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Oocyte growth and fecundity regulation by atresia of Atlantic herring (*Clupea harengus*) in relation to body condition throughout the maturation cycle

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Abstract

Oocyte growth, fecundity regulation by resorption of vitellogenic oocytes (atresia), and condition effects on fecundity for repeat spawners (≥ 32 cm in total length (TL)) of Norwegian spring-spawning (NSS) herring, *Clupea harengus,* were examined using samples collected periodically from July 1998 to February/March 1999. This period almost covered the maturation cycle of the fish, i.e., 67% (30/45) of the examined fish had started vitellogenesis as early as in July and 18% (7/40) showed hydrated oocytes in February/March. Oocyte diameter increased linearly over time. Average fecundity of 34 cm TL fish decreased by about 56% from 113 000 in July to 49 200 in February/March. Both prevalence of atresia (portion of fish with atresia) and average relative intensity of atresia (prevalence multiplied by geometric mean of relative intensity of atresia among only fish with atresia) were highest in October and November, i.e., following the summer feeding season when fish started to rely on accumulated body reserves. Estimated duration of atresia was 4.5, 6.8, 6.1 and 7.2 d for July–October, October–November, November–January and January–February/March, respectively. Atresia seemed to be limited to oocytes smaller than 1100 μm, which had lipid and solids (protein, ash and carbohydrates) contents that were only half of the values observed for fully matured oocytes (1400–1550 μm). Both the timing of intensive resorption and size of atretic oocytes seemed to optimise fecundity given available energetic reserves. There appeared a highly significant, positive correlation between ovary dry weight, a proxy of reproductive investment, and muscle dry weight condition factor (MDCF; 100 × muscle dry weight/TL^3) in the later maturation cycle. Relative fecundity also showed a significant, positive correlation with MDCF in February/March. In conclusion, this study demonstrates important energetic and cellular mechanisms for regulation of reproductive investment in NSS herring females, a long-lived, temperate capital breeder.

Keywords: Herring; Oocyte growth; Fecundity regulation; Atresia; Condition effects; Maturation cycle

1. Introduction

While there is little doubt that variation in early life mortality plays a major role in the recruitment dynamics of fish populations, relatively little attention has been paid to the effects of reproductive traits of

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spawning fish on recruitment. A weak or non-existent relationship is commonly observed between spawning stock biomass (SSB) and subsequent recruitment (Hilborn and Walters, 2001). This is often interpreted as evidence that the spawning stock has little or no influence on subsequent recruitment. However, recent reports suggest that reproductive traits of spawning fish are likely to affect recruitment dynamics, e.g., diversity of age composition of spawning fish for Atlantic cod, *Gadus morhua* (Marteinsdottir and Thorarinsson, 1998), and portion of older Atlantic cod (Kjesbu et al., 1996). Spawning grounds of Norwegian spring-spawning herring (NSS herring), *Clupea harengus,* show fish of different body condition, and larval mortality is related to specific spawning grounds (Slotte and Fiksen, 2000). In addition, SSB may not be proportional to total egg production by the population because relative fecundity (fecundity/body weight) may change according to age composition and/or nutritional condition of spawning fish, e.g., northern anchovy, *Engraulis mordax* (Hunter et al., 1985) and Atlantic cod (Trippel et al., 1997; Kjesbu et al., 1998; Marshall et al., 1998). Thus, accurately estimating total egg production is critical to improving the spawning fish-recruitment relationship.

The NSS herring is the largest stock of Atlantic herring in the North Atlantic Sea. The SSB of NSS herring fluctuated from 16 million metric tonnes in 1945 to 50 thousand metric tonnes in the late 1960s, then dropped to close to zero (2000 metric tonnes), followed by a gradual, successful rebuilding in the 1980s and 1990s up to about 5–10 million tonnes (Toresen and Østvedt, 2000; Óskarsson et al., 2002). NSS herring spawn once a year from late February to early April on stony or rocky bottom below 250 m depth along the Norwegian coast (58–70°N). They first spawn (recruit spawners) at 3–5 years of age, and 27–31.5 cm in total length (TL), after which ($\geq$32 cm TL) they spawn every year (repeat spawners) (Slotte et al., 2000). Maximum age can reach 20 years (15 spawnings), but individual growth after onset of sexual maturity is very low (0–1 cm per year, or less than 20% of final total length) (Dragesund et al., 1980; Holst, 1996). In contrast, large variation in condition (length-specific weight) exists between years (Holst, 1996; Óskarsson et al., 2002). After spawning, the fish feed in the Norwegian Sea in summer and migrate to a restricted coastal overwintering area within Vestfjorden, northern Norway (67–68°N) in September (see Óskarsson et al., 2002 and references therein). They stay in this area until mid-January without feeding, followed by spawning migration. During overwintering and spawning migration, body reserves accumulated during summer feeding are the only source of energy for reproduction, migration, and routine metabolism (Slotte, 1999). Thus, this species is truly a so-called capital breeder (Sterns, 1992).

Potential fecundity of NSS herring was examined by Baxter (1959) in 1958, Lyamin (1966) in 1954–63, and Belikov et al. (1996) in 1985–95. However, as resorption of atretic oocytes occurs in this population (Óskarsson et al., 2002), potential fecundity may differ substantially from realised fecundity. Although some studies have examined the intensity of atresia in herring, they covered only the later part of the maturation cycle (Pacific herring, *Clupea harengus pallasi*, Hay and Brett, 1988; Atlantic herring, Óskarsson et al., 2002). Intensity of atresia may vary outside these stages. It is therefore necessary to examine the seasonal dynamics of fecundity regulation through the whole maturation cycle. In addition, fecundity of both Atlantic herring (Ma et al., 1998) and Pacific herring (Hay and Brett, 1988) is known to vary due to nutritional condition of the spawning fish. Condition probably affects not only fecundity but also egg quality (Brooks et al., 1997), which may influence early life mortality. Differentiating between recruit and repeat spawners is potentially important because the timing of oocyte growth, and seasonal change in intensity of atresia can be different between the two (Slotte et al., 2000; Óskarsson et al., 2002).

The objectives of this paper are (1) to clarify how the fecundity of repeat spawning NSS herring ($\geq$32 cm TL) is regulated throughout the maturation cycle, (2) to study the role of atresia in the regulation of fecundity, and (3) to determine how fecundity variation is influenced by fish condition using chemical analyses of fatty acid and water content.

2. Materials and methods

2.1. Sampling

The present fish were collected in the Norwegian Sea at the following places and times: the active

feeding area, 9–25 July 1998 (6 locations, water temperature at 20 meter depth varied between 4.2 and 11.3 °C); the overwintering area (Vestfjorden), 28–29 October (2 locations), 24 November 1998 (2 locations, 6.8–7.2 °C), and 15 January 1999 (3 locations, 5.8–6.6 °C); the spawning ground, 21 February–3 March 1999 (4 locations, 5.8–6.7 °C) (Fig. 1). All samples except those collected in October were collected with a mid-water trawl by research vessel. In October, the fish were caught by commercial trawl boat at night and placed in ice. Morphometric measurements and dissections were conducted just after sampling, or in the laboratory the following morning in the case of the commercial catch samples. Total lengths (TL, to 0.5 cm) and body weight (BW, to 1 g) were examined for at least 100 randomly sampled females at each sampling time. Half of the ovary, i.e. one of the ovary lobes, was dissected out and fixed in 3.6% phosphate-buffered formaldehyde. The rest of the fish with the other half of the ovary still in place was wrapped in aluminium foil and stored in a freezer at −20 °C for a few weeks (the July samples) or −80 °C. Each ovary lobe was weighed to 0.01g in the laboratory. The weight of the one preserved in formaldehyde

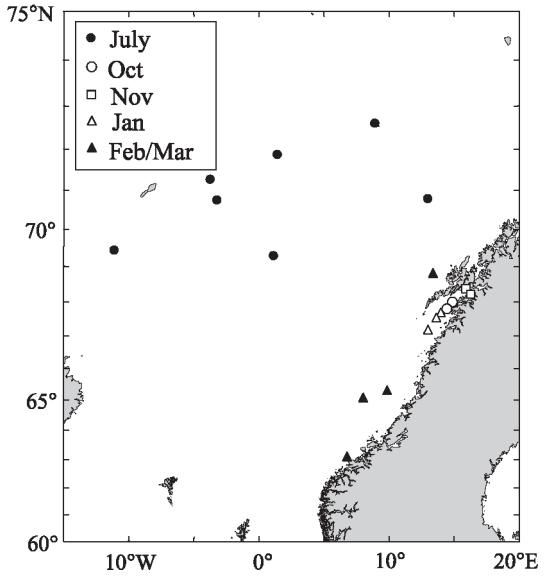


Fig. 1. Location of sampling sites of Norwegian spring-spawning herring.

