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A COMPARISON OF EGG QUALITY AND LARVAL VIABILITY BETWEEN CULTURED COASTAL COD AND WILD ARCTO-NORWEGIAN COD

BY

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ABSTRACT

Experiments on cod eggs revealed an inverse relationship between egg diameter and mortality. Further, larvae hatching from small eggs showed a redeuced feeding ability. Other methods to characterice larval viability included behaviour studies of activity and a test to evaluate learning in the feeding ability of cod larvae and the egg parameters size, dry weight and the RNA and DNA relations. The results are discussed in relation to the problem of reproductive failure of the Arcto-Norwegian cod during cold years.

INTRODUCTION

In recent investigations on stock and recruitment of fish populations the variability in both the quantitative and qualitative aspects on eggs and larvae have been stressed (Rothchild 1986, Kjørsvik et al.,1990, Serebryakov, 1990, Kjesbu et al.,1991.) The condition of the mother fish affects the fecundity in cod (Gadus morhua) (Kjesbu et al.,1991)

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and the quality of the eggs and larvae of the capelin (Mallotus villosus) (Chambers et al. 1989). Other factors of the spawning fish have also been demonstrated to influence upon viability of eggs and larvae:
1.Different populations of the same species .
2.The age of the spawning female (Nikolskii 1962)
3.The protracted spawning of the batch spawners, with progressively reduced egg size, as demonstrated both in the field and under experimental conditions (Hiemstra, 1962, Solemdal, 1970 and Kjesbu, 1989.)
In field studies point 3 makes it very difficult to perform the correct comparisons between egg mortality and larval viability of spawning females of different conditon and age. A special method to describe the degree of spentness has been devloped on cod (Kjesbu et al. 1990).
Various criteria of larval viability have been put forward. Survival experiments of larvae throughout a certain period should be the most reliable, though shortcomings in the experimental design and the variation in the quality of the food organisms and feeding procedure can introduce large variability in the results. Long term experiments to describe survival of larval Psudopleuronectes americanus are given by Buckley et al. (in press). He demonstrated that dry weight of the larvae described viability better than other parameters, including the RNA/DNA- ratio. But the results are somehwat conflicting. Zastrof et al. (1989) performed short term viability experiments on larval striped bass (Morone saxatilis) He indicates the hatchability of the egg and the viability of the larvae are independent variables.
The present paper compares various short cut methods describing larval viability on the background of egg mortality and the parameters of the mother fish.

MATERIAL AND METHODS

Spawning fish.

Coastal cod from the Austevoll Aquaculture Research Station, Norway, were transported to the Institute of Marine Research, Bergen, in the autumn 1989, atr an age of nearly two years. They were fed wet pellets in outdoor tanks at approximately 0.5 % bodyweight per day (Kjesbu et al. 1991). The cod females spawned individually for the first time in spring 1990. One female and one male were kept in each special spawning tank. The present experiments were carried out in March-April 1991, removing the separate egg batches by an eggnet from the spawning tanks.
Arcto-Norwegian cod were caught by Danish seine in Lofoten, Northern

Norway during the spawning season in March-April 1991. Age and length of the spawning females were recorded.
Eggs were stripped and artificially fertilized according to the wet method. Egg groups were transported by air to the Institute of Marine Research, Bergen, and incubated in commercial refrigerators at 5 centigrades.
Eggs were sent to Austevoll and kept at 5 centigrades. Larvae from these groups were used for behavoir studies, feeding experiments and measuring the content at DNA and RNA.

Egg parameters.

Egg diameters from 50 eggs were measured manually according to Kjesbu (1989).
Egg dry weight was calculated on the basis of 50 eggs dried for at least 48 hours at 70 centigrades. Weighing was performed on a Cahn microbalance.
Egg mortality.
Eggs sampled from the spawning tanks, with temperatures of 8-9 centigrades, were kept in a refrigator at 5 centigrades untill egg developmental stage Iaβ according to Westernhagen (1970).
The eggs were sterilized in 1% Buffodin for 10 minutes and rinsed in seawater with chemicals added. Buffodin is a fish farming disinfectant, based on an iodine complex used against major fish viruses (Evans vanodine International LTD).
Seawater used for the experiments was filtered and UV-irradiated. In addition the following compounds were added: Mycostatin (2500 IU/liter, streptomycin sulphate (0.05 gram/liter), doktacillin (0.2 gram/liter).
200 eggs were chosen for each experiments, and all experiments were made in parallel. Pipette and vial to count the eggs were rinsed in 4 % formaldehyde.
30-40 eggs were inspected under a low power stereomicroscope, and rejected on the basis of the following criteria: unfertilized, activated, dead and irregular egg development and eggs from earlier batches
The selected eggs were transferred to a 1 liter jar with medicine seawater, and put in a refrigerator at 5 centigrades. Temperature was read manually every day. Each day dead eggs were removed with a glassrod specific for each jar, counted and staged. Developmental stage was checked on live eggs each third day.

Larval parameters.

Dry weight.

