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The Propagation of Cod *Gadus morhua* L.

MORPHOLOGICAL, PHYSIOLOGICAL AND GENETICAL STUDIES OF EGG
QUALITY IN COD (*Gadus morhua* L.)

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ABSTRACT

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The development of cod eggs from various parent fishes was studied. Fertilization success, and proportion of normally developing embryos and larvae varied according to the quality of the eggs. Eggs from poor quality cultures had a significant increase in yolk osmolarity during the first 24 h after fertilization, and maintained higher osmotic values throughout the development than eggs of good quality. Fertilization in different salinities affected yolk osmolarity for all egg cultures, whereas a transfer to different salinities 24 h after fertilization did not. The chorion of eggs from good quality cultures was stronger than that from poor cultures. Heavy infection weakened the chorion.

Cod eggs collected from the plankton were compared with eggs incubated in the laboratory. Chorion strength was similar to that of good quality eggs, and varied greatly in both groups. The percentage of morphologically abnormal cod eggs from the plankton samples were as high as 20 % when early developmental stages were predominant, but decreased to about 5 % when embryos at a more advanced stage were more common.

Chromosome studies of early embryos from good egg cultures revealed few abnormal mitoses. High percentages of abnormal mitoses were often found in bad laboratory cultures, even though the early embryos appeared normal. A surprisingly large number of abnormal mitoses was also found in normal looking embryos from the plankton samples.

INTRODUCTION

In rearing experiments with marine fish species relatively little attention has hitherto been paid to possible effects of varying egg quality. Gamete quality may be affected by environmental factors concerning the parent fishes. Food availability, stress from handling and transfer, temperature, light and water quality are such factors (Billard et al., 1981). Results from parallel experiments with fish eggs from different females often seem to vary considerably, this may be due to small variations in incubation techniques. As conditions that may affect gamete quality can be difficult to control, early information on egg quality is important.

In a previous study, storage of cod eggs and characteristic early differences due to varying quality of egg cultures were outlined (Kjørsvik and Lønning, 1983). The present study is an attempt to achieve a further characterization of egg quality, and a better understanding of the implications these early egg quality criteria have for the further embryonic and larval development. Eggs from several females were followed from fertilization to hatching. The eggs were exposed to various salinities, in order to determine whether the response to these could be correlated with egg quality. Finally, eggs incubated in the laboratory were compared with eggs collected from the plankton.

MATERIALS AND METHODS

Adult cod (*Gadus morhua* L.) were collected by trawling in Balsfjorden near Tromsø in northern Norway. They were held for a few days in large seawater tanks (34 o/oo, c. 5°C). Ripe gametes were obtained by gentle stripping of the fish, and only apparently healthy, mature fish were used. The eggs were fertilized in seawater with excess sperm, and transferred to clean seawater after about 1 h. Egg cultures were held in 4 l beakers containig filtered seawater (34 o/oo). They were

inspected daily for dead eggs which were removed, and the filtered seawater was changed every 2 days. Care was taken to keep the experimental conditions as similar as possible for all experiments. The experiments were carried out at about 5 °C.

Fertilization and development in different salinities

Egg cultures were exposed to salinities between 14 and 48 o/oo in two ways:

- One batch was fertilized and reared in the different salinities.
- Another batch was fertilized in normal seawater (34 o/oo), and only normal looking eggs were transferred to the different salinities 24 h after fertilization.

Each batch consisted of eggs which were incubated in 100 ml glass dishes, otherwise they were treated as the larger egg cultures. Fertilization percentage was noted 24 h after fertilization, the number of dead and abnormally developing embryos was noted every second day.

Yolk osmolarity during development

The yolk osmolarity of dry unfertilized eggs, and that of fertilized eggs at various times during development was measured. The measurements were carried out on eggs fertilized and reared in 20, 34 and 41 o/oo. Measurements on eggs transferred to these salinities 24 h after fertilization were carried out after 10 days. The yolk samples were analyzed by measuring the freezing point depression on a Clifton Nanoliter Osmometer (Riis-Vestergaard, 1982; Kjørsvik et al., in press), and only eggs which developed normally were used.

Egg diameter and chorion strength

Diameter and mechanical strength of normal looking eggs were measured according to Kjørsvik and Lønning (1983) and Lønning et al. (in press). Samples were taken from unfertilized dry eggs,

and of fertilized eggs for up to 5 days after fertilization. Eggs collected from the plankton were also measured.

Egg collected from plankton

During the spawning season in 1983, plankton samples were collected once a week in the inner part of Balsfjorden (see also Falk-Petersen, 1982). In each haul, a Juday net (mesh size 540 μm) was towed near the surface for 15 minutes, at a speed of c. 0.5 knot. The amount of cod eggs in each sample was estimated, percentages of cod embryos younger than 5 days (embryos not visible), and also percentages of abnormally developing embryos were determined. Eggs from the plankton samples were always treated as soon as possible (by return to the laboratory, about 3 h after capture), to ensure that the observed abnormalities in cleavage and development were present before capture. Only live, floating eggs were examined for abnormalities.