($OW_{h,fm}$) was transformed to thawed weight ($OW_{h,fr}$) by this previously established equation:

$$OW_{h,fr} = (OW_{h,fm} - 0.21)/1.06$$
$$(r^2 = 0.99, n = 35).$$

Thus, total fresh ovary weight (OW) was set as the sum of the $OW_{h,fr}$ for each of the two lobes assuming that fresh and thawed ovary weight was similar (Kjesbu et al., 1998).

A random subsample of about 40 repeat spawners (32–37.5 cm TL) was, as far as possible (see numbers of analyses given in Result Section), taken from the main sample of 100 females for subsequent chemical and reproductive analyses. These subsamples demonstrated a rather wide range in individual condition, as characterised by the somatic condition factor (SCF; $100 \times (BW-OW)/TL^3$).

2.2. Whole-mount preparations

2.2.1. Oocyte diameter

Diameters of oocytes ((short + long axis)/2) fixed in 3.6% phosphate-buffered formaldehyde were measured with an image analyser (NIH Image, provided free of charge on the internet by the National Institute of Health, US). Each fish was represented by the mean of 50 oocytes taken randomly from a group of developing oocytes, a method found to give good accuracy (Ma et al., 1998). Standard deviation (SD) and coefficient of variation (CV) of oocyte diameter were also calculated for each individual. The average and SD of individual mean oocyte data were calculated for all the fish collected in each month and for each stage within a month supplemented by information on minimum and maximum oocyte diameter.

2.2.2. Potential fecundity

Developing oocytes were counted by the gravimetric method based on two replicates and average number was used as potential fecundity of each individual. Each subsample, which contained more than 200 developing oocytes, was taken from the middle part of the ovary. Oocytes are reported to distribute homogenously in ovaries of Atlantic herring (Ma et al., 1998). For the July sample only ovaries in

Table 1
Oocyte diameter (μm) at both population level and individual level for Norwegian spring-spawning herring of 32–37.5 cm TL from July 1998 to February/March 1999

Month	Oocyte developmental stage	Number of females	Oocyte diameter at population level				Oocyte diameter at individual level	
			Average	SD	Minimum	Maximum	Average SD	Average CV (%)
July	all combined	24	457	81.8			36.4	7.9
	cortical alveoli	6	359	30.5	314	398		
	vitellogenic	18	489	65.8	360	589		
October	vitellogenic	40	853	71.7	699	970	42.3	5.0
November	vitellogenic	45	928	105.3	506	1155	43.9	4.8
January	vitellogenic	39	1137	77.4	1021	1291	44.9	3.9
Feb/Mar	vitellogenic	32	1283	54.3	1134	1390	48.0	3.7
	hydrated	6	1466	54.9	1391	1548	54.9	3.7

which the most developed oocytes were at vitellogenic stage were used for fecundity counting. Oocytes in both cortical alveolus and vitellogenic stage coexisted in these ovaries. They were separated under the binocular microscope based on transparency and size as outlined by Kjesbu (1991) supplemented by histological verification (see below). The latter showed, however, that the proportion of oocytes at the cortical alveolus stage to all developing oocytes was only 4% in average (range, 0–11%; $n=19$). In October and later on, all developing oocytes were vitellogenic except for 7 fish in February/March, which had hydrated oocytes.

As expected, fecundity was found to be positively influenced by TL (Óskarsson et al., 2002). Monthly values of fecundity were standardised to 34 cm, which was approximately midway in the length distribution using simple linear regressions. Relative fecundity (=fecundity/(BW−OW)) was uncorrelated with TL; therefore the average for each month was used.

2.3. Histological observations

2.3.1. Proportion of atresia

A piece of the middle part of about 40 fixed ovaries from each month (see above) was dehydrated in ethanol in a progressive series up to 95% and embedded in Historesin for two days. Thin sections (1–4 μm) were made and stained with 2% toluidine blue and 1% borax, which stains structures such as nucleus, yolk granule, and chorion in different degrees of blue. The numbers of normal and α-stage atretic vitellogenic oocytes (Hunter and Macewicz, 1985) were counted in histological sections. All oocytes both with and without nuclei were counted because diameters of developing oocytes were considered homogeneous (see above). When counting oocytes of different true diameters in histological sections, the degree of bias should in principle be inversely proportional to oocyte diameter. Thus, atresia could be underestimated but not overestimated because atretic oocytes are smaller

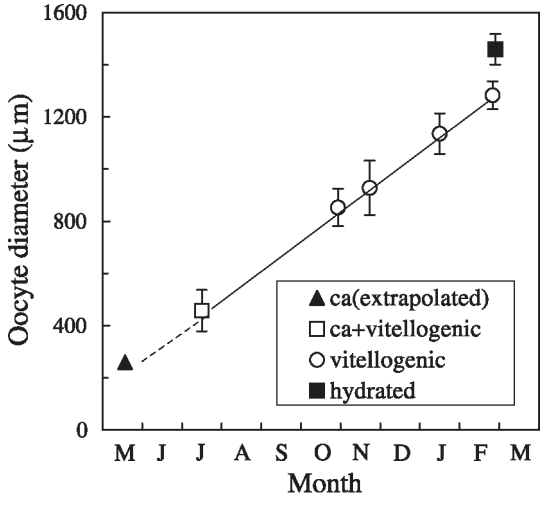


Fig. 2. Oocyte growth in mean diameter for Norwegian spring-spawning herring of 32–37.5 cm TL from July 1998 to February/March 1999. Bars show ± SD. Closed triangle indicates that the cortical alveolus stage (>240 μm) will start to appear in the middle of May, estimated by extrapolation (broken line) from the growth line (solid line).

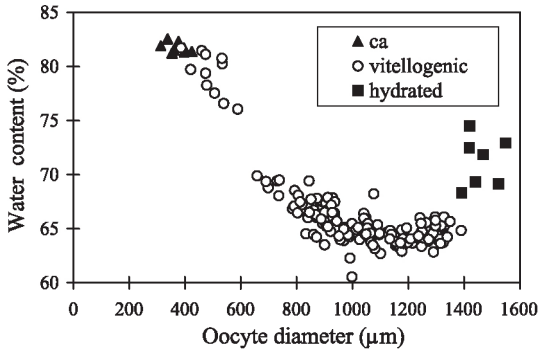


Fig. 3. Relationship between oocyte water content and mean oocyte diameter for three oocyte developmental stages for Norwegian spring-spawning herring.

than non-atretic oocytes, either because of size-specific selection (Witthames and Greer Walker, 1995) or shrinkage or both. To test this, unbiased stereological methods, more specifically the disector principle as reviewed in Mayhew (1992), were applied on 5 ovaries having relative intensities ranging from 6.3–87.2%. A total of 17–18 histological sections were analysed from each fish by this method. The two results on atresia per fish, traditional (profile counting) vs. stereometric figures, were subsequently compared directly (see Result Section).

