Oogenesis, fecundity and condition of Baltic herring (Clupea harengus L.): A stereological study

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\textbf{A B S T R A C T}

Herring (Clupea harengus) is a capital breeder that stores energy reserves in muscle tissue. Individual potential fecundity relies on the size and weight of female fish. Poor condition during the maturation process can lead to a heavy down-regulation of fecundity through atresia and, in the extreme, cause skipped spawning. Herring in the Central Baltic Sea exist in a variable environment where food availability fluctuates substantially. Compared to other herring populations their condition is generally poor. In the present study, the oocyte dynamics and fecundity in relation to the condition of Central Baltic herring was investigated. A modern stereological method, the physical fractionator, was used to quantify the number of oocytes in previtellogenic (PG), cortical alveoli (CA) as well as successive vitellogenic (VT1 and VT2) stages in central Baltic herring during ovarian maturation. The potential fecundity, i.e. the number of VT2 oocytes, was low compared to other Atlantic stocks but the relative potential fecundity was higher. The latter decreased by 71% when comparing early-maturing individuals with CA oocytes and late-maturing individuals with VT2 oocytes, suggesting a substantial down-regulation of fecundity. Although determined as spring spawners by otolith hatch type, 15% of the randomly sampled females were characterized by oocytes in CA stage in the prespawning period, indicating skipping spawning. The condition of these females was poor, which might have resulted in skipped spawning. Ovary weight was a good predictor of potential fecundity within maturing stages of females. Combined with estimates of skipped spawning, this ovary weight could be used to estimate egg production thereby improving Central Baltic herring stock-recruitment models.

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1. Introduction

Herring (Clupea harengus L.) has been characterized as a capital spawner (Kennedy et al., 2010). In capital spawners, reproductive costs are subsidized from energy reserves such as fat accumulated in muscle tissue (Bull and Shine, 1979; Jager et al., 2008; Slotte, 1999). By contrast, income spawners, e.g. sprat (Sprattus sprattus L.) meet the costs of an extended reproductive period from resources acquired continuously from feeding (Jönsson, 1997; Stearns, 1989). Capital and income spawner strategies in female iteroparous fish often relate to determinate and indeterminate fecundity type, which is reflected in differences in oocyte recruitment dynamics. In the determinate fecundity type, all oocytes to be spawned are recruited early in the reproductive cycle and potential fecundity is determined before the spawning season, whereas recruitment of oocytes in the indeterminate fecundity type can continue for a long period into the spawning season if food supplies suffice (Murua and Saborido-Rey, 2003).

Determinate spawners often start out optimistically recruiting large numbers of oocytes at the onset of maturation, but tend to adjust numbers of developing oocytes by resorption, i.e. atresia, as maturation proceeds (e.g. Kjesbu et al., 1991; Kurita et al., 2003; Ma et al., 1998). As a consequence of such down-regulation, the individual realized fecundity during spawning will be lower than the potential fecundity at any time during the ovarian maturation process. The degree of individual fecundity down-regulation has been linked to the condition of female fish showing an increase in frequency of atresia at lowered condition (Kennedy et al., 2011a; Kjesbu et al., 1991; Kurita et al., 2003; Ma et al., 1998). In the extreme, fish can entirely skip a spawning season (Rideout and Tomkiewicz, 2011). Oocyte development will normally stop in the late primary growth stage; however some species, e.g. winter flounder (Pseudopleuronectes americanus) and Northeast Arctic cod (Gadus morhua L.), may arrest oocyte growth later in development, i.e. during the subsequent maturation stage characterized by cortical alveoli. These individuals seem to skip a reproductive
season, while others continue the maturation process (Burton, 1994; Skjørraesen et al., 2009).

Herring is characterized by determined fecundity with synchronous oocyte development and total spawning, i.e. all matured oocytes are spawned as part of one short spawning event (Murua and Saborido-Rey, 2003). Recruitment and development of oocytes for the following spawning season may be initiated only a few months after the end of the previous season (Kurita et al., 2003). Oocyte development from the appearance of the earliest developing stage to ovulation last 8–10 months (e.g. Hay et al., 1987; Kennedy et al., 2011a; Kurita et al., 2003; Ma et al., 1998). The mean individual potential fecundity prior to spawning ranges from about 30,000 to 70,000 eggs in different herring populations (Kennedy et al., 2011a; van Damme et al., 2009); fecundity immediately prior to the spawning season tends to relate better to weight than to length (Kennedy et al., 2010, 2011a; Kändler and Dutt, 1958; Öskarsson et al., 2002; Parmann and Kuittinen, 1991).

Down-regulation of fecundity during maturation can be substantial in herring. Estimates of down-regulation in Icelandic summer-spawning herring (Öskarsson and Taggart, 2006), North Sea autumn- and winter-spawning herring (van Damme et al., 2009), and Norwegian spring-spawning herring (Kennedy et al., 2011a; Kurita et al., 2003; Ma et al., 1998; Öskarsson et al., 2002) span from 6 to 59% of potential fecundity depending on stock and study. Skipped spawning has been discussed in the case of Norwegian spring-spawning herring, in particular. Based on spawning check analyses of scales sampled between 1935 and 1973, Engelhard and Heino (2005) found that 10% of North Sea spring-spawning herring at stock level skipped spawning, while Kennedy et al. (2011a, 2011b), based on maturity staging of ovaries sampled between 2006 and 2008, argued that skipped spawning was negligible with only 1.1% skippers.

The present study focuses on Central Baltic Sea herring (Clupea harengus membras). Although a few fecundity studies were carried out in the last century (Kosior and Strzyzewska, 1979; Kändler and Dutt, 1958), little is known about oocyte dynamics, fecundity and its regulation of this herring stock. The Central Baltic herring stock includes a large spring-spawning population, spawning from March to June and a small autumn spawning component, spawning from August to November (Aro, 1989). These herring populations, living in the estuarine Baltic Sea, are subjected to a variable environment caused by changing hydrographic conditions. Environmental changes include changes in food availability and composition, affecting life history characteristics. In particular, changes in zooplankton composition concurrent with high sprat abundance, and thereby increased food competition, resulted since the 1970s in reduced growth and condition of herring (Casini et al., 2010, 2011; Möllmann et al., 2005). The reduced condition of herring may lead to reduced potential fecundity, increased down-regulation as well as higher prevalence of skipped spawning.

This study used histology and modern stereological methods to quantify oocyte dynamics, fecundity and occurrence of skipped spawning of Central Baltic herring. The physical fractionator (Gundersen, 1986; Sterio, 1984) was applied to herring ovaries, and the number of both primary growth and developing oocytes in female specimens ranging from juvenile to late maturation stage were quantified. A detailed description of the physical fractionator method is provided and calculation examples are included, as this method has not previously been utilized in studies of fish oocyte dynamics and fecundity. Specific objectives of the study were to (1) quantify oocyte recruitment and dynamics during ovarian maturation, (2) estimate individual potential fecundity in relation to female maturity stage, size and condition, (3) assess fecundity down-regulation through progressive maturity stages, (4) evaluate the likelihood of skipped spawning in relation to condition (5) evaluate the applicability of the physical fractionator for oocyte quantification.

2. Materials and methods

2.1. Sampling of herring for quantitative analysis

Herring were sampled during a trawl survey with R/V Dana in the Central Baltic Sea (54.9–55.8° N; 15.0–17.6° E) in March 2008. A random sample of 30–60 herring was obtained from each of 11 trawl hauls using randomized procedures. The sampled herring were length measured, sex identified and for females, the maturity stage was visually staged (Bucholtz et al., 2008). Among a total of 259 females, ovaries for stereological analysis were sampled according to a length and maturity based sampling scheme including six 2.5 cm length groups and four maturity stages MI–MIV (Bucholtz et al., 2008) (Table 1). Within each length group, the aim was the collection of one female of each maturity stage per haul in order to distribute sampling over a larger area as well as to minimize handling time and decay of ovarian tissue. In order to randomize sampling within hauls, the first female per length group and maturity stage within the sample was selected. In total, 89 ovaries were sampled. For each female, length (total length, TL, cm) and weight (body mass, MB, g) was recorded. Each ovary was gently dabbed on both sides and weighed (fresh ovary mass, M0F, 0.1 g). The ovary was ID-tagged, wrapped in gauze to keep NB intact and preserved in 4% phosphate-buffered formaldehyde solution (Lilly’s fixative). Small ovaries (below 0.4 g) that could not be accurately weighed at sea were preserved and weighed in the laboratory after washing in phosphate buffer. Ovaries were removed from the carcass for hatch type analysis.

