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Measurements of condition and growth of cod larvae reared in mesocosms: individual variability as a function of environmental condition or genetic inheritance

Catriona Clemmesen, V. Bühler, G. Carvalho, R. Case, G. Evans, L. Hauser, W. Hutchinson, H. Mempel, E. Moksness, H. Otteraa, H. Paulsen and T. Svaasand

Abstract

Size, dry weight and RNA/DNA measurements of 3876 cod larvae from 26 different families of recruit and repeat spawners reared in two mesocosms (2500m³ and 4400m³) under natural conditions were analysed. To be able to relate individual data to parental background, DNA microsatellite analysis was performed. The larvae from the two groups (recruit, repeat) already differed significantly in size and weight at hatching with the larvae from the recruit spawners being larger and heavier at the start of the experiment. Growth curves fitted for the larvae from the recruit spawning groups showed a trend of greater sizes at given ages. For all sampling dates offspring of recruit spawners had significantly higher sizes and dry weights than the repeat spawners. RNA/DNA ratios from recruit spawners showed a trend to higher ratios compared to the repeat spawners. The 2500m³ mesocosm was characterized by low plankton density during the transition from exogenous to endogenous feeding followed by a higher density during the metamorphosis period, while the 4400m³ mesocosm showed the opposite situation. The change in the food density occurring in the mesocosm was reflected in the growth rates. Survival was slightly higher in the mesocosm with the higher food density in the beginning, survival between recruit and repeat is assumed to be the same. Estimates of non-parametric probability distributions of the RNA/DNA ratios differed in the amount of scatter (variability) between mesocosms indicating that the higher food density lead to more better conditioned and fewer badly growing extremes of individual larvae in the first three weeks. When the feeding conditions changed the larvae from the low food environment could compensate and reach similar conditions, but were lacking some of the bad conditioned extremes. RNA/DNA analysis within each mesocosm showed that the individual fish exhibited very different growth and condition responses under the same environmental conditions. These different growth responses in both mesocosms were not related to being offspring of first or recruit spawners but seem to be caused by the environmental conditions.

C. Clemmesen, H. Mempel: Institute für Meereskunde an der Universität Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany [tel: +49 431 6004558, fax: +49 431 6001515, email: cclemmesen@ifm.uni-kiel.de]. V. Bühler, E. Moksness: Institute of Marine Research-Flodevigen Research Station, N-4817 His, Norway , H. Otteraa, T. Svaasand: Institute of Marine Research, P.O. Box 1870 Nordnes, 5817 Bergen, Norway, G. R. Carvalho, R. Chase, L. Hauser, W.F. Hutchinson: The University of Hull, Molecular Ecology & Fisheries Genetics Group, Dept. of Biological Sciences, Cottingham Road, HU6 7RX Hull, UK, G. Evans: Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, Newfoundland, Canada, H. Paulsen: Danish Institute for Fisheries Research, P.O. Box 101, 9850 Hirtshals, Denmark.

Introduction

One of the effects of fishing is a reduction in average age and body size in fish stocks being exploited. One consequence of this is an increasing proportion of recruit spawners in the stock. Time series analysis of data of Northeast Arctic cod from 1930 to 1990 have shown a decrease in age of first maturity and size (Jorgensen 1990, Pope *et al*. 2001). Current management practice assumes equal viability of offspring from first time spawners and from repeat spawners, despite the fact that first time spawners often produce smaller eggs than older spawners (Chambers & Leggett, 1996, Chambers & Waiwood 1996, Kjesbu *et al*. 1996, Trippel 1998). The characteristics of the mother (the maternal effect) will largely determine the size and quality of the eggs. These characteristics are partly inherited, but feeding and other environmental conditions the mother has experienced before spawning are also important. In addition, the mother's age, earlier spawning experience, and the spawning period duration (female Arto Norwegian cod spawn in 15-20 batches per year) will influence egg quality. One might also expect similar paternal effects, but these are probably confined to the genetic component, and are likely to be of little importance for the egg and early larval stage, although such effects have not been investigated in the same detail as maternal effects (Chambers and Leggett 1992, Trippel & Nielsen 1992, Evans & Geffen 1998).

There is substantial information on maternal effects on egg and early larval stages in marine fish in general, and in cod in particular. However, the importance of these maternal effects on later stages has been little investigated, because it is nearly impossible to follow individuals from egg to recruitment in a natural environment, even though otolith microstructure analysis can give valuable information about selective mortality in the field (Meekan and Fortier 1996). Therefore the idea came up to follow offspring of different females with different maternal spawning experiences in a mesocosm setup, by measuring growth and condition indices ((Buckley 1984, 1999, Clemmesen 1988, 1994, Moksness *et al.* 2000) and determining the parental background by DNA microsatellite approaches (O´Reilly and Wright 1995; Estoup *et al.* 1998; Estoup and Angers 1998, see EU-Project MACOM, Svaasand *et al.* 2000) to compare the viability of offspring between the two experimental groups: recruit female spawners (first-time female spawners) and repeat female spawners (elder female spawners).

The advantage of using the combination of mesocosm and DNA-fingerprinting was that all fish experienced the same environmental conditions and that comparisons of groups of fish that were hatched and released at the same time could be made. The powerful technique of DNA microsatellite analysis made it possible to identify the maternal and paternal origin of each individual produced in the mesocosms. The use of RNA/DNA ratios in addition to size and weight measurements allowed to compare individual growth and condition estimates, since RNA/DNA ratios have proven useful as indicators of nutritional condition and growth in larval fish up to metamorphosis. RNA/DNA ratios are capable of reflecting differences in metabolic activity and protein metabolism and are able to differentiate between well-growing, intermediate and poorly-growing larvae (Buckley 1984, 1999, Clemmesen 1994).

The aim of the study was to analyse whether size and condition differences among larvae of equal age that have experienced the same environmental conditions may be due to:
a) differences in growth characteristics – a genetic component based on the spawning experience. b) a different start position – different egg size and quality c) the stochastics of encountering food particles (environmental condition) or d) a combination of any of these factors.

The novelty of this study is the combination of mesocosm rearing with DNA fingerprinting which makes it possible to compare the viability of offspring of different parental origin over an extended period of time without bias from unknown variations in laboratory experiments or natural environments.

Material and Method

The broodstock for the experiments performed in 2000 were collected from the Barents sea during August 1998. More than 200 fish were collected and transferred to net cages at the Parisvatn experimental station near Bergen, Norway. Their maturing status was determined by biopsy during the 1999 spawning season. All fish were individually tagged by internal PIT-tags and small pieces of fin tissue was collected for genotype determination.

The fish that were immature in the 1999 spawning season were then considered as recruit spawners if they matured in the 2000 spawning season. The broodstock was by February 2000 reduced to about 80 individuals due to mortality. From the survivors 15 recruit spawning females and 15 repeat spawning females were selected, and placed individually in one of 30 spawning tanks. The spawning tanks were 3m ∅ tanks, each divided into three similar spawning compartments by vertical walls. Each female was accompanied by one randomly selected male (but of similar size). Eggs were collected daily from the 30 spawning compartments in the period 18th February to 25th April, 2000 with the amount of eggs produced, egg size and fertilisation rate of each egg batch monitored. 62 batches were incubated and samples for egg quality analysis were frozen. Egg quality parameters investigated included: percent fertilisation, size, lipids (lipid groups and individual HUFA), free amino acid content, dry weight and energy content. Incubated eggs for transfer to the mesocosms were selected based on the following criteria: 1. As many families as possible with eggs hatching within one or two days. 2. Eggs of best quality. 3. Eggs produced relatively early during the spawning period, preferably around 5th spawning. Based on these criteria it was possible to have newly hatched yolk-sac larvae from 26 of the 30 families transferred from Parisvatn to mesocosms at Flødevigen near Arendal, Norway by air on March 28th , 29th and 30th, 2000. 4000 larvae per family were counted and released at various spots in the 2500m^3 mesocosm (600 m^2 surface area, maximum depth 5.0 m), while 8000 larvae per family were released in the 4400 m^3 mesocosm (1700 m^2 surface area, maximum depth 4.5 m). Priority was given to the small basin (2500 m^3), i.e. when the total number of larvae per family was lower than the ideal number of 12,000, larvae were first released into the small basin. A total of 82,285 larvae were released into the 2500 m^3 and 134,314 into the 4400 m^3 basin (see Figure 1).