The following three indices of atresia were calculated:

Relative intensity of atresia (I): the number of α-atresia divided by total number (n>100) of normal and atretic vitellogenic oocytes in an individual fish.

Prevalence of atresia: the number of fish with atresia divided by total number of examined fish.

Average relative intensity of atresia: the geometric mean of relative intensity of atresia only among fish with atresia multiplied by prevalence. Thus, this index indicates relative intensity of atresia at the group (population) level.

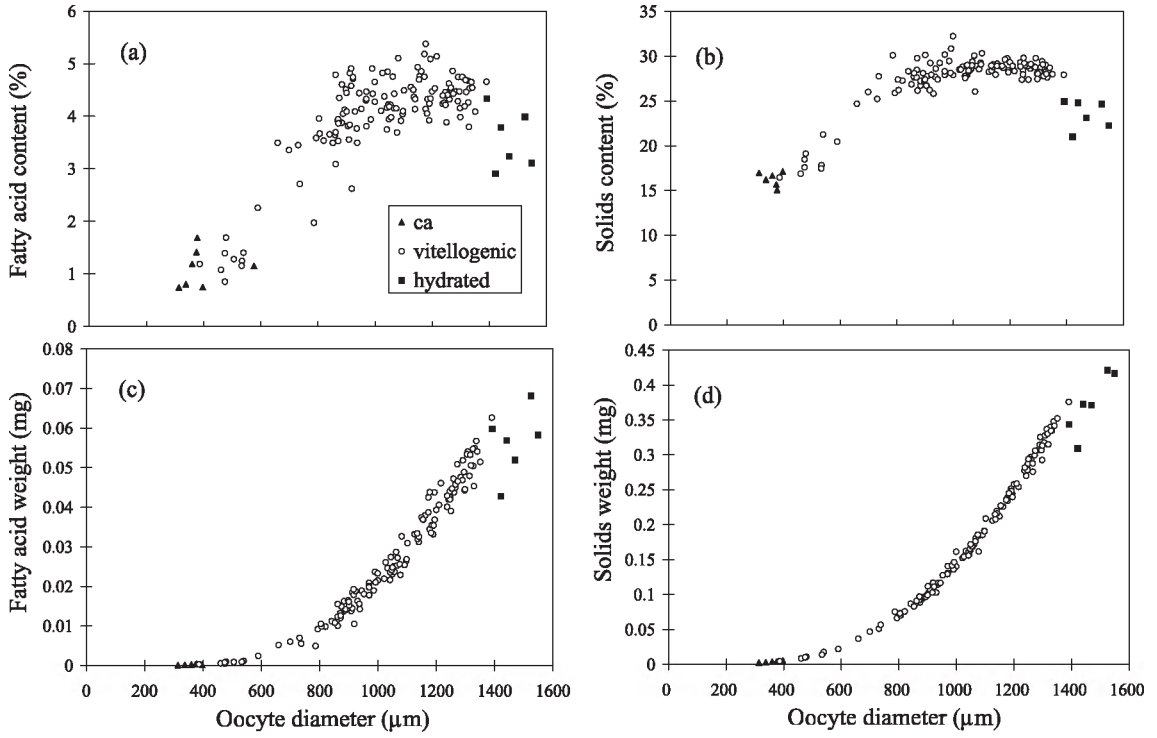


Fig. 4. Relationship between (a) oocyte diameter and fatty acid content of oocyte, (b) solids content of oocyte, (c) fatty acid weight per oocyte, and (d) solids weight per oocyte for three oocyte developmental stages of Norwegian spring-spawning herring.

The latter two indices were calculated for each month. They were also calculated for fish grouped according to their observed average normal oocyte diameter using intervals of 50 μm for each month. Average relative intensity and prevalence of atresia were subsequently related to oocyte diameter.

2.3.2. Estimating duration of α-atresia

Assuming that relative intensity of atresia (see above) is constant between two sampling points, fecundity should theoretically decrease exponentially as

$$Fec_2 = Fec_1 \times \exp((-A) \times T) \quad (1)$$

where Fec_1 and Fec_2 are fecundity at day 1 and day 2, respectively, T is time interval in days between the two sampling dates, and A is the coefficient of decrease. Thus

$$A = (\ln(Fec_1) - \ln(Fec_2))/T. \quad (2)$$

Fecundity decreases by I, the relative intensity of atresia, during one cycle of duration of atresia as:

$$Fec_{t+D}/Fec_t = 1 - I \quad (3)$$

where D is duration of atresia in days, and Fec_t and Fec_{t+D} are fecundity at day t and day (t+D), respectively. According to Eq. (1)

$$Fec_{t+D} = Fec_t \times \exp((-A) \times D). \quad (4)$$

Substituting both Fec_{t+D} in Eq. (3) and A in Eq. (2) to Eq. (4),

$$D = T \times \ln(1 - I)/(\ln(Fec_2) - \ln(Fec_1)). \quad (5a)$$

Standardised fecundity at 34 cm TL was used as reference point for each month. Representative relative intensity of atresia (I_R) between the two sampling periods was determined as the mean of the average relative intensity of atresia (see above) at the two periods, and used as substitute for I in Eq. (5a),

$$D = T \times \ln(1 - I_R)/(\ln(Fec_2) - \ln(Fec_1)). \quad (5b)$$

Duration of atresia was calculated for July–October, October–November, November–January, and