Before drying ten larvae were rinsed twice in distilled water. Drying and weighing procedure as for eggs.
Developmental stages.
The early larvae were staged according to the system by Fossum (1986).

Behaviour studies.

Activity

Behaviour studies of cod larvae were conducted using a computer-aided video system, allowing three-dimensional registration of the larvae's position in 15 liter observation chambers. For a more detailed description see Huse & Skiftesvik (1990). All groups of larvae were observed two times, at day 3 (before time of start-feeding) and at day 6 (after time of start-feeding)The larvae were unted. Each of ten randomly chosen larvae was tracked for five minutes and position and behaviour, swimming and resting, were logged for each second. The temperature was maintained at 5 centigrades and the light intensity was 30 lux at the surface.

Feeding ability.

In order to investigate the possible differences between early larval groups in their feeding ability, a test with inert polystyrene spheres developed at SINTEF, Trondheim, Norway, was developed. The main idea is taken from Yin & Blaxter (1987), using rotifer as food particles. Using the inert plastic spheres the size was equal, 150 µm, and it would be easier to count the numbers in the stomach. The relatively small size compared to Calanus nauplii, 200-400 µm (Ellertsen et al. 1989) would enable the larvae to attack and swallow more items before the gut was filled, giving a more exact description of its feeding ability. No social hierarchies or gradients according to light would develop during the experiment using the present method. Using a short test period, 1 hour,it was intended to separate slow and fast learning larvae groups.
The inert polystyrene spheres were produced with similar specific gravity to seawater of 34 per mille salinity, neither sinking nor rising to the surface during the experiment.
The experiments were performed in two cylindrical plexiglass jars of 12 litres each. They were placed in a thermostatic waterbath at 8-9

centigrades. The equipment was placed in a tent of black polyethylene film to control light variations. Light level was kept at 20 lux at the surface which is in the optimum range for feeding of early cod larvae. Preliminary experiments revealed that a density of spheres of 10000 per liter significantly increased feeding ratio compared to 1000 or 5000. The same density of rotifers was used by Yin & Blaxter (1987) for feeding ability studies of early cod larvae.
A standardized mixing, rotating a laboratory spoon 10 times from bottom to the top, was performed before introducing 20 larvae into the suspension. After one hour the larvae were removed by pipette, preserved in 4 % formalin, and the number of spheres in the digestive tract counted under a low power stereomicroscope. The larval groups were tested 6-7 times during the larval stages 7 and 8, according to the system of Fossum (1986). At lastthe mean numbers of spheres in the larvae containing spheres were calculated.

Feeding experiments with rotifers.

The feeding units were 6 liter incubators with airation, 1o ml per minute. The temperature was 9 centigrades and the light intensity was 30 lux. The number of larvae per liter were about 5. Rotifers, Brachionus plicatilis at amount at 2 per ml, were fed to five days old cod larvae. The feeding experiment lasted for 24 hours. The larvae were conserved in neutral 4% formaldehyde. From each sample the number of rotifers in the intestine were counted in 15 larvae. The number og rotifers per larvae were calculated from the total numbers of rotifers eaten and the number of larvae that had eaten.

DNA and RNA were determined according to Raae et al. (1988).

RESULTS

Fig. 1 shows the correlation between parallels from the egg mortality experiments performed in 1991. Experiments for other purposes than the present are included. The correlation is highly significant. Correlation between parallels in the experiment with feeding polystyrene spheres to cod larvae is poor, Fig 2. In Fig. 3 egg diameter is plotted against egg mortality from the 9 Lofoten cod and 3 coastal cod used in the experiments. Egg diameter and egg dry weight from the 9 Lofoten cod and 3 coastal cod are plotted against egg mortality in Fig. 4. Note especially the low dry weight of one of the coastal cod (indicated with an arrow). This coastal cod were spawning in captivity, and this was the last batch (no. 17).

In Fig.5. number. of polystyrene spheres and nos. of rotifers , given as a mean per larvae containing spheres, are plotted against egg mortality. The coastal cod indicated with an arrow shows very low value on polystyrene spheres eaten, but nos. of rotifers eaten seems to be normal. A general trend for lower number of rotifers eaten according to increased egg mortality is seen. Fig. 6 demonstrates the content of RNA and DNA in the different egg groups and the ratio between them. Fig. 7 demonstrates 2 of the polystyrene spheres experiment series, including the series giving highest numbers of spheres and one of those giving low values.
The activity of the same cod larval groups, as swimming period in percentage of total observed period, is demonstrated in fig.8. The arrow indicates the same larval group as in figs. 4 and 5. Compared to the other larval groups a reduced difference in activity between day 3 and day 6 are found.

DISCUSSION

The methods.