From these newly hatched larvae a total of 20 individuals from each family were sampled, individually frozen in Eppendorf vials and stored at – 70°C for later biochemical (RNA/DNA analysis) and genetic analysis. The cod larvae were sampled after one week, three weeks, four weeks, five weeks and ten weeks between April 6th and June 9th, 2000 by hauling a two-chambered plankton net (500-µm mesh) diagonally across the mesocosms. After metamorphosis light attraction or food attraction was used to catch larvae. The captured larvae and juveniles were immediately frozen at –70°C. The experiment was terminated by draining the mesocosm on June 8th, 2000, at age approx. 10 weeks when 2927 fish were caught in the 2500 m^3 basin and transferred to indoor tanks. On June 9th, 2000 the 4400 m^3 basin was emptied and 11,400 fish were caught and transferred to indoor tanks. From these fish samples for growth and condition analysis were taken at random.

Prior to the nucleic acid analysis standard length and dry weight after 24 hours freeze-drying were taken. RNA and DNA content was determined based on a fluorimetric method using ethidiumbromide as a nucleic acid specific dye (Clemmesen *et al* in prep., Moksness *et al.* 2000). To perform the RNA/DNA analysis whole fish were homogenised for samples up to 5th week , whereas for older larvae only part of the fish (dorsal muscle) was used.

DNA was extracted from fin-clippings taken from the first year's broodstock using a standard phenol-chloroform method. Conditions were then developed to PCR-amplify the broodstock-DNA using the three cod microsatellite loci originally chosen from simulations to assign parentage to the larvae. Tissue homogenates from RNA/DNA analysis were used for the genetic screening of offspring, thus reducing the effort required for DNA extraction. Preliminary experiments had already demonstrated the suitability of these homogenates for microsatellite analysis (Clemmesen & Hutchinson in prep.). The extracted DNA was amplified using published microsatellite primers and the fragment analysed on a designated automated DNA sequencer (ALFexpress, Pharmacia).

Although the three originally selected loci could be used in the adult broodstock, scoring was difficult and time consuming due to severe stuttering and occasional weak amplification. As DNA from larval samples was of poorer quality and quantity than that from fin clippings of adult fish, these loci were rejected for routine analysis of larvae. An additional five microsatellite loci were optimised and the first year's broodstock was re-screened. The three most variable and reliable loci, GMO2, GMO8 and GMO19 were then selected for routine screening. The program PROBMAX was used to create 4500 simulated progeny from the parental genotypes to test how many larvae could be assigned to specific parents using the chosen loci. These simulations showed that the three loci allowed the family identification of 99.3% of the larvae. For the remaining individuals, a further locus GMO132 ensured full identification. PCR conditions for the three loci were subsequently re-optimised to amplify DNA from a selection of the larvae release groups, of which the parental genotype was already known, thus allowing the accuracy of the parental assignment to be tested.

The environmental conditions in the mesocosm were monitored regularly from March 1st, 2000 until June 5th, 2000. Weekly estimates of zooplankton density were obtained from pump samples taken from depths of 0, 0.5, 1, 2, 3, 4, 4.5 and 5m. Water was pumped from each depth for a short period prior to filtering a 100 liter sample through a 90µm plankton net. Samples were preserved in formalin and later examined using a microscope and counting chamber. A similar procedure was performed for phytoplankton, using a 10 µm plankton net. Temperature was measured every day at the same depths and the mean temperature of the water column was calculated. Salinity and oxygen were measured at the same depths once a week.

Statistical analysis was performed using STATISTIKA software. Means were compared using Student's t-test, if requirements were fulfilled. To compare the variability in the data set in the absence of any theory, either about the form or the distribution or about the form of the dependence, probability distribution functions were estimated (Evans, 2000, Pepin *et al.* 1999).

Results

The size and condition of the broodstock and the larvae at hatch is given in Table 1 showing that the recruit spawners had more or less reached the size of the repeat spawners after one year in captivity. There was no significant difference in size, weight and Fulton's condition factor prior to the start of the spawning season. The larvae hatching from the two groups (recruit- and repeat spawners) already differed significantly in size and weight with the larvae from the recruit spawners being larger and heavier at the start of the experiment (Student's t-test, $p < 0.05$, Table 1, 3). Nutritional condition based on RNA/DNA ratios was not significantly different at the introduction to the mesocosms (Table 3).

Table 2 shows the numbers of cod larvae analysed from the different sampling dates and the separation into offspring of recruit and repeat spawners based on the DNA microsatellite analysis. In total 1926 fish from recruit spawning females and 1950 fish from repeat spawners were analysed for growth and condition analysis. Numbers from both groups showed approximately a 50 to 50 ratio.

The environmental situation within the two mesocosms was monitored by measuring temperature and by determining food availability for the larval and juvenile fish. The temperature experienced in the two mesocosms is given in Figure 2 including the temperature situation before the larvae were introduced to the mesocosms on March 28th, 2000. The temperature in the 4400m³ mesocosm started at approx. 6 °C and raised to 16°C at the end of May and then dropped to about 13°C shortly before termination. Temperature within the 2500m³ mesocosm was only 4°C when the larvae were introduced to the mesocosm and was always lower except for the beginning of May, when both mesocosms had the same temperature of 10.25°C. The maximum temperature values were measured in the second half of May with nearly 15°C (Figure 2).

Food density available in the mesocosms is shown in Figure 3 as total zooplankton numbers (individuals/liter). At the end of March when the newly hatched larvae were introduced to the mesocosm zooplankton density was nearly 6 times higher in the 4400m³ mesocosm. The densities showed great fluctuations varying between 20 and 110 organisms/liter and were always higher in the 4400m³ mesocosm until the middle of May. After that the numbers of organisms found in the pump samples decreased steadily and at the end of May no food organisms could be observed in the 4400m³ mesocosm. The 2500m³ showed variations between 1 and 20 organisms/liter with a trend of increasing numbers from the beginning of April to the middle of May. The biggest change in food density in the 2500m³ mesocosm was found from the middle to the end of May when densities changed from 20 to 60 organisms/liter and stayed significantly higher than in the 4400m³ mesocosm until termination of the experiment (Figure 3).

Survival rates within the two mesocosms were estimated from bongo samples twice a week based on the amount of larvae per water volume sampled. After one week in the mesoscosm the survival rates for cod larvae from the 2500m³ mesocosm were only about 12% in comparison to a survival rate of 27% in the 4400m³ mesocosm (Figure 4). The survival rate was always higher in the 4400m³ mesocosm except for the estimates based on samplings on May 1st, 2000 and on June 20th, 2000 when the survival rates in both mesocosms were the same. At termination of the experiments on June 8th , 2000 survival from the 4400m³ mesocosm was still slightly higher but reached the same final survival rate on June 20th, 2000 based on mortality measures from the indoor tank experiments. Since no DNA microsatellite measurements were performed on these samples, a separation of survival rates due to repeat

or recruit spawners could not be analysed. There is reason to conclude that the mortality between the two groups should have been similar, since the distribution of the recruit and repeat spawners in the Bongo samples taken for biochemical analysis from the mesocosms was more or less a 50 to 50 ratio (Table 2).

