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**ORIGINAL ARTICLE**

# Effects of female spawning experience and larval size on feeding and growth of cod larvae *Gadus morhua* L. reared in mesocosms

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**Abstract**

The viability, defined in terms of growth and feeding success, of cod larvae from first-time and repeat spawners of similar sizes was studied in two mesocosms. The growth rate of larvae from first-time and repeat spawners were similar from hatching through to 41 days old, when the experiment was terminated. Larvae from first-time spawners were heavier throughout the experiment than larvae of repeat spawners, a difference that might be explained by a difference (although non-significant) in egg size between the two groups. There was a significant difference in growth rate between the two mesocosms, which may be explained by the difference in temperature between the mesocosms. In both mesocosms larvae fed mainly on copepod nauplii during the first weeks of life and started to actively select copepodites at age 20 days post hatch. There was no significant difference in the energy content of the ingested prey at a given age between offspring of first-time and repeat spawners, although there was a significant difference between the two mesocosms. The dry mass of the ingested prey items compared to larval dry mass increased until age 17–27 days post hatch and decreased thereafter.

**Key words:** *Maternal effects, feeding success, fish larva, microsatellites, temperature-dependent growth, energy content*

## Introduction

The quality of fish larvae of first-time versus repeat spawners is an important issue both in fishery management and for the aquaculture industry. In 1973, Ponomarenko noted that in years where first-time spawners dominated the broodstock the likelihood of a strong year-class appearing was small. He hypothesized that poor year-classes may be produced in such years due either to a narrower distribution of offspring in time and space or a low quality of offspring from first-time spawners.

One way in which female spawning experience can influence offspring is through the size of the eggs. The increase in egg size with increasing size and age of female spawners has been well documented for a number of species (see reviews by Brooks et al. 1997; Solemdal 1997; Trippel et al. 1997; Kamler 2005) and for different cod, *Gadus morhua* Linnaeus, 1758, populations (Chambers & Waiwood 1996; Kjesbu et al. 1996; Vallin & Nissling 2000; Marteinsdottir & Begg 2002). Female age or size might also influence the chemical composition of eggs which in turn may lead to differences in larval performance. Most studies of the effect of maternal age and size on offspring quality have focused on egg size and only a few investigators have linked maternal age or size to other factors such as offspring survival or growth. Berkeley et al. (2004) found that older females of *Sebastes melanops* Girard, 1856 gave rise to larvae with a growth rate three times higher than those of the youngest females, and suggested that these differences were caused by the larger oil globule volume of larvae from the oldest females. This study indicates that maternal age may influence

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larval energy reserves, which in turn may lead to notable differences in growth.

Starvation and predation are the major causes of mortality during early life stages (Hunter 1981; Leggett & Deblois 1994), hence the ability to search for and successfully capture food is of the utmost importance in order to survive the critical larval stages (Cowan et al. 1997; Pepin & Penney 1997). It is generally believed that a larger size at hatch will be favourable, as a larger larva will have a larger yolk supply, better searching and swimming ability (Miller et al. 1988; Cowan et al. 1997), and a larger gape size will allow the larva to capture larger food items (Economou 1991; Knutsen & Tilseth 1985; Rowlands et al. 2006). Furthermore, Kamler (2005) concludes in her review that embryonic survival is dependent on the nutritional composition of the eggs. Houde & Scheckter (1980) observed that some individuals were able to capture twice as much prey as the average larva, and suggested that only 'exceptional larvae' manage to capture a sufficient amount of food during their first days of life to survive in the natural environment. Based on these findings and observations we consider feeding success a suitable indicator of larval viability.

In order to study the effect of female spawning experience on larval feeding, growth and survival, larvae from first-time and repeat spawning females were reared in a common environment. Two earlier studies from the same mesocosm experiment have reported the long-term effects of parental origin on growth and condition of cod larvae (Clemmesen et al. 2003; Paulsen et al. in press). In this study, feeding success measured as gut content (number, size and type of prey items) and growth were studied from hatching through metamorphosis. This design formed the basis for testing the null hypothesis of no difference in viability between larvae of first-time and repeat spawners.

## Materials and methods

The experiments were conducted in two mesocosms located at Flødevigen, Norway during spring of 2000. The first mesocosm (M1) had a volume of 2500 $m^3$ and the second mesocosm (M2) had a volume of 4400 $m^3$. Both mesocosms were emptied prior to the experiment in order to remove predators and refilled with seawater. The larvae fed upon the natural production of phyto- and zooplankton in the mesocosms and no additional food supplements were given. Water was exchanged during the experiment with the nearby fjord at a rate of 6% $day^{-1}$ in M1 and 1% $day^{-1}$ in M2.

North-East Arctic cod were captured in the Barents Sea in 1998 to provide the broodstock. After capture the broodstock was placed in sea cages at Parisvatn Field Station located in Norway at Bergen. The maturity status of each female was determined by biopsy during the 1999 spawning season. Females that were immature in 1999 and spawned in 2000 were described as first-time spawners and females that spawned in 1999 and 2000 were repeat spawners. Before the 2000 spawning season, 13 first-time and 13 repeat spawning females of a similar size were placed in separate spawning compartments and crossed with randomly chosen males (Table I). Egg production was monitored during the spawning period, and when the eggs reached a suitable quality and size, batches were collected from each spawning pair and incubated in separate incubators. The diameter of the eggs in each batch was measured. The batches used in the experiment hatched within the time period between 27 March and 30 March, and thus 28 March was chosen as age 0 days post hatch (DPH) for all larvae. Where available, 4000 larvae from each family were released in M1 and 8000 in M2. If less than 12,000 larvae were available from a family, M1 was prioritized. Altogether 41,128 larvae of first-time spawners and 41,157 larvae of repeat spawners were released in M1, and 68,764 larvae of first time spawners and 65,550 larvae of repeat spawners in M2. A more detailed description of the mesocosms, the broodstock and the families is given in Clemmesen et al. (2003).

