Effects of ration on the maturation and fecundity in captive Atlantic herring (*Clupea harengus*)

Y. Ma, O.S. Kjesbu, and T. Jłrgensen

Abstract: We examined the effects of amount of food on vitellogenic oocyte size and number (fecundity) between two experimental groups of Atlantic herring (*Clupea harengus*) kept in separate but identical compartments of a large seawater tank. Growth and maturation were monitored for 1.5 years. Each group of 200 fish was fed exclusively on krill. At the end of the experiment, a high-ration group, fed 12 mg·g fish^{-1}·day^{-1}, had similar weights-at-length as wild specimens of the same stock. A low-ration group fed about half the amount of the high-ration group had significantly lower weights-at-length. Ration had no effect on oocyte size and relative potential fecundity (oocytes per gram), but mean potential fecundity (oocytes per fish) of the low-ration fish was 26% lower than that of the high-ration fish. The relative intensity of early stages of atresia was quantified histologically. Atresia is a common phenomenon in Atlantic herring and increases markedly with suboptimal feeding. Mean relative atretic intensities were estimated at about 3 and 6% for the high- and low-ration groups, respectively, but with large intragroup variation. Modeled realized fecundity (spawned eggs per fish) indicated a 9 and 40% reduction compared with the measured potential fecundities, respectively.

Résumé : Nous avons étudié les effets de la quantité de nourriture sur la taille et le nombre (fécondité) d'oocytes vitellogènes chez deux groupes expérimentaux de hareng (*Clupea harengus*) maintenus dans deux compartiments séparés mais identiques d'un grand réservoir d'eau de mer. Nous avons suivi la croissance et la maturation des poissons durant 1,5 ans. Chaque groupe de 200 poissons était nourri exclusivement de krill. À la fin de l'expérience, les poissons du groupe ayant reçu une forte ration alimentaire, soit 12 mg·g de poisson^{-1}·jour^{-1}, avaient des poids selon la longueur similaires à ceux de poissons sauvages du même stock. Les poissons du groupe ayant reçu une ration alimentaire faible (environ la moitié moins de celle de l'autre groupe) montraient des poids selon la longueur significativement inférieurs. La ration n'a pas eu d'effet sur la taille des oocytes et la fécondité potentielle relative (oocytes par gramme), mais la fécondité potentielle moyenne (oocytes par poisson) des poissons à faible ration a été de 26% inférieure à celle des poissons à forte ration. L'intensité relative des premiers stades d'atrésie a été quantifiée de façon histologique. L'atrésie est un phénomène courant chez le hareng et son intensité s'accroît de façon marquée quand l'alimentation est sous-optimale. Les intensités relatives moyennes de l'atrésie ont été estimées à environ 3 et 6% dans les groupes à forte ration et à faible ration, respectivement, mais il y avait une grande variation intra-groupe. La fécondité réalisée modélisée (oeufs pondus par poisson) a montré une réduction de 9 et 40%, respectivement, par rapport aux fécondités potentielles mesurées.
[Traduit par la Rédaction]

Introduction

Environmental conditions and nutritional status may have strong modifying effects on maturation and fecundity in fish (Wootton 1990). Most studies on the relationship between reproductive output and resulting abundance of progeny are based on theoretical models assuming egg production to be directly proportional to spawning stock biomass, an assumption seldom verified in the field (Cook and Armstrong 1986). These efforts have generally met with little success in explaining the observed variability in recruitment (Hilborn and Walters 1992). Several studies recently reviewed by Trippel et al. (1997) have pointed to the importance of maternal status for the resulting number and survival of eggs and larvae and ultimately for recruitment to the stock.

There are a number of field studies on herring reproduction in the literature, but very few experimental ones. Feeding captive Pacific herring (*Clupea pallasi*) in the later maturity stages advanced spawning time and likely increased egg weight, while starvation led to lower fecundity (Hay et al. 1988). For wild populations, Winters and Wheeler (1994) found that length-specific weight of Northwest Atlantic herring (*Clupea harengus*) varied significantly among years, while growth in length was more stable. Ovary weight was positively correlated with interannual variation in fish condition which appeared to be strongly density dependent. No clear density-dependent effect on reproductive investment has yet been shown for Northeast Atlantic herring (Almatar and Bailey 1989; Bailey and Almatar 1989). These articles give, however, examples of substantial annual changes, i.e., by a factor of up to 1.75 over a period of 10 years, in relative fecundity (i.e., per unit body weight). Tanasichuk and Ware (1987) underlined variation in ambient water temperature 2–3 months before

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Y. Ma and T. Jørgensen.[1] Department of Fisheries and Marine Biology, University of Bergen, HiB, N-5020 Bergen, Norway.
O.S. Kjesbu.[2] Department of Marine Environment, Institute of Marine Research, P.O. Box 1870, N-5024 Bergen, Norway.

[1] Present address: Department of Marine Resources, Institute of Marine Research, P.O. Box 1870, N-5024 Bergen, Norway.
[2] Author to whom all correspondence should be addressed.
e-mail: olav.kjesbu@imr.no

Fig. 1. Temperature regime during the experiment.

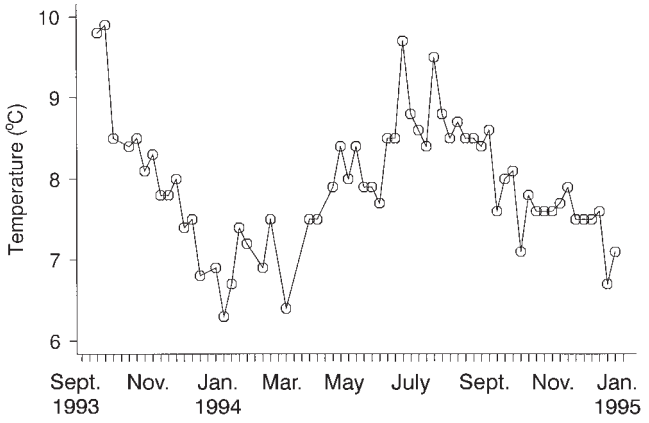
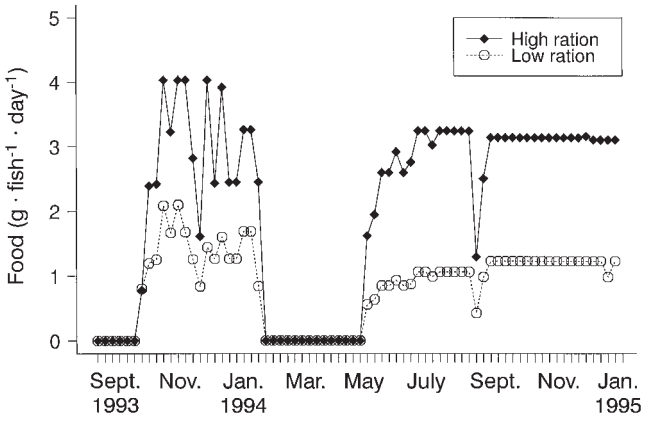