Standard length measurements of the larvae from both mesocosms and the classification to the recruit and repeat spawning groups are shown in Figure 5. The growth curves fitted show that the larvae caught from the 4400m³ mesocosm grew faster and reached a greater size at age in the first part of the experimental period. Larvae from the recruit spawning groups showed a trend of greater sizes at given ages. The change in the food density occurring in the middle of May (see Figure 3) was reflected in the growth rates. Larvae from the 4400m³ mesocosm were smaller at the end of the experiment reaching a mean size of 38.4 mm compared to 42.8mm in the 2500m³ group (Table 5). Means and standard deviations of standard lengths and dry weight measurements in relation to sampling date and spawning experience are given in Table 3 and Table 4. During the whole experimental phase in the 4400m³ mesocosm recruit spawners were significantly larger than repeat spawners (Student's t-test , $p < 0.05$, Table 4). Larvae from the 2500m³ mesocosm showed larger standard lengths in the recruit spawning groups and were statistically different except for the samples taken in the 1st week (Table 3). The effect of the mesocosms with their different environmental situation was already seen by April 6th (1st week sample).The higher temperature and food density in the 4400m³ mesocosm already resulted in significantly larger and heavier larvae after one weeks (Student's t test, $p < 0.05$, Table 5). The reduction in food density starting from the middle of May in the 4400m³ mesocosm led to reduced growth in the fish being significantly smaller than the fish from the 2500m³ in the beginning of June (10th week) (Table 5). The comparison of dry weights of the larvae confirmed the results shown for the standard lengths (Table 5). For all sampling dates offspring of recruit spawners had significantly higher dry weights than the repeat spawners, this was seen in both mesocosms.

RNA/DNA ratios in relation to sampling date, mesocosm and spawning experience are shown in Table 3-5. From the beginning of the experiment until the beginning of May RNA/DNA ratios were significantly higher in the larvae sampled from the 4400m³ mesocosm. At the end of the experiment the RNA/DNA values were significantly lower in the 4400m³ mesocosm reflecting the limited food availability in that basin. RNA/DNA ratios from recruit spawners showed a trend to higher ratios compared to the repeat spawners (Figure 6). RNA/DNA ratios of larvae from the 4400m³ mesocosm in relation to their individual dry weight for offspring of recruit and repeat spawners are presented in Figure 7. The data are based on samples taken at the beginning of the experiment, at 1st week, 3rd week, 4th week and 10th week. To analyse the distribution pattern of the RNA/DNA ratios in relation to spawning experience of the mothers, probability distribution functions were fitted to the data and the 10th, 50th and 90th percentiles of these distributions were analysed. Figure 7a shows the individual data points and the fitted percentiles, Figure 7b only shows the percentiles. There is a striking similarity between the distribution patterns of the recruit versus the repeat spawners. Althought an enormous variability in the RNA/DNA ratios is seen, this variability is the same for both spawning groups and shifts to higher or lower values cannot be related to the spawning experience. The 10th percentile as a measure for the worst growing larvae gives stable RNA/DNA values of about 1.8 up to 1 mg dry weight (log =0). After that some of the lower conditioned larvae are not found in the samples any more. For weights higher than 1 mg up to about 3 mg (log = 0.5) the percentiles increase to higher values showing that the condition of the whole population increased. The sharp decline in the RNA/DNA values for samples caught at the termination of the experiment is seen in all percentiles.

The same kind of analysis was performed for the larvae and juveniles from the 2500m³ mesocosm. Figure 8 gives the RNA/DNA ratios in relation to the individual dry weights for offspring of recruit and repeat spawners from the 2500m³ mesocosm with the percentiles fitted to the data. The distribution patterns show a continues increase in all percentiles with significant changes in RNA/DNA ratios during the course of the experiment. Striking again is the similarity of the distribution patterns and the percentiles for the recruit and repeat spawners. There is no significant difference in the pattern of RNA/DNA ratios in relation to maternal background. The variability in the data found cannot be attributed to the spawning experience of the mothers, since the patterns are more or less the same. Whereas the different environmental conditions experienced by the larvae in the two mesocosms resulted in significantly different viability measures for the larvae.

Discussion

The aim of the study was to analyse whether a genetic component, the spawning experience of the female fish (maternal effect) could lead to differences in size and condition among larvae of equal age that have experienced the same environmental conditions. Maternal effects on fish were first investigated in the Soviet Union in the 1930s (Nikolskii 1962), where differences in egg size, spawning period, and fecundity could be tracked back to the size and condition of the mother. Eggs size increased significantly from first to second spawning season for fish kept at same level of condition (Kjesbu *et al*. 1996). Solemdal (1997) reviewed the literature on maternal effects on fish. Several maternal factors, like size and earlier spawning experience, have been thoroughly investigated in cod during the last decade (Kjesbu *et al*. 1991; Solemdal *et al.* 1992; Solemdal *et al.* 1995, Kjesbu *et al.* 1996), and have shown to be of significant influence on the egg parameters (egg diameter, specific gravity, mortality). Similar results have been obtained from studies of other species, including herring, capelin and turbot (Chambers & Leggett 1996).

The importance of the difference in size at hatch for later growth and survival has been a relatively neglected field in fisheries science, and such differences have rarely been incorporated into predictive models of fish recruitment. Nevertheless it is believed that the difference in egg size and quality influences the viability of the larvae. It is also well established that small eggs develop into small larvae (e.g. Knutsen & Tilseth 1985; Kjesbu *et al*. 1992) and that the sizes of the larvae are significantly related to egg size in many species including cod (Pepin *et al*. 1997, Trippel 1998).

Large larvae are believed to have a competitive advantage over smaller larvae under otherwise similar environmental conditions (e.g. Hunter 1981). Large larvae are better developed in their sensory and swimming abilities, making them more efficient at capturing food and avoiding predators (Bailey and Houde 1989). Fast-growing larvae will also grow faster through stages at which predation risks are high, and will thereby experience lower cumulative mortality (Cushing 1975). In addition to these general relationships, factors such as the size distribution of prey, prey preferences, and predator-prey contact ratios are also significant (Leggett & Deblois 1994).

Despite the fact that many studies have shown that large females with spawning experience produce large eggs and again many studies were able to detect significant correlations between egg size and viability parameters of the larvae , only few were able to reveal direct relationships between female and larval characteristics. Martinsdottir & Steinardson (1998) were able to relate larval growth to female length but not female weight.

The effect that repeat spawners should provide larger and more viable larvae is mainly based on the fact that these fish produce larger and higher quality eggs (Kjesbu *et al.* 1996). However, controversaly to the expectations the results in this study showed that the newly hatched larvae of the recruit spawners were larger and heavier at the start of the experiment and that this results carried throught to the early juvenile stage. Analysis of the egg size and quality of the egg batches used in this study have shown that first time spawners had bigger eggs in size and dry weight and higher energy content than repeat spawners (Svaasand *et al* 2001). Consequently the situation at the start of the experiment is contradictory to the assumptions in the literature. The spawning experience of the adult fish did not create the signal that was expected, but the effect already seen in the egg sizes was followed in the larvae leading to better growing fish based on the eggs sizes, confirming the idea of egg size effecting size of the larvae.