Chromosome studies

The mitoses of approximately 3 day old embryos were observed, both from cultures fertilized and reared in the laboratory and from eggs collected from the plankton. Normal looking eggs were fixed in Carnoy (6:3:1 absolute ethanol:chloroform:acetic acid) for 24 h and stored in 96 % ethanol at 5°C. Before examination, the embryo was dissected from the egg with a needle under a low power microscope. The embryo was stained in aceto-orcein (Darlington and La Cour, 1969), squashed on a microscope slide, and cells and dividing chromosomes were examined in the light microscope. In each sample, between 12 and 17 eggs were examined, i.e. 1300-2000 mitoses were observed per sample.

In chromosome studies of fish embryos, 3 types of chromosomal aberrations are generally encountered (see Fig. 1, and also Crosby Longwell, 1977):

a) Delayed anaphases - i.e. delayed divisions of some of the centromeres, but the chromosomes will in most cases reach the

poles at late telophase.

b) Fragments - chromosomes, or parts of these remain in or near the equatorial plane.

c) Anaphase bridges - some of the chromosomes do not divide, but remain in the equatorial plane; others do not divide properly, but form a bridge between the dividing chromosomes.

Such fragments and bridges are indicative of severe chromosomal damage in fish embryos, and can result in irregular distribution of chromosome material to the daughter cells (Crosby Longwell, 1977).

If an embryo had more than 25 % delayed anaphases it was classified as being of poor quality. This limit was decided since embryos with less than 25 % aberrations had few or no fragments and bridges.

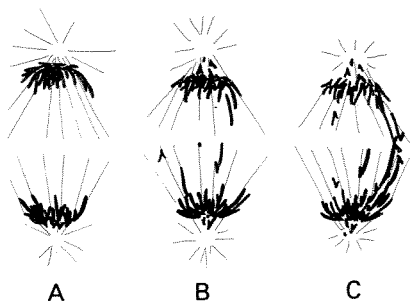


Fig. 1. Examples of chromosome aberrations in fish embryos. A: normal anaphase, B: fragments, C: anaphase bridges and fragments.

RESULTS

Egg cultures were classified as of good quality or of poor quality according to the criteria earlier outlined (Kjørsvik and Lønning, 1983). To point out some major differences that are due to quality, we followed the best and the poorest egg cultures during development to hatching.

Fertilization and development in different salinities

Differences in fertilization percentage between the egg cultures are illustrated in Fig. 2, and these differences are also reflected in the curves showing hatching success. In all cultures, gastrulation started 3-4 days after fertilization, the blastopore closed after 8 days, and 50 % of the larvae had hatched after 18 days. One culture had an incomplete cortical reaction. Traces of cortical alveoli were visible throughout the blastula stage, but the fertilization percentage was rather high, and the blastulae appeared normal. However, from the curve showing hatching success (same figure), it is obvious that the condition of this culture was rather poor. The fertilization process thus seems to reflect the subsequent "health condition" of an egg culture.

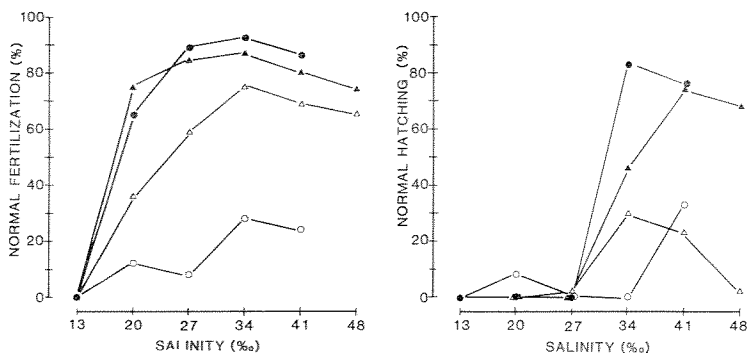


Fig. 2. Fertilization and hatching of eggs from four female cod. The eggs were fertilized and reared in various salinities. ●, ▲ : good quality cultures, ○ : poor quality culture, △ : culture with incomplete cortical reaction.

Fertilization percentage varied both between eggs from different females and between eggs fertilized in different salinities (see also Fig. 2). All cultures had fertilization maxima in 34 o/oo, but the proportion of fertilized eggs in 34 o/oo ranged from 30 to 90 %.

In diluted seawater, the fertilization % decreased for all cultures. In good cultures, most unfertilized eggs were unactivated (i.e. the cortical reaction had not taken place). In the poor quality cultures on the other hand, there was a large proportion of activated eggs among the unfertilized eggs.

No eggs could be fertilized in 14 o/oo. If eggs were transferred to 14 o/oo 24 h after fertilization, they developed slowly and rather irregularly, and died within 4 days (i.e. before gastrulation). Fertilization and subsequent development in 48 o/oo seemed lethal for poor cultures, whereas good quality eggs survived. We describe therefore, development in salinities between 20 and 41 o/oo. All eggs incubated in salinities lower than 34 o/oo, developed on the bottom of the containers.