Temperature was measured daily, and oxygen and salinity weekly at 0, 0.5, 1, 2, 3 and 4 m depths. Samples of zooplankton were collected twice a week at the same depths by filtering 80 l of water pumped from each depth into a plankton net with a mesh size of 90 μm. The zooplankton samples were analysed under a dissecting microscope. The species, stage and size of the plankton were determined, and species that accounted for less than 1% of the total abundance in both mesocosms were counted as 'others'.

Table I. Size of broodstock and egg diameter of first time and repeat spawners.

| | First-time | Repeat | *t-test* *P*-value |
|---|---|---|---|
| Mean female length (cm) | 79.6 (SD 5.7) | 80.3 (SD 4.91) | 0.37 |
| Mean female K | 1.25 (SD 0.11) | 1.24 (SD 0.23) | 0.46 |
| Mean male length (cm) | 79.6 (SD 5.7) | 80.1 (SD 4.02) | 0.44 |
| Mean male K | 1.16 (SD 0.17) | 1.19 (SD 0.19) | 0.34 |
| Mean egg diameter (mm) | 1.47 (SD 0.08) | 1.43 (SD 0.05) | 0.21 |

The spawning experiences of the males are unknown and they are grouped according to the spawning experience of their mates.

Larvae were sampled at night between 20:00 and 02:30 hours to minimize net-avoidance, using a two-chambered net with a 0.3 $m^2$ opening. The net was towed at 2 m depth across the ponds at a speed of 1 m $s^{-1}$. Samples were taken twice a week during the first three weeks and once a week thereafter until day 41. Each sampled larva was put into a separate tube with salt water and stored in a Forma Scientific −86°C Freezer. The number of larvae caught in the hauls was used to estimate survival assuming no net-avoidance.

*Analysis of larvae*

In total 288 larvae were analysed (Table II). Standard length ($L_S$), defined as the distance from the end of the notochord to the tip of the upper jaw, was measured on thawed larvae to the nearest 0.1 mm under a dissecting microscope. The larvae without the gut were then freeze-dried and the dry mass ($M_D$) was obtained using a Sartorius micro M3P microbalance with an accuracy of 1 μg. The larvae were then stored in 96% alcohol for DNA analyses. The specific growth rate ($G_S$) and daily length increase ($L_D$) at a given age was found by deriving the second-degree polynomial describing the ln $M_D$ (μg) versus Age (DPH) and $L_S$ (mm) versus Age (DPH) relationships, respectively.

The guts from the larvae were split open and the content analysed under a dissecting microscope. For the youngest larvae the content of the undifferentiated gut was analysed, whilst with older larvae the content of the stomach and intestine was analysed. In the following text 'gut' will be used to describe both the undifferentiated gut and the stomach and intestine. The number of prey items in the gut was counted. Adult copepods were identified to species level and the cephalothorax lengths ($L_C$) were measured to the nearest 0.1 mm. Copepodites were identified to the level of order and the $L_C$ was measured to the nearest 0.1 mm. Copepod nauplii were not assigned to species, but the total lengths ($L_T$) were measured to the nearest 0.1 mm. In addition to copepods some gastropods ( $<0.2$ mm) and rotifers were found, but the identity of the species was not determined. The total energy content in the gut (later referred to as 'energy content') was estimated using energy conversion factors for copepods (Blom et al. 1991). Rotifers, gastropods and harpaticoid copepods were excluded from the energy estimates as they numerically accounted for less than 0.5% of the gut items. As *Centropages hamatus* accounted for more than 99% of the adult copepods in the guts, all nauplii and copepodites were counted as *C. hamatus* when estimating the energy content. The gut index ($I_G$) was defined as:

$$I_G = (M_{DG} * 100)/M_{DL}$$

where $M_{DG}$ is the calculated dry mass of the gut and $M_{DL}$ is the larval dry mass. The gut filling rate was measured by eye, using a scale in 10 steps from 0 to 1, where 0 represents empty gut and 1 full gut. In order to study the larvae's selective predation, Chesson's α was calculated for copepod nauplii, calanoid copepodites, *C. hamatus* and harpacticoids. It was assumed that larval feeding on a given day did not influence the zooplankton concentration and hence α could be estimated as the maximum likelihood estimator (Chesson 1978, 1983).

$$\alpha i = \frac{\frac{ri}{ni}}{\sum_{j=1}^{m} \frac{rj}{nj}}, i = 1, \ldots\ldots, m \text{ (Chesson 1987)}$$

where $r_i$ is the number of items of food type $i$ in the larval diet, $n_i$ is the number of food type $i$ in the environment and $m$ is the number of food types in the environment. If α is equal for all prey types, that is $\alpha = 1/m$ for all prey types, no selection has taken place, but if α is higher than $1/m$ for any prey type, it follows that this type has been selected by the larvae.