Temperature influences metabolic processes and besides prey availability is the most important factor that drives growth rates in fish. Temperature differences of 1-2 °C as experienced in the mesocosm in the first month of the experiment can already effect the standard length, dry weight and growth rate of larval fish as shown in Otterlei *et al.* (1999). For all temperatures tested (4- 14°C) growth rate initially increased with larval size and peaked at a pre-metamorphosis size of 0.1 to 1.0mg dry weight and then declined during the juvenile stage leading to a dome-shaped relationship between weight specific growth rate and body size. A positive significant relationship between cod larval length and temperature was also shown by Pepin at al. (1997). Higher temperatures and higher food density during the initial conditions in the 4400m³ mesocosm led to better growing and better conditioned larvae. But there was no selection for better growth and condition based on the spawning experience since recruit and repeat spawners showed the same trends in their viability measures and the same pattern in extreme values (percentiles). Blom *et al.* (1994) found that the average growth during the larval and juvenile stages in a study on two strains of Atlantic cod throught the early life stages in a marine pond did not differ between progeny of large and small females, indicating that the size of the female does not always have to be responsible for the size of the larvae.

Results on growth and condition estimates from the 2500m³ mesocosm, where the food situation was less favourable in the beginning of the experiment and afterwards improved indicated that the surviving fish were able to outgrow the group of fish from the 4400m³ mesocosms and reached higher growth rates and better condition and were able to compensate for the growth deficit they had experienced earlier. The question of compensatory growth is of importance in the discussion of long-term maternal effect (Solemdal 1997). The duration of maternal effect on size differences of fish is practically unknown. These effects will be further analysed by studies of the otolith microstructure analysis, first preliminary studies have already been able to see compensatory growth effects in the increment width analysis (Bühler *et al.* in prep.).

Some of the results of this study seem to contradict the findings described in the literature, but have to be viewed under the special situation found in this study. Although the offspring of the recruit spawners showed a tendency for better growth and higher RNA/DNA values compared to the repeat spawners mainly due to the differences already seen in the egg sizes and sizes at hatch the patterns and variability found in the size, weight and RNA/DNA ratio estimates did not differ significantly between the parental spawning experiences. Striking differences in viability estimates specially the RNA/DNA distribution functions and the patterns of the percentiles were largely influenced by the environment the larvae and juveniles had experienced in the two mesocosms. Differences in temperature and food density

encountered gave a clear signal on the size, weight and nutritional condition determined in the fish.

Data analysis so far suggests that the food signal is responsible for the great differences found in both mesocosms, but further analysis of the data by incorporation into a temperature dependent growth model (Folkvord in prep.) will further test on the separation of both effects. Concluding the observed variability in growth and condition of cod larvae reared in mesocosms seems to be more influenced by the environmental conditions than by the genetic inheritance described by the spawning experience.

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Table 1: Comparison of size, weight (in January 1999 and in January 2000) and condition measures of the broodstock and the newly hatched larvae used in the mesocosm experiments.

Broodstock							**Newly hatched larvae**		
parents	numbers	mean age (years)	mean length (cm)	mean weight (g) 1999	mean weight (g) 2000	mean Fulton's K	mean size (mm)	mean dry weight (mg)	mean RNA/DNA ratio
recruit	13	6.9	79.7	2208	6400	1.25	4.44	0.0615	2.61
repeat	13	7.8	80.9	3697	6618	1.26	4.39	0.0581	2.65

Table 2 : Number of samples analysed for viability measures from both mesocosms and from the start samples (known families before introduction into the mesocosm). Percentage values give the ratios between numbers of offspring of recruit and repeat spawners based on the DNA-microsatellite fingerprints.

	Hatching		**1st week**		**3rd week**		**4th week**		**5th week**		**10th week**	
sample site	28.03.00		6/7.04.00		21.4.00		27.04.00		3.05.00		7/8.06.00	
	recruit	repeat	recruit	repeat	recruit	repeat	recruit	repeat	recruit	repeat	recruit	repeat
2500m³			281	270	123	102			174	142	168	125
%			51	49	54.7	45.3			55.1	44.9	57.3	42.7
Start	232	249										
%	48.2	51.8										
4400m³			236	320	288	293	286	290			138	159
%			42.4	57.6	49.6	50.4	49.7	50.3			46.5	53.5
Total recruit: n = 1926 Total repeat: n = 1950												

Table 3: Comparison of means and standard deviations of standard lengths, dry weight and RNA/DNA ratios from samples taken from the 2500m³ mesocosm separated into offspring of recruit and repeat spawners. P- values are determined by Student's t-test.

Date	Spawning group	Number (N) (SL/DW)	Length (SL) [mm] mean, sd	Dry weight (DW) [mg] mean, sd	Number (N)	RNA/DNA
Start sample (28.03.00)	Recruit	232/232	mean = 4.44 sd = 0.37	mean = 0.0615 sd = 0.017	232	mean = 2.61 sd = 0.67
			p = 0.012	p = 0.023		p= 0.413
	Repeat	249/249	mean = 4.39 sd = 0.28	mean = 0.058 sd = 0.015	249	mean = 2.65 sd =0.51
1st week (6/7.04.00	Recruit	281/281	mean = 5.03 sd = 0.63	mean = 0.097 sd = 0.123	281	mean = 2.34 sd = 0.49
			p= 0.147	p = 0.560		p=9.64E-05
	Repeat	270/270	mean = 4.93 sd = 0.999	mean = 0.107 sd = 0.294	270	mean = 2.17 sd = 0.52
3rd week (21.04.0)	Recruit	123/123	mean = 7.34 sd = 0.78	mean = 0.4 sd = 0.133	123	mean=2.75 sd=0.52
			p= 0.00060	p = 0.00012		p = 0.195
	Repeat	102/102	mean = 6.98 sd = 0.76	mean = 0.333 sd = 0.12	102	mean = 2,65 sd = 0.53
5th week (27.04.00	Recruit	174/174	mean=12.39 sd = 1.61	mean = 2.71 sd = 0.87	174	mean = 3.79 sd = 0.66
			p= 1.74E-05	p = 4.97E-07		p=0.472
	Repeat	142/142	mean=11.62 sd = 1.50	mean = 2.22 sd = 0.79	142	mean = 3.73 sd = 0.63
10th week (7/8.06.00	Recruit	168/168	mean=42.80 sd = 5.30	mean = 132.46 sd = 59.69	168	mean = 6.08 sd = 1.71
			p = 0.00094	p = 0.0044		p = 0.097
	Repeat	125/125	mean=40.74 sd = 5.83	mean = 113.68 sd = 48.74	124	mean = 5.78 sd = 1.49

Table 4: Comparison of means and standard deviations of standard lengths, dry weight and RNA/DNA ratios from samples taken from the 4400m³ mesocosm separated into offspring of recruit and repeat spawners. P-values are determined by Student's t-test.