2.2. Histological preparation for stereological analysis and maturity validation

A random sub-sample of 29 out of the 89 ovaries representing different maturity stages (MI–MIV) and length groups was obtained for stereological oocyte quanification (Table 1). All sampling was conducted using systematic uniform random sampling (SURS). Prior to histological preparation the two ovary lobes were

<table>
<thead>
<tr>
<th>Length group (cm)</th>
<th>I. Juvenile</th>
<th>II. Early development</th>
<th>III. Early vitellogenesis</th>
<th>IV. Late vitellogenesis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15</td>
<td>0(2)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(2)</td>
</tr>
<tr>
<td>15.0–17.5</td>
<td>3(4)</td>
<td>2(3)</td>
<td>2(4)</td>
<td>1(1)</td>
<td>8(12)</td>
</tr>
<tr>
<td>17.5–20.0</td>
<td>0(0)</td>
<td>2(7)</td>
<td>2(55)</td>
<td>2(7)</td>
<td>6(69)</td>
</tr>
<tr>
<td>20.0–22.5</td>
<td>0(0)</td>
<td>2(14)</td>
<td>2(71)</td>
<td>2(34)</td>
<td>6(119)</td>
</tr>
<tr>
<td>22.5–25.0</td>
<td>0(0)</td>
<td>2(10)</td>
<td>28(8)</td>
<td>1(32)</td>
<td>5(50)</td>
</tr>
<tr>
<td>&gt;25</td>
<td>0(0)</td>
<td>2(4)</td>
<td>1(1)</td>
<td>1(2)</td>
<td>4(7)</td>
</tr>
<tr>
<td>Total</td>
<td>3(6)</td>
<td>10(38)</td>
<td>9(139)</td>
<td>7(76)</td>
<td>29(259)</td>
</tr>
</tbody>
</table>
separated and weighed individually (i.e. preserved ovary lobe mass, \( M_{OL1} \) and \( M_{OL2} 0.1 \text{ g} \). One lobe was randomly selected for stereology, ID-tagged, wrapped in gauze and dehydrated using standard procedures; applying an optimized time table for varying ovary sizes to enhance dehydration and embedding of tissue (Table 2). The entire ovary lobe was embedded in paraffin and sectioned into blocks for stereological analysis following the fractionator method (Gundersen, 1986). Thus, each ovary was divided into a series of equidistant parallel blocks (Fig. 1). The distance between blocks i.e. block thickness, \( T \), corresponded to dehydrated lobe length divided by 8. Each block was sectioned using a calibrated microtome and three sections were obtained from each block using a microtome block advance of 5 \( \mu \text{m} \). Two initial consecutive sections were obtained followed by a section at 30 \( \mu \text{m} \) or 60 \( \mu \text{m} \) from the onset of the first section. The three sections were placed on a glass slide, stained with standard H&E and mounted using Eukitt®; the two initial sections representing a 5 \( \mu \text{m} \) disector and the first and the third section representing a 30 \( \mu \text{m} \) or 60 \( \mu \text{m} \) disector, respectively. The distance between sections was based on the nuclei diameters of oocytes in progressive developmental stages (Section 2.3).

Histological characteristics were used to classify oocyte development and verify maturity stages. Oocytes categories included primary growth (PG), cortical alveoli stage (CA) and vitellogenic (VT1 and VT2). In addition atretic oocytes were identified (Bucholtz et al., 2008). Histological maturity stage was defined by the development of the most progressed oocytes, including juvenile stage (MI) characterized by PG, early development (MII)

Table 2: Size-specific dehydration schemes for embedding of herring ovary lobes.

<table>
<thead>
<tr>
<th>Solution and concentration</th>
<th>Duration &lt;3.5 cm</th>
<th>Duration ≥3.5 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>70% alcohol</td>
<td>2 h</td>
<td>4 h</td>
</tr>
<tr>
<td>96% alcohol</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>96% alcohol</td>
<td>2 h</td>
<td>2 h</td>
</tr>
<tr>
<td>99% alcohol</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>99% alcohol</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Xylen</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Xylen</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Paraffin</td>
<td>2 h</td>
<td>4 h</td>
</tr>
<tr>
<td>Paraffin</td>
<td>2 h</td>
<td>5 h</td>
</tr>
</tbody>
</table>

Duration defines residence time in different solutions using length of lobe as indicator of size category.

characterized by CA, early vitellogenesis (MII) characterized by VT1 and late vitellogenesis (MIV) characterized by VT2. In the stereological analysis, the 5 \( \mu \text{m} \) disector was used to quantify PG oocytes, the 30 \( \mu \text{m} \) disector quantified CA oocytes and the 60 \( \mu \text{m} \) disector VT1 and VT2 oocytes (Table 3). In addition, the thickness of the ovarian wall (tunica albuginea) was used to distinguish first-time (MII) and repeat spawners (MIIr) in Stage MI (Fig. 2a–b).

2.3. Stereological analysis for oocyte quantification

Stereological analysis was performed using newCAST™ and Autodisector™ (Visiopharm), an Olympus BX 51 microscope, a Prior 8 slide motorized stage H138 and an Olympus DP70 digital camera. An overview image of the three sections per slide was captured using a low magnification (1.25×) objective. The section pairs within blocks i.e. 5 and 30 or 60 \( \mu \text{m} \) apart, respectively, were linked and the section pairs automatically aligned. The region of interest of the sections was then delineated, and a systematic uniform random sampling (meander sampling) started with a sampling fraction of 1 (100%). A counting frame of 200 \( \mu \text{m} \times 200 \mu \text{m} \) and step lengths of 200 \( \mu \text{m} \) were used to count PG oocytes in stage MI ovaries, a 500 \( \mu \text{m} \times 500 \mu \text{m} \) counting frame and step lengths of 500 \( \mu \text{m} \) for PG and CA oocytes in MII ovaries, and 1000 \( \mu \text{m} \times 1000 \mu \text{m} \) counting frame and step lengths of 1000 \( \mu \text{m} \) were used for PG and VT1 or VT2 oocytes in MIII and MIV ovaries. Higher resolution images (fields of view) containing counting frames were automatically acquired using a 4× objective. The procedure resulted in a set of corresponding images, disector pairs, with counting frames from each of the two section pairs. The nucleus was chosen as the counting unit to quantify oocytes, as it is easily recognizable and cannot be mistaken for any other structure. Nuclear sizes of different oocyte types were measured in an initial study to assess disector height i.e. the distance between sections. Sample settings and expected sizes of embedded oocytes and their nuclei in different developmental categories are provided in Table 3.

Quantification of oocytes of different sizes followed standard stereological counting procedures using the disector pairs as both reference and look-up sections (Gundersen, 1986; Sterio, 1984) (Fig. 3a). Oocytes (nuclei) sampled by the reference section were counted only if they were not present in the look-up section. Subsequently, the look-up section served as reference and vice versa, i.e. we counted both ways as described in Sterio (1984). All oocyte developmental stages were counted in disector pairs: 5 \( \mu \text{m} \) and 30 \( \mu \text{m} \), or 60 \( \mu \text{m} \) within the eight blocks per ovary lobe. The total number of oocyte nuclei per developmental stage per ovary was estimated using the fractionator principle (Dorph-Petersen and Lewis, 2011; Gundersen, 1986):

\[ N_\text{c} = \frac{1}{bsf} \cdot \frac{1}{ssf} \cdot \frac{1}{asf} \cdot \Sigma Q - \frac{1}{2} \]

where \( N_\text{c} \): Total number of oocyte nuclei i.e. oocytes, \( bsf \): Block sampling fraction, i.e. of blocks used from each individual. All eight blocks from one randomly chosen ovary lobe were used, and none from the other. As the two ovary lobes, within the individuals studied, were not significantly different in weight (t-test; \( P = 0.744; n = 29 \)), we used \( bsf = 1/2 \), \( ssf \): Section sampling fraction, fraction of sections used from each individual – generally given by: \( ssf = \frac{N}{2} \), in this study BA equals disector height (BA normally refers to the block advance of the cutting device i.e. microtome—see Section 2.2), \( asf \): Area sampling fraction, fraction of area analyzed given by: \( asf = \frac{\text{step length}_x \times \text{step length}_y}{\text{area of counting frame}} \). In this study, the counting frames were all tilted up to give an area sampling fraction of \( asf = \frac{1}{1(100\%)} \) in order to achieve sufficient number of counts, \( Q \). \( Q \) is a disector count i.e. number of nuclei counted. As the procedure for disector counting

Fig. 1. Herring ovary lobe randomly selected for stereological analysis and embedded in paraffin. Blocks are cut 7 units apart corresponding to dehydrated lobe length divided by 8. The arrow indicates the randomized starting point for sectioning the lobe, which is found using a random number table, within the interval 0–T from the anterior ovary tip. The full line indicates the anterior ovary tip and the broken line indicates the posterior ovary tip.
involved counting in both directions, the total disector count was divided by 2. For a calculation example see Appendix A.

