until June (i.e. new oocytes recruited from the reserve pool throughout the spawning season) confirms the indeterminate fecundity of Baltic sprat, which has been described before (Heidrich, 1925; Polivaiko, 1980; Alheit, 1993).

The mean number of cortical alveolar (CA) oocytes was always higher compared to the number of vitellogenic oocytes (VIT), which was, in turn, higher than that of oocytes in the germinal vesicle migration stage (GVM) in all four months. If it is assumed that the recruiting batch develops continuously to the next development stage until final hydration is reached, then the observed differences in numbers between stages give evidence for stage-specific relative development durations. Thus, in Baltic sprat CA oocytes would last more than twice as long as VIT oocytes and more than four times as long as GVM oocytes.

However, the numbers of hydrated oocytes (HYD) seem to be overestimated when using the stereometric method. Since GVM oocytes and HYD oocytes were never observed in the same ovary, it can be concluded that all GVM oocytes belong to the batch to be spawned. Thus, GVM oocytes are also overestimated using the stereometric method as their numbers

were similar or even higher compared to the HYD oocytes. This may be due to the non-spherical shape of HYD oocytes and advanced GVM oocytes.

The observed overestimation of batch fecundity using the stereometric method is in contrast to findings for hake (*Merluccius merluccius*; Domínguez-Petit, 2007) and Brazilian sardine (*Sardinella brasiliensis*; Isaac-Nahum et al., 1988), for which good agreement has been reported between the stereometric and gravimetric method. However, 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. Nevertheless, the stereometric approach proved to be a valuable, time-saving tool to estimate the number of developing oocytes, especially the CA and VIT stages in a number of sprat ovaries. For the estimation of batch fecundity direct counting of hydrated oocytes is still preferable, because it is more correct compared to the stereometric approach.

4.3. Batch fecundity

Seasonal variability in batch fecundity has been described for a number of clupeoid fish (e.g. *Engraulis encrasicolus*, Motos, 1996; *Sardina pilchardus*, Zwolinski et al., 2001) and also for Baltic sprat (Heidrich, 1925; Alekseev & Alekseeva, 2005). In the present study, batch fecundity was found to be lower in the beginning of the spawning season and increased towards its end. Peak spawning of Baltic sprat might be delayed by extremely cold temperatures in spring, following a hard winter (Karasiova, 2002). Thus, inter annual comparisons of sprat batch fecundity might be biased by seasonal variability (Alheit, 1988). However, data of the present study make it reasonable to assume that batch fecundity increases at the beginning of the spawning season, reaches a plateau at peak spawning, and decreases rather 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, since batch fecundity will be rather stable over several months during spring and early summer.

The low batch fecundity observed in early spawning season (January 2005, March 2008) compared to the peak spawning period might indicate a regulation of fecundity through lower egg production. This might reflect the adjustment of reproductive output to the

prevailing environmental conditions, e.g. food availability, on the population level early in the season.

4.4. Spawning fraction

The proportion of actively spawning female sprat only varied over the course of peak spawning period in 2008, which is in line to previous findings for Baltic sprat. However, 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 in 1999, with somewhat lower values towards the end of spawning season. The exceptionally high values in April were explained as being an early phase of spawning season, with ongoing migration of actively spawning females into the studied area. The values observed for May to July by Kraus and Köster (2004) come close to the observed values of the present study. Possibly, spawning activity in 1999 was delayed compared to the year 2008, where spawning was fully established already in March. Thus, this estimate of spawning fraction 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 fraction. Kraus and Köster (2004) estimated spawning fraction solely by macroscopic inspection of ovaries applying the hydrated oocyte method (Hunter and Macewicz, 1985), whereas the present findings are based on histology and the additional incorporation of recent POF stages. However, it is argued in literature that the use of hydrated oocytes or recent POF for estimation of spawning fraction is problematic because of clupeoid fish forming spawning aggregations and might be more vulnerable to the fishing gear (Alheit, 1985). But, the observed spawning stages in the present study can be used to compare the relative rate of spawning during spawning season. For other indeterminate species the use of hydrated females and recent POF were shown to be a good estimate of spawning fraction (Isaac-Nahum et al., 1988; Korta et al., 2010; Domínguez-Petit, 2007).

4.5. Atresia in Baltic sprat

Prevalence of 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. Ganius (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 also be a mechanism for indeterminate spawners to react quickly to short-term changes in environmental conditions, especially a sudden shortage of food supply, and to regulate the number of oocytes recently recruited. Thus, the high levels of atresia found in March and in November can be interpreted as a re-arrangement of energy allocation due to sub-optimal environmental conditions outside the main spawning season.