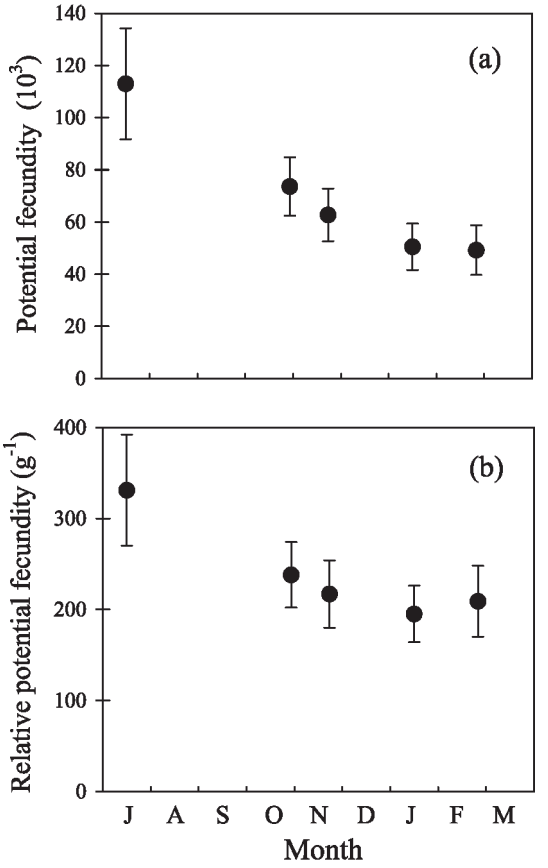
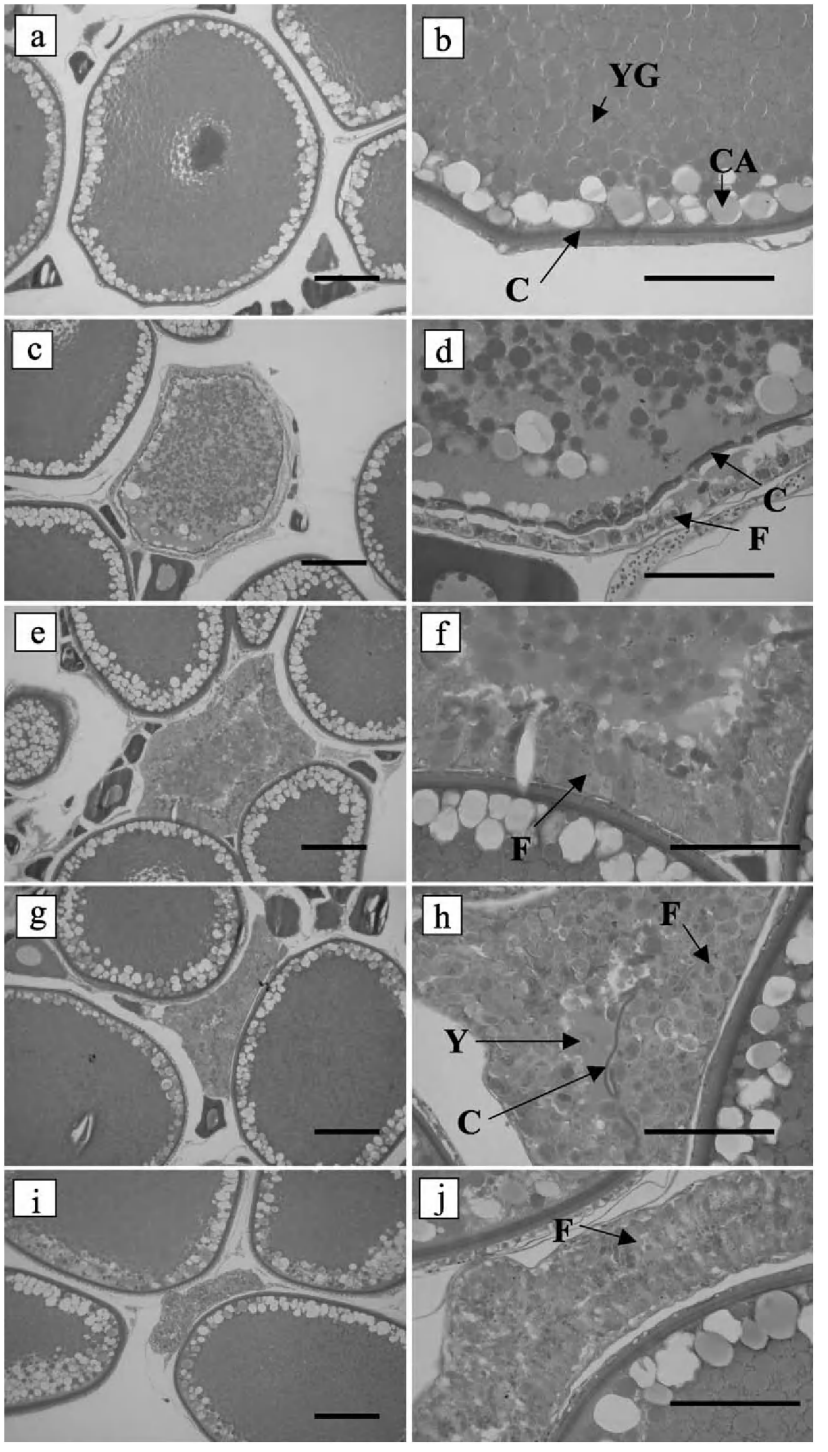


Fig. 5. Changes in (a) potential fecundity, and (b) relative potential fecundity both standardised to 34 cm TL fish through the maturation cycle (July 1998–February/March 1999) for Norwegian spring-spawning herring.

Fig. 6. Light micrographs of normal and different phases of α- and β-atretic oocytes for Norwegian spring-spawning herring. (a) A normal vitellogenic oocyte. (b) Numerous clear yolk granules (YG) are densely distributed, surrounded by cortical alveoli (CA). Chorion (C) is intact. (c) A very early phase of α-atresia. (d) Some yolk granules are fused and discharged. Chorion (C) is distorted and fragmented, but in position. Follicle cells (F) become enlarged. (e) An advanced α-stage of atresia. (f) Follicle cells (F) phagocytised yolk granules, yolk and chorion are resorbed, and oocyte size shrinking. (g) A late stage of α-atresia. (h) Little yolk (Y) and chorion (C) remain, surrounded by follicle cells (F). (i) A β-atresia. (j) No yolk and chorion remain. Bars for (a), (c), (e), (g), (i) = 200 μm, for (b), (d), (f), (h), (j) = 100 μm.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January–February/March. This approach, Eqs. (1)–(5b), was designated as Method 1.

Duration of α-atresia (D_{conv}; designated as Method 2) was also calculated in the conventional way as follows (Kjesbu et al., 1991; Witthames and Greer Walker, 1995):

$$D_{conv} = T/(((fec_1 - fec_2)/fec_1)/I_R).$$

2.4. Proximate body composition

2.4.1. Water, fatty acid, and solids contents of muscle and ovary

Fillets of each fish were homogenised with a blade cutter. Muscle tissue weighing approximately 50 mg excluding skin and bone was taken carefully into a 15-ml thick-walled glass tube containing ca.1.00 mg of C19:0 fatty acid as standard. The fatty acids from the samples and the standard were transformed into methyl esters with anhydrous methanol containing 2 M HCl in an oven at 100 °C for 2 h (modified from Viga and Grahl-Nielsen, 1990). After the methanolysis, all fatty acid methyl esters were extracted with hexane and the fatty acid composition was analysed (according to Joensen and Grahl-Nielsen, 2000) with a HP-5890A gas chromatograph equipped with a HP-7673A autosampler and flame ionisation detector. The total weight of fatty acid was calculated using the weight of standard C19:0. The fatty acid content of ovary samples weighing approximately 100 mg was examined in the same way, except that the weight of the C19:0 standard was set at ca. 0.50 mg. The portion of fatty acid content (FA (%)) was estimated as:

$$FA(\%) = 100 \times \text{fatty acid weight}/\text{sample wet weight}.$$

Dry weight of about 1–3 g (to 0.01 mg) in wet weight of muscle and 0.5–3 g in wet weight of ovary was weighed after drying at 100 °C for 48 h. The portion of water (Water (%)) was calculated as

$$\text{Water}\ (\%) = 100 \times (\text{sample wet weight} - \text{dry weight})/\text{sample wet weight},$$

then averaged for 3 replicate samples.

The lipid content (Lipid (%)) of muscle and ovary tissues was estimated with formulae, which were the relationship between FA (%) examined by direct-methanolise and Lipid (%) by the method of Folch et al. (1957) for 6 samples from July to March:

$$\text{for muscle, Lipid}\ (\%) = 1.117 \times \text{FA}\ (\%) + 0.330 \quad (r^2 = 0.99, n = 6)$$

$$\text{for ovaries, Lipid}\ (\%) = 1.558 \times \text{FA}\ (\%) - 0.030 \quad (r^2 = 0.98, n = 6).$$

The portion of solids content (Solids (%)) was calculated as:

$$\text{Solids}\ (\%) = 100 - \text{Lipid}\ (\%) - \text{Water}\ (\%),$$

and taken to represent the sum of protein, ash, and carbohydrates. Since ash and carbohydrates in Atlantic herring were considered to be low and approximately constant seasonally, changes in solids primarily represent changes in protein (Bradford, 1993).

Single oocyte wet weight was approximated to ovary wet weight (OW) divided by fecundity. The amounts of fatty acid and solids per single oocyte were calculated as each content (%) multiplied by estimated oocyte wet weight. Then changes in the amount of fatty acid and solids against oocyte diameter were examined.

2.4.2. Balance between dry, fatty acid, and solids weight of muscle and ovary

The relationship between head and bone weight (HBW) and TL was established as follows:

$$HBW = 6.81 \times TL - 155.9 \qquad (r^2 = 0.88, n = 24).$$

Then weight of muscle (MW) was calculated as

$$MW = BW - HBW - OW - (\text{gut weight}).$$

Gut weight was set to be constant at 11 g referring to the average weight of gut, including mesenteric fat, for captive NSS herring (31.5–34.5 cm) from November (11.2 g) to February (8.9 g) (first author, unpubl. data).