2.4. Estimation of oocyte size and prevalence of atresia

Concurrently with counting, the area of each individual oocyte counted was estimated using the 2D nucelarator (Gundersen et al., 1988) (Fig. 3b) in order to establish oocyte size distributions for each oocyte type within the ovaries and their maturity stages. Oocyte diameters were derived from area measurements assuming spherical shapes to facilitate comparison with previous studies. Oocyte measurements were later corrected for shrinkage.

The prevalence of atresia among the individuals in developing stages (MI–MIV) was estimated by screening the first histological section of each of the eight blocks of embedded ovaries lobes for the presence of atretic oocytes in the leading cohort; if one or more atretic oocytes were present in at least one section, the individual was considered to undergo down-regulation of fecundity.

2.5. Estimation of oocyte shrinkage during tissue processing

Fresh herring from the same stock were obtained from local fishermen on Bornholm in April 2009. The fish stored on ice were sexed and females of different maturity stages were sampled. Their ovary was removed, the two ovary lobes were disconnected, and their lengths recorded. One randomly chosen lobe was preserved in 4% buffered formaldehyde (Lilly’s fixative), while the other was immediately embedded in agar using the following procedure: Agar at 45 °C was poured into a cooled-off cutting box with a paper scale (1 mm intervals) at the bottom. Immediately prior to the agar hardening, the fresh lobe was placed in the box and stored in a refrigerator until hardening was completed. While placed in the cutting box, a series of parallel slabs were cut through the ovary a fixed distance apart corresponding to 1/8 of the lobe length, using the now embedded paper scale and the methods described at Section 2.2 above. A 2 mm section was cut from the beginning of each slap and a smear of the section was photographed.

The preserved ovary lobe was sampled for analysis as follows: After approximately ten days in 4% buffered formaldehyde, the entire ovary lobe was embedded in paraffin and processed using the methods described at Section 2.2. From the beginning of each of the eight blocks, a section was collected using a microtome with a 5 μm block advance. These sections were stained with standard H&E and mounted. Using an Olympus MVX10 macroscope mounted with an Olympus DP71 digital camera, photographs were taken of each section. The stereological analysis was performed using newCAST™ (Visiopharm) and the same procedure used on smears from the fresh lobe and sections from the preserved lobe. Photographs of the fresh smears were imported into newCAST™ software and calibrated using a ruler.

Oocyte sizes of different developmental categories were estimated using the following procedure: A point grid was randomly placed over each of the smears or sections and the area of oocytes hit by a point was estimated using the 2D nucelarator (Fig. 3b). Point grid density varied according to tissue condition, i.e. smear or embedded section, maturity stage and number of oocytes in smears and sections. In the case of the paraffin-embedded sections, only oocytes sectioned through the nucleus were used. The oocyte areas were converted to diameters to enable comparison with previous studies. Shrinkage of oocytes of a specific developmental category was calculated by subtracting the mean embedded diameter from the mean fresh diameter divided by the mean fresh diameter (Fig. 4a).

The mean fresh diameter was plotted against the mean embedded diameter (Fig. 4b) and a linear regression analysis was used to calculate the equation below, which in turn was used to back-calculate oocyte diameters (OD) measured in embedded ovary lobes for size frequency distribution and fecundity estimation: $OD_{\text{fresh}} = 1.0882 \times OD_{\text{embedded}} + 48.191$
Due to differential shrinkage of the various oocyte types (PG, CA, VT1 and VT2), oocyte sizes within the categories of oocytes counted using the fractionator were corrected to represent fresh oocyte size.

2.6. Data analysis

Oocyte size frequency distributions corrected to fresh oocyte sizes were established as the relative number of oocytes per 20 µm size intervals per oocyte type per ovary. The estimated total number of developing oocytes $N$ of CA, VT1 or VT2 per ovary equals the individual potential fecundity ($F_P$). The relative potential individual fecundity ($F_{RP}$) was obtained by dividing $F_P$ by $M_B$. Fulton’s condition factor, $K$, was calculated for each fish according to the equation: $K = \frac{M_B}{L^2} \times 100$ and gonadosomatic index ($GSI$) was calculated according to the equation: $GSI = \frac{M_B}{L^2} \times 100$.

The cumulated relative oocyte size frequency distributions were fitted with Weibull five parameter curves. One-way ANOVA was used to test the various maturity stages for significant differences in mean values of the number of PG oocytes, developing oocytes (CA, VT1 and VT2) and $K$, respectively, and the a posteriori Holm–Sidak pairwise comparison was used to detect between which specific maturity stages the differences were significant. The relationship between $F_{RP}$ and $TL$, $M_B$ and $K$ were fitted to a linear function $f = y_0 + ax$, a multiple linear function $f = y_0 + a_1x_1 + a_2x_2$ and a power function $f = ax^b$. The correlation coefficient between $K$ and mean oocyte diameter of the developing cohort as well as GSI was calculated as a Pearson’s product moment correlation. The statistical analyses were all conducted using statistical analysis software SigmaPlot Version 12.0.

Otoliths were analyzed using a combination of otolith microstructure and otolith shape to identify hatch type (Clausen et al., 2007). The individual females were categorized as spring hatched or autumn hatched depending on the identified time of larval hatch.

To estimate the proportion of possible skipped spawners among the 253 developing (stage MI–MIV) females collected (Table 1), the fraction of females in MI was calculated. To distinguish and estimate the number of first-time and repeat spawners in stage MI in the only macroscopically maturity-stage part of the sample, a knife edge length of 20 cm was established, based on the length ranges of the histologically analyzed females in MI (15–19.5 cm for MIff and 19.5–26 cm for MIfr and MIir). Females with a $TL$ above 20 cm were regarded as repeat spawners.

2.7. Estimation of precision of stereological quantification

The coefficient of error (error variance) of the fractionator estimate $CE(\Sigma Q^{-})$ was estimated (Gundersen et al., 1999; Nyengaard, 1999). $CE(\Sigma Q^{-})$ is the variance introduced by the stereological method on the number estimate and consists of two major
components, counting noise ("Noise") and \( \text{VAR}_{\text{SURS}} (\text{Area}) \). Noise is the independent variance of a stereological counting procedure, in this case object counting: \( \text{Noise} = \Sigma Q^- \). The contribution to the \( CE(\Sigma Q^-) \) caused by the systematic uniform random cutting of blocks, \( \text{VAR}_{\text{SURS}} (\text{Area}) \), is calculated as: \( \text{VAR}_{\text{SURS}} (\text{Area}) = \left( \frac{2}{n} \right) \text{Area} \frac{\sum_{i=1}^{n-1} Q_i - Q_i^2}{\sum_{i=1}^{n-1} Q_i^2 - Q_i^2} \), where \( A = \sum_{i=1}^{n-1} Q_i - Q_i^2 \), \( B = \sum_{i=1}^{n-1} Q_i^2 - Q_i^2 \) and \( C = \sum_{i=1}^{n-1} Q_i^2 - Q_i^2 \); \( i \) is the block number. The error variance of the fractionator estimate of oocyte numbers (PG and CA, VT1 or VT2, respectively) is calculated as:

\[
\text{CE}(\Sigma Q^-) = \sqrt{\frac{\text{Total variance}}{\Sigma Q^-}} = \sqrt{\frac{\text{Noise} + \text{VAR}_{\text{SURS}} (\text{Area})}{\Sigma Q^-}}
\]

Subsequently, the mean total variance, \( CV_{\text{tot}} = \frac{\text{SD}_{\text{est}}}{\text{meanest}} \), as well as the mean variance added to \( CV_{\text{tot}} \) by the stereological method, \( CE_{\text{ste}} \), of the estimate of mean number of oocytes per ovary for a given maturity stage were estimated to evaluate the precision of the number estimate and whether adjustments could be made to improve the method in future studies (Gundersen et al., 1999). \( CV_{\text{tot}} \) includes biological variance, \( CV_{\text{bio}} \), and the variance added by the stereological method: \( CV_{\text{bio}} = CV_{\text{bio}}^2 + CE_{\text{ste}}^2 \). Biological variance is an inherent trait of the population and as such cannot be minimized by the method. However, including more individuals in a study will increase the precision of the \( CV_{\text{bio}} \) estimate. As biological variation is a fixed quantity, the important issue is whether or not the variance introduced by the stereological method, \( CE_{\text{ste}} \), significantly contributes to \( CV_{\text{tot}} \). As a rule of thumb, \( CE_{\text{ste}} \) does not contribute significantly to \( CV_{\text{tot}} \) if \( 0.2 < \frac{CE_{\text{ste}}^2}{CV_{\text{tot}}^2} < 0.5 \). The ratio can be used to evaluate whether adjustments could be made to the method: If the ratio is below 0.2 one should reduce efforts (less blocks and/or smaller sampling area), and if it is higher than 0.5 one should increase efforts (more blocks and/or higher sampling area). \( CV_{\text{tot}} \) is calculated as: \( CV = \frac{\text{SD}_{\text{est}}}{\text{meanest}} \), where \( \text{SD}_{\text{est}} \) is the standard deviation and \( \text{meanest} \) is the mean number of estimated oocytes (PG and CA, VT1 or VT2 oocytes), within maturity stages M1–MIV. Mean \( CE_{\text{ste}} \) was calculated using the following equation: \( CE_{\text{ste}} = \sqrt{\frac{\sum_{i=1}^{n-1} CE(\Sigma Q^-) - \sum_{i=1}^{n-1} Q_i}{n}} \), where in this case, \( n \) is the number of ovaries in the given maturity stage. For calculation examples, see Appendix B.