Table II. Number of offspring of first-time and repeat spawners of family identified larvae in M1 and M2.

| | | M1 | | M2 | |
|---|---|---|---|---|---|
| Date | Age (DPH) | First-time | Repeat | First-time | Repeat |
| 3 April | 6 | 11 | 7 | 8 | 15 |
| 6 April | 9 | 9 | 10 | | |
| 7 April | 10 | | | 12 | 5 |
| 10 April | 13 | 7 | 13 | 4 | 9 |
| 13 April | 16 | 12 | 10 | 5 | 4 |
| 17 April | 20 | 6 | 12 | 12 | 11 |
| 24 April | 27 | 15 | 6 | 11 | 8 |
| 1 May | 34 | 5 | 1 | 6 | 13 |
| 8 May | 41 | 10 | 12 | 8 | 11 |
| Total | | 75 | 71 | 66 | 76 |

Genetic microsatellite markers were used to find the family identity of each larva (Wright & Bentzen 1995; Hutchinson et al. 2001) using a combination of the following loci: Gmo8, Gmo19, Gmo2 and Gmo34 (Brooker et al. 1994; Miller et al. 2000).

One-way ANCOVAs (analysis of covariance) were used to test for differences between offspring of first-time and repeat spawners in each mesocosm for the following relationships: mass-at-age, length-at-age, length-at-mass, energy-at-age and energy-at-mass. All comparisons included larvae from all sampling days. If the relationship to be tested was non-linear, residual analyses of common relationships were performed. The residuals of offspring of first-time and repeat spawners were then compared using a one-way ANCOVA to detect linear trends or mean differences in residuals. The level of significance was set to $P<0.05$ in all analyses.

The number of survivors was estimated by multiplying the average number of sampled larvae per haul by the total volume of the mesocosm divided by the sampled volume. The instantaneous daily mortality rate ($Z$) was calculated as:

$$Z = \frac{\ln N_1 - \ln N_2}{t2 - t1}$$

where $N_1$ is the estimated number of larvae at time 1 (DPH) and $N_2$ is the estimated number of larvae at time 2 (DPH). $N$ was calculated by an exponential regression fitted to the estimated number of larvae in the mesocosms at each sampling day.

## Results

### *Environmental conditions*

The average daily temperature over the experimental period was 0.9°C higher in M2 than in M1. On the day of release, 29 March 2000, the mean temperature was 4.0°C in M1 and 4.9°C in M2, it increased throughout the experiment and reached 11.6°C in M1 and 12.5°C in M2 by the last sampling day, 8 May 2000. No stratification was observed in M1, while a marked shift in temperature between 1 and 2 m depth developed towards the end of the experiment in M2. The oxygen level was 94% or higher throughout the experiment in both mesocosms. Average salinity in M1 was 33.1 psu and 32.9 psu in M2.

The zooplankton concentration was highly variable in M1 with a minimum of 1.5 $l^{-1}$ on 10 April and a peak of 32.3 $l^{-1}$ on 2 May. The zooplankton concentration in M2 ranged from 10.6 to 30.5 $l^{-1}$ and was higher than that of M1 in the first half of the experiment, but somewhat lower towards the end of the experiment (Figure 1).

The density of copepod nauplii at the start of the experiment was lower in M1 than in M2 and varied during the experiment (Figure 1). *Centropages hamatus* was the dominating species on all sampling days. *Temora longicornis* was present in the beginning of the experiment, but was almost absent after age 30 DPH in both mesocosms. Harpacticoids, mostly *Zeus* sp., but also *Trigioptus fulvus*, were present in both mesocosms throughout the experiment (Figure 1).

### *Survival and growth*

The overall mean instantaneous mortality rate ($Z$) from the start of the experiment to the end of the experiment (41 DPH) was 0.065 in M1 and 0.050 in M2. The loss of larvae between the day of release and the first sampling day was high, and hence the estimated $Z$ for the period from age six DPH to age 41 DPH was relatively low (0.01) in both mesocosms. Of the larvae that were assigned a family, there was an even distribution of progeny from first-time and repeat spawners in M1 ($\chi^2=0.11$, $P=0.7$) and in M2 ($\chi^2=0.704$, $P=0.4$) (Table II).

There was a non-significant difference in egg size between the groups, eggs of first-time spawners being slightly larger than eggs of repeat spawners ($t$-test, $P=0.21$; Table I). Offspring of first-time spawners were significantly heavier at a given age than offspring of repeat spawners in both mesocosms (ANCOVA, $P<0.05$; Figure 2). The $G_S$ increased throughout the experiment for both groups in both mesocosms. The mean $G_S$ was higher in M2 than in M1, but was similar for offspring of first-time and repeat spawners in both mesocosms (Table III). The mean larval $M_D$ on the last sampling day was 4.6 mg in M1 and 8.5 mg in M2, and hence larvae in M2 were 85% heavier than larvae in M1 at this point.

The $L_S$ of offspring of first-time spawners in M1 increased from 4.5 mm at age 6 DPH to 15.9 mm at age 41 DPH while offspring of repeat spawners were 4.3 mm long at age 6 DPH and 15.4 mm at age 41 DPH. In this mesocosm offspring of first-time spawners tended to be longer at a given age compared to offspring of repeat spawners although not significantly so (ANCOVA, $P=0.06$), but the average $L_D$ of offspring of first-time and repeat spawners were similar (Table III). In M2 offspring of first-time spawners increased in $L_S$ from 4.3 mm at age six DPH to 18.5 mm at age 41 DPH while offspring of repeat spawners increased from 4.1 mm at age six DPH to 18.5 at age 41 DPH. Offspring of first-time spawners were significantly longer at age in this mesocosm (ANCOVA, $P<0.01$), while the average $L_D$ was the same for both groups (Table III). Larvae in M2 were 19% longer than larvae in M1 at the last sampling day.