Measuring egg mortality in a stagnant system seems to be a sufficent precise method for the present purpose. Very few occasions with bacterial infection occurred during the investigations, and these experiments are omitted from the material. The parallels are usually very similar.
The parallels in the larval experiments on feeding ability, using polystyrene spheres, are more variable.The environmental conditions, temperature, light intensity and density of spheres were measured to be equal within each experiment. Between experiments a variation of 1 centigrade occurred. The variability between parallels are probably caused by the short feeding period, one hour, which is significantly shorter than the 4 hours used by Tilseth & Ellertsen (1984), 5-6 hours by Yin & Blaxter (1987), and the feeding experiments with rotifers given in the present study. The feeding ratio increased steadily through the first 3 hours in the experiments by Tilseth & Ellertsen (1984). One hour was supposed to be enough time in the high particle concentration, 10000 per liter, used in the present experiments. One of the reasons to introduce this kind of behaviour test was to reduce time to be able to bring a large number of groups through the test. The inert polystyrene spheres are eagerly eaten by the cod larvae: the maximum number eaten by one larvae during one hour experiment was 19. Hjelmeland (1988) used inert polystyrene spheres eaten by herring larvae to investigate their effect on the production of trypsin in the digestive tract.

It is concluded that the present method could be a short cut method to indicate viability, as feeding ability, in early cod larvae, by improving the environmental control, especially temperature, and optimizing the test period.

The spawning females.

On the basis of otholith characteristics Rollefsen (1933) divided the cod along the Norwegian coast in the migrating Arcto-Norwegian cod and coastal cod. The growth pattern and age of spawning vary considerably between these populations. However, it has been demonstrated that the populations have an equal genetic constitution (Mork 1985) due to migration patterns and intermingling (Godø 1986). The adaptive significance of growth and age at spawning was demonstrated experimentally by Godø and Moksness (1985).
Though genetically similar, in practice they should be considered as two populations, both for management purpose and for reproductive comparisons.
The present material of coastal cod was reared from egg at the Aquaculture Station, Austevoll, south of Bergen,Norway, and reach sexual maturty at an age of two years at a size of about 30 centimeters. The majority of the Arcto-Norwegian cod reach maturity at the age of 6-9 years and a size of 6o-80 centimeters (Jørgensen 1989).
Egg size.
Correlating egg diameter to the length of mother fish of the two groups a positive trend is indicated for Arcto-Norwegian cod in contrast to the smaller sized coastal cod. As mentioned earlier the eggs from the coastal cod are obtained from spawning tanks, and the material included in the figure are the first batch, consisting the larger eggs. On the contrary, eggs from the Arcto-Norwegian cod are stripped from wild fish, not knowing anything of batch number and degree of egg size reduction. There is all reasons to believe that most the egg batches are not the first one, since cod spawns 20 batches througout the season, and most of the samples were taken in April, which is past peak spawning in Lofoten (Solemdal 1982) . No special visual classification method of gonad maturation stages is reliable for the present purpose. Samples have been taken from the gonads of the Arcto-Norwegian cod egg batches to be analyzed according to the method of Kjesbu et al. (1990). Is concluded that there is a large span of egg diameter from the Arcto-Norwegian cod, the smallest one obviously being from the latest, smallsized, batches. Normally a reduction in eggdiameter of .2 mm occurs throughout spawning from an individual

cod females (Kjesbu 1989). The egg diameter range within separate egg batches is approximately .02 mm (Solemdal 1970).
In contrast to Ware (1975) we will also conclude that the reduction in egg size in a batch spawner is the result of the exhaustion of the female, and not an adaptation to smaller prey organisms.The exhaustion of the batch spawning cod is well documented by Kjesbu et al. (1991). In Arcto-Norwegian cod the only prey for the first feeding larvae is nauplii of Calanus finmarchicus (Ellertsen et al. 1989). During the 2 months of first feeding of the cod larvae in Lofoten the nauplii grow larger, not smaller.

Egg mortality.

Investigations on the effect of egg size on the egg mortality are very few in the literature. Batch spawners with reduces egg size throughout their spawning and hence be of less quality. This is documented on different marine species (Hislop & Bell 1987), and especially for haddock (Melanogrammus aeglefinus), spawning in captivity (Hislop et al.1978)
The same is found for wild Arcto-Norwegian cod. Assuming that the smallest eggs are coming from the latest spawned batches, this would be of importance for the reproductive success of the cod. Ellertsen & Solemdal (1990) put forward a hypothesis combining the year to year variation in peak spawning of Calanus and the cod egg size reduction throughout the spawning season. During warm years peak spawning of Calanus are ahead of the peak hatching of cod larvae, giving the best start feeding to the largest, presumably most viable larvae. On the contrary, during cold years peak spawning of Calanus is significantly delayed and coincides with the small sized, probably less viable, larvae hatched at the end of the season. The present material on increased egg mortality from the small, late spawned eggs, adds a further negative factor to the reproduction success in cold years, the potential egg number being significantly reduced through inferior egg quality.

Larval viability.

The feeding experiments demonstrate an inverse relation between egg mortality and the number of rotifers eaten. Since size of eggs, and hence size of larvae, is also negatively correlated with egg mortality, the results on stomach content could be the result of different stomach capacity.
The results of the feeding ability tests with inert polystyrone spheres indicate that the test period was too short. The learning period is probably longer with inert particles. However, one of the groups is learning very fast. This method seems promising, but needs further improvements and developments.