3. Results

3.1. Validation of herring maturity stages

Sampled female herring for stereological analysis covered successive ovarian developmental stages from juvenile to late vitellogenesis (stage M1–MIV) and a range of size groups from 15 to larger than 25 cm. The histological evaluation excluded a few juvenile specimens (M1) with signs of early development (CA), but otherwise confirmed the macroscopic determination of maturity stages leaving 29 samples for analysis of ovarian development and fecundity (Table 1). The histological analysis of stage M1 ovaries identified three first-time spawners with thin ovarian wall (stage MILf) and seven repeat spawners with thick ovarian wall (stage MIIr).

3.2. Ovarian development and oocyte recruitment dynamics

Herring oocyte recruitment and development during successive maturity stages progressed in a group synchronous way (Fig. 5). Relative size frequency distributions based on estimated total numbers of different oocyte types and back-calculated to fresh oocyte sizes exemplify five different stages of ovarian development from juvenile to late vitellogenesis (Fig. 5a–e). The juvenile stage (M1) was defined by the presence of PG oocytes only (Fig. 5a). As ovarian development proceeded into the early development stage (MII), oocytes with CA were present within the range of the larger PG oocytes and oocyte growth had commenced (Fig. 5b). Among repeat spawners, three specimens were in this recruiting sub-stage and are later referred to as recruiting repeat spawners (MIIr). By the end of maturity stage MII recruitment was completed, CA oocytes were significantly increased in size and a hiatus had developed between the PG and CA distributions (Fig. 5c). As oocytes grow and enter the vitellogenic phase (VT1) in stage MIII, the synchronous...
advancement of the developing oocyte cohort is obvious concurrent with a decrease in the relative number of developing, VT1, to PG oocytes (Fig. 5d). During the second vitellogenic phase, VT2, in stage MIV, oocytes increased further in size, approaching 1000 µm, and the range of final oocyte maturation onset (Fig. 5e). However, final oocyte maturation was not observed in any of the analyzed ovaries.

PG oocytes were present in all stages of ovary development (MI–MIV) and the cumulated oocyte size distributions of PG oocytes, based on all ovaries analyzed, were very similar throughout stages MI–MIV. Overall PG oocyte diameter ranged from 50 to 270 µm, while the 50th percentile ranged from 110 to 123 µm with a tendency toward smaller sizes in juvenile ovaries (MI) and larger sizes in the early developing stage (MI) (Fig. 6a).

Cumulated oocyte size distributions for PG oocytes combined, and for developing oocytes in progressive stages CA, VT1 and VT2 showed substantial overlap in size range among oocyte types (Fig. 6b). The CA oocyte frequency distribution in stage MII ovaries overlapped with the combined distribution of PG oocytes in the diameter range 190–270 µm, while oocytes larger than 270 µm were all CA, VT1 or VT2. Accordingly, oocyte recruiting could be considered complete, if all oocytes in the developing cohort exceeded 270 µm. The median oocyte diameters of CA, VT1 and VT2 oocyte distributions were 291.2 µm, 646.1 µm and 817.4 µm and their ranges 190–610 µm, 430–850 µm and 630–950 µm, respectively. The deviation from the expected curvilinear relationship in stage MII resulted from a bimodal appearance of the CA oocyte size frequency distribution. The bimodality was due to the fact that stage MII combined two groups of individuals, one in early MII with a diameter range of 190–430 µm and another in late MII with a diameter range of 330–610 µm.

### 3.3. Estimated numbers of oocytes

The estimated total numbers of oocytes, i.e. N for PG, CA, VT1 and VT2 within maturity stages MII–MIV, with a subdivision of MI into fully recruited first-time spawners, MIIF, not fully recruited repeat spawners, MIrr and fully recruited repeat spawners, MIrr, showed variation in numbers among maturity stages (Table 4). Although numbers of PG oocytes did not differ significantly between maturation stages (ANOVA; P = 0.267, df = 28), estimates tended to be lower in stage MI than in developing stages (MI–MIV). In developing stages, the mean number of PG oocytes was more or less equal in MIIF, MIrr and MIV, while it tended to be higher in MIrr and slightly lower in MIIF. Mean number of developing oocytes (CA in stage MI, subdivided into MIIF, MIrr and MIrr, VT1 in MII and VT2 in MIV) corresponding to mean potential fecundity, was significantly higher in MIIF than in MIIF (ANOVA; P = 0.003, df = 25; a posteriori test, MIIF and MIrr: P = 0.010). Likewise mean potential fecundity was significantly higher in stage MIrr than in MIIF and MIV (ANOVA; P = 0.003, df = 25; a posteriori test, MIrr and MIV: P = 0.010, MIrr and MIV: P = 0.005). Although not significant, mean potential fecundity in stage MIrr tended to be higher than in MIIF, MII and MIV, and lower than in MIIF.

### 3.4. Individual size and fecundity

TL, Mg and MOF of females in the fecundity analysis varied substantially among maturity stages (Table 4). Females in stage MI and MIIF were smallest in length and weight, while MIrr and MIrr were largest. Ovary masses were lowest in stage MI and developing first-time spawners, MIIF, increasing to a maximum in MIV. Individuals in stage MIrr were not fully recruited (Section 3.2), and they were

### Table 4

<table>
<thead>
<tr>
<th>Stage</th>
<th>n</th>
<th>TL (cm)</th>
<th>Mg (g)</th>
<th>K</th>
<th>MOF (g)</th>
<th>PGOD (µm)</th>
<th>nPG</th>
<th>OD (µm)</th>
<th>Fp</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>3</td>
<td>15.2</td>
<td>19.8</td>
<td>0.57</td>
<td>0.07</td>
<td>124.1</td>
<td>55.670</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MIIF</td>
<td>3</td>
<td>17</td>
<td>29.6</td>
<td>0.58</td>
<td>0.52</td>
<td>115.1</td>
<td>111.577</td>
<td>442.3</td>
<td>19.313</td>
</tr>
<tr>
<td>MIrr</td>
<td>3</td>
<td>23</td>
<td>67.3</td>
<td>0.55</td>
<td>0.87</td>
<td>137.4</td>
<td>111.800</td>
<td>265.7</td>
<td>48.539</td>
</tr>
<tr>
<td>MIrr</td>
<td>4</td>
<td>23</td>
<td>66.1</td>
<td>0.53</td>
<td>1.50</td>
<td>144.5</td>
<td>144.609</td>
<td>350.1</td>
<td>74.519</td>
</tr>
<tr>
<td>MIIF</td>
<td>9</td>
<td>20.4</td>
<td>55.1</td>
<td>0.61</td>
<td>4.60</td>
<td>126.1</td>
<td>99.2800</td>
<td>669.5</td>
<td>30.056</td>
</tr>
<tr>
<td>MI</td>
<td>7</td>
<td>20.1</td>
<td>55.3</td>
<td>0.63</td>
<td>7.41</td>
<td>134</td>
<td>108.113</td>
<td>808.2</td>
<td>24.742</td>
</tr>
</tbody>
</table>

MI, juvenile; MIIF, early developing first-time spawners fully recruited; MIrr, early developing repeat spawners not fully recruited; MIrr, early developing repeat spawners fully recruited; MIIF, early vitellogenesis; MIV, late vitellogenesis; number of specimens (n).
therefore not included in the comparison of individual fecundities below.