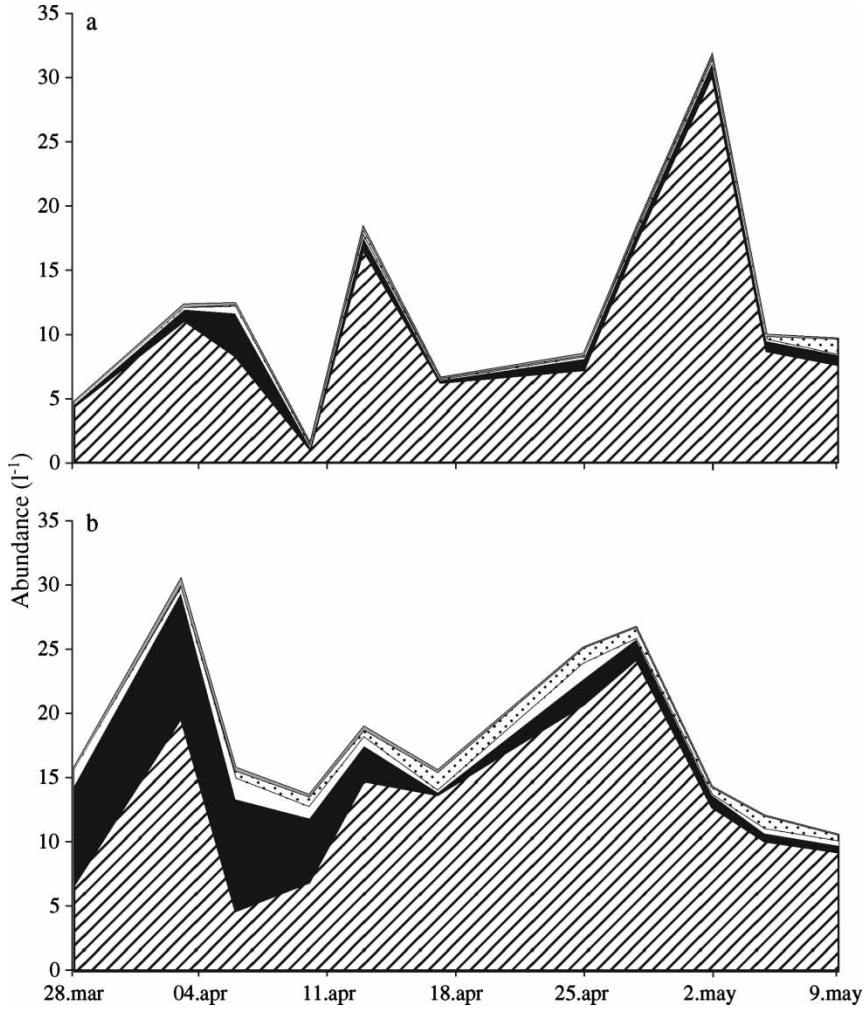


Figure 1. Mean zooplankton concentration in M1 (a) and M2 (b) throughout the experiment. ▨ copepod nauplii, ■ copepodites, □ *C. hamatus*, ⬚ harpacticoids, ▩ miscellaneous.

There was no difference in the length–mass relationship between larvae from the two mesocosms, neither was there a difference between offspring of first-time and repeat spawners in any of the two mesocosms (ANCOVA, $P > 0.6$; Figure 3), indicating a similar morphometric condition of larvae of first-time and repeat spawners.

*Feeding*

The average gut-filling rate of cod larvae at age six DPH was 0.28 in M1 and 0.52 in M2 and most larvae older than 16 DPH had full guts in both mesocosms. The larvae in M2 had a higher energy content of prey in their guts at a given age compared to larvae in M1 (ANCOVA, $P < 0.05$). There was no difference in the energy contents of prey in the guts of offspring of first-time and repeat spawners at a given age in any mesocosm (ANCOVA, $P < 0.9$). At a given mass there was a non-significant tendency that offspring of repeat spawners had a higher energy content of prey in their guts than offspring of first-time spawners in both mesocosms (ANCOVA, $P < 0.2$; Figure 4).

The number of prey items in the gut increased with increasing larval age until 27–34 DPH in both mesocosms, and declined towards the end of the experiment (Figure 5). In the beginning of the experiment (age 6–13 DPH) the larvae showed a clear preference for copepod nauplii (Chesson's $\alpha > 0.9$), but by day 20 copepodites were preferred ($\alpha > 0.8$) and at the end of the experiment (age 34 and 41 DPH) adult *C. hamatus* were chosen ($\alpha > 0.5$). The harpacticoids were seldom selected ($\alpha < 0.1$).

The mean length of prey items and the lengths of the smallest and the largest prey item found in the guts of larvae increased with increasing larval length (Figure 6). We calculated the larval gape size based on larval length/gape size relationships given by Rowlands et al. (2006). Comparing the calculated