Similar to observations by Kraus et al. (2003), the observed oocyte recruitment in November 2008 may be a second spawning peak, triggered by exceptionally favourable environmental conditions late in the year. If conditions abruptly changed to be sub-optimal, a large proportion of already recruited oocytes could have been reabsorbed by atresia to attain energy. Further, indeterminate species are also known to re-allocate energy of surplus oocyte production at the end of the spawning season (Murua and Saborido-Rey, 2003). However, these questions cannot be finally solved here, because further observations on adult sprat covering the end of the spawning season or the following autumn and winter months are lacking.

4.6. 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. towards 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 oocyte recruitment and batch fecundity of the following spawning season in Baltic sprat, which seems to be in accordance with the observation commented above on atresia and batch fecundity at the beginning of the season. 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 might affect sprat egg production negatively.

4.7. Oocyte dry weight

The decrease in hydrated oocyte dry weight during the course of spawning season is well in line with the increasing trend of batch fecundity. It is discussed in literature that a trade off exists between egg weight and fecundity in fish, and that egg weight is a maternal response to the variability in environmental conditions (Castro et al., 2009). Larger or heavier eggs often seem to be of better quality, and larval size is directly correlated to egg weight. Hence, producing larger eggs may increase larval survival probability because more nutrients are supplied to the developing embryo.

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 for 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 because first feeding larvae are at once in an environment where sufficient prey organisms are available. Thus, female fish can then produce more eggs to 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 weight over the spawning season may also be in response to changes in physical factors of the ambient environment, in order to maintain neutral buoyancy.

4.8. Conclusion

The seasonal dynamics of important sprat fecundity traits have been revealed in the present study. This is important for a number of applications, *e.g.* the Daily Egg Production Method or individual and population based modelling approaches to better understand sprat population dynamics. However, which processes or environmental parameters drive these dynamics is still not well understood in the case of Baltic sprat and should be addressed in future research activities. Especially when investigating the impact of a changing environment on the reproductive potential of a fish stock it is a prerequisite to understand the critical mechanisms which drive variability of spawning and fecundity traits. The combination of histology and stereometric methods is recommended to differentially investigate the dynamics of oocyte growth, fecundity, and atresia in future studies on sprat maturation dynamics.

5. Acknowledgements

This study was partly conducted during a Short Term Scientific Mission (STSM) at the Institute of Marine Research Vigo, Spain (IIM-CSIC) supported by the COST action Fish Reproduction and Fisheries (FRESH, FA0601). This research was also supported by the German Science Foundation (DFG) cluster project RECONN2 #CL126/3-3 (Resolving Trophodynamic Consequences of Climate Change) within the priority program 1162 AQUASHIFT. We acknowledge all people involved in sprat sampling on the several surveys. For technical support in the laboratory we thank M. Fabeiro Quinteiro and D. Domínguez Vázquez, and especially R. Callazo Araujo for her help in preparing the histological sections of sprat ovaries. We would like to thank two anonymous referees for their valuable comments, suggestions, and constructive criticisms which indeed helped to improve the manuscript.

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Tables

Table 1: Sampling details for determination of sprat ovary developmental stages and batch fecundity for the years 2005 and 2008. DA = research vessel (RV) "Dana", AL = RV "Alkor", WH = RV "Walther Herwig III". P = pelagic trawl, B = bottom trawl. n = number of 2-kg sub-samples taken.

cruise	gear	date	n	size range (cm)	number of females analysed	
					ovarian development phase	fecundity
AL 251	P	26 th – 28 th January 2005	none	11.4 – 14.0	n.a.	25
AL 255	P	20 th – 23 th April 2005	none	10.1 – 14.9	n.a.	45
WH 275	P	16 th – 19 th May 2005	none	9.6 – 15.3	n.a.	102
AL 262	P	22 nd – 25 th July 2005	none	n.a.	n.a.	0
DA 0208	B	13 th - 15 th March 2008	5	9.9 - 14.5	110	54
AL 318	P	19 th - 21 th April 2008	5	10.5 - 13.4	104	44
WH 312	P	12 th - 18 th May 2008	5	10.6 - 14.4	101	55
AL 320	P	7 th - 10 th June 2008	3	11.2 - 14.2	60	31
AL 324	P	27 th August 2008	none	13.3 - 13.8	5	0
DA 0808	B	15 th November 2008	1	9.8 - 14.6	26	0

Table 2: Definition of ovarian development phases according to the most advanced oocyte stage (after Brown-Peterson et al., 2011) and mean diameter and range (µm) of different oocyte stages measured from histological sections.