Fig. 2. Food regime during the experiment. The values given refer to weekly averages and wet weight after thawing.



spawning as important for fecundity and egg size in Pacific herring, being positively and negatively influenced, respectively. This effect was, however, not found in Atlantic herring (Bradford and Stephenson 1992).

The demonstrated effects of food supply on maturation and fecundity in fish raise the following question concerning the causal mechanisms: does food supply modulate fecundity by affecting female growth rate/condition or by affecting the rate of resorption of developing oocytes (atresia) during the maturation cycle, as suggested by many authors (e.g., Hunter and Macewicz 1985; Hay et al. 1988). Even though atresia has often been mentioned as one of the determinants of fecundity in fish, only a very limited number of quantitative studies on the incidence of atresia at various stages of oocyte development have been undertaken (Witthames and Greer Walker 1995: wild sole (*Solea solea*)). To our knowledge, no such data have yet been published for herring.

This paper presents the results of a controlled laboratory study of a local population of spring-spawning Atlantic herring. The experiment lasted 1.5 years and covered a complete maturation cycle. In a series of pilot studies, we established the daily ration that produces fish of normal condition. Then, based on this ration size, we set two experimental rations to determine how amount of food affects fish growth, maturation (vitellogenic oocyte size), level of atresia, and fecundity. In subsequent analysis of collected ovaries, special emphasis was placed on methodological aspects including precision and accuracy in fecundity estimation and intraovarian variation in oocyte size and density. Finally, we quantified histologically the intensity of vitellogenic atresia hypothesized to be an important regulatory mechanism of egg production in Atlantic herring.

Material and methods

Experimental protocol

The Atlantic herring used in the experiment were caught by purse seine on August 31, 1993, at Foldnes, west of Bergen, Norway, and transferred to a 30-m^3 outdoor concrete tank at the Institute of Marine Research. The tank was divided vertically by a fine-meshed net into two compartments, each 15 m^3. The compartments were stocked randomly with 199 and 193 fish, corresponding to the high- and low-ration experimental group, respectively. Due to initial mortality caused by scale loss/injuries, these numbers were reduced to 184 and 167, respectively. No natural mortality was observed later in the experiment. Sex ratio established at the completion of the experiment showed 52 and 50.5% females, respectively. The water supply to the tank was 200 $L \cdot min^{-1}$ provided from an intake at 120 m depth in the fjord. Temperature was measured weekly (Fig. 1).

After 6 weeks of adapting to the captive conditions, the fish were handled food. Each group was fed for about half an hour during daytime from Monday to Friday. In 1994, feeding was completely stopped from February 7 to May 15, likely including the duration of the local Atlantic herring spawning season (A. Slotte, Institute of Marine Research, Bergen, Norway, unpublished data). This was done to reflect the wild situation, where spawning Atlantic herring are assumed to feed very little (Henderson and Almatar 1989). The Atlantic herring were fed exclusively on krill, mainly *Meganyctiphanes norvegica*, collected and immediately frozen (–20°C) during cruises in the northeastern Norwegian Sea. Before use, the food was thawed. Temporal changes in energy content measured as lipids were noticed (S. Meier, University of Bergen, Bergen, Norway, unpublished data), but the two experimental groups received in every case food of identical origin.

Ration sizes (Fig. 2) were establish to produce fish of either good or poor condition. The high ration was about 3 g $fish^{-1} \cdot day^{-1}$ based on the results of two pilot studies, each 6 months long, aimed at producing fish with a condition typical of the wild, local population. The low ration was initially set at half of this amount from an experiment with Atlantic cod (*Gadus morhua*) (Kjesbu and Holm 1994) but reduced to one third after the first spawning season in spring 1994 to increase the difference in condition between the two groups. This low ration was slightly increased from September 6, 1994, onwards, as several fish appeared to be in a very poor condition. Thus, the low-ration fish were fed at 38% of the high ration during the last 4 months of the experiment. The overall yearly (1994) mean ration (i.e., including the 14-week starvation period) was 2.1 and 0.8 g $fish^{-1} \cdot day^{-1}$ for the high- and low-ration groups, respectively.

Samples of Atlantic herring from the two groups were collected at the start of the experiment, at intervals four times later on, and when the experiment was ended (Table 1). Total length (TL) was measured to the nearest 0.1 cm and whole-body wet weight (W) to the nearest 0.1 g. Gonad, liver, and viscera for both males and females were removed and weighed to the nearest 0.1 g and all ovaries preserved in 3.6% phosphate-buffered formaldehyde. For purposes of comparison, parallel measurements were made on wild Atlantic herring caught in early February 1996 in the same geographical area where the experimental fish were caught.

Seasonal changes in gonadal development were described using the gonadosomatic index (GSI), i.e., $10^4 \cdot W_g/TL^3$, where W_g is the wet weight of the fresh gonad. The hepatosomatic index (HSI) was used for describing the relationship between liver weight (W_h) and TL,

i.e., $10^4 \cdot W_h/TL^3$. Somatic condition factor (SCF) was defined as $10^2 \cdot (W - W_g)/TL^3$.