$F_F$ estimated for stage MIIr + r, MIII and MIV from CA, VT1 and VT2 oocytes, respectively, increased with female length within the various maturity stages (Fig. 7a). $F_F$ in early development, stage MIIr + r, was substantially higher than in MIII and MIV, while the length-fecundity relationships for the latter two were similar. $F_F$ estimates within maturity stages MIIr + r, MIII and MIV were regressed against length, TL, and weight, $M_B$, respectively, using linear and power functions. Additional potential improvements of the coefficient of determination, including Fulton’s condition factor, $K$, in multiple linear regressions, were tested (Table 5). Generally, relationships were highly significant, explaining a high proportion ($r^2 = 0.77–0.95$) of the variability in $F_F$. Looking at the linear regression models, $F_F$ as a function of TL best described the relationship in stage MIIr + r ($r^2 = 0.91$) with $K$ adding marginally to the explained variation ($r^2 = 0.92$), while it was $M_B$ in MIII ($r^2 = 0.90$), and $M_B$ ($r^2 = 0.94$) again with a marginal addition from $K$ ($r^2 = 0.95$) in MIV. The variation in explanation for the power functions was low ($r^2 = 0.87–0.95$). In stage MIIr + r as well, MIV, power functions of either TL or $M_B$ were the best predictors of $F_F$ ($r^2 = 0.87$ in MIIr + r and $r^2 = 0.91$ in MIII), while for MIV, $M_B$ ($r^2 = 0.95$) was marginally better than TL ($r^2 = 0.93$).

$F_F$ was linearly related to fresh ovary weight, $M_{G_0}$, within maturity stages MIIr + r, MIII and MIV (Fig. 7b; Table 5). $F_F$ of ovaries with recruiting oocytes, stage MIIr, was similar to MIIr + r in this respect. The fecundity-ovary weight regressions showed high coefficients of determination in all maturity stages (MIIr + r: $r^2 = 0.77$, MIII: $r^2 = 0.77$ and MIV: $r^2 = 0.90$) with $K$ added to the explained variation ($r^2 = 0.83$) only in MIIr + r.

$F_F$ showed a significant negative relationship with mean oocyte diameter of the leading cohort ($n = 23, r^2 = 0.3423, P = 0.0034$). The reduction in $F_F$ between early MIII (avg. 289 μm) and late MIV (avg. 854 μm) was 76%, if calculated according to the equation $F_F = 87,811.73 – 84.66D$.

Residuals from the linear relationship between $F_F$ and $M_B$ were plotted against TL for each of the fully recruited development stages. The slope was not significantly different from zero in any of the maturity stages (MIIr + r: $P = 0.8884$; MIII: $P = 0.9974$; MIV: $P = 0.8645$) indicating that individual relative potential fecundity, $F_{RP}$, was constant with length.

$F_{RP}$ showed a significant negative relationship with mean oocyte diameter of the leading cohort ($n = 23, r^2 = 0.6131, P < 0.0001$) (Fig. 8). The highest $F_{RP}$ was found at small mean oocyte sizes in stage MIII (avg. 1.092 μm) and MIIr (avg. 554 μm) ovaries; and $F_{RP}$ was, on average, lower in MIV (avg. 453 μm) than in MIII (avg. 325 μm).

### Table 5

Coefficients of determination ($r^2$) for herring fecundity relationships to length, body weight and fresh ovary weight of herring within maturity stages (MIIr + r, early development first-time and repeat spawners fully recruited; MIII, early vitellogenic; MIV, late vitellogenic) and considering individual condition calculated as Fulton’s condition factor ($K$).

<table>
<thead>
<tr>
<th>Regression model</th>
<th>$n$</th>
<th>Linear, $r^2$</th>
<th>Power, $r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$M_B$</td>
<td>$M_{G_0}$</td>
</tr>
<tr>
<td>MIIr + r</td>
<td>7</td>
<td>0.91”</td>
<td>0.21’</td>
</tr>
<tr>
<td>$y_0$: 132971.5</td>
<td>$a$: 8998.7</td>
<td>$y_0$: -47466.9</td>
<td>$y_0$: -18285.9</td>
</tr>
<tr>
<td>MIII</td>
<td>9</td>
<td>0.85’</td>
<td>0.90’</td>
</tr>
<tr>
<td>$y_0$: -74418.2</td>
<td>$a$: 5110.1</td>
<td>$y_0$: -124815.8</td>
<td>$y_0$: -8950.2</td>
</tr>
<tr>
<td>MIV</td>
<td>7</td>
<td>0.88’</td>
<td>0.94’</td>
</tr>
<tr>
<td>$y_0$: -69718.6</td>
<td>$a$: 4659.1</td>
<td>$y_0$: -98787.8</td>
<td>$y_0$: -6128.5</td>
</tr>
</tbody>
</table>

Regression models, $y = y_0 + a x_1 + b x_2$, include individual potential fecundity ($F_F$) as dependent variable and as independent variables: TL: Total length, TLK: Total length ($x_1$) and condition ($x_2$), $M_B$: Body weight, $M_{G_0}$: Body weight ($x_1$) and condition ($x_2$), $M_{G_0}K$: Ovary weight, $M_{G_0}K$: Ovary weight ($x_1$) and condition ($x_2$). Number of samples ($n$).

* Significance level ($p < 0.05$).

** Significance level ($p < 0.001$).

*** Significance level ($p < 0.0001$).
518 g⁻¹. The reduction in F_RP between early MI (avg. 289 µm) and late MIV (avg. 854 µm) was 71%, if calculated according to the equation F_RP = 1420.51 − 1.310D.

3.5. Ovarian development and female condition

Female condition expressed as Fulton's condition index, K, varied according to mean oocyte diameter of the developing cohort, GSI and maturity stage (Table 4). Mean oocyte diameter of the developing cohort and K were positively correlated (n=26, r=0.6954, P<0.0001) (Fig. 9a). Accordingly, GSI and K correlated positively (n=29, r=0.6673, P<0.0001) with progressive maturation (Fig. 9b) and differences in mean K among some of the maturation stages were significant (Fig. 9c). Stage MIIrr (K=0.55) individuals had a significantly lower mean K than MIV (K=0.63) (ANOVA; P<0.001, df=28; a posteriori test, MIIrr and MIV; P=0.05) individuals. Likewise, stage MIrr (K=0.53) individuals had a significantly lower mean K than MII (K=0.61) and MIV (ANOVA; P<0.001, df=28; a posteriori test, MIrr and MII: P<0.018, MIIrr and MIV; P=0.004) individuals. Although not significant, there was a tendency toward higher mean K in stage MI (K=0.57) and MII (K=0.58) compared with MIrr and MIIr, and a tendency toward lower mean K compared with MII and MIV.

3.6. Potential down-regulation and skipped spawning

Screening the histological sections revealed that the prevalence of atresia among females with developing oocytes, VT1 oocytes in stage MII and VT2 oocytes in MIV ovaries, was 100% and 86%, respectively. In stage MII, the prevalence was estimated to be app. 50%, but atretic CA oocytes were difficult to distinguish. Encapsulated hydrated oocytes were also observed in one MIrr individual, and one MIrr individual.

A considerable proportion of the total number of developing female herring (stage MII–MIV) caught for this study was possible skipped spawners. 38, or 15%, of 253 females were at stage MI, based on a visual inspection of macroscopic characters of ovaries. The 253 females were randomly collected from hauls, and thus representative of the population. 79% (30 out of 38) of females in stage MI were repeat spawners (MIrr and MIIrr) based on histological observations or length; this figure corresponding to 12% of the total number of developing females sampled.

3.7. Stock identity

Otolith analysis indicated that of the 29 females in the stereological study the majority belonged to Baltic Sea spring-spawning herring stock. Three specimens, two in stage MI and one in MIrr had otoliths of the autumn hatched type, whereas 26 fish had otoliths of the spring hatched type. The stage MIrr female with encapsulated
hydrated oocytes was the one with autumn hatched type otoliths.

3.8. Precision of estimated oocyte numbers

Fractionator estimates of total number of PG and CA, VT1 or VT2 oocytes \( N \) in the individual ovary, ranging from 5590 to 187,720, was obtained by counting between 34 and 644 nuclei profiles \( \Sigma Q^j \) – see Section 2.3, and had corresponding error variances \( CE(\Sigma Q^j) \) ranging from 0.04 to 0.17 (see Section 2.7). The relationship \( CV_{\text{st}/CV_{\text{tot}}} \) within each of the maturity stages MI–MIV ranged from 0.02 to 0.92 (Table 6). In the case of PG and CA or VT oocytes in stages MI–MIV, the ratio \( CV_{\text{st}/CV_{\text{tot}}} \) was below 0.2, meaning the error introduced by the stereological method, \( CE_{\text{st}} \), did not significantly contribute to the total variance, \( CV_{\text{tot}} \). In the same stages, the biological variance, \( CV_{\text{bio}}, \) was dominating. In one case, PG oocytes in stage MI, the ratio \( CV_{\text{st}/CV_{\text{tot}}} \) was above 0.5, meaning the error introduced by the stereological method significantly contributed to the total variance.