Dry weight, fatty acid weight, and solids weight of both muscle and ovary were calculated for each month using MW, OW, Water (%), Fatty acid (%), and Solids (%). Figures for each variable were standardised to 34 cm TL by linear regression based on natural log-

Table 2
Characteristics of atresia from July 1998 to Feb/Mar 1999 for Norwegian spring-spawning herring of 32–37.5 cm TL

Month	Number of females	Prevalence (%)	Average relative intensity (%)	
			Among fish with atresia[a]	Among all fish[b]
July	31	0	0	0
October	40	98	3.9	3.8
November	45	91	4.8	4.3
January	39	33	2.1	0.7
Feb/Mar	38	11	1.7	0.2

Refer to the text for definition of each characteristic.
[a] Geometric mean of relative intensity of atresia for fish with atresia.
[b] Geometric mean of relative intensity of atresia for fish with atresia multiplied by prevalence.

transformed values followed by studies of seasonal changes. Assuming all oocytes at sampling could develop to final maturation without resorption, expected muscle dry weight in February/March ($EMDW_{Feb}$), again standardised to 34 cm, was also calculated for each month:

$$EMDW_{Feb} = MDW - (0.000449 \times Fecundity - ODW) - (metabolic\ losses),$$

where MDW (muscle dry weight), Fecundity, and ODW (ovary dry weight) were values at sampling, 0.000449 g was the average dry weight of individual hydrated oocytes in February/March (n = 6), and metabolic losses were calculated as the difference between the amount of loss in MDW and the amount of gain in ODW, both from each sampling time to February/March. Thus, loss of muscle dry weight was assumed to be allocated entirely to ovary and metabolism.

2.4.3. Analyses of condition effects on reproductive investment

Because Water (%) of muscle and ovary varied from 60.1 (July) to 71.1% (February/March) and from 80.6 (July) to 64.4% (January), body reserves and reproductive investment were evaluated on dry weight basis. To examine seasonal changes in condition effects, multiple regression analysis was conducted for each month using ODW, which was considered to represent reproductive investment, as the dependent variable and both muscle dry weight condition factor (MDCF; $=10^2 \times MDW/TL^3$) and TL as independent variables. In cases where size effects were not significant, simple linear regression analysis between ODW and MDCF was conducted.

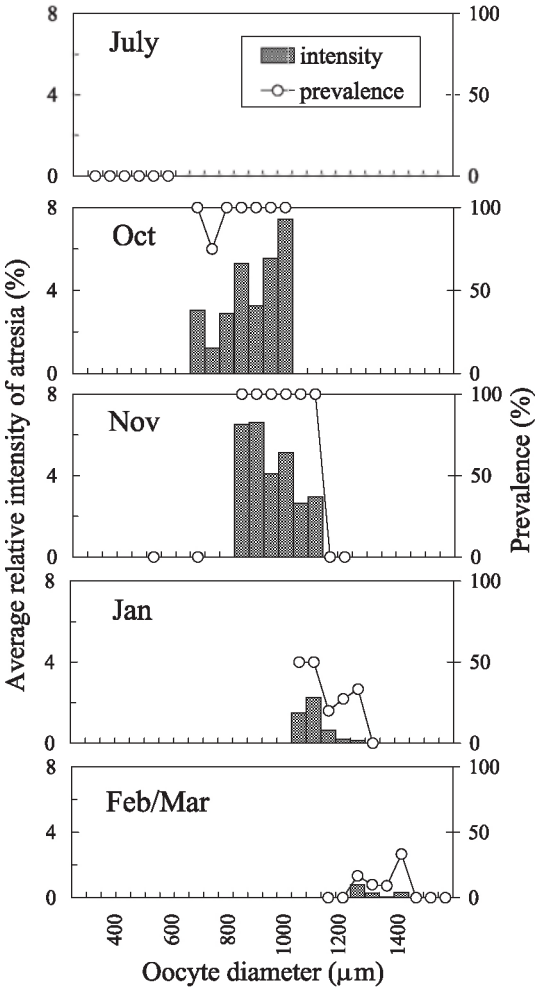

Fig. 7. Changes in average relative intensity of atresia and prevalence of atresia against each 50-μm interval of average oocyte diameter through the maturation cycle (July 1998–February/March 1999) for Norwegian spring-spawning herring. Both indices were calculated for fish grouped according to their observed average normal oocyte diameter using intervals of 50 μm for each month. One extreme fish in November with 87% atresia of vitellogenic oocytes (average diameter, 862 μm) was excluded.

ODW can also be approximated as the product of single oocyte dry weight and fecundity. To indicate which of the reproductive traits were regulated by condition, the relationships among fecundity, MDCF, and TL, and among oocyte dry weight, MDCF, and TL were examined for each month with multiple regression analysis. Relationships among relative fecundity, MDCF, and TL, and among either fecundity or relative fecundity, SCF, and TL were also examined for February/March. Lastly, the relationship between intensity of atresia and MDCF was examined.

3. Results

3.1. Oocyte growth

In July 67% (30/45) of the examined fish had entered vitellogenesis. From October to February/March all fish had vitellogenic oocytes except for 7 fish in February/March, which had hydrated oocytes. Thus, the histological data indicated that the sampling schedule almost covered the complete maturation cycle of NSS herring.

Developing oocytes formed a group having similar diameters. Average SD and CV of 50 oocyte diameters for individuals in each month were very small and stable (SD, 36.4–48.0 μm; CV, 3.7–7.9%; Table 1) throughout the maturation cycle. The most developed oocytes from 6 out of 24 fish in July were in cortical alveolus stage with an average diameter of 359 μm (minimum–maximum: 314–398). The other 18 fish had vitellogenic oocytes with larger diameter (*t*-test, $p<0.001$) of 489 μm (360–589 μm). In February/March the 7 fish with hydrated oocytes had an average diameter of 1466 (1391–1548) μm, which were statistically larger (*t*-test, $p<0.001$) than the other 32 members with vitellogenic oocytes of 1283 (1134–1390) μm. Monthly mean developing oocyte diameter (OD, 314–1390 μm), excluding hydrated oocytes, varied according to the relationship:

$$\mathrm{OD} = 3.75 \times \mathrm{ED} + 402 \quad (r^2 = 1.00, n = 5), \qquad (6)$$

where ED is elapsed days from 1 July (Fig. 2). Standard deviations of reported mean oocyte diameter at the population level were approximately stable from July to January (54.3–105.3 μm) (Table 1).

Oocyte water content decreased from ca. 82% at a diameter around 360 μm to ca. 64% at a diameter around 1000 μm, then remained constant at 64–65% up to 1400 μm. As reported above, hydrated oocytes

Table 3
Estimated duration of α-atresia for Norwegian spring-spawning herring of 32–37.5 cm TL by two methods

Date[a]	Interval (day)	Temperature at 20 m deep	Method 1						Method 2[c]
			Number of females	Estimated fecundity[b]	A (day)	Average relative intensity of atresia (%)		Duration (day)	Duration (day)
						For each month	Average between successive months		
20 Jul		4.2–11.3	31	113000		0 (0)[e]			
	100				0.0043		1.92 (2.43)[e]	4.5 (5.8)[e]	5.5
28 Oct		(9–10)[d]	40	73600		3.83 (4.85)[e]			
	26				0.0062		4.09 (5.19)[e]	6.8 (8.7)[e]	7.2
24 Nov		6.8–7.2	45	62700		4.34 (5.51)[e]			
	52				0.0042		2.53 (3.21)[e]	6.1 (7.8)[e]	6.7
15 Jan		5.8–6.6	39	50500		0.71 (0.90)[e]			
	42				0.0006		0.45 (0.57)[e]	7.2 (9.1)[e]	7.3
26 Feb		5.8–6.7	38	49200		0.18 (0.23)[e]			

Refer to the text for explanation of each method.
A = coefficient of decrease.
[a] Midpoint of the range of sampling dates.
[b] Standardised to fish of 34 cm TL and rounded to hundreds.
[c] Calculating with the same estimated fecundity and relative intensity of atresia data as in Method 1.
[d] Sea surface temperature informed by fishermen.
[e] Figure in parenthesis indicates relative intensity or duration of atresia when relative intensity is adjusted according to the results of the stereological method.

appeared above ca. 1390 μm and water content was found to increase rapidly (Fig. 3). Both fatty acid and solids content in an oocyte increased with diameter up to around 800 μm, from ca. 1% to 4% and from ca. 15% to 26% for fatty acid and solids, respectively. After that, the levels remained approximately constant, between ca. 4 and 5% for fatty acid and between ca. 27 and 30% for solids, up to around 1400 μm (Fig. 4a, b). Thus, in terms of total amounts, both lipid and solids increased slowly to 800 μm, then increased rapidly according to the third power of diameter (Fig. 4c, d). The rate of incorporation of fatty acid increased from 2.0×10^{-5} (mg/μm) between 360 and 800 μm to 8.3×10^{-5} (mg/μm) between 800 and 1400 μm, and for solids from 1.4×10^{-4} (mg/μm) between 360 and 800 μm to 5.0×10^{-4} (mg/μm) between 800 and 1400 μm.