Ovarian homogeneity

To study if developing oocyte size varied systematically among different locations of the ovary, three subsamples were taken from four different locations in the ovary (anterior, middle, and posterior part of the right lobe and middle part of the left lobe). For each subsample, 40 developing oocytes were measured under a binocular microscope (see below). The samples were taken from six individuals of the high-ration group in stages 3, 4, and 5 based on the Hjort maturity scale (Bowers and Holliday 1961). The data were analyzed using a nested ANOVA, and the analysis was run separately for each fish. The model was

$$(1) \qquad d_{ijk} = \mu + \mathrm{loc}_i + s_{ij} + \varepsilon_{ijk}$$

where d_{ijk} is the diameter of the kth oocyte of the jth subsample at location i, μ is the overall mean oocyte diameter, loc_i is the effect of the ith location, s_{ij} is the effect of the jth subsample at the ith location, and ε_{ijk} is the error term. Partition of the variance was obtained by the varcomp function in S-Plus, using the maximum likelihood estimation method (Venables and Ripley 1994). The partition of the variance in measured oocyte diameter showed a consistent trend for all fish studied; about 80–90% of the variance was among oocytes from the same subsample, and the remaining variance was equally distributed among locations and subsamples. Thus, the variation in oocyte size among different locations of the ovary was marginal compared with the variation in size of neighboring oocytes, and for the estimation of mean oocyte diameter, it was decided to take one subsample from each ovary. For the purpose of consistency, this subsample was always taken from the middle part of the right lobe.

Oocyte size in whole-mount preparations

Developing oocyte diameter was used as a criterion of maturity status. Using a binocular microscope (50×, precision ±10 μm), the diameter was taken as the average of the shortest and the longest axes of the oocyte. A pilot study was undertaken to test for the number of cells required to be analyzed to get reliable descriptive statistics. For each of three fish selected from the January 1995 samples, 100 vitellogenic oocytes were initially measured. Bootstrapping, i.e., 2000 iterations and bias- and acceleration-corrected percentiles (Efron and Tibshirani 1993), was then used to estimate 95% confidence intervals of the estimated mean diameter for different numbers of oocytes included. The interval width for all three fish studied decreased only marginally with sample sizes above 30–40 oocytes. Consequently, a conservative sample size of 40 was adopted for the developing oocyte diameter measurements in the main study.

Maximum previtellogenic oocyte diameter, thought to reflect minimum developing oocyte diameter, was also determined under the binocular microscope. Twelve high-ration fish were selected according to the mean diameter of developing oocytes in an effort to cover the different Hjort stages from 2–3 to 5. From each chosen ovary, besides 40 randomly selected developing oocytes, 10 of the most advanced previtellogenic oocytes were measured. Any abnormally distorted oocytes were excluded.

Histological examination of oocyte size and atresia

Before sectioning, the ovaries were dehydrated (90%) with ethanol and embedded in Historesin. Cross sections 4 μm thick were made with a metal knife. The sections were stained with toluidine blue and dried before being mounted on glass slides with Eukitt. The sections were then analyzed with an image analyzer (Olympus Micro Image) with 40 and 100× magnification for counting and measuring, respectively.

For the purpose of (*i*) contrasting measurements made of previtellogenic oocytes under the image analyzer with similar measurements made on the same fish under the binocular microscope and (*ii*) a similar comparison for vitellogenic oocytes, the following series of calibration tests were performed. Three fish from each ration group were used for determining the maximum previtellogenic oocyte size histologically; the circumferences of 10 of the most advanced, normal-looking previtellogenic oocytes were measured and the diameter calculated assuming the cell to be spherical. Only oocytes whose nucleus was sectioned were measured. Similarly, the circumferences of 40 randomly chosen developing oocytes (or the total number present if less than 40) were measured and used to estimate the corresponding diameter for a total of 43 females (both groups) sampled at various times of the year.

Table 1. Sampling dates and number of fish sampled by sex from each of the two experimental groups.

	High ration		Low ration	
Date	Females	Males	Females	Males
Sept. 1, 1993	4[a]	7[a]		
Feb. 9, 1994	27	16	17	18
May 4, 1994	7	9	7	8
Sept. 5, 1994	7	18	9	12
Dec. 12, 1994	6	1	—	—
Jan. 19, 1995	40	42	50	46

[a]Sample taken on September 1, 1993, was representative of the overall experimental population at the start of the experiment.

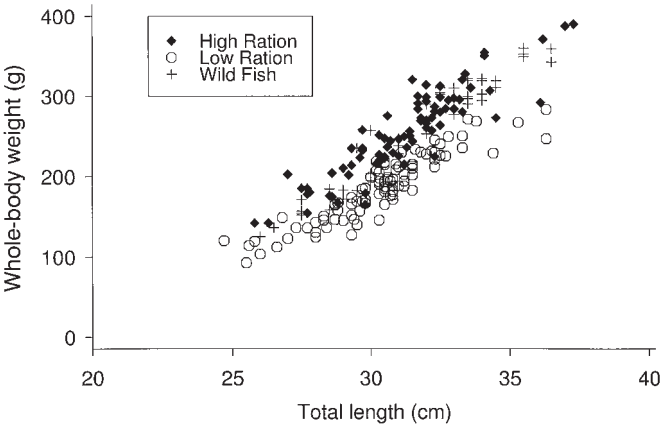
During oocyte development, some vitellogenic oocytes might be resorbed and are called atretic. By using the present potential fecundity estimation method, including counting of vitellogenic oocytes under the binocular microscope (detailed below), any early atretic oocytes were most likely included along with normal ones due to the difficulty in distinguishing these two types. More specifically, those that microstructurally would be characterized by showing a disrupted, but still peripheral, chorion and the granulosa cells of the surrounding follicle layer having just begun to invade the oocytic cytoplasm, i.e., the early α-atretic stage (Hunter and Macewicz 1985). To correct for these possible examples of atresia during further estimation of fecundity, histological cross sections were prepared to estimate the relative intensity of early α-stage atresia. This intensity was defined as the percentage of early α-stage atretic oocytes relative to the sum of normal vitellogenic and early α-stage atretic oocytes. The ovaries were selected from the high- and low-ration fish sampled in January 1995, 20 and 15 ovaries, respectively. To indicate the level of precision of the method chosen, three of the fish in different developmental stages from each group were first analyzed extensively. The number of vitellogenic and early α-stage atretic oocytes in fixed-size, non-overlapping fields (snapshots from a video camera) were counted. Subsequent use of bootstrapping (described above) demonstrated that the number of fields needed to obtain stable estimates and acceptable precision varied considerably among fish. Also, the actual size of sections and thereby the number of fields varied notably due to the variation in ovarian size. It was thus decided to base the estimates of the relative intensity of early α-stage atresia on counts for the whole area of the section.