Date	Spawning group	Number (N) (SL/DW)	Length (SL) [mm] mean, sd	Dry weight (DW) [mg] mean, sd	Number (N)	RNA/DNA
Start sample (28.03.00)	Recruit	232/232	mean = 4.44 sd = 0.37	mean = 0.0615 sd = 0.017	232	mean = 2.61 sd = 0.67
			p = 0.012	p = 0.023		p= 0.413
	Repeat	249/249	mean = 4.39 sd = 0.28	mean = 0.058 sd = 0.015	249	mean = 2.65 sd =0.51
1st week (6/7.04.00	Recruit	236/236	mean = 5.01 sd = 0.54	mean = 0.113 sd = 0.033	236	mean = 2.97 sd = 0.68
			p= 1.54E-06	p = 6.45E-16		p=0.011
	Repeat	320/320	mean = 4.78 sd = 0.54	mean = 0.093 sd = 0.026	319	mean = 2.82 sd = 0.68
3rd week (21.04.0)	Recruit	288/288	mean = 8.06 sd = 0.85	mean = 0.693 sd = 0.166	288	mean=2.98 sd=0.91
			p= 6.73E-10	p = 1.03E-13		p = 0.303
	Repeat	293/293	mean = 7.61 sd = 0.88	mean = 0.588 sd = 0.168	292	mean = 2,91 sd = 0.82
4th week (27.04.00	Recruit	286/286	mean=11.09 sd = 0.107	mean = 1.863 sd = 0.454	286	mean = 3.79 sd = 0.75
			p= 2.04E-09	p = 6.35E-11		p=0.122
	Repeat	290/290	mean=10.49 sd = 1.29	mean = 1.592 sd = 0.522	290	mean = 3.69 sd = 0.84
10th week (7/8.06.00	Recruit	138/138	mean=38.38 sd = 1.88	mean = 76.56 sd = 13.92	138	mean = 2.54 sd=0.61
			p = 0.0019	p = 0.0126		p = 0.104
	Repeat	159/159	mean=37.57 sd = 2.49	mean = 72.52 sd = 13.76	159	mean = 2.68 sd = 0.87

Table 5: Comparison of means and standard deviations of standard lengths, dry weight and RNA/DNA ratios from samples taken from the 2500m³ and 4400m³ mesocosm separated into offspring of recruit and repeat spawners. P- values are determined by Student's t-test.

Date	Spawning group	Number (N) (SL/DW)	Length (SL) [mm] mean, sd	Dry weight (DW) [mg] mean, sd	Number (N)	RNA/DNA
1st week (6/7.04.00	Recruit 2500m³	281/281	mean = 5.03 sd = 0.63	mean = 0.097 sd = 0.123	281	mean = 2.34 sd = 0.49
			p= 0.678	p = 0.042		p=1.57E-30
	Recruit 4400m³	236/236	mean = 5.01 sd = 0.54	mean = 0.113 sd = 0.033	236	mean = 2.97 sd = 0.68
1st week (6/7.04.00	Repeat 2500m³	270/270	mean = 4.93 sd = 0.999	mean = 0.107 sd = 0.294	270	mean = 2.17 sd = 0.52
			p= 0.026	p = 0.366		p=0.0000
	Repeat 4400m³	320/320	mean = 4.78 sd = 0.54	mean = 0.093 sd = 0.026	319	mean = 2.82 sd = 0.68
3rd week (21.04.00	Recruit 2500m³	123/123	mean= 7.34 sd = 0.78	mean = 0.4 sd = 0.133	123	mean = 2.75 sd = 0.52
			p= 5.78E-15	p = 0.0000		p=0.0075
	Recruit 4400m³	288/288	mean= 8.06 sd = 0.85	mean = 0.693 sd = 0.166	288	mean = 2.98 sd = 0.91
3rd week (21.04.00)	Repeat 2500m³t	102/102	mean= 6.98 sd = 0.76	mean = 0.333 sd = 0.12	102	mean = 2.65 sd=0.53
			p =4.41E-10	p = 0.0000		p = 0.0036
	Repeat 4400m³	293/293	mean= 7.61 sd = 0.88	mean = 0.588 sd = 0.168	292	mean = 2.91 sd = 0.82
10th week (7/8.06.00)	Recruit 2500m³	168/168	mean=42.80 sd = 5.30	mean = 132.46 sd = 59.69	168	mean = 6.08 sd = 1.71
			p= 2.27E-18	p = 4.29E-23		p=0.0000
	Recruit 4400m³	138/138	mean=38.38 sd = 1.88	mean = 76.56 sd = 13.92	138	mean = 2.54 sd = 0.61
10th week (7/8.06.00	Repeat 2500m³	125/125	mean=41.00 sd = 5.83	mean = 113.68 sd = 48.74	124	mean = 5.78 sd=1.49
			p =1.28E-10	p = 7.74E-21		p = 0.0000
	Repeat 4400m³	159/159	mean=37.57 sd = 2.49	mean = 72.52 sd = 13.76	159	mean = 2.68 sd = 0.87

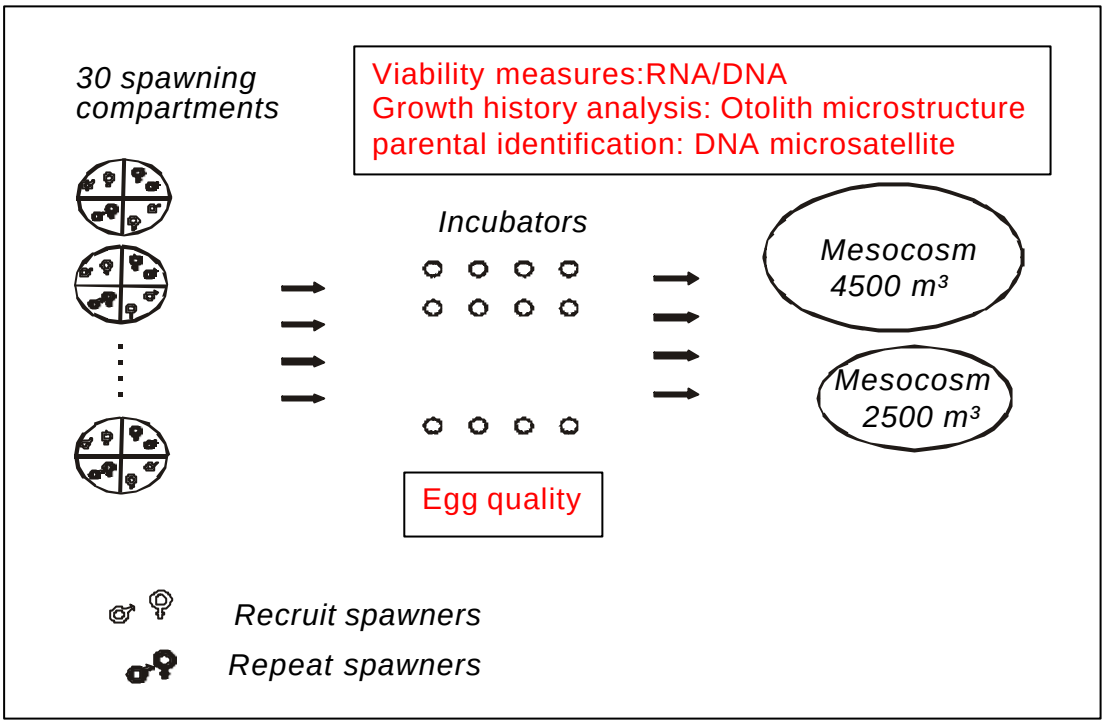

Figure 1: Setup for the experiments performed in this study (taken from Svaasand *et al* 2001)