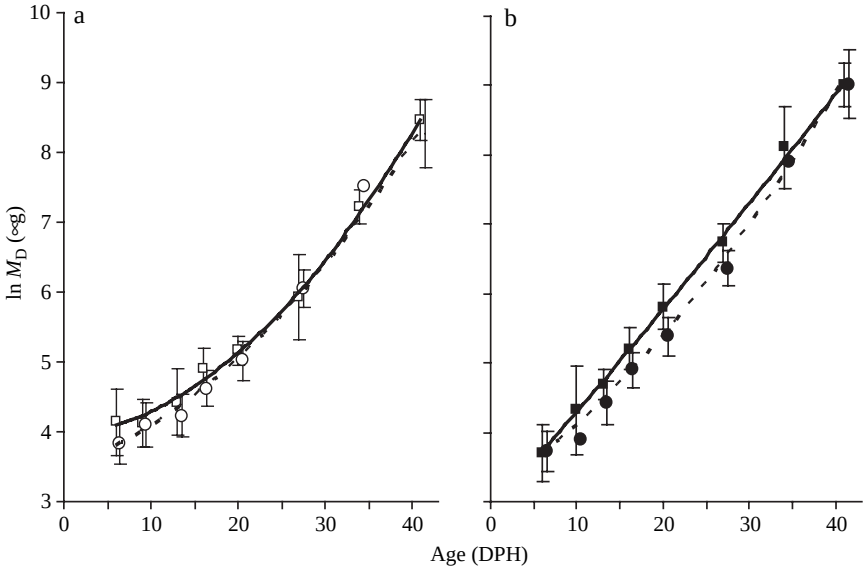


Figure 2. Weight-at-age of cod larvae of first-time (squares and solid lines) and repeat (circles and dotted lines) spawners in M1 (a) and M2 (b). Circles and squares: mean values, whiskers: ±standard deviations. The *x*-values of the boxes are shifted to make the graph more readable, but the lines are fitted to the original *x*-values. The fitted lines are: First-time M1: ln $M_D$ (μg) = 3.92 + 0.0130 * Age + 0.00238 * Age$^2$ ($R^2$ = 0.92, $N$ = 72); Repeat M1: ln $M_D$ (μg) = 3.51 + 0.0391 * Age + 0.00190 * Age$^2$ ($R^2$ = 0.96, $N$ = 69); First-time M2: ln $M_D$ (μg) = 2.88 + 0.139 * Age + 0.000289 * Age$^2$ ($R^2$ = 0.98, $N$ = 64); Repeat M2: ln $M_D$ (μg) = 3.12 + 0.0830 * Age + 0.00154 * Age$^2$ ($R^2$ = 0.96, $N$ = 75).



gape size and the ingested prey length, we found the gape size/mean prey item size to be relatively constant during the experiment ranging from 0.32 (SD 0.10) to 0.48 (SD 0.10). The relationship between the biggest prey and gape size was 0.46 (SD 0.27) at age 6 DPH. It increased steadily to 0.80 (SD 0.20) at age 16 DPH and was relatively constant from age 16 DPH to age 27 and decreased thereafter to 0.58 (SD 0.11) at age 41 DPH.

The mean $M_D$ of each prey item measured as a percent of larval $M_D$ found in the guts of cod larvae was relatively constant throughout the experiment ranging from an average of 3.5% (SD 1.9) on age 7 DPH, and gradually increasing to 5.8% (SD 1.3) at age 41 DPH. The proportion of gut $M_D$ compared to larval $M_D$ increased to 12% in M1 and 18% in M2 at age 27 DPH and decreased thereafter (Figure 7). At this age the offspring of first-time and repeat spawners were 432 μg (SD 201) and 437 μg (SD 121), respectively, in M1 and 876 μg (SD 261) and 603 μg (SD 175) in M2.

## Discussion

### *Broodstock*

The broodstock that was used in this experiment had to be captured more than one year prior to the experiment in order to determine the spawning status of the females. After one year in captivity, recruit and repeat spawners were of similar sizes. Two factors may have influenced the size of the spawners. First, they all received formulated feed, and even though they were fed moderately the feeding conditions were likely to be better than in the wild, which probably enhanced growth. Second, the females that did not spawn during their first year in captivity continued to grow during the first spawning season, while those that spawned stopped growing, or even lost weight during spawning. This may explain why first-time and repeat spawners were of a similar size. In this experiment we were thus able to compare the effect of spawning experience with no confounding size effects.

Table III. Specific growth rate ($G_S$) and daily length increase ($L_D$) for offspring of first-time and repeat spawners in M1 and M2.

| | M1 | | M2 | |
|---|---|---|---|---|
| | First-time | Repeat | First-time | Repeat |
| $G_S$ at age 6 DPH | 4.2 | 6.2 | 14.2 | 10.2 |
| $G_S$ at age 41 DPH | 20.8 | 19.5 | 16.2 | 20.9 |
| Mean $G_S$ | 12.5 | 12.8 | 15.2 | 15.5 |
| $L_D$ (mm) at age 6 DPH | −0.06 | −0.01 | 0.06 | 0.00 |
| $L_D$ (mm) at age 41 DPH | 0.69 | 0.64 | 0.74 | 0.81 |
| Mean $L_D$ (mm) | 0.31 | 0.31 | 0.40 | 0.40 |

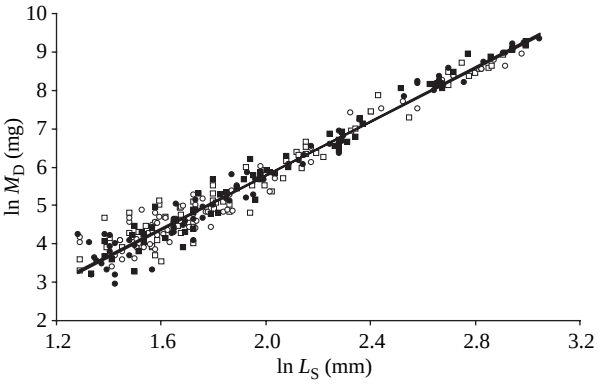


Figure 3. Weight-at-length of offspring of first-time (squares) and repeat (circles) spawners in M1 (open symbols) and M2 (filled symbols). The fitted non-discernable lines are: ln $M_D$ (µg) = –1.154 – 3.47 * ln $L_S$ (mm) ($R^2$ = 0.97, $N$ = 278).