Fecundity analysis

Definitions

Potential fecundity (F_P), defined as the number of vitellogenic oocytes in the ovary prior to spawning, was calculated using the gravimetric method (Bagenal 1978), i.e., $(W_o/W_S) \cdot N$, where W_o is the weight of the preserved ovary, W_S is the weight of the fixed subsample taken for counting, and N denotes the number of vitellogenic oocytes in the subsample. The term W_o/W_S is defined as the raising factor. Realized fecundity (F_R), defined as the number of eggs (fully developed cells) spawned in the current season, was calculated as $F_R =$

Fig. 3. Relationship between length and weight of the experimental fish at the end of the experiment (January 19, 1995). Also shown are data for wild fish caught in early February 1996 in the same area where the experimental fish were taken.



$F_P - N_{res}$, where N_{res}, the number of oocytes resorbed due to atresia, is given by

$$(2) \qquad N_{res} = F_P \cdot A \cdot D/T$$

where A is the relative atretic intensity, T is the turnover rate (i.e., life span in days of an early α-stage oocyte), and D is the estimated number of days from the sampling date until the assumed final (maximum) oocyte diameter is reached. Relative potential fecundity (RF_P) and relative realized fecundity (RF_R) were defined as $F_P/(W - W_g)$ and $F_R/(W - W_g)$, respectively.

Estimations of F_P and F_R

Estimates of F_P were made for fish in Hjort maturity stages 3–5 collected in January 1995. All estimated F_P values were finally corrected for cumulative atresia up to the expected time of spawning, thus indicating F_R. These calculations were all based on specimen-specific A and D values but a general T value. Variable A was assumed to be constant during the period from sampling until spawning for each individual fish (see Discussion).

To decide the minimum ovarian subsample weight needed to achieve stable counts prior to starting on the main F_P study, three subsamples of about 100, 150, and 200 mg, respectively, were taken from the middle part of the right ovary lobe of each of three specimens. Before weighing the ovaries, excess moisture was removed using absorbent paper. The weights of the preserved ovaries and the subsamples were recorded to the nearest milligram. Counts were made separately for each subsample size. The results showed no consistent trend and only minor differences (<2%) between the highest and lowest F_P recorded within a fish. Therefore, in the main study, a conservative subsample weight of 150 mg was used and two subsamples were taken from each fish at the middle part of the right lobe.

The turnover rate T in eq. 2 was set at 10 days assuming an experimental water temperature of 8°C. This value was based on the following estimates: 7–9 days (16°C) in northern anchovy (*Engraulis mordax*) (Hunter and Macewicz 1985), 10–13 days (8°C) in Atlantic cod (Kjesbu et al. 1991), and 7.5 days (temperature not given) in Atlantic mackerel (*Scomber scombrus*) (Anonymous 1993).

The variable D in eq. 2 was deduced from the following oocyte growth model. By assuming a final oocyte size (i.e., at commencement of spawning) of 1200 µm for the local Atlantic herring, a value found to vary little among years (A.-L. Agnalt, Institute of Marine Research, Bergen, Norway, unpublished data), the number of days it will take before a fish spawns can be estimated using the exponential equation

$$(3) \qquad Y = Y_{min} + ae^{bX}$$

where Y is mean oocyte diameter, Y_{min} is the minimum developing oocyte diameter, X is the number of days (starting from May 1 to the sampling day), and a and b are constants. Both diameters (micrometres) refer to fixed values in whole-mount preparations.

The available time series of diameter measurements were used in the model. Assuming the oocyte development rate being consistent between years, the diameter measurements reported in February 1994 were used to represent the same period in 1995. The model parameters were estimated using the nonlinear regression function in S-PLUS (Venables and Ripley 1994).

Results

Condition of experimental versus wild Atlantic herring

The use of ANCOVA on fish weight–length relationships at the end of the experiment (Fig. 3) demonstrated no significant differences in slope ($P = 0.08$) but in intercept ($P < 0.001$) (log–log transformed data). The latter result was due to the low-ration fish; the high-ration group was not significantly different (Tukey, pairwise comparative probability, $P = 0.08$) from the wild fish, while weight-at-length for the low-ration group was markedly lower ($P < 0.001$). The variance in weight within a length-class was large for both groups, but only a few fish of the high-ration group had weight-at-length similar to that of the low-ration group.