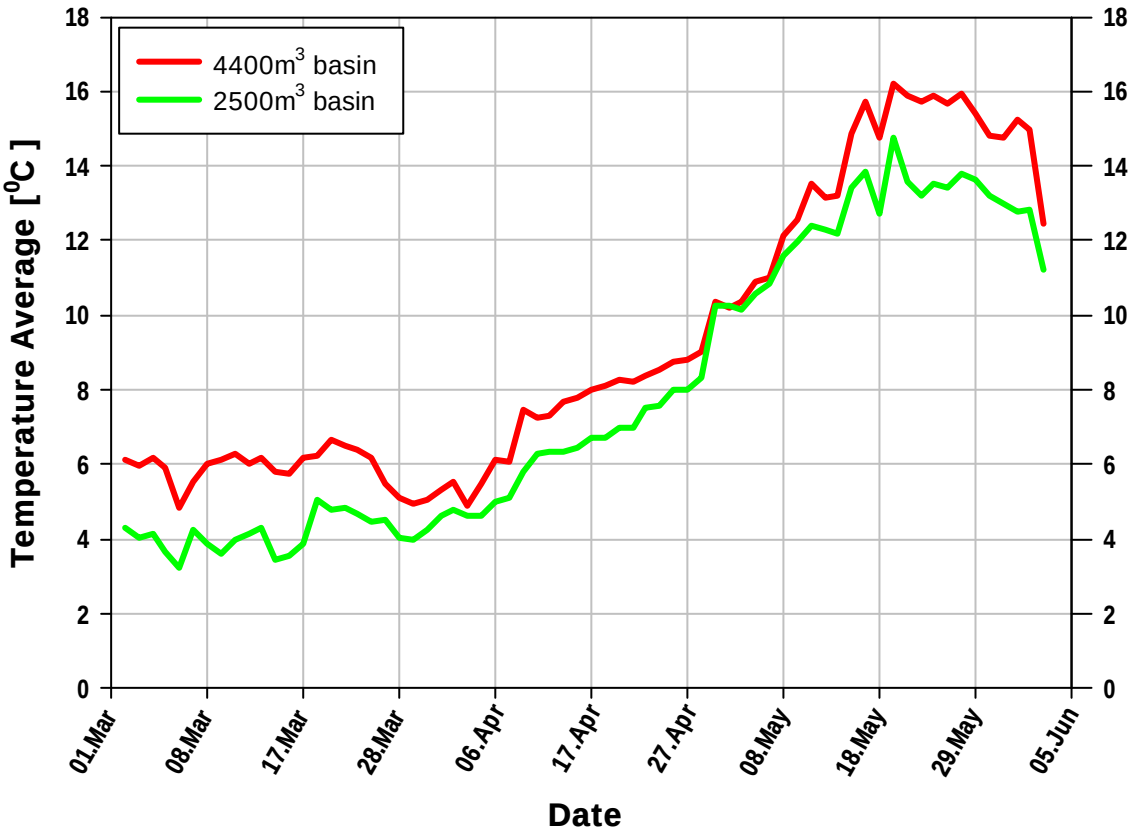

Figure 2: Average temperature in the two mesocosms during the course of the experiment as a mean of measurements from 7 different depths (0, 0.5, 1, 2, 3, 4, 4.5, 5) m. Newly hatched larvae were introduced into the basins on March 28th, 2000.

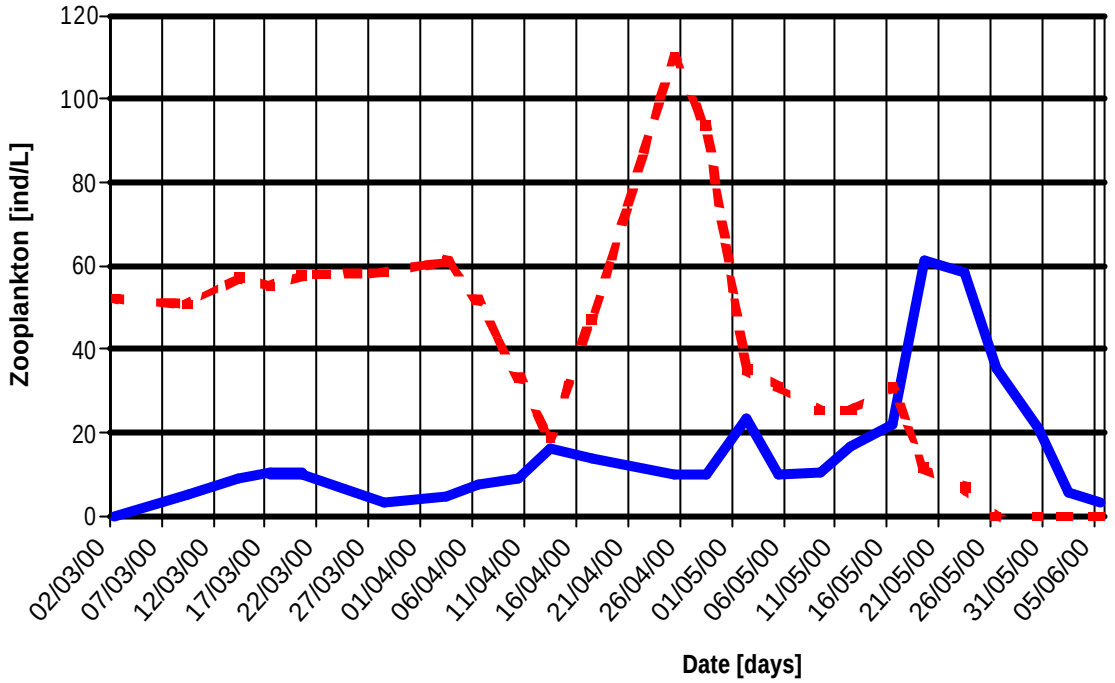

Figure 3: Total zooplankton distribution in the two mesocosms (solid line 2500m³, dashed line 4400m³ from March to June 2000. Newly hatched larvae were introduced to the basins on March 28th 2000.

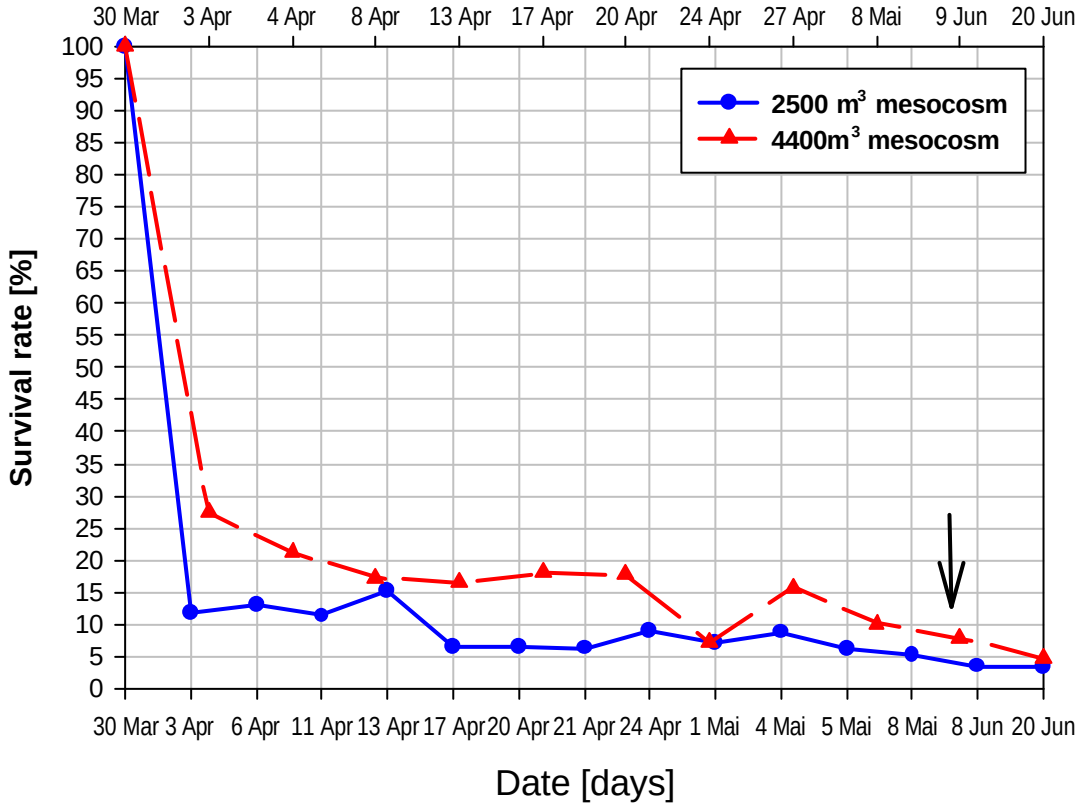

Figure 4: Estimated survival rates of cod in the 2500m³ mesocosm (solid line) and the 4400m³ mesocosm (dashed line) (calculations based on amount of larvae sampled per water volume.) Arrow indicates date, when the mesocosm was emptied. Later survival estimates are based on indoor tank experiments.