Ovarian development phase	Most advanced oocyte stage	Mean oocyte diameter	Oocyte diameter range	n
Immature	primary growth (PG)	82	37-139	182
Early developing	cortical alveolar (CA)	218	143-302	199
Developing	vitellogenic (VIT)	377	228-553	102

Spawning capable	germinal vesicle migration (GVM)	550	429-706	49
Actively spawning	hydrated (HYD)	782	599-991	55

Table 3: Ovary samples of different developmental phases according to the most advanced oocyte stage analysed with stereology for the spawning season in 2008. CA = cortical alveolar, VIT = vitellogenic, GVM = germinal vesicle migration, HYD = hydrated.

month	size range (cm)	number of processed fish				
		CA	VIT	GVM	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

Table 4: Number of samples processed with histology (n) and proportions (%) of different ovarian development phases based on the most advanced oocyte stage, post ovulatory follicles (POF), and atretic oocytes. Number in brackets give the samples size processed with stereology. PG = primary growth, CA = cortical alveolar, VIT = vitellogenic, GVM = germinal vesicle migration, HYD = hydrated.

month	n	most advanced oocyte stage					POF		atresia
		PG	CA	VIT	GVM	HYD	<24h	all	
March	110	0.0	9.1	32.7	24.5	33.6	15.5	52.7	16.4
April	104	0.0	1.0	37.5	25.0	36.5	5.8	89.4	1.0
May	101	0.0	3.0	36.6	25.7	34.7	7.9	81.2	4.0
June	60	0.0	0.0	26.7	38.3	35.0	1.7	100.0	0.0
August	5	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
November	26	23.1	57.7	19.2	0.0	0.0	0.0	0.0	38.5

Table 5: Mean values of the oocyte size distribution correction factor C obtained for ovaries (n) in different developmental phases according to the most advanced oocyte stage. CA = cortical alveolar (early developing), VIT = vitellogenic (developing), GVM = germinal vesicle migration (spawning capable); SD = standard deviation.

Oocyte stage	C	n	SD
CA	1.04	6	0.02
VIT	1.12	19	0.03
GVM	1.19	9	0.05

Figure captions

Fig. 1: Sampling locations in the Bornholm Basin, Baltic Sea. Different symbols present different research cruises. Cruise abbreviations and detailed sampling dates given in table 1.

○ = AL 251 January 2005; △ = AL 255 April 2005; □ = WH 275 May 2005; ■ = DA 0208 March 2008; ▲ = AL 318 April 2008; + = WH 312 May 2008; ● = AL 320 June 2008; ◆ = AL 324 August 2008; ▼ = DA 0808 November 2008.

Fig. 2: Histological image of sprat ovary processed with the used ImageJ application. Displayed are the colour coded point counts to estimate the partial area (V_i) of cortical alveolar oocytes (blue points), vitellogenic oocytes (green points), and oocytes in the germinal vesicle migration stage (red points). Colour coded crosses represent the count of numbers of oocytes in different developmental stages (N_a).

Fig. 3: Linear relationship between ovary weight and ovary volume ($n=21$, $r^2=0.99$, $p<0.05$).

Fig. 4: (a) Relationship between nucleus diameter (ND) and oocyte diameter (OD). Error bars denote the standard deviation. (b) Correction factor D in relation to oocyte size class.

Fig. 5: Stereology results. Mean number of developing oocytes (NDO) of spawning capable female sprat in relation to fish length class. (a) White circles = cortical alveolar, (b) grey squares = vitellogenic oocytes, (c) grey triangles = germinal vesicle migration, (d) white diamonds = hydrated oocytes. Error bars denote standard deviation.

Fig. 6: Stereology results. Relative number of developing oocytes ($RNDO$) of spawning capable female sprat corrected for ovary free body weight over the spawning season. (a)

White circles = cortical alveolar, (b) grey squares = vitellogenic oocytes, (c) grey triangles = germinal vesicle migration, (d) white diamonds = hydrated oocytes. Error bars denote standard deviation.

Fig. 7: Relationship between total fish length and absolute batch fecundity in Baltic sprat for different months in 2005 (a, b, c) and in 2008 (d, e, f, g). Solid black lines show significant linear regressions ($p < 0.05$).

Fig. 8: Seasonal variability in mean relative Baltic sprat batch fecundity for the years 2005 (grey triangles) and 2008 (white squares). Error bars denote the standard deviation. Numbers of analysed sprat are listed in table 1.

Fig. 9: Comparison between batch fecundity obtained by the gravimetric method and stereology.

Fig. 10: Change in condition index K (white circles) and gonadosomatic index GSI (grey triangles) of female sprat over the course of the spawning season in 2008. Error bars denote the standard deviation for K . Given numbers of samples (n) are equal for GSI and K .

Fig. 11: Seasonal variability in hydrated oocyte mean dry weight of Baltic sprat for the year 2008. Displayed is the number of females (n), error bars denote the standard deviations.