Seasonal and temporal changes in fish length, weight, GSI, and HSI

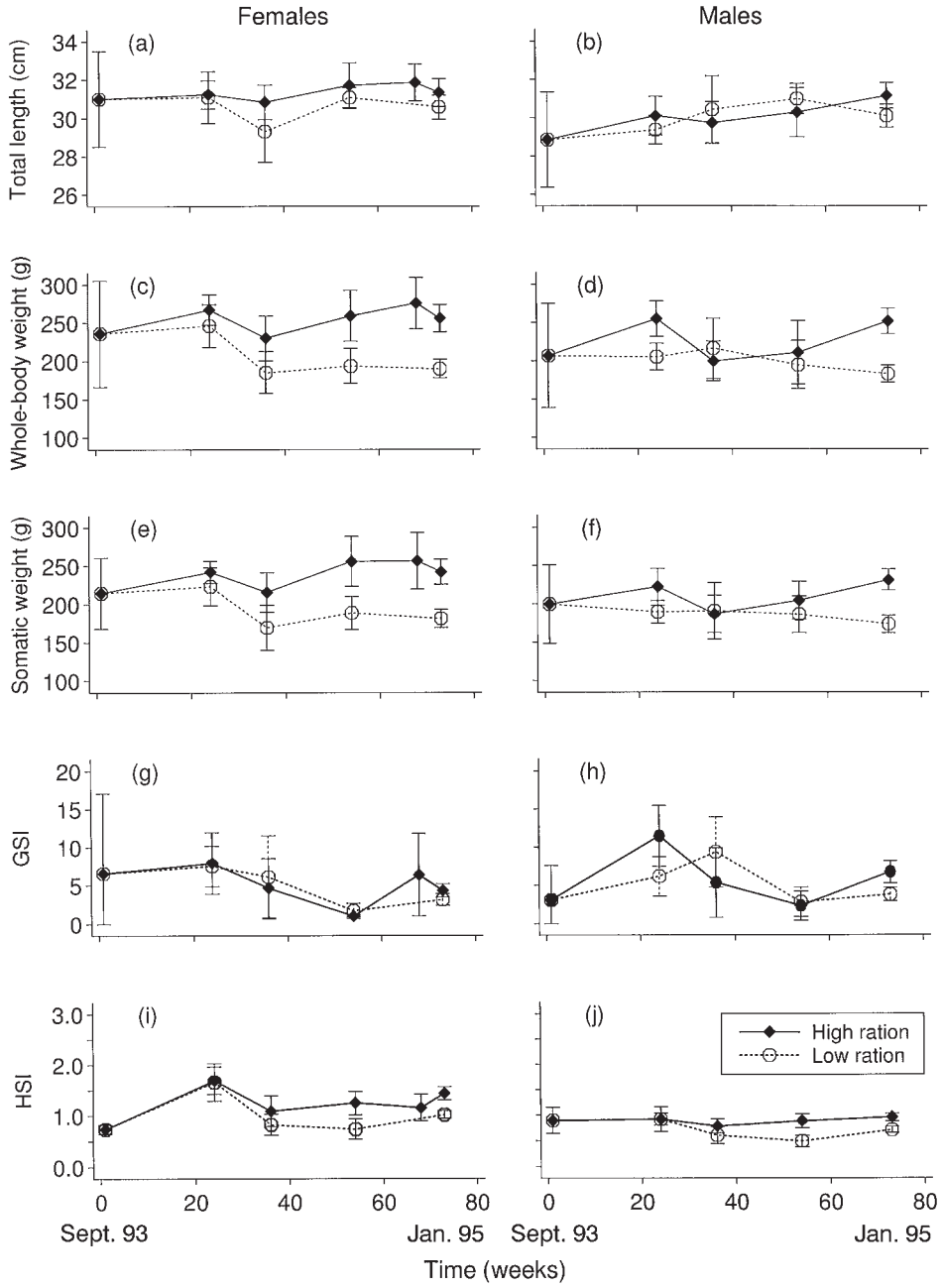
A significant overall increase in length during the experimental period was observed for all but the low-ration females ($P = 0.02$ and 0.008 for high-ration females and males, respectively, and 0.007 for low-ration males) (Figs. 4*a* and 4*b*). The estimated overall growth increment during the experimental period was 2–2.5 cm. Only at the end of the experiment was there evidence (*t*-test, $P = 0.02$) of a ration effect on mean length, and only for males.

The time series of whole-body and somatic weight (Figs. 4*c*–4*f*) showed indications of a seasonal cycle for the high-ration group. Females of the low-ration group showed a similar trend to that of the high-ration group for the first spawning season in captivity, although the weight loss was larger. However, contrary to the high-ration group, they did not regain this weight loss and from May 1994, there was evidence (*t*-test, $P < 0.05$) for a lower weight (both somatic and whole-body weight) of the low-ration group than that of the high-ration group. For the low-ration males the somatic and whole-body weight decreased marginally and no seasonal pattern was found.

There was evidence for a difference in GSI between the two ration groups of females at the last sampling date (*t*-test, $P = 0.04$) but not elsewhere ($P > 0.20$) (Fig. 4*g*). For males the difference in GSI was significant in both February 1994 ($P = 0.004$) and January 1995 ($P = 0.001$) (Fig. 4*h*). The data indicated that the seasonal maximum for males is reached later for the low-ration than for the high-ration fish.

Females of both groups had higher HSI than males (*t*-test, $P < 0.05$), except at the start of the experiment and in May 1994 (Figs. 4*i* and 4*j*). There were indications of a seasonal cycle in HSI for females. For males, this pattern was less clear (low-ration group) or nonexistent (high-ration group).

Fig. 4. Temporal changes in total length, whole-body and somatic weight, GSI, and HSI of the experimental fish and a comparison between the two ration groups and sexes. Mean values and 95% confidence limits are given.



Evidence for a ration effect on the values of the HSI was found for both sexes in the September 1994 (females: $P = 0.005$; males: $P < 0.001$) and January 1995 samples (females: $P < 0.001$; males: $P < 0.001$), i.e., after the first spawning season.

Oocyte diameter

Whole-mount diameter measurements under the binocular microscope (Y_{bino}) were closely correlated with those reported histologically with the image analyzer (Y_{image}):

(4) $Y_{bino} = -4 + 1.090 \cdot Y_{image}$ ($n = 49$, $r^2 = 0.984$, $P < 0.001$, $180 < Y_{image} < 1150$ μm).

The process of dehydration, embedding, and sectioning on average resulted in a total shrinkage of 7%. There were no indications of any oocyte size-specific differences in this degree of shrinkage.

As maturation advanced, i.e., vitellogenic oocytes examined under the binocular microscope grew in mean diameter from about 375 to 925 μm, the gap between the size–frequency distributions of vitellogenic oocytes and previtellogenic oocytes gradually increased from about 100 to 550 μm. For fish with mean vitellogenic oocyte diameter above 550 μm, the maximum previtellogenic oocyte diameter remained stable at 240 μm. In the less advanced maturity stages (mean vitellogenic oocyte diameter from 375 to 550 μm), it was estimated at 250 μm. From histological sections the maximum

Fig. 5. Comparison of mean vitellogenic oocyte diameter at whole-body weight between the first and second year in captivity for the two ration groups.