3.2. Fecundity and atresia

3.2.1. Dynamics of fecundity through the maturation cycle

The fecundity of 34 cm fish decreased significantly from 113 000 ± 21 300 (mean ± SD, $n = 31$) in July to 49 200 ± 9500 ($n = 38$) in February/March, about 44% of that in July (Fig. 5a). A major decrease was also found in terms of relative fecundity from 331 ± 61 oocytes g^{-1} ($n = 31$) in July to 195 ± 31 oocytes g^{-1} ($n = 38$) in January, 59% of the July value (Fig. 5b).

3.2.2. Morphology of α-stage atresia

The atretic process was basically similar to that of other fish species (Fig. 6). Briefly, studying high quality light micrographs (Historesin-embedded material), in the earlier phase of α-stage atresia, chorion was distorted and fragmented, but in position. Follicle cells became enlarged and yolk granules disintegrated. Chorion apparently moved into deeper layers and follicle cells phagocytised yolk granules; yolk and chorion were resorbed and oocyte size shrinking.

3.2.3. Dynamics of atresia through the maturation cycle

The observed decrease in fecundity was consistent with seasonal changes in oocyte resorption (Table 2, Fig. 7). Both the prevalence and average relative intensity of atresia were zero in July when fish had just started vitellogenesis. Active resorption of developing oocytes occurred in October and November, i.e., almost all fish had atresia and average relative intensity of atresia for the population reached around 4%. Resorption declined in January with prevalence decreasing to 33% and average relative intensity of atresia to only 0.7%, and even more in February/March, to 11% and 0.2%, respectively (Table 2). Average relative intensity of atresia for each developing normal oocyte diameter at 50-μm intervals was high in October and in November. Particularly, all fish with oocytes between 800 and 1000 μm had atresia and average relative intensity exceeded 3% (Fig. 7).

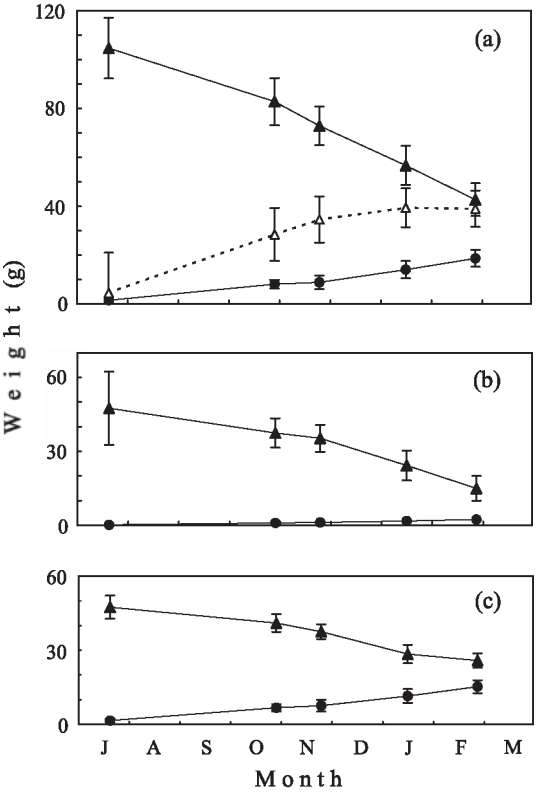

Fig. 8. Changes in (a) dry weight, (b) fatty acid weight, and (c) solids weight for muscle (closed triangles) and ovary (closed circles) of Norwegian spring spawning herring of 32–37.5 cm TL through the maturation cycle from July 1998 to February/March 1999. All figures are standardised to 34 cm TL fish. Expected muscle dry weight in February/March (see text, open triangles) are also shown in panel (a). Bars show ± SD.

3.2.4. Duration of atresia

The duration of α-stage atresia was estimated to be 4.5, 6.8, 6.1, and 7.2 d between July and October, October and November, November and January, and January and February/March, respectively (Table 3). Relative intensity of atresia estimated by the present traditional simple counting of profiles in histological sections was 63.9–107.8% (79.0 ± 17.1%, mean ± SD; n = 5) of those found by the stereological method, indicating that our figures were, as expected, underestimated. If all relative intensities of atresia were underestimated to the same degree as the 5 samples calibrated by the stereological method, then the relative intensity should generally increase by a factor of 1.27. Thus, the duration should increase to 5.8 d for July–October, 8.7 d for October–November, 7.8 d for November–January, and 9.1 d for January–February/March. It seems reasonable to expect that active resorption of developing oocytes began sometime between July, when no resorption was observed, and October, when there was active resorption. Estimated duration by the conventional method (Method 2) was longer, 0.1–1.0 day, than that by the new method (Method 1). Differences were larger when the time interval between two samplings was long and/or the intensity of atresia was high.

3.3. Condition effects on reproduction

3.3.1. Balance between dry, fatty acid, and solids weight through maturation

Loss of muscle solids weight of 34 cm standardised fish during fasting and the second half of the maturing period, October–February/March, was 15.3 g, which was about 2 times larger than the corresponding gain in ovary weight (7.6 g) (Fig. 8c). In contrast, loss of muscle fatty acid weight during this period was 23.1 g while gain in ovary was only 1.29 g (Fig. 8b). In total, 40.1 g of dry weight was lost from muscle and 10.5 g of dry weight gained in ovary from October to February/March. The variable $EMDW_{Feb}$, expected muscle dry weight in February/March assuming no atresia later on, showed values of only 4.6 g in July, but increased to 39.4 g in January, and then remained constant (Fig. 8a).

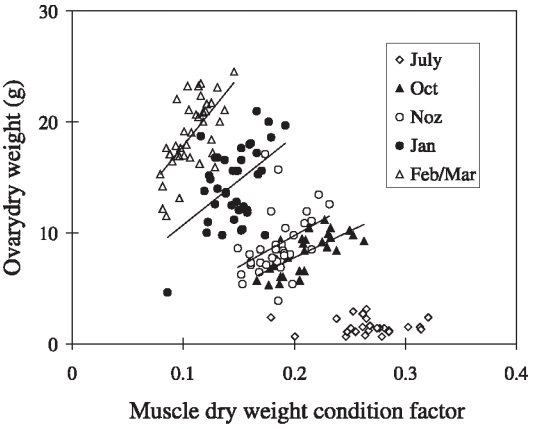

Fig. 9. Changes in relationships between ovary dry weight standardised to 34 cm TL fish and muscle dry weight condition factor (100 × muscle dry weight/TL^3) through the maturation cycle (July 1998–February/March 1999) for Norwegian spring spawning herring of 32–37.5 cm TL. Each line shows the linear regression line for each month. Slopes are shown in Table 4.