4. Discussion

4.1. Oocyte recruitment

Stereological quantification of all oocyte types, including PG, in the ovaries documented oocyte dynamics during development, which followed the suggested pattern of herring as an iteroparous total spawner with determinate fecundity and group synchronous oocyte development (Murua and Saborido-Rey, 2003). This included the generation of a hiatus between PG and developing oocytes (CA, VT1 and VT2) in size frequency distributions, which increased during maturation as oocyte development progressed.

The recruitment of CA oocytes from the PG oocyte pool documented the initiation of ovarian development, followed by oocyte growth through the vitellogenic stages VT1 and VT2. We found that in Central Baltic herring CA oocytes first appeared in oocytes in the size range 190–200 \( \mu m \) and the threshold for fully recruited CA was 270 \( \mu m \). The size range of overlap between PG and CA oocytes in other Atlantic herring stocks has not been investigated, however, a few studies have looked at the recruitment threshold and size ranges of CA oocytes (Kurita et al., 2003; Ma et al., 1998). In North Sea spring-spawning herring Ma et al. (1998) found a threshold of 250 \( \mu m \). Although the maximum previtellogenic oocyte diameter was used to reflect minimum developing oocyte size without identification of CA oocytes, the threshold sizes of Central Baltic and North Sea spring-spawning herring appears relatively similar. However, the upper limit of CA oocyte diameter range (610 \( \mu m \)) observed in Central Baltic herring was substantially higher than in a study of North Sea spring-spawning herring by Kurita et al. (2003), who found an upper limit at 398 \( \mu m \). Central Baltic herring vitellogenic oocyte size range (430–950 \( \mu m \)) was within the size range found in Norwegian spring-spawning herring: 375–925 \( \mu m \) according to Ma et al. (1998) and 360–1390 \( \mu m \) according to Kurita et al. (2003). The reason for the somewhat higher maximum diameter in Kurita et al. (2003) is that the final oocyte maturation stage oocytes were included in this study under the term vitellogenic. Generally, oocyte size comparisons across studies should be interpreted with caution as measurements can differ, depending on the preservation method applied.

The physical fractionator allowed quantification of oocyte numbers in PG, CA as well as vitellogenic stages. Resulting oocyte size frequency distributions separating these stages within individual ovaries made it possible to distinguish between females that were still in the recruitment phase (MI1r) and females that had finished recruitment of oocytes from the PG to the CA oocyte pool (MI1f and MI2f), thereby enhancing the accuracy of fecundity estimates in stage MI1. Full recruitment of the CA stage marked the earliest stage at which potential fecundity is quantifiable hence the stereological method provided a tool to accurately identify MI1 ovaries for use in the fecundity analysis.

4.2. Fecundity length, weight and condition relationships

Total length, \( TL \), body mass, \( M_B \), and condition, \( K \), were all good predictors of potential fecundity, \( F_P \), within the three maturation stages, MI–MIV, with high explanatory powers. Overall, \( TL \) tended to be a better predictor of \( F_P \) in stage MI1f and MI2f, while, as maturation progressed in stages MI1 and MI2, \( M_B \) with a marginal addition from \( K \) became the better predictor. Kennedy et al. (2011a) similarly found that fecundity becomes more related to weight as development proceeds, and most studies on Atlantic herring (Kennedy et al., 2010, 2011a; Öskarsson et al., 2002), including the Baltic (Kändler and Dutt, 1958; Parmanne and Kuijittinen, 1991) have found that just before the spawning season weight is the better predictor of potential fecundity. This could indicate an increasingly higher dependence on weight and condition as fecundity is adjusted during ovarian maturation.

Central Baltic herring fecundity estimates differ among studies on a temporal and spatial scale. The linear relationship between weight and fecundity \( (F_P = 588.4M_B - 6900) \) for herring caught north of Öland (Kändler and Dutt, 1958) is comparable to our findings \( (F_P = 558.4M_B - 6128.5 \text{ in stage MIIV}) \), the relationships being almost parallel with only a slightly higher fecundity in the 1958 data. However, the fecundity-length and the length-weight relationships in the 1958 data suggest a higher fecundity and better condition in larger fish and increasingly lower fecundity and poorer condition in smaller fish than observed in the present study. In contrast, the fecundity–weight relationship of herring sampled in the G'Dansk area by Kosiór and Strzyżewska (1979) \( (F_P = 390.94M_B - 2420.59) \) was substantially lower for all sizes than

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>n</th>
<th>Oocyte type</th>
<th>( \bar{N} ) ± SD</th>
<th>CV_{int}</th>
<th>CV_{int}/CV_{tot}</th>
<th>CE_{int}/CV_{tot}</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>3</td>
<td>PG</td>
<td>55,670 ± 6348</td>
<td>0.11</td>
<td>0.11</td>
<td>0.09</td>
</tr>
<tr>
<td>MI</td>
<td>10</td>
<td>PG</td>
<td>124,857 ± 36,955</td>
<td>0.30</td>
<td>0.09</td>
<td>0.10</td>
</tr>
<tr>
<td>MI</td>
<td>9</td>
<td>CA</td>
<td>50,163 ± 32,337</td>
<td>0.64</td>
<td>0.08</td>
<td>0.02</td>
</tr>
<tr>
<td>MI</td>
<td>10</td>
<td>VT1</td>
<td>99,280 ± 47,235</td>
<td>0.48</td>
<td>0.13</td>
<td>0.08</td>
</tr>
<tr>
<td>MIV</td>
<td>7</td>
<td>VT2</td>
<td>108,113 ± 57,048</td>
<td>0.54</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>MIV</td>
<td>7</td>
<td>VT2</td>
<td>24,742 ± 15,921</td>
<td>0.64</td>
<td>0.09</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Number of specimens \( n \), primary growth (PG), cortical alveoli (CA), early vitellogenic oocytes (VT1) late vitellogenic oocytes (VT2), mean and standard deviation of estimated oocyte numbers within ovaries of same maturation stage \( \bar{N} \pm SD \), and total variance \( CV_{tot} \), biological variance \( CV_{bio} \), variance added by the stereological method \( CV_{st} \) and relationship between variance added by the stereological method and total variance \( CE_{st}/CV_{tot} \) among ovaries of the same maturation stage.
in the present study as well as in Kändler and Dutt (1958), possibly due to that herring in their study belonged to a local coastal population (Aro, 1989).

Potential fecundity, \( F_p \), and relative fecundity, \( F_{rg} \), estimated in stage MIV specimens were similar to other Baltic spring-spawning populations (e.g. Anokhina, 1971, TL: 10–21 cm, \( F_p: 4000–28,000; \) Kändler and Dutt, 1958, TL: 19.6–25.2 cm, \( F_p: 23,600–58,000, F_{rg}: 460–530 \, g^{-1} \); Parmanne and Kuittinen, 1991, TL: 14–24 cm, \( F_p: 2100–42,800 \)), while Baltic autumn-spawning populations had a higher fecundity, especially in the upper length-range (e.g. Oyaveyer, 1974, TL: 12.4–22.1 cm, \( F_p: 4800–112,800 \)). The higher fecundity appears a general trait of autumn-spawning herring, and has been proposed to be an adaptation to the harsher environment, lower food availability and thereby higher mortality experienced by autumn-hatched larvae (Kosior and Strzyzewski, 1979). However, the proportion of autumn hatched specimens was negligible in the present study, allowing no fecundity estimation.

The mean potential fecundity, \( F_p \), was lower in the Central Baltic spring-spawning population than any of the Atlantic stocks and similar to Pacific herring, however, the mean \( F_{rg} \) was considerably higher. Thus, the mean \( F_p \) (24,742) compared to that of the Pacific spring-spawning population (Hay et al., 1987, \( F_p: 26,772 \)), while the North Sea winter-spawning population had a moderately higher mean \( F_p \) (van Damme et al., 2009, \( F_p: 30,894 \)) and Norwegian spring-spawning (Kennedy et al., 2011a, \( F_p: 68,000; \) Kurita et al., 2003, \( F_p: 49,200 \)) and North Sea autumn-spawning populations had a substantially higher mean \( F_p \) (van Damme et al., 2009: \( F_p: 67,341 \)). This is not surprising given the larger size of adult herring in the latter populations. On the contrary, the mean value of \( F_{rg} \) was considerably higher in the Central Baltic spring-spawning population (426 g\(^{-1}\)) than any of the Atlantic stocks (Kurita et al., 2003, \( F_{rg}: 195 \, g^{-1}; \) Öskarsson et al., 2002, \( F_{rg}: 147 \, g^{-1} \) in 1997 and 161 g\(^{-1}\) in 1998; van Damme et al., 2009, \( F_{rg}: 343 \, g^{-1} \) for autumn-spawners and 178 g\(^{-1}\) for winter-spawners) or the Pacific (Hay et al., 1987, \( F_{rg}: 199 \, g^{-1} \)) populations. A possible explanation could be that the relatively high \( F_{rg} \) of Central Baltic herring is a compensatory adaptation to counteract increased egg and larvae mortality experienced by this smaller subspecies in its variable environment, meaning a possible trade-off between body growth and reproductive investment, in particular at times characterized by poor growth conditions.