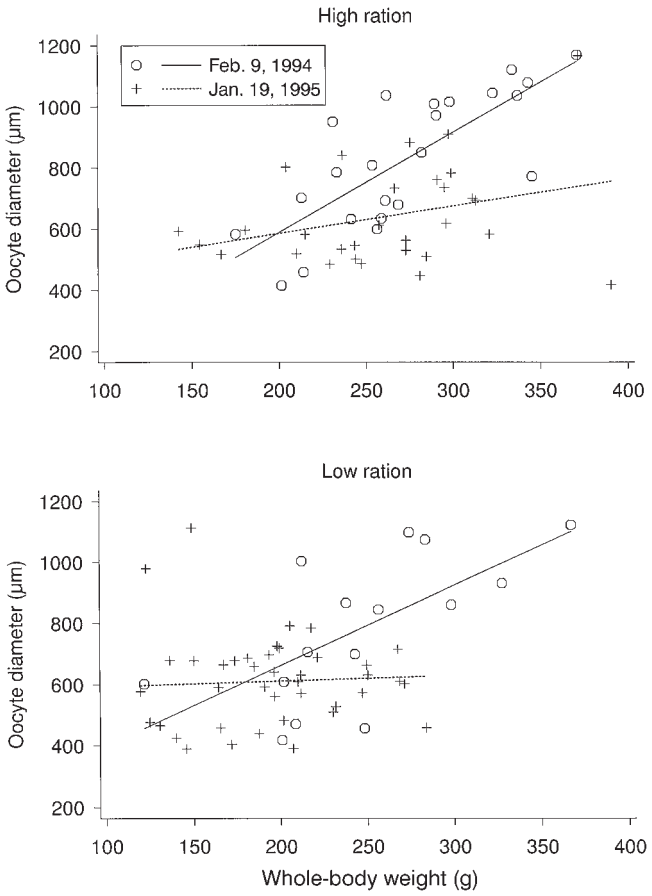
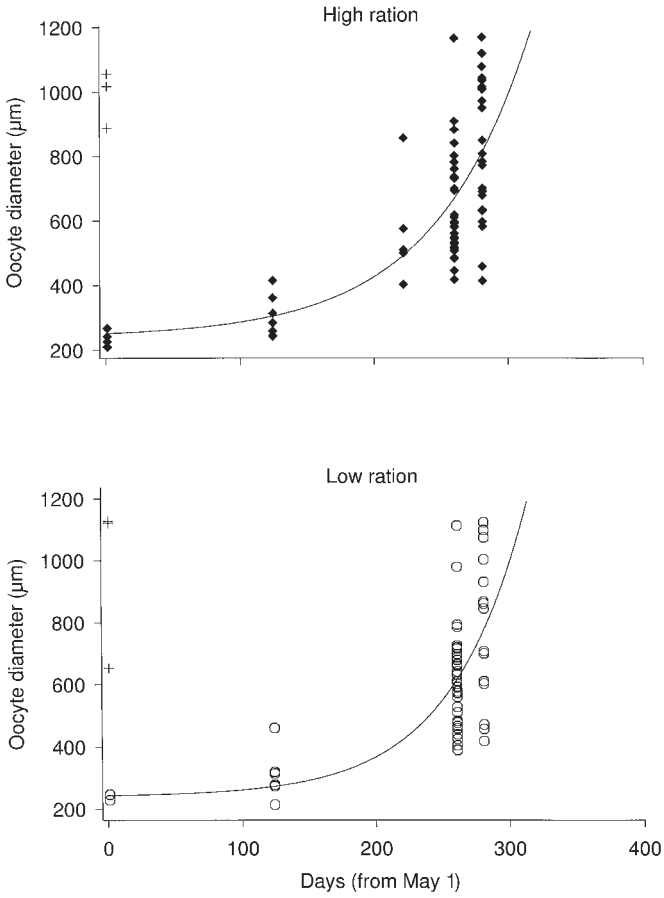


Fig. 6. Oocyte growth curve for the two ration groups. Fish excluded from the analysis are indicated by plus signs.



previtellogenic oocyte diameter was estimated at 230 µm after correcting for shrinkage using eq. 4. Thus, oocytes above 240 µm were considered developing.

There was no strong evidence for a relationship between whole-body weight or total length and mean vitellogenic oocyte diameter based on the data collected in January 1995, either for the high- or the low-ration group (Fig. 5). Omission of extreme values did not change this conclusion. Moreover, there was no evidence for a difference in oocyte diameter of the two ration groups. However, by studying the data collected 1 year earlier, in February 1994, oocyte diameter was found to increase with increasing weight for both groups (high ration: $r^2 = 0.59$, $P < 0.001$; low ration: $r^2 = 0.43$, $P < 0.01$) and also with increasing total length for the high-ration group ($r^2 = 0.34$, $P < 0.01$).

Oocyte growth pattern

Using a minimum developing oocyte diameter of 240 µm, the estimated growth curves were

$$(5) \quad Y = 240 + 11.59 \cdot e^{0.014x}$$

for the high-ration group and

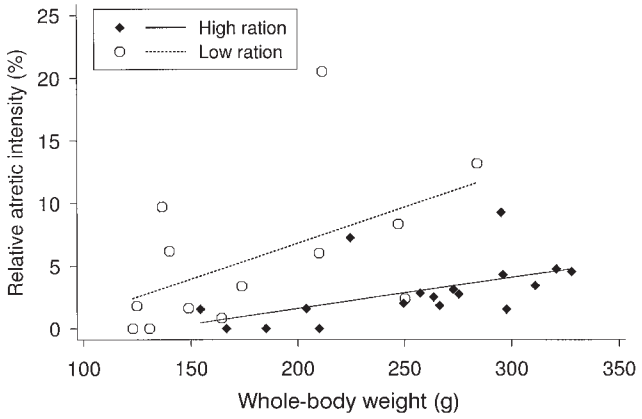
$$(6) \quad Y = 240 + 3.67 \cdot e^{0.018x}$$

for the low-ration group (Fig. 6). The variation in oocyte diameter was very large in May (i.e., at the starting point) due to the mixture of spent and spawning fish. For the purpose of studying the developing oocyte growth pattern, the maturing and spawning fish found in this sample were excluded. Nevertheless, the among-fish variation in oocyte diameter was considerable. However, the estimated time needed to reach the assumed final oocyte diameter of 1200 µm was found to be very similar for the high- and low-ration females: 318 and 314 days, respectively.