*Survival*

The total mortality from the release of larvae to the last sampling day was high compared to other mesocosm experiments (Blom 1995), although most of this mortality seems to have taken place at the beginning of the experiment, before the first day of sampling. Blom (1995) summarized mortality rates until metamorphosis of cod in 10 different mesocosm experiments and reported a mean $z$ of 0.042 (SD 0.019). The early mortality rates observed in this experiment are higher than the average mortality reported, but within the range of previously published mortality rates (Blom 1995). This mortality occurred earlier than would have been expected if it were due to starvation, thus it may have been caused by stress from transportation and counting of individual larvae before release. However, the observed mortality from age 6 DPH to age 41 DPH was lower than the mortality reported by Blom (1995). Hence, neither starvation nor predation was likely to have influenced the larvae markedly during this period. That the larvae did not starve was further indicated by the prey densities in the mesocosms, which were never below 1.5 $l^{-1}$ and thus above the threshold of 1 $l^{-1}$ that has been reported to support growth and survival in mesocosms (Blom 1995). In addition, the average gut-filling rate was high throughout the experiment, indicating no signs of starvation. Since cannibalism has not been confirmed in larvae shorter than 12 mm (Blom & Folkvord 1997) and predators were removed prior to the experiment, predation is not likely to have influenced survival.

*Growth and feeding*

The growth of larvae was higher in M2 compared to M1, which might be explained by the difference in temperature and/or zooplankton concentration between the two mesocosms. To evaluate the effect of temperature we compared the observed growth rates with estimated growth rates in the two mesocosms. Folkvord (2005) developed a model that estimates the growth of cod larvae under favourable feeding conditions at different temperatures. We ran the model based on the observed temperatures in the

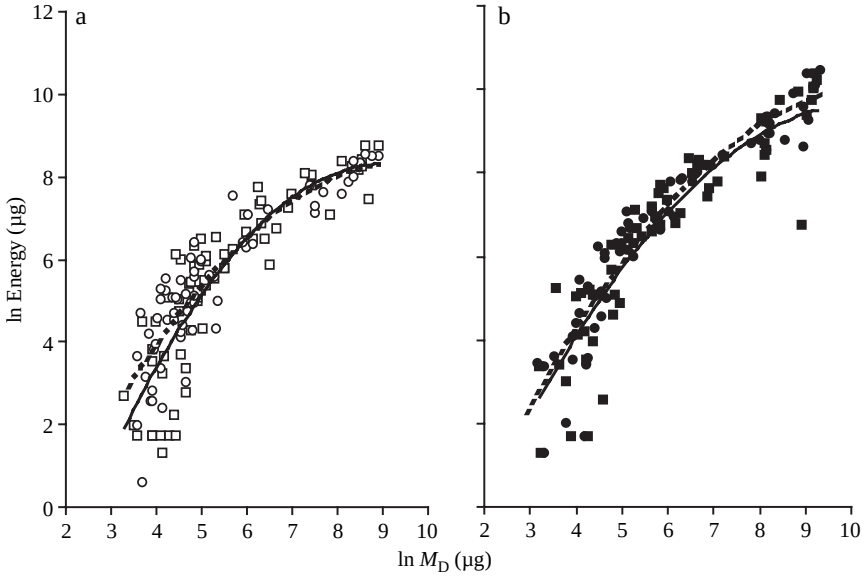


Figure 4. Energy contents in guts of cod larvae of first-time (squares and solid lines) and repeat spawners (circles and dotted lines) at a given mass in M1 (a) and M2 (b). First-time M1: ln Energy (µJ) = –9.36 + 4.10 * ln $M_D$ (µg) – 0.238 * ln $M_D$ (µg)$^2$ ($R^2$ = 0.79, $N$ = 71); Repeat M2: ln Energy (µJ) = –6.00 + 3.14 * ln $M_D$ (µg) – 0.173 * ln $M_D$ (µg)$^2$ ($R^2$ = 0.80, $N$ = 65). First-time M1: ln Energy (µJ) = –6.71 + 3.34 * ln $M_D$ (µg) – 0.172 * ln $M_D$ (µg)$^2$ ($R^2$ = 0.84, $N$ = 63); Repeat M2: ln Energy (µJ) = –5.96 + 3.13 * ln $M_D$ (µg) – 0.155 * ln $M_D$ (µg)$^2$ ($R^2$ = 0.91, $N$ = 68).