Cod larvae activity increased significantly from day 3 after hatching to day 6. A tendency of increased activity according to increased egg mortality was observed.
The inverse relation between egg size, dry weight and content of DNA and egg mortality is clearly noticed.
The eggbatch from one of the coastal cod spawning in captivity indicated with an arrow, showed divergent results. This is the last batch spawned from this cod female, the number 17. The egg diameter is relatively large compared to dry weight. The values of RNA and DNA are very low. The results on the activity and feeding ability tests are also very low, while the numbers of rotifers eaten are more like the other groups.
These results demonstrate the reduced viability of eggs and larvae occurring at the end of spawning of a batch spawner.

The viability of an early fish larvae is a very complex term, and is extremely difficult to investigate experimentally, with a certain amount of realism. The most realistic experiments are to bring the larvae through a certain period under the most natural environmental and feeding conditions. The different growth and survival relations of the groups are then related to various characteristics of the early larvae: dry weight, standard length, RNA/DNA, and chemical and biochemical parameters. In this way Buckley et al. (in press), on the basis of experiments with Pseudopleuronectes americanus, concluded that high dry weight of the newly hatched larvae increased the survival. In general dry weight described the survival and growth relations in his material better than the other parameters. On the other hand Moodie et al.(1989) demonstrated a better survival of small sized eggs coming from a special stock of the freshwater fish walleye (Stizostedion vitreum)
The normal short cut method for describing viability is to keep larvae under constant environmental conditions from hatching to death of starvation (Blaxter & Hempel 1963, Chalmers et al. 1989). This is also a viability test in favour of larger size at hatching. Houde (1987) reduced the viability test to the survivors three days posthatching. On the basis of this test he did not find any influence of egg size, female weight or hatchability on the viability of the larvae of striped bass (Morone saxatilis)
By including behaviour studies and feeding experiments similar to Yin & Blaxter (1987) the present investigations has demonstrated an inverse relationship between egg mortality and the viability of the cod larvae. This means that small eggs having a high mortality producing inferior larvae. Since small eggs come from late spawning the present

investigation indicate that the larvae from these eggs are of lover viability, as hypotesized by Ellertsen & Solemdal (1990). This can be one of the explanations to the fact that no good year classes occur during cold years.

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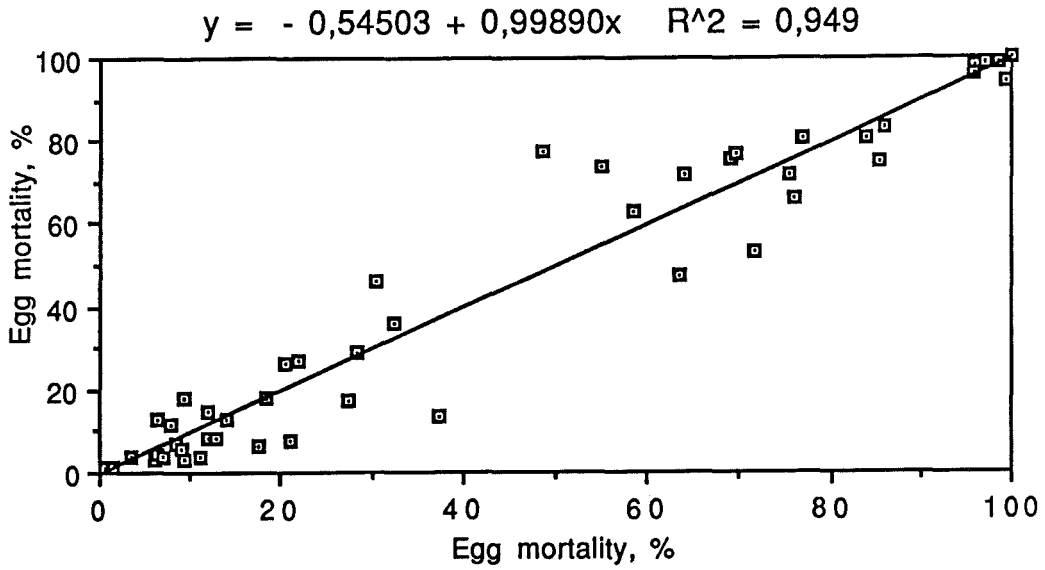