4.3. Down-regulation of fecundity

The mean individual potential fecundity, \( F_p \), was lower at stages MIII and MIV than in the fully recruited stages MIII+r, indicating that fecundity is down-regulated during maturation in Central Baltic herring. Interestingly, it appears that the main regulation occurs between stages MIII+r and MIII, while only a smaller adjustment takes place between stages MIII and MIV. Hence it appears that the number of CA oocytes in stage MIII+r represents the maximum potential fecundity, while numbers of VT1 and VT2 oocytes in stages MIII and MIV, respectively, reflects to which extend this potential is utilized. Similarly, it appears that the ability to maintain a high \( F_p \) from stage MIIIf+r to stage MIV depends on individual female condition.

This down-regulation is supported by the presence of atretic oocytes in the herring studied. Atretic oocytes were found in all three developing stages with a prevalence of 50% in stage MII, 100% in MIII and 86% in MIV specimens. Thus there does not appear to be a specific “window” of oocyte diameter range where fecundity regulation occurs; however, there appear to be a period during the maturation process when it is more prevalent. In Atlantic herring, Öskarsson et al. also found atretic vitellogenic oocytes within a wide oocyte size range (400–1200 \( \mu \text{m} \)), furthermore Öskarsson et al. and Kurita et al. found that prevalence and intensity of atresia declined close to spawning (Öskarsson et al., 2002; Kurita et al., 2003). We found that prevalence of atresia was lowest in the 190–610 \( \mu \text{m} \) oocyte diameter range, corresponding to ovaries with CA oocytes (stage MIII), highest in MIII and declining again in MIV. This indicated that the size range between that of CA oocytes and late VT2 oocytes, i.e. the VT1, was the most important for fecundity regulation, corresponding to the high down-regulation occurring from stage MIII to MII. Energy availability in terms of stored lipid during this transition may be critical to later potential fecundity.

In general, down-regulation of fecundity can be substantial in herring. The reduction in \( F_p \) and \( F_{rg} \) from early stage MII to late stage MIV was estimated to 76% and 71%, respectively, considering the entire range of oocyte development stages from CA to VT2 oocytes. Previous studies on fecundity down-regulation in herring have focused on the oocyte reduction from the early vitellogenic to late vitellogenic oocyte developmental stages (e.g. Kennedy et al., 2011a; Kurita et al., 2003; van Damme et al., 2009). van Damme et al. and Kurita et al. found substantial reductions in fecundity in North Sea winter-spawning herring (53% in \( F_p \) and 59% in \( F_{rg} \) from an average oocyte diameter of 400–965 \( \mu \text{m} \)) and Norwegian spring-spawning herring (56% in \( F_p \) of and 41% in \( F_{rg} \) from an average oocyte diameter of 489 \( \mu \text{m} \) to 1466 \( \mu \text{m} \) standardized to a 34 cm individual) (van Damme et al., 2009; Kurita et al., 2003). However, the magnitude of down-regulation in these studies might have been even higher, if the potential fecundity in the CA stage had been considered as starting point. The strength of the stereological method is in this context that all oocytes and development stages can be quantified during the entire maturation process.

4.4. Skipped spawning or spawning fidelity

Herring staged MII with CA as the most advanced oocyte development stage were observed in the pre-spawning period, implying that they will not reproduce in the coming spawning season. 15% of the total number of females caught in March 2008 were at stage MII. 12% of these were repeat spawners (MIIr and MIIrr) and 3% first-time spawners (MIIIF). Histological identification of females recruiting for the coming spawning season is generally related to the appearance of CA oocytes in their ovaries (Tylor and Sumpter, 1996) and in herring being a determinate spawner, CA only appears in the earliest development stage. The maturation period from the CA to hydrated oocyte stage was estimated in Norwegian spring-spawning (Kennedy et al., 2011a; Kurita et al., 2003; Ma et al., 1998) and in North Sea herring (van Damme et al., 2009) to last about 8 months. Hence, it is neither probable that first-time nor repeat spawners with oocytes in this stage in March would be able to complete maturation and spawn within the coming spawning period (March–June).

The question is therefore whether adolescent females and repeat spawners would continue oocyte maturation and spawn during summer (July) or the autumn spawning season (August–November), or whether they would remain in the CA stage, postponing spawning until the following spring (i.e. skip spawning).

The first explanation would imply a switch of spawning season from spring spawning to autumn spawning for females with otoliths of the spring hatch type; or, less drastic, that Central Baltic herring are part of a spawning continuum rather than two distinct spawning populations. Switching of spawning season and spawning continuums between two consecutive spawning seasons has been observed in Newfoundland herring (McQuinn, 1997) as well as Baltic herring (Aneer, 1985; Anokhina, 1971; Rajasila, 1992), and suggested as theoretically possible in North Sea autumn- and winter–spawning herring (van Damme et al., 2009). This indicates that herring are not necessarily confined to spawning only in the same season they were hatched; and although McQuinn (1997)
assumes that once an individual herring has spawned for the first time, it remains fixed as that spawner type for the rest of its life, others have suggested a more plastic approach to spawning type fidelity (Rajasila, 1992).

The second explanation, as mentioned, would be that the observed adolescent and repeat spawning females in stage MII skip spawning and do not reproduce until the following spring. Adolescent and resting stage females generally do not produce secondary growth oocytes, referring collectively to CA and VT oocyte stages. However, it is suggested for e.g. wolfish (Anarhichas lupus) that not only PG but also CA oocytes can remain inactive for extended periods of time (Gunnarsson et al., 2006). Skipped spawning has previously been reported in a number of fish species (Möllmann and Tomkiewicz, 2011); among herring, however, only in Norwegian spring-spawning herring (Engelhard and Heino, 2006; Kennedy et al., 2011b). The present study suggests that herring can remain in the CA stage for an extended period in a preparation or resting condition.

The cumulative size frequency distribution of CA oocytes in stage MII individuals showed a bimodal curve, due to a combination of two groups of individuals, one in early and another in late MII with particularly large oocytes. This bimodality reflected neither fish size nor first-time and repeat spawners, but might reflect a mixture of females that either postpone spawning or spawn in autumn. Characteristic for all stage MII specimens were that they were characterized by low condition, K.

4.5. Condition and spawning probability

All three types of spawning behavior, i.e. skipped spawning, spawning continuums and switching of spawning season, have been suggested to be a response to poor feeding and body condition during or prior to the initiation of maturation, either in larval, juvenile or adult phases (Aner, 1985; Engelhard and Heino, 2006; Hay and Brett, 1988; Kennedy et al., 2011a; McQuinn, 1997). Hence it is possible that the condition of stage MII individuals was too poor to enable them to continue maturation within the reproductive period they were caught. In the present study, K ranged from 0.5 to 0.6 in stage MII individuals and from 0.55 to 0.7 in stages MIII–MIV. Öskarsson et al. found a sharp rise in atresia in wild Norwegian spring-spawning herring with K <0.65–0.7; and based on an experimental study of the same population, Kennedy et al. argued that there appeared to be a threshold of 0.7, which needed to be reached before commencement of ovarian maturation (Öskarsson et al., 2002; Kennedy et al., 2010). It is possible that a similar, but lower, threshold K between 0.55 and 0.6 is required for the initiation of vitellogenesis in Central Baltic herring.

Overall, the condition, K, correlated positively with oocyte size, GSI and ovarian development. This falls in line with the hypothesis of Hay and Brett, who found that individuals of better condition mature and spawn earlier than individuals of poorer condition (Hay and Brett, 1988). The Central Baltic herring feed intensively during autumn and gain weight, while condition generally reduces during winter when zooplankton and bentipelagic prey is scarcer (Möllmann et al., 2004). In capital spawners, reproductive costs are primarily allocated from fat accumulated in muscle tissue, and the positive relationship between K and ovarian development in these spring spawners may reflect the individual energy reserves available at the onset of maturation during autumn.

It appears that the maturation progression and potential fecundity in spring spawning Central Baltic herring depend on environmental conditions, in particular food availability, at two levels; firstly, for accumulation of a threshold level of stored energy reserves to determine initial potential fecundity and initiate vitellogenesis and, secondly, for the capability to exploit maximum potential fecundity depending on the wintering conditions. Consequently, the annual egg production of the population is influenced by environmental changes in particular food availability and composition (Möllmann et al., 2004, 2005, 2008). Thus the presently low condition and fecundity of the Central Baltic herring agrees with the present state of the ecosystem (Casini et al., 2010, 2011; Möllmann et al., 2005).