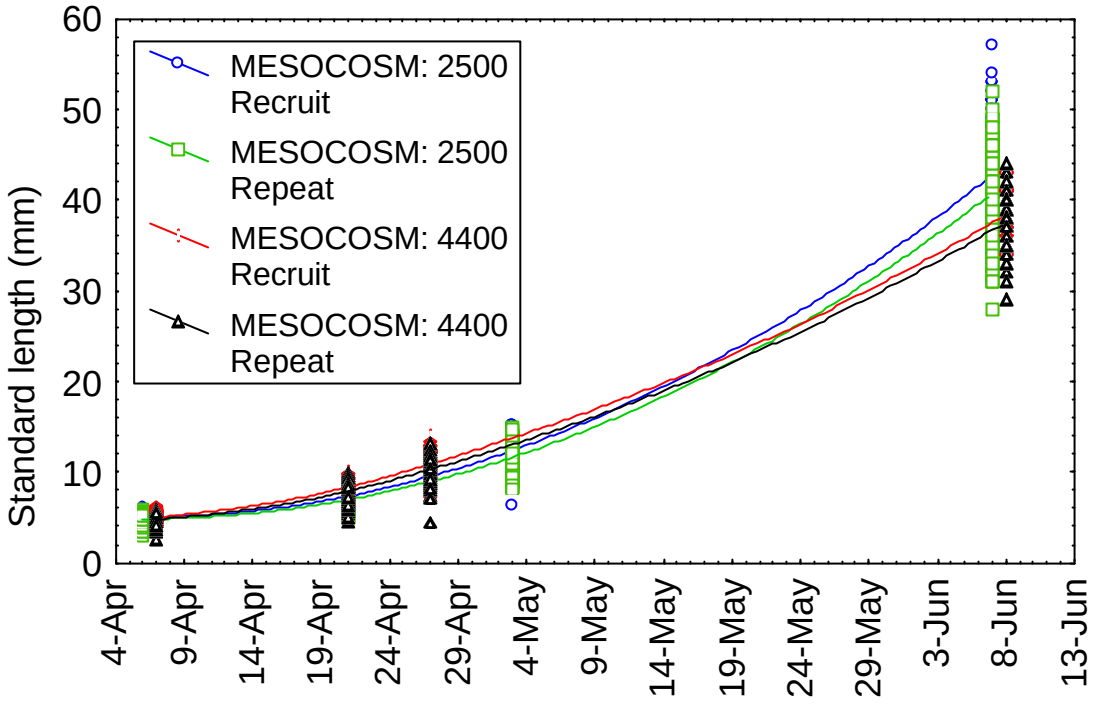

Figure 5: Standard length versus sampling date (age) of cod larvae from both mesocosms divided into offspring from recruit versus repeat spawners. Growth curves were fitted by a least square smoothing function.

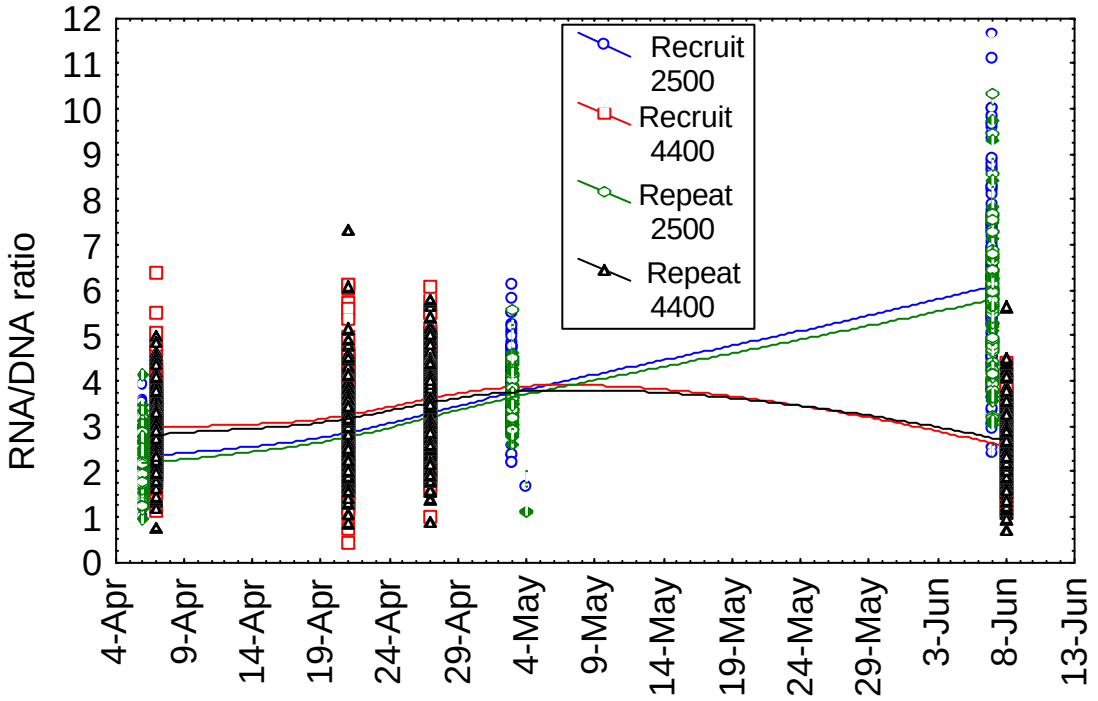

Figure 6: RNA/DNA ratio versus sampling date (age) of cod larvae from both mesocosms divided into offspring off recruit versus repeat spawners. Curves were fitted by a least square smoothing function.