Fig.1. Egg mortality experiments on cod eggs.
The results of parallels

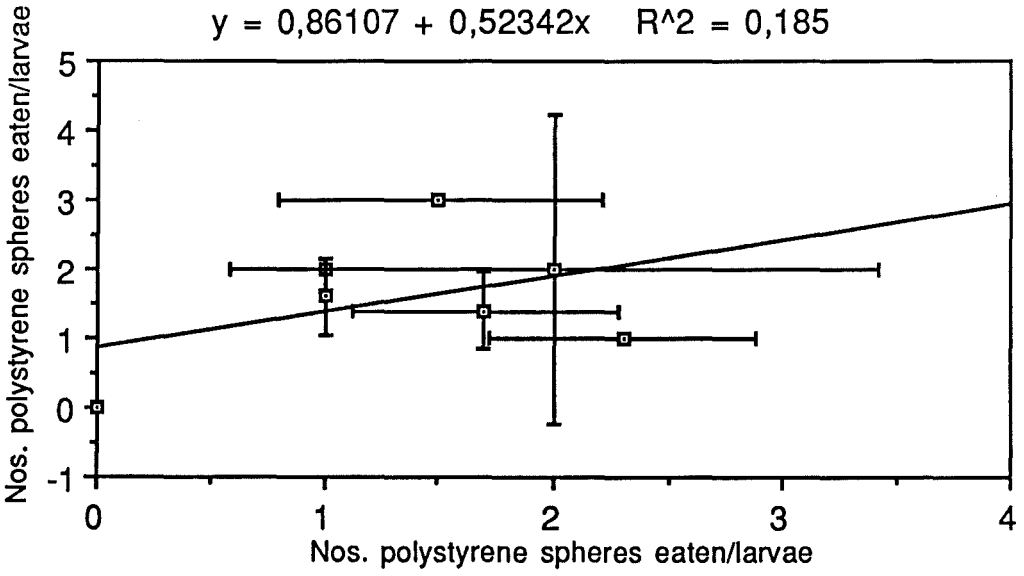

Fig.2. Feeding experiments on cod larvae using inert polystyrene spheres.
The results of parallels.

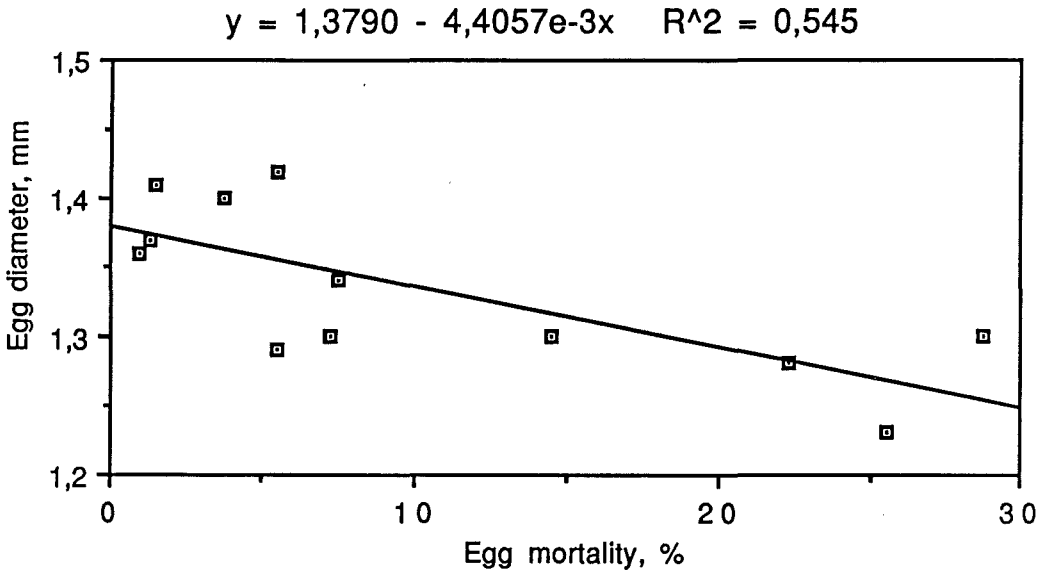

Fig.3. Egg diameter of 9 Lofoten cod and 3 coastal cod correlated to egg mortality.