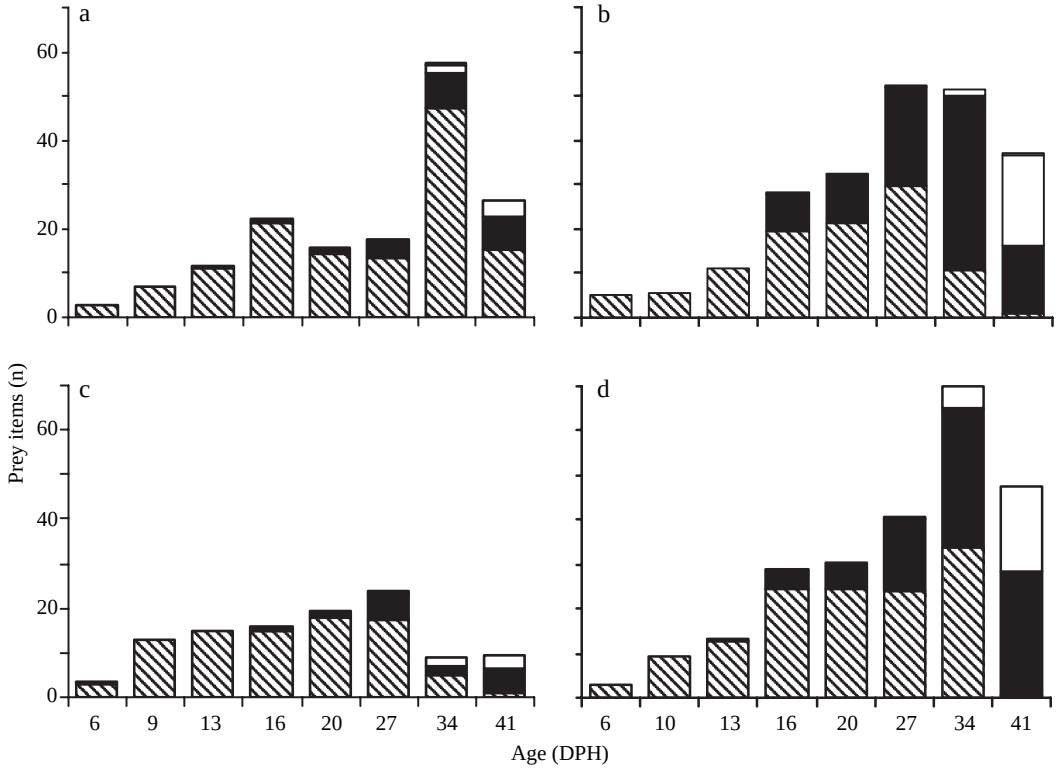


Figure 5. Average number (*n*) of prey items in the guts of cod larvae that had food in the guts (a) offspring of first-time spawners in M1 (b) offspring of first-time spawners in M2 (c) offspring of repeat spawners in M1 (d) offspring of repeat spawners in M2. ▨ copepod nauplii, ■ copepodites, □ adult *C. hamatus*, ▩ miscellaneous.

two mesocosms with an initial $M_D$ of 0.045 mg (Folkvord 2005). Larvae in M1 grew at a rate similar to the growth estimated by the model. On the last sampling day the observed $M_D$ in this mesocosm was 14% higher than the estimated $M_D$, corresponding to less than one day of growth. The observed growth in M2 was 37% higher than the estimated $M_D$ on the last sampling day, corresponding to 2 days of growth. In M2 temperatures varied with depth and when running the model with maximum temperatures we found that that the observed $M_D$ was 19% lower than the estimated $M_D$. It is known that cod larvae swim towards a preferred temperature when offered a choice (Jobling 1988), and in M2 larvae may have moved towards the maximum temperature, which would increase their growth rates.

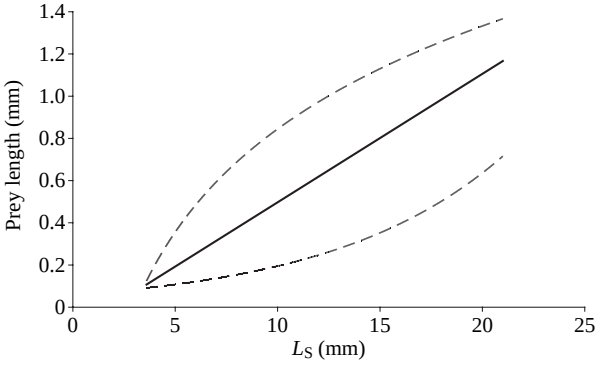


Figure 6. Length of zooplankton (mm) found in stomachs of cod of different lengths (mm). The upper dashed line shows the largest prey items found in the guts of cod, the solid line shows the mean size of prey items, the lower dashed line shows the smallest prey items. The lines are given by the following equations:
Largeprey (mm)=0.7042*ln($L_S$ (mm)) – 0.7773 ($R^2$=0.82).
Meanprey (mm)=0.0609* $L_S$ (mm) – 0.1114 ($R^2$=0.81).
Smallprey (mm) =0.0598*exp(0.1182)* $L_S$ (mm) ($R^2$=0.57).

Throughout the experiment, the zooplankton density was higher in M2 than in M1 and as such the feeding conditions were better in M2. Many factors in addition to density and size distribution are important, however, in determining the encounter rates between larvae and prey. Small-scale turbulence and light will influence the encounter rate between cod larvae and their prey (Sundby et al. 1994; Fiksen et al. 1998). In addition, the patchy distribution of zooplankton and the density of larvae will influence the feeding conditions (Blom 1995). Tilseth & Ellertsen (1984) state that feeding conditions are good when the feeding incidence (percentage of larvae with food in their guts) is >90% and the number of prey items in the guts are >3 per larvae. Following this definition, the feeding conditions were generally good in both mesocosms; however, in a parallel study of larval condition Clemmesen et al. (2003) found that the RNA:DNA ratio was lower in M1 than in M2 one week post hatch, which may indicate that food was a