Level of atresia

ANCOVA showed relative atretic intensity to increase with total length ($P = 0.007$) and whole-body weight ($P = 0.007$) (Fig. 7), but there was no evidence for a difference in slopes between groups ($P = 0.582$ for length as covariate, $P = 0.237$ for weight as covariate). The analysis further showed evidence for an overall higher level of atresia in the low-ration than in the high-ration group ($P = 0.021$ with length as covariate, $P = 0.003$ for weight as covariate). The above conclusions were not changed by excluding the notable outlier for the low-ration group. The average relative atretic intensities for the high- and low-ration groups were 3.0 and 5.7%, respectively. No evidence for a relationship between oocyte diameter and relative atretic intensity existed based on separate correlation analyses of fish from the high-ration group ($P = 0.581$) and the low-ration group ($P = 0.389$). Inclusion of fish condition measured

Fig. 7. Relationship between whole-body weight and relative atretic intensity for the two ration groups.



as SCF failed to significantly improve the understanding of within-group variation in atresia.

Fecundity analysis

Within each of the two ration groups, F_P and RF_P were found to be independent of whole-body weight (Fig. 8), total length, and SCF. The experiment provided strong evidence for higher F_P in the high- versus low-ration group (t-test, $P = 0.002$) (Table 2). However, RF_P was not found to differ significantly between the two groups (t-test, $P = 0.940$).

The F_R (i.e., after correction for atresia) was not significantly correlated with total length or whole-body weight for either of the two ration groups. High-ration fish had higher F_R than low-ration fish (t-test, $P < 0.001$), but there was no clear statistical evidence for a difference in RF_R between the groups (t-test, $P = 0.212$), although the low-ration group had a lower average value than the high-ration group (Table 2). The F_R was significantly lower (40% reduction) than F_P for fish in the low-ration group ($P < 0.01$), but no clear difference was found for the fish in the high-ration group (9% reduction). For the two low-ration fish with the highest relative atretic intensities (13.2 and 20.5%) the calculated F_R was zero (negative), i.e., all vitellogenic oocytes were likely resorbed before spawning. Both fish were in extremely poor condition.

Discussion

Two phenomena likely attributable to the impoundment and feeding regime were discernible. First, compared with the wild situation, the present way of feeding obviously reduced within-year variation in accumulated body reserves; wild Atlantic herring store significant amounts of body reserves (lipids) in the summer peak feeding period for subsequent use during winter fast (Henderson and Almatar 1989). Nevertheless, for the high-ration group, weight-at-length in January was comparable with that of wild fish across the whole length range. Second, a significant relationship between vitellogenic oocyte size and fish size was found based on the data collected in February 1994, i.e., after about half a year in captivity. However, at a similar stage in the following maturation cycle, no such relationship was found. Oocyte diameter is generally found to increase with fish size, and large fish spawn earlier than smaller ones (Lambert 1987; Ware and Tanasichuk 1989). It is suggested that this trend in wild fish is caused by fish of different sizes experiencing slightly different environmental conditions (e.g., temperature). Therefore, they mature at different rates (Kjesbu 1994). However, under identical environmental conditions, the fish size effect on oocyte size should become undetectable, as noted.

Fig. 8. Relationship between whole-body weight (W) and potential fecundity (F_P) for the two ration groups and wild Atlantic herring caught in the same geographical area as the experimental fish (A.-L. Agnalt, Institute of Marine Research, Bergen, Norway, unpublished data). The linear regression between F_P and W for the combined high-ration and wild Atlantic herring data was $F_P = 19\,046 + 267.7 \cdot W$ ($r^2 = 0.415$, $P < 0.001$).

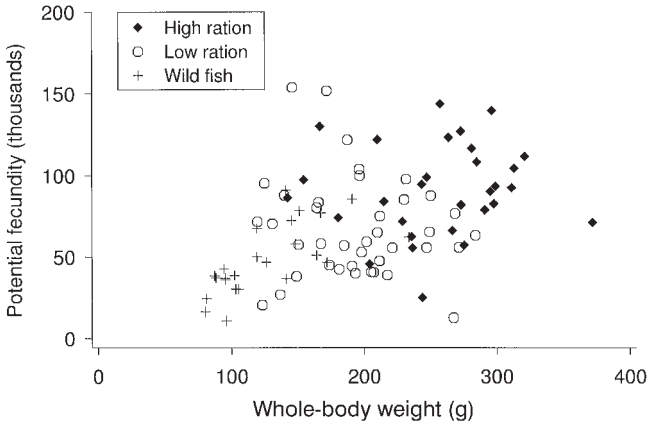


Table 2. Mean and standard error (in parentheses) of potential fecundity (F_P), realized fecundity (F_R), relative potential fecundity (RF_P), and relative realized fecundity (RF_R) for the two ration groups in the January 1995 sample.