■ Lofoten cod

▨ Coastal cod

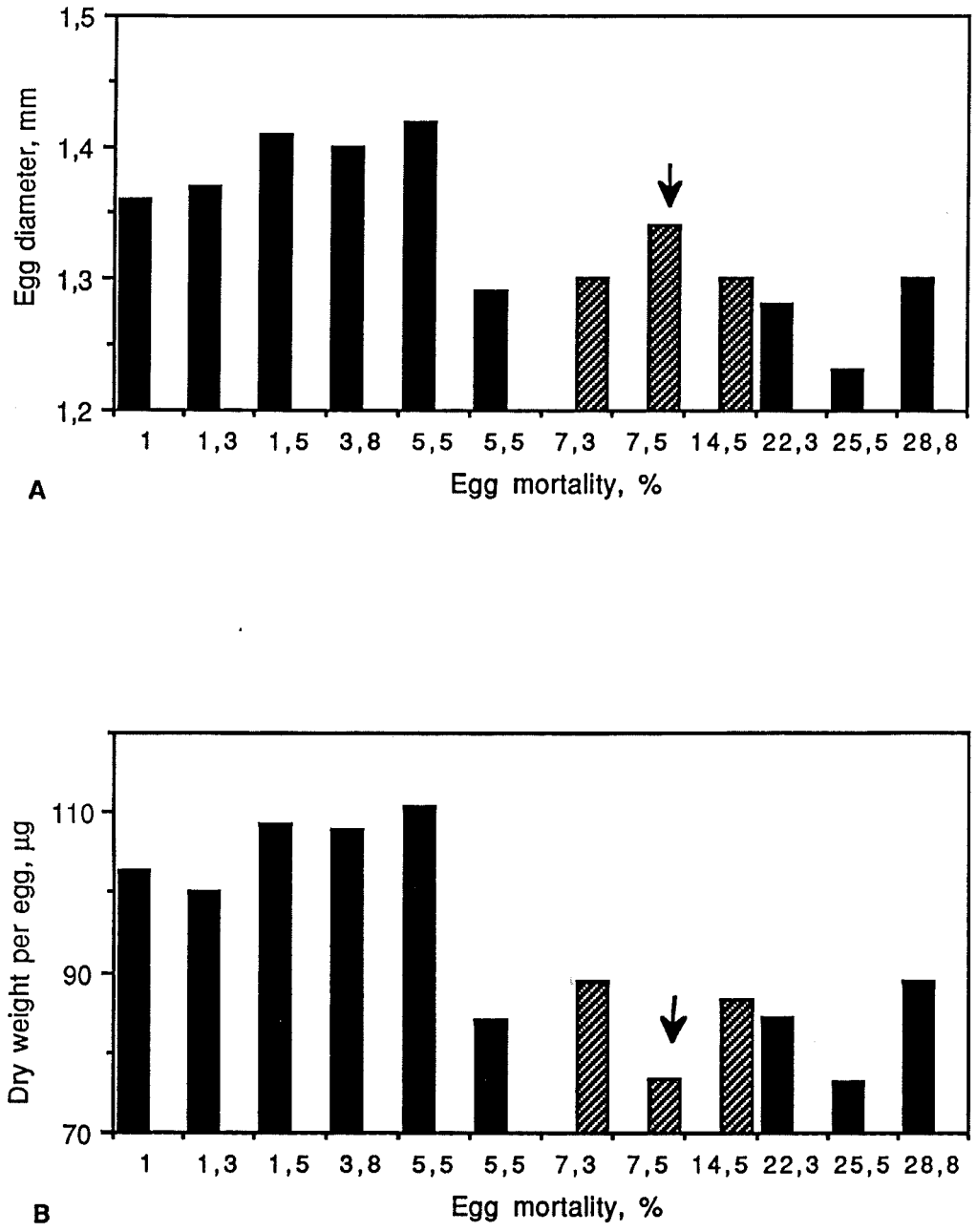

Fig.4. Egg diameter plotted against egg mortality from the 9 Lofoten cod and 3 coastal cod (A).
Egg dry weight plotted against egg mortality from the same cod specimens as A, (B). The arrow indicates the results from the last egg batch from a cod spawning in captivity.