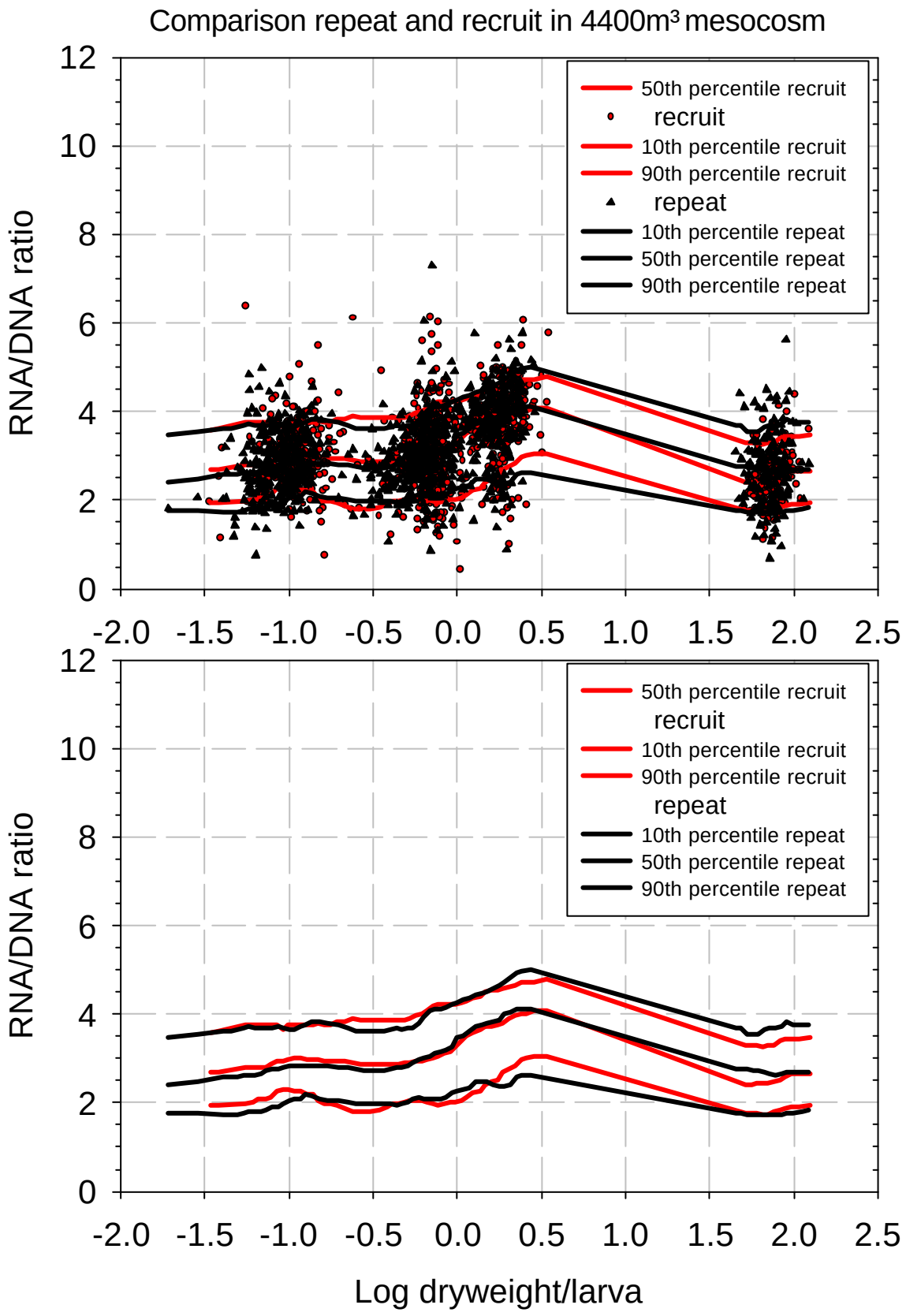

Figure 7: Comparison of RNA/DNA ratios in relation to dry weight of cod larvae and juveniles off offspring from recruit and repeat spawners reared in the 4400m³ mesocosm. Lines fitted to the data are percentiles calculated based on probability distribution functions. Upper panel shows the individual data and the percentiles fitted. Lower panel only shows the percentiles fitted.

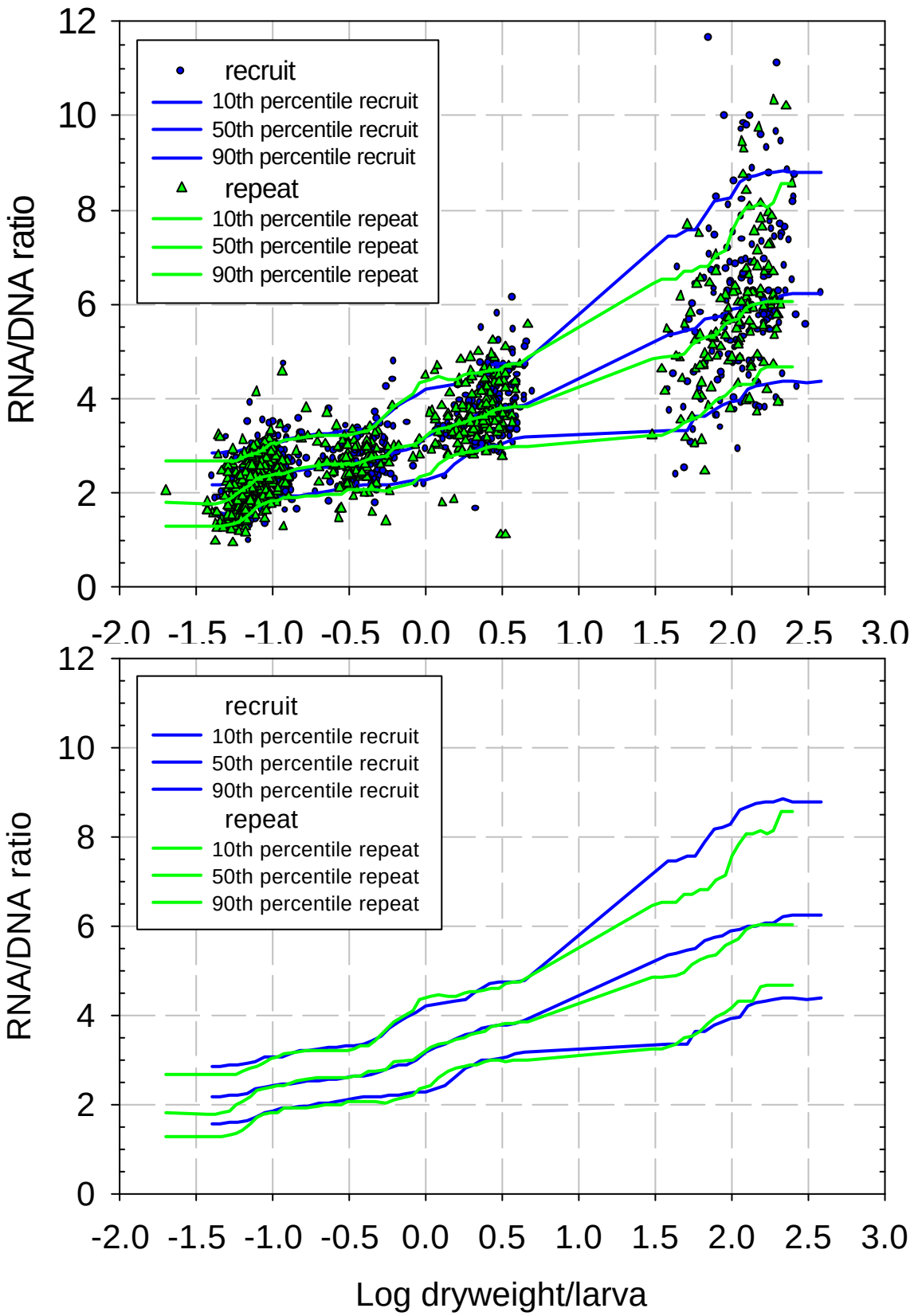

Figure 8: Comparison of RNA/DNA ratios in relation to dry weight of cod larvae and juveniles from recruit and repeat spawners reared in the 2500m³ mesocosm. Lines fitted to the data are percentiles calculated based on probability distribution functions. Upper panel shows the individual data and the percentiles fitted. Lower panel only shows the percentiles fitted.