Table 4
Coefficients of total length (TL) and muscle dry weight condition factor (MDCF; 100 × muscle dry weight/TL^3) in multiple regression analysis against ovary dry weight, fecundity, and oocyte dry weight

Month	N	Ovary dry weight				Fecundity				Oocyte dry weight			
		TL		MDCF		TL		MDCF		TL		MDCF	
		Coefficient	p	Coefficient	p	Coefficient ($\times 10^3$)	p	Coefficient ($\times 10^5$)	p	Coefficient	p	Coefficient ($\times 10^{-4}$)	p
July	10	–	n.s.	–	n.s.	–	n.s.	–	n.s.	–	n.s.	–	n.s.
	25[a]	–	n.s.	–	n.s.								
Oct	30	0.16	0.001	47.8	<0.001	1.41	<0.001	–	n.s.	–	n.s.	4.88	0.030
Nov	35	–	n.s.	55.7	0.017	4.59	0.014	–	n.s.	–	n.s.	–	n.s.
Jan	39	1.90	<0.001	79.9	0.003	5.27	<0.001	2.54	<0.001	–	n.s.	–	n.s.
Feb	38	1.89	<0.001	123.4	<0.001	4.29	<0.001	2.96	<0.001	–	n.s.	–	n.s.

[a] Result when 15 available data sets are added, including ovary dry weight, TL, and MDCF.

3.3.2. Condition effect on reproductive investment through maturation

The slope of the relationship between ODW (dependent variable) standardised to 34 cm fish and MDCF (independent) increased from July (not significant) to February/March (123.4) (ANOVA, $p<0.001$) (Table 4, Fig. 9). In addition, the slope in February/March was larger than that in July (ANOVA, $p<0.001$), October ($p<0.003$), and November ($p<0.044$). These results indicate that the condition effect on reproductive investment increased from the beginning to the end of the maturation cycle.

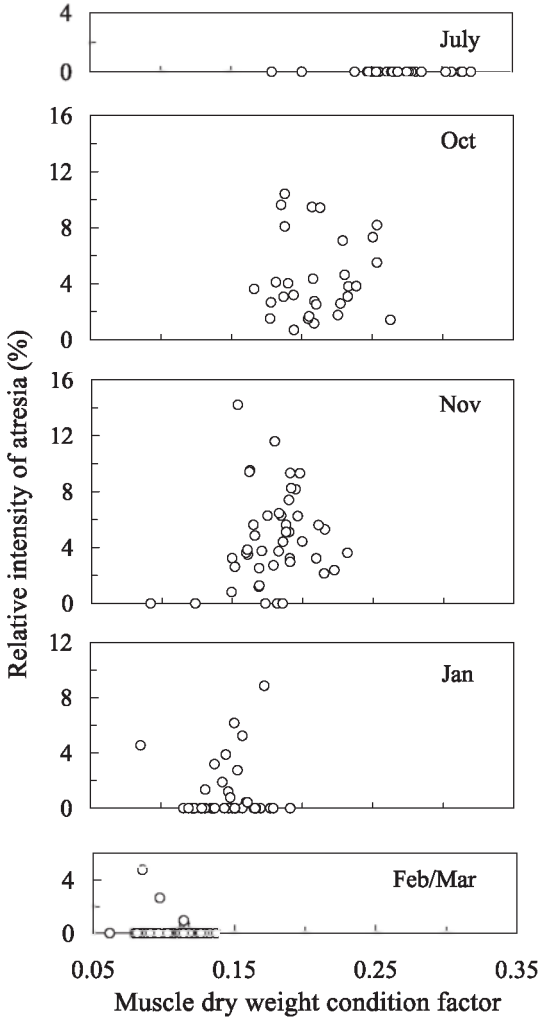

Fig. 10. Changes in relative intensity of atresia against muscle dry weight condition factor (MDCF; 100 × muscle dry weight/TL^3) through the maturation cycle (July 1998–February/March 1999) for Norwegian spring-spawning herring of 32–37.5 cm TL. One extreme fish in November with a 0.182 MDCF and 87% atresia was excluded.

3.3.3. Trade-offs between fecundity and oocyte size during maturation

The condition (MDCF) effect on fecundity appeared in the later maturation cycle (January and February/March) (Table 4). In contrast single oocyte dry weight appeared to be affected only in October with weak coefficient of determination ($r^2=0.148$). Taken together, the results indicate reproductive investment was mainly regulated through fecundity, at least in the later part of the maturation cycle.

3.3.4. Relationship between relative intensity of atresia and condition

No clear relationship between relative intensity of atresia and MDCF appeared from October to February/March (Fig. 10). One fish with an extreme intensity of atresia of 87% was observed in November; however, it had a medium-level MDCF of 0.182 (data point not shown).

3.3.5. Relationships between fecundity and condition in February/March

Standardised fecundity for a 34 cm fish in February/March varied from 29 300 to 69 700, and positive condition effects were observed; 27% and 16% of the variation in fecundity was explained by MDCF ($r^2=0.27$, $p<0.001$, $n=38$) and SCF ($r^2=0.16$, $p=0.014$, $n=38$), respectively. Moreover, MDCF influenced relative fecundity (134–303 g^{-1}) ($r^2=0.12$, $p=0.035$, $n=38$), while SCF did not.

4. Discussion

If the present oocyte growth trajectory (Eq. (6), Fig. 2) is valid for the period before July, oocytes in the cortical alveoli stage (>240 μm, Ma et al., 1998) would start to appear in the middle of May, i.e., at the beginning of the feeding season. Thus, adult females of NSS herring seem to have a very short spent-recovery period and enter the early stages of vitellogenesis one or two months after spawning in March.

Assuming that NSS herring stop feeding in September (Slotte, 1999), ovary dry weight (ODW) of 34

cm fish accumulated before and after fasting was estimated to be 4.4 g and 14.3 g, respectively (Fig. 8). Thus, about three quarters of the final ODW was allocated from body reserves during fasting. If all developing oocytes in July reached final maturation, ODW would be 50.7 g and muscle dry weight (MDW) in February/March would be less than 5 g. This is very much in contrast with realised MDW in February/March, 42.7 g. Thus, body reserves at the start of fasting seem to be far too low to support all developing oocytes until full maturation. In other words, fecundity should in principle be reduced according to nutritional status via atresia.

At the population level intensive resorption of developing oocytes started to occur between July and October and almost stopped between November and January (Table 2). According to the oocyte growth formula (Eq. (6)), oocyte diameter in September is estimated to be ca. 650 μm (range: 450–800 μm). Since relative intensity of atresia of greater than 1% was observed between 650 and 1100 μm in October and November (Fig. 7), oocytes between 450 and 1100 μm would be the possible target size range of atresia. On the other hand, even greater (>3%) losses were observed for oocytes between 800 and 1000 μm. However, oocytes are estimated to remain in this size range for only 53 d between mid-October and mid-December (Eq. (6)). Thus, two conclusions are possible. First, target size range was wide (450–1100 mm), but a great loss was experienced by smaller (<800 μm) vitellogenic oocytes between September and October. Second, the main target size was restricted to 800–1000 μm and even far greater (>>3%) intensities of atresia occurred just prior to the October sample. In both cases, oocyte size in volume is still small before November and even oocytes of 1100 μm have accumulated only half of the yolk that will be accumulated until final maturation (Fig. 4). Thus, resorption of smaller oocytes should require less energy than for larger oocytes. The season of intensive resorption also seems to be reasonable. Since feeding begins in April/May, but peaks in July and stops in September (Slotte, 1999), fecundity can be regulated according to level of accumulated body reserves after September. Including also other results (Fig. 9), fecundity regulation of NSS herring can probably be expressed as follows. During summer feeding, when the amount of materials to be invested in reproduction has not yet been decided, fish produce high quanta of small vitellogenic oocytes independently of condition, while in autumn, when the possible amount of investment is determined, much more material is needed for oocyte growth and thus the number of oocytes is regulated depending on nutritional condition. During this active regulation period, obviously only 'selected' oocytes can be supported by surplus nutrition to reach final maturation and be spawned.