Group	F_P (thousands)	F_R (thousands)	RF_P (g^{-1})	RF_R (g^{-1})
High ration	91.2 (5.2)	83.0 (7.8)	373 (27)	352 (52)
Low ration	67.2 (5.0)	40.4 (7.9)	369 (34)	254 (55)

Lasting for 1.5 years, this study clearly demonstrated that Atlantic herring can maintain a normal development on an average diet of 12 mg wet weight·g fish^{-1}·day^{-1} (mean whole-body weight of high-ration fish was about 250 g and food intake was 3 g·fish^{-1}·day^{-1}). In contrast with other studies, this experiment covered the full maturation cycle. The estimate is lower than the indirect maintenance ration of 17 mg·g fish^{-1}·day^{-1} for Pacific herring calculated by Hay et al. (1988) and comparable with the lower range of the maintenance estimates (9.1–28.6 mg·g fish^{-1}·day^{-1}) for Atlantic herring found by Blaxter and Holliday (1963). However, estimates of required food rations based on controlled laboratory experiments may not fully apply to natural conditions, especially as wild Atlantic herring tend to undertake annual migrations which would increase their metabolic requirements. Actual food composition and ambient temperature may also be different. The present feeding regime of 5 days per week instead of 7 days per week is believed to have had minor impact. The gastric evacuation rate for krill is in the order of about 2 days at the ambient temperature, extrapolated from data on copepods assuming an evacuation constant for krill half that of

copepods (Temming 1995; A. Temming, personal communication). Thus, our Atlantic herring did not likely suffer from periodic starvation. Based on the comparison of the length–weight relationship between the experimental fish and wild fish, the present estimate is assumed to give a realistic value of the required food requirements of wild Atlantic herring.

The low-ration group showed clear signs of inadequate food. For females belonging to this group, the fall in somatic weight following the first spawning in captivity was larger than for the high-ration females. Also, the ration fed was apparently insufficient to subsequently restore their somatic energy deposits depleted for reproductive investment. The low-ration males showed no seasonal pattern in somatic weight at all, but an overall weak decline. Investment in reproduction is generally larger for females than for males of the same size (Wootton 1990). This may explain why low-ration females were the only group to show no overall change in mean length. The temporal pattern of HSI supports these conclusions.

Presented female data on GSI, oocyte growth, and final mean vitellogenic oocyte size indicated no significant effect of ration size on time of spawning. GSIs for males indicated, however, delayed spawning for males within the low-ration group. During the late maturity stages (February 1994), GSI of males was significantly higher than that of females. Testes growth precedes ovary growth and males mature earlier than females (Iles 1964; Ware and Tanasichuk 1989). In contrast with our observations, Gillis et al. (1990) found that the mean GSI values for males were always lower than those for females from the time of population ovulation until the end of the spawning season. This might indicate that the maximum GSI for females may be underestimated. As pointed out by Gillis et al. (1990), GSI increases rapidly during ovulation, and more frequent sampling would have been needed to determine the GSI pattern at this time. For spring spawners, oocyte development, starting somewhere between May and September (Bowers and Holliday 1961; Iles 1964; Ware and Tanasichuk 1989), has been found to slow down or halt during part of the winter (Iles 1964). Thus, there might be a plateau somewhere on the oocyte growth curve. The length of the overwinter period differs among stocks. Besides, herring have been shown to be able to hold off spawning for several days, perhaps awaiting environmental cues (Iles 1964; Ware and Tanasichuk 1989). Thus, the present oocyte growth curve might fail to determine the exact spawning time. Despite this, it still should serve the purpose of a comparison of the two ration groups. Considering the large among-fish variation in oocyte diameter, the difference in estimated time at spawning is marginal.

The low-ration fish had a significantly lower F_P than the high-ration fish. RF_P was not different, which can be explained by the much lower somatic weight of the low-ration fish. It thus appears that the number of developing oocytes is determined by the nutritional status of the female. This statement is confirmed for a wide range of fish species (Bromage et al. 1991; Karlsen et al. 1995). Food restriction might also produce greater stress on the fish through increased competition, and this stress may lead to lower fecundity through changes in endocrine activity (Bagenal 1969). The F_P was unrelated to fish size in both ration groups. However, when similar data from samples of wild fish collected in the same geographical area (Fig. 8) were included in the analysis, this variable was significantly related to whole-body weight. Thus, given the large variation in potential fecundity-at-size, a larger contrast in size was needed to show the relationship that is clearly found in wild specimens (Almatar and Bailey 1989).

The histological analysis of the data collected at the end of the experiment indicated that atresia was a common phenomenon in these Atlantic herring. The low-ration fish had on average twice as high relative intensity (5.7%) of atresia as the high-ration fish (3.0%). The variation within each group was large, especially within the low-ration group. The significantly higher mean value in the low-ration fish is in agreement with other studies showing a progressive increase in atresia as the ration decreases below an optimal level (Bagenal 1969; Tyler et al. 1990; Kjesbu et al. 1991). An increase in atresia with fish size was also noted in both ration groups. The reason for this is unclear. When calculating F_R, relative intensity of atresia from the time of sampling until final maturation was assumed constant and the turnover rate set at 10 days. No relevant detailed study of the variation in relative intensity of atresia throughout the maturation cycle is known to the authors. However, no indications for a significant relationship between maturity stage (oocyte diameter), condition, and relative atretic intensity were found based on the individuals sampled in January 1995, even though these showed a wide range of oocyte sizes. Thus, the assumption of a constant relative intensity within the period of interest seems warranted but, obviously, further studies are needed on this topic as well as determination of the atretic turnover rate for Atlantic herring.

For the high-ration group, estimated mean F_R was 9% lower than the similar F_P as estimated at the end of the experiment. Due to the large variation in both potential fecundity-at-size and relative atretic intensity, the reduction was not significant. The reduction for the low-ration group was as high as 40%. The difference between RF_P and RF_R for the low-ration group indicated that the low-ration fish had to get energy by resorbing some vitellogenic oocytes to maintain normal development of the remaining oocytes. Although atresia was not an extensive phenomenon in their study, Hay and Brett (1988) observed a 20% reduction in mean vitellogenic counts during a 3-month period prior to spawning.

In summary, this study suggests a maturation strategy whereby Atlantic herring at the beginning of the maturation cycle start out with a number of developing oocytes that is in excess of what the fish can bring to full maturation and the fish will later resorb some vitellogenic oocytes and likely reallocate a part of this energy to other growing oocytes. At the group level the relative intensity of atresia varies negatively with food ration, but due to large variance, any negative effect of condition could not be shown statistically at the individual level. Size and growth rate of developing oocytes do not seem to be significantly affected by the nutritional status of the fish. Further laboratory studies are needed to determine the effect of within-year changes in ration size, particularly during the summer-recovering, peak feeding period, on energy allocation and potential fecundity coupled with a detailed monitoring of temporal variation in relative atretic intensity.

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