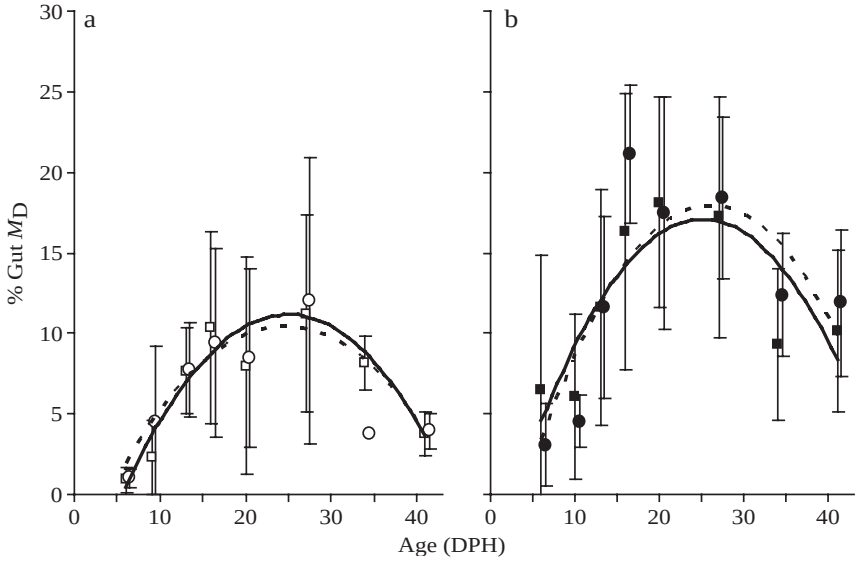


Figure 7. Estimated gut $M_D$ on larval $M_D$ in larvae of first-time (squares and solid lines) and repeat spawners (circles and dotted lines) in M1 (a) and M2 (b).



limiting factor at this time. In conclusion, the difference in temperature may explain most of the difference in growth rate between the two mesocosms, while food limitation may to some extent have influenced larval growth in M1 at the beginning of the experiment.

The difference in mass-at-age between offspring of first-time and repeat spawners might be explained by an initial small, but non-significant, difference in egg size. Although offspring of first-time spawners were heavier at a given age compared to repeat spawners, the $G_S$ did not differ. This is in line with previous results from experiments on *Melanogrammus aeglefinus* (Linnaeus, 1758), cod, and *Morone saxatilis* (Walbaum, 1792) where it has been shown that under favourable feeding conditions an initial difference in egg size prevails several weeks after hatch, but no difference in growth rates were observed (Monteleone & Houde 1990; Zhao et al. 2001; Rideout et al. 2005). The growth rate of larvae of first-time and repeat spawners are further discussed by Clemmesen et al. (2003).

In this experiment the zooplankton community was dominated by *C. hamatus* and *Harpacticoida* spp. Heath & Lough (2007) summarized 40 studies of gut content in cod larvae. They found some evidence that *C. hamatus* is not the preferred prey of cod larvae. In our experiment, however, *C. hamatus* was clearly selected in favour of *Harpacticoida* spp. Small nauplii were the dominant prey item during the first sampling days, whilst from age 20 DPH, corresponding to $L_S$ 6.5 mm, copepodites were actively selected. This finding is in line with previous studies on cod larval prey, which indicate that cod switch from nauplii to copepodites at $L_S$ 6 mm (Heath & Lough 2007). The prey size ingested by larvae was relatively smaller compared to gape size at the beginning of the experiment than at later ages. This might indicate that gape size is not a major limitation during the first weeks of life, and hence swimming speed and capture ability may be the major constraints to feeding at this age (Pepin & Penney 1997).

Cod larvae seem to eat a larger amount of prey compared to their body size between the ages of 17–27 DPH than before and after this period. The increase in gut index from start of feeding to age 17–27 DPH might to some extent be explained by an underestimation of the gut content in the earliest samples. Gut items like rotifers, which lack a carapace, will rapidly be degraded and are difficult to recognize in the gut content. However, the increase in gut index coincides with an increase in $G_S$ (%) and it is known that the larval ability to catch food increases with age, as the search time and handling time of prey decreases as larvae grow (Houde & Schekter 1980). The decrease in gut index from age 27 to 41 DPH may be explained in part by an increasing gut passage rate of older larvae (Kjørsvik et al. 2004). The digestive system develops as the larva grow and around day 17 the gut of cod forms a loop and the inner surface of the gut increases (Kjørsvik et al. 1991; Hunt von Herbing et al. 1996a,b), whilst the ability to digest proteins also increases after this day (reviewed in Falk-Petersen 2005). It may follow from this that the amount of food compared to larval size (gut index) needed to support growth is reduced as the intestine develops. It thus seems reasonable to

suggest that the decrease in gut index to some extent reflects an increased digestion efficiency.

The higher gut index in M2 compared to M1 might to a large extent be explained by the difference in growth rate in the two mesocosms, growth rate itself partly being a function of temperature. In order to support a higher growth rate, a higher energy intake is required. The low zooplankton concentration in M1 might, however, explain some of the difference in gut index in the very beginning of the experiment (see discussion above).

Despite the difference in mass and length between offspring of first-time and repeat spawners, no difference in energy content-at-age was found between the two groups. Previously it has been shown that large larvae feed more at a given age than small larvae (Knutsen & Tilseth 1985; Marteinsdottir & Steinarsson 1998). Offspring of repeat spawners tended to have higher energy content in both mesocosms. As offspring of repeat spawners were smaller at a given age than offspring of first-time spawners, they would also be older at a given mass. This difference in age could influence their ability to detect and catch prey, as their visual and possibly swimming ability could be more developed than their younger counterparts of the same size. In conclusion, given favourable feeding conditions, it could not be shown that there are any notable qualitative differences in the offspring of first-time and repeat spawners as has been suggested by Ponomarenko (1973) and Reznick (1991).

The findings in this study may indicate that the spawning experience of female cod does not per se influence the size and viability of her offspring when the larvae experience favourable conditions. Hence, the size and condition factor of the spawning females, which is easier to obtain than the spawning experience, could yield sufficient information about the reproductive potential of the population. However, environmental factors and maternal phenotypes are more variable in nature (Chambers & Waiwood 1996) and young females are usually smaller than older females (Kjesbu et al. 1996). Small cod yield a significantly smaller egg biomass than larger individuals (Chambers & Waiwood 1996; Kjesbu et al. 1996; Scott et al. 1999) and hence fishery management should promote spawning stocks composed of multiple year-classes.



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