4.6. Applications in management

A highly significant relationship between ovary weight, M0F, and potential fecundity, Fp, in stage MIV was documented. Assuming that atretic activity has leveled off in stage MIV, potential fecundity is quite close to realized fecundity in this stage. Weighing stage MIV ovaries and calculating fecundity based on the relationship provided in this study could be a quick and easy method of estimating fecundity.

Furthermore, Central Baltic herring specimens caught in the pre-spawning period staged as MII specimens should not be considered spawners in maturity keys used to assess the spawning stock biomass. These specimens tend to be adolescent specimens or adults that skip spawning, as indicated by their low condition. Considering that the present study found that 15% of the females caught in March were in stage MII and that fecundity was lower than in previous times the stock egg production and reproductive potential may be overestimated in present assessments of the central Baltic herring.

4.7. Stereology

The fractionator method was successfully implemented for quantification of PG, CA, VT1 and VT2 oocytes in herring ovaries. We found that the error introduced to the total variance of the estimated potential fecundity by the stereological method was negligible, as the contribution from the biological variance was clearly dominating in all but one case. Consequently, in future studies, the workload within each maturity stage can be reduced by studying fewer blocks, or a smaller area fraction could be sampled (asf), while more ovaries could be analyzed to achieve an even more precise estimate of the respective oocyte categories, i.e. “do more less well” (Gundersen and Østerby, 1981). It was expected that the biological variance would dominate since we applied a length-based sampling schedule, including not only small individuals with fewer oocytes but also larger individuals with relatively more oocytes. However, in one case, namely PG oocytes in stage MI, the variance added by the stereological method dominated, as the biological variance was low. This was probably due to the fact that only three stage MI individuals were analyzed and that the size of juvenile fish and their ovaries do not vary much. In future studies, more ovaries should be included in this stage to ascertain whether the estimated low biological variance was real. If that is the case, more blocks from each stage MI ovary should be analyzed.

Among biologists studying fish reproduction, stereological methods have been judged as labor intensive and not efficient, as a considerable number of females are normally required in fecundity studies in order to take account of natural variation (Kjesbu et al., 2010; Murua et al., 2003). However, the advantages of design-based stereological methods are that they are unbiased, assumption-free and allow for a statistical evaluation of the method. This study shows that, when using the physical fractionator method and a length- and maturity-based sampling schedule, only few (7–9) ovaries per maturity stage are required to achieve highly significant relationships with total length, total weight and ovary weight of the fish and thus explaining a high proportion of the variation in fecundity. Using stereology, number and size estimates of primary growth and developing oocytes as well as registration of atresia and signs of previous spawning can
be completed in one working procedure. Furthermore, the histological nature of stereological analysis makes it easy to recognize early-stage atresia as well as distinguish primary growth oocytes from early developing (CA) oocytes.

5. Conclusion

In conclusion, we found that (1) the oocyte recruitment of Central Baltic herring followed the typical pattern of a group-synchronous, determinate, total spawner, (2) the individual potential fecundity related strongly to maturity stage as well as the size of the individual female and ovary; it was relatively higher in the earliest developmental stage (MII) and increased with increasing fish and ovary size in all developing stages (MII-MIV). Length appeared to be the better predictor of fecundity in early development, while weight was the better predictor later in the maturation process. Ovary weight predicted fecundity almost 100% in late maturation, and this relationship could be used to estimate fecundity in a quick and easy manner, (3) a potential down-regulation of fecundity seemed to occur through atretic resorption of developing oocytes. Atretic activity is possibly at its highest between the cortical alveoli stage and the late vitellogenic stage. (4) we found a relatively high proportion of possible skipped spawners which had CA as the most advanced oocyte developmental category. Low condition factor may be a reason for the arrested or delayed development of these fish, (5) the physical fractionator gave an unbiased and assumption-free estimate of PG, CA as well as VT oocytes.

Acknowledgments

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Appendix A.

Calculation example of number of CA oocytes in a stage MII ovary; the length of the analyzed lobe was 28 mm ($T=28$ mm)/8 = 3.5 mm), the disector height (equal to BA in this study) was 30 μm, the total count, $\Sigma Q^-$, was 255 and $bsf = 1/2$:

$$ssf = \frac{BA}{T} = \frac{30 \mu m}{3.5 \text{ mm}} = 8.57 \times 10^{-3}$$  \hspace{1cm} (A.1)

$$asf = \frac{\text{area of counting frame}}{\text{step length}(x) \times \text{step length}(y)} = \frac{500 \ \mu m \times 500 \ \mu m}{500 \ \mu m \times 500 \ \mu m} = 1$$  \hspace{1cm} (A.2)

$$N = \frac{1}{bsf} \times \frac{1}{ssf} \times \frac{1}{asf} \times \Sigma Q^- / 2$$

$$= \frac{1}{1/2} \times \frac{1}{8.57 \times 10^{-3}} \times \frac{1}{1} \times \frac{225}{2} = 29,750 \ \text{CA oocytes}$$  \hspace{1cm} (A.3)

Appendix B.

Calculation example of CE ($\Sigma Q$) for a stage MII ovary – data available in Table B.1:

<table>
<thead>
<tr>
<th>Block (i) #</th>
<th>$Q^-$</th>
<th>$Q_i$, $Q_{i+1}$</th>
<th>$Q_i$, $Q_{i+2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>9</td>
<td>102</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>1156</td>
<td>1938</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>3249</td>
<td>2394</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>1764</td>
<td>1848</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>1936</td>
<td>1452</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>1089</td>
<td>891</td>
</tr>
<tr>
<td>7</td>
<td>27</td>
<td>729</td>
<td>405</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>225</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$\Sigma Q^- = 255$</td>
<td>$A=10,157$</td>
<td>$B=9030$</td>
</tr>
</tbody>
</table>

$$\text{Noise} = \sum Q^- = 255$$  \hspace{1cm} (B.1)

$$\text{VAR}_{\text{SURS}} \left( \sum \text{area} \right) = \frac{3(\text{A} - \text{Noise}) - 4B + C}{240}$$

$$= \frac{3(10157 - 255) - 4 \times 9030 + 7176}{240} = 3175$$  \hspace{1cm} (B.2)

$$CE \left( \sum Q \right) = \sqrt{\text{Total variance}} = \sqrt{\text{Noise} + \text{VAR}_{\text{SURS}} \left( \sum \text{area} \right)}$$

$$= \sqrt{\frac{255 + 3175}{255}} = 0.063$$  \hspace{1cm} (B.3)

Calculation example (PG oocytes estimate (N) in stage MIV ovaries) of $ CV_{\text{tot}}$, $ CV_{\text{ste}}$ and $ CV_{\text{bio}}$ – data available in Table B.2:

<table>
<thead>
<tr>
<th>Fish</th>
<th>$N$</th>
<th>$CE$</th>
<th>$CE^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>73,740</td>
<td>0.134</td>
<td>0.018</td>
</tr>
<tr>
<td>2</td>
<td>56,022</td>
<td>0.172</td>
<td>0.030</td>
</tr>
<tr>
<td>3</td>
<td>203,786</td>
<td>0.103</td>
<td>0.011</td>
</tr>
<tr>
<td>4</td>
<td>152,744</td>
<td>0.120</td>
<td>0.014</td>
</tr>
<tr>
<td>5</td>
<td>176,765</td>
<td>0.101</td>
<td>0.010</td>
</tr>
<tr>
<td>6</td>
<td>87,871</td>
<td>0.148</td>
<td>0.022</td>
</tr>
<tr>
<td>7</td>
<td>54,215</td>
<td>0.172</td>
<td>0.029</td>
</tr>
<tr>
<td>Total</td>
<td>805,143</td>
<td>0.134</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>115,020</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>61,555</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$$CV_{\text{tot}} = \frac{SD_{\text{est meanest}}}{115,020} = 0.535$$  \hspace{1cm} (B.4)

$$CE_{\text{ste}} = \sqrt{\frac{\sum_{i=1}^{n} CE(\Sigma Q^-)^2}{n}} = \sqrt{\frac{0.134^2}{7}} = \sqrt{0.019} = 0.138$$  \hspace{1cm} (B.5)

$$\frac{CE_{\text{ste}}^2}{CV_{\text{tot}}^2} = 0.138^2 / 0.535^2 = 0.067$$  \hspace{1cm} (B.6)

$$CV_{\text{bio}}^2 = CV_{\text{tot}}^2 - CE_{\text{ste}}^2 = 0.535^2 - 0.138^2 = 0.267$$  \hspace{1cm} (B.7)

$$CV_{\text{bio}} = \sqrt{0.267} = 0.517$$  \hspace{1cm} (B.8)