Energetically reasonable regulation of fecundity by atresia has been observed for captive northern anchovy following starvation (Hunter and Macewicz, 1985) and in sole, *Solea solea* (Witthames and Greer Walker, 1995). Fecundity of northern anchovy is indeterminate, i.e., they can make new vitellogenic oocytes during the spawning season according to food supply and body condition. Atretic oocytes increased rapidly in numbers when the fish stopped feeding, then decreased rapidly when the fish started feeding again. Massive atresia, >50% of vitellogenic oocytes affected, occurred in a short period at the end of the spawning season. Atretic oocyte diameters for sole, which should be considered to have more of a determinate fecundity, are the smaller ones among the developing oocytes, which are to be spawned in the current spawning season. Resorbing smaller oocytes requires less metabolic investment than larger oocytes. For NSS herring, it was difficult to decide whether certain size classes of oocytes among a group of normal developing oocytes were preferentially degenerated or not, because diameters of normal developing oocytes within each individual were distributed homogeneously with low SD (Table 1), moreover, α-stage atretic oocytes themselves had already shrunk to some extent at measurement due to degeneration.

Reported levels of fecundity reduction during the spawning season for other fish species are 8% for Dover sole, *Microstomus pacificus* (Hunter et al., 1992), 30.5% for sole in Bristol Channel (Horwood, 1993), 12.4% for North Sea sole (Witthames and Greer Walker, 1995), and 6–13% for western mackerel, *Scomber scombrus* (Greer Walker et al., 1994). Thus, the level of resorption of about 56% for NSS herring reported in this study is generally higher than in the other studies; however, this seems to be the only study examining almost the complete maturation

cycle. A 56% reduction of potential fecundity was supported by data on seasonal changes in relative intensity and prevalence of atresia. Duration of atresia between October and November, and between November and January was estimated to be 6.8 d (water temperature at 20 m deep being 6.8–7.2 °C in November) and 6.1 d (5.8–7.2 °C in November and January), respectively (Table 3). Taking into account the underestimation introduced by the applied method of counting atresia, estimated duration should be about 25–30% longer, i.e., 8.7 and 7.8 d, respectively. Estimated duration of α-atresia for NSS herring is comparable to 8 d for northern anchovy (15.5–16.5 °C, Hunter and Macewicz, 1985) and 10–13 d for Atlantic cod (8 °C, Kjesbu et al., 1991), but neither of these works corrected for any likely underestimation of atresia levels. In addition, given that resorption of oocytes started from 1 September and intensity of atresia occurs at the same level as for October (3.83%) instead of 100 d of medium resorption (1.92%) as in Table 3, duration of α-atresia is calculated to be 5.3 d, which is a reasonable figure compared to the others, because of smaller oocyte sizes.

In this study we estimated the duration of atresia from temporal information on relative intensity of atresia and fecundity. When the intervals between samplings are short or the intensity of atresia is low, the estimated value of duration will, logically, be very sensitive to variation in the estimated value of fecundity. The formula we adopted is robust when intensive resorption occurs. In this sense, the duration between October and November will be the best estimate among the four periods. To specify when resorption starts and ends using shorter intervals of sampling seems to be very important to acquire a more precise picture of fecundity regulation.

ODW, an indicator of reproductive investment, or fecundity, was more strongly affected by MDCF, an indicator of body reserves, as maturation progressed (Fig. 9) to such a degree that even relative fecundity was positively affected by MDCF. Since reproductive investment for the present type of fish is mainly regulated around October and November, body reserves in February/March should in principle have little influences on reproductive investment or fecundity. Nevertheless, the influence of MDCF on ODW was stronger in February/March, indicating that fish with more body reserves at the beginning of overwintering in September could invest more in their ovaries and at the same time be in a good body condition at the end of maturation. Fish that are in a better condition at the start of spawning migration are also known to migrate longer distances, reaching spawning grounds that are presumably more favourable for larval survival (Slotte, 1999; Slotte and Fiksen, 2000). Thus, a population of NSS herring in a better condition at the beginning of overwintering may spawn more eggs at a better spawning ground, possibly leading to the production of a larger year class.

There was no clear condition effect on intensity of atresia in October and November in which intensive resorption occurred (Fig. 10). This is probably due to asynchronous resorption among individuals; for some fish, sampling was conducted during peak resorption, for others during pre- or post-peak resorption. However, larger resorption of potential fecundity probably occurred for individuals with low MDCF because potential fecundity was not related to MDCF from July to November, while a strong relationship appeared in January and February/March (Table 4). In agreement with this, some studies could not find any relationship between individual body condition and intensity of atresia at sampling (Atlantic cod, Kjesbu et al., 1998; Atlantic herring, Ma et al., 1998) although some did find ration size effect at the group level on the amount of resorption (rainbow trout, *Oncorhyncus mykiss*, Bromage and Cumaranatunga, 1987; Atlantic cod, Kjesbu et al., 1991; Atlantic herring, Ma et al., 1998). Thus, condition effects negatively influencing the amount of resorption throughout maturation cycle are expected, but at a time section out of relevant focus such condition effects might be hard to detect.

In our study oocytes grew linearly over time. This is in conflict with the assumptions made in Ma et al. (1998) and Óskarsson et al. (2002) adopting an exponential growth curve for Atlantic herring. We think it is important to examine oocyte growth through the maturation cycle of the same year because oocyte growth rates may differ from year to year due to ambient water temperature (Ware and Tanasichuk, 1989; Kjesbu, 1994) and nutritional condition (Hay and Brett, 1988; Kjesbu, 1994). Ours is the only field study covering most of the maturation cycle for Atlantic herring in the same year. We used only repeat spawners, which have a much more uniform devel-

opmental cycle among individuals than recruit spawners (Óskarsson et al., 2002) and excluded hydrated oocytes, which start to swell abruptly. This obviously presents a clearer picture. Sampling bias due to selection of sampling locations was not investigated in this study. However, our sampling schedule is believed to reduce this kind of bias, i.e., studying 6 locations in the Norwegian Sea in July, 2–3 locations in the narrow overwintering area from October to January, and 4 locations from north to south in the spawning grounds in February/March. Our result is also supported by Hay (1985), who reported that oocytes of Pacific herring grow almost linearly from September to February during vitellogenesis.

As a final point, it might be argued that the use of a constant, standard length (34 cm) in several of the present calculations excludes the detection of any possible growth effects on reproductive traits. As oocyte diameter is uncorrelated with length of repeat spawners, an effect is not expected here (Óskarsson et al., 2002). However, possible influences on fecundity required further considerations. Assuming that individual growth happens during the feeding season, this was set to be 0.5 cm (our observations referring approx. to half of this season, i.e., 1 cm/2: Dragesund et al., 1980). This would imply that the correct standard length in the subsequent autumn and winter should be changed to 34.5 cm. Based on Tables 3 and 4, this would increase fecundity by 2100 for February/March, or about 2% of July-value and 4% of February/March-value. Such an error in fecundity was considered negligible. Furthermore, we were unable to locate any detailed information on the timing of growth during the year in the literature.

Reproductive investment is influenced by the amount of body reserves and somatic growth (Rijnsdorp, 1994; Nash et al., 2000). In this study ODW increased with body condition close to spawning while, based on Dragesund et al. (1980), little positive somatic growth is expected for this species at any stage following sexual maturity. Thus, according to the model of surplus production of Rijnsdorp (1994), repeat spawning NSS herring is rather extreme in the sense that surplus production is mainly allocated to metabolism and gonad growth only, or stated in another way, these specimens may be rather sensitive to variations in annual surplus production leading to variations in body condition and in reproductive investment (= fecundity). However, the body condition in the present 1999 spawning season (SCF = 0.59 in February/March) was rather poor in a historical perspective (Óskarsson et al., 2002). Thus, a similar type of study in a year with fish of better condition will provide further insight into the energy allocation patterns of this species.

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