■ Lofoten cod
▨ Coastal cod

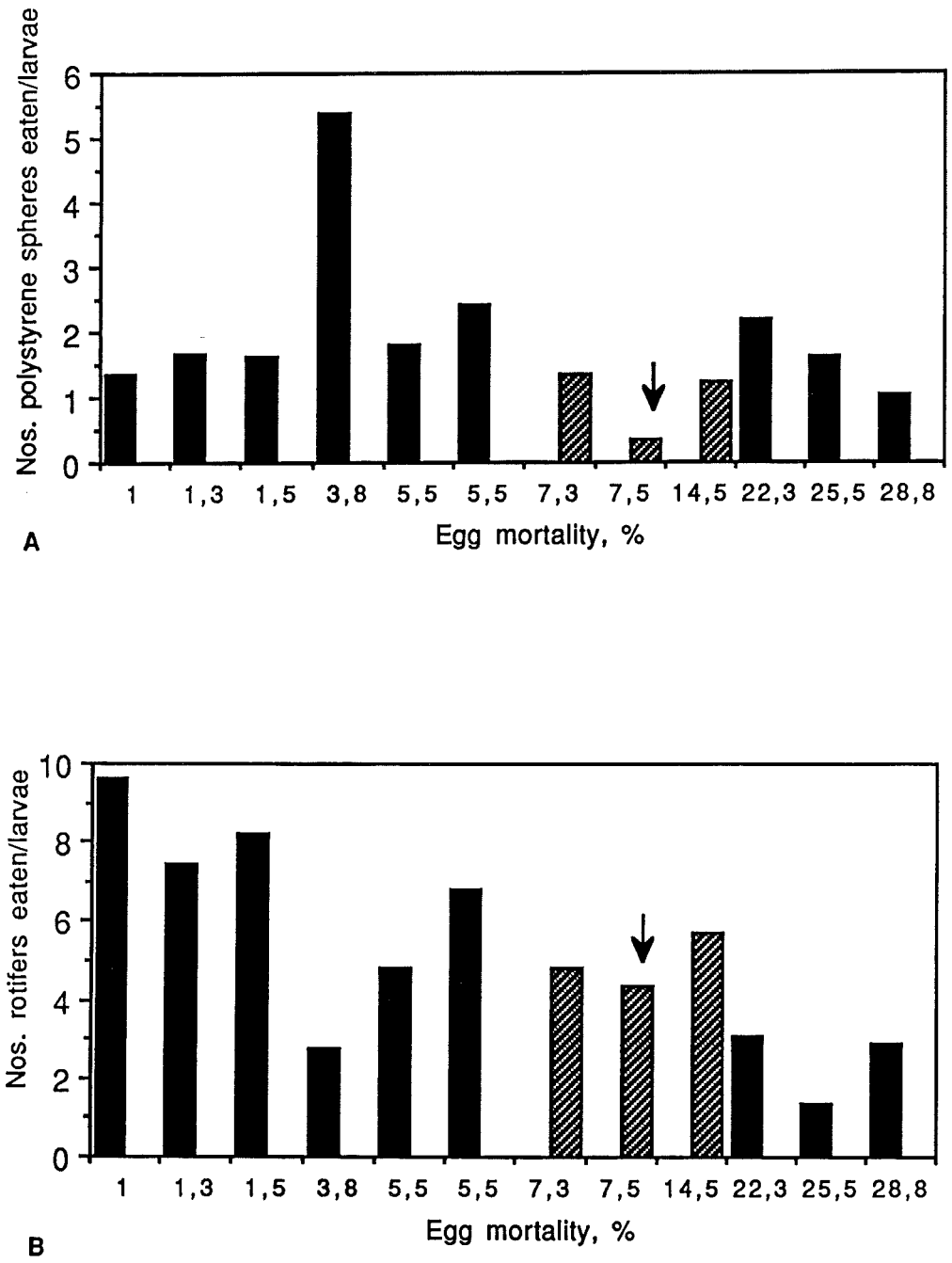

Fig.5. Numbers of inert polystyrone spheres eaten by cod larvae, plotted against egg mortality (A).
Numbers of rotifers eaten by cod larvae plotted against egg mortality (B). Arrow as in fig.4.

■ Lofoten cod
▨ Coastal cod

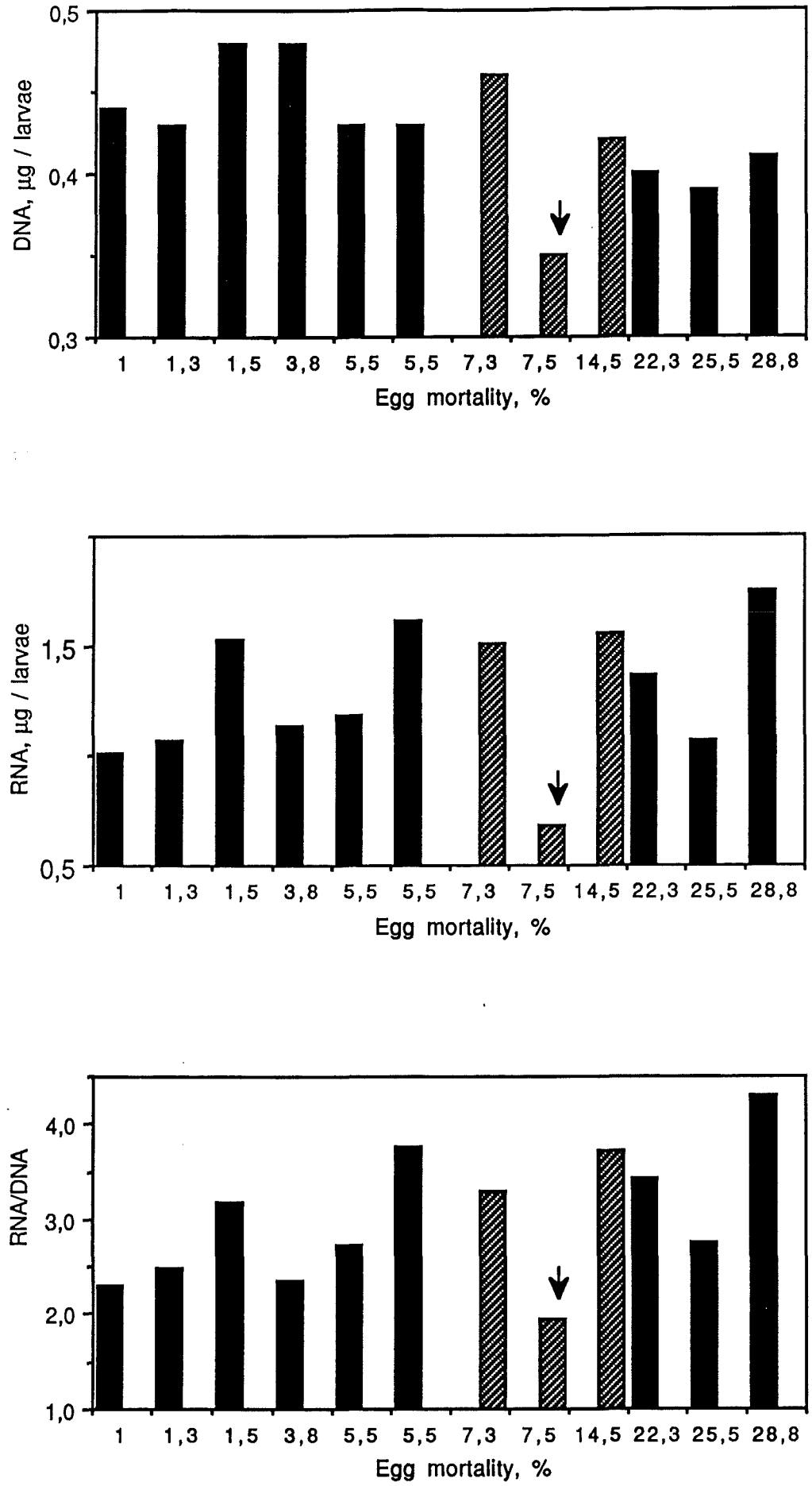

Fig.6. The content od DNA (A) , RNA (B) and the ratio RNA/DNA (C) from the 9 Lofoten cod and 3 coastal cod.

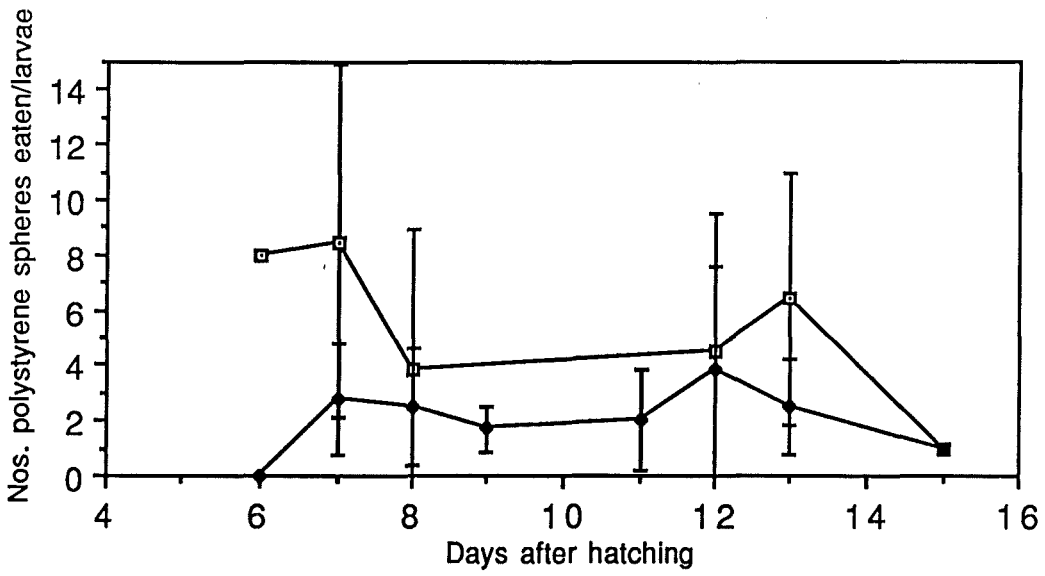

Fig.7. Experiments with inert polystyrone spheres eaten by cod larvae. The figure shows the test series from the highest scoring larvae group and one of the low scoring groups.

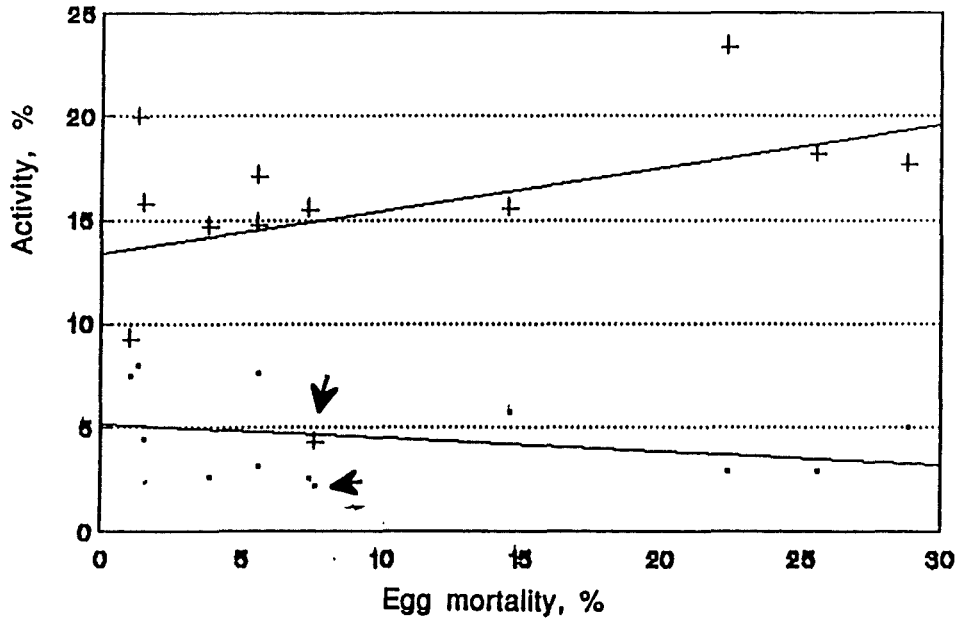

Fig.8. Activity, defined as % active time of the total observation period. Experiments were performed on Day 3 (•) and Day 6 (+) after hatching. Arrow as in figs. 4 and 5.