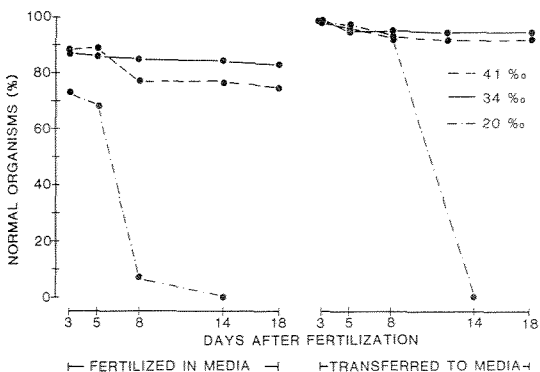


Fig. 3. Development of cod eggs from a good quality culture in three different salinities. The eggs were either fertilized in the incubation media, or fertilized in normal seawater (34 o/oo) and transferred to the media 24 h after fertilization.

In a good quality culture (Fig. 3), there was not much difference between eggs fertilized in different salinities, and those transferred to them 24 h after fertilization. In high and normal salinities, there was little mortality and abnormal development. Eggs fertilized in 20 o/oo developed apparently normally up to mid-gastrula, thereafter their development was

retarded and somewhat irregular. Eggs transferred to this salinity seemed to develop normally almost up to closure of the blastopore. However, all eggs in 20 o/oo died before closure of the blastopore.

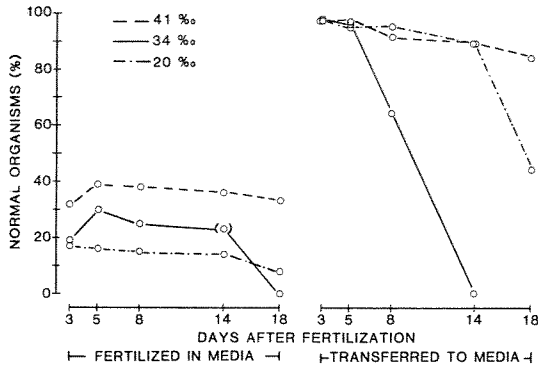


Fig. 4. Development of cod eggs from a poor quality culture. (○): somewhat irregular embryos.

The poor quality culture (Fig. 4) showed a marked difference between eggs fertilized in, and eggs transferred to the various salinities. This difference was due to a low fertilization percentage, but the largest mortality also occurred during the first 24 h. The percentage of normal development was higher in 41 o/oo than in 34 o/oo, probably due to greater buoyancy in the higher salinity. No eggs in the small glass containers survived in 34 o/oo, and during development a decreasing buoyancy was observed, as well as bacterial contamination. Oddly enough, eggs could survive in 20 o/oo, but eggs in this salinity did not have many bacteria attached to the chorion. In general, we have observed that eggs from poor quality cultures are more subjected to decreasing buoyancy and bacterial contamination than are eggs from good cultures. The survival in 20 o/oo in this culture seems therefore rather an exception.

The culture which showed an incomplete cortical reaction (Fig. 5) also showed an increasing proportion of abnormalities

during development. Although most of the blastulae seemed normal, the more advanced embryos were irregular and often bent. In 34 o/oo, only 30 % of the embryos hatched normally. Most of the larvae were bent, and many did not survive hatching. A tendency to sink was observed during development also in this culture.

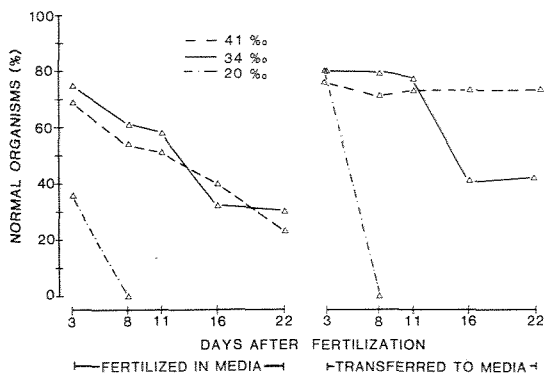


Fig. 5. Development of cod eggs from a culture with incomplete cortical reaction.

Yolk osmolarity during development

Yolk osmotic values for eggs fertilized and reared in 20, 34 and 41 o/oo are shown in Fig.6. A significant difference in yolk osmolarity was observed according to egg quality. In 34 o/oo, eggs from the good culture had a minor increase in yolk osmolarity during the first hour after fertilization, but thereafter values decreased somewhat during development. Eggs of poor quality had a larger increase in yolk osmolarity after fertilization. Like the good quality eggs, eggs of poor quality also had a decrease in osmotic values during subsequent development. However, values were always higher than for eggs of good quality.

Fertilization in different salinities affected yolk osmolarity for both good and poor quality eggs, and the osmotic

difference between the cultures seemed to increase with increasing salinity. Transfer to different salinities 24 h after fertilization did not seem to affect yolk osmolarity much, as measurements made 10 days after transfer did not reveal significant differences between eggs in the various salinities (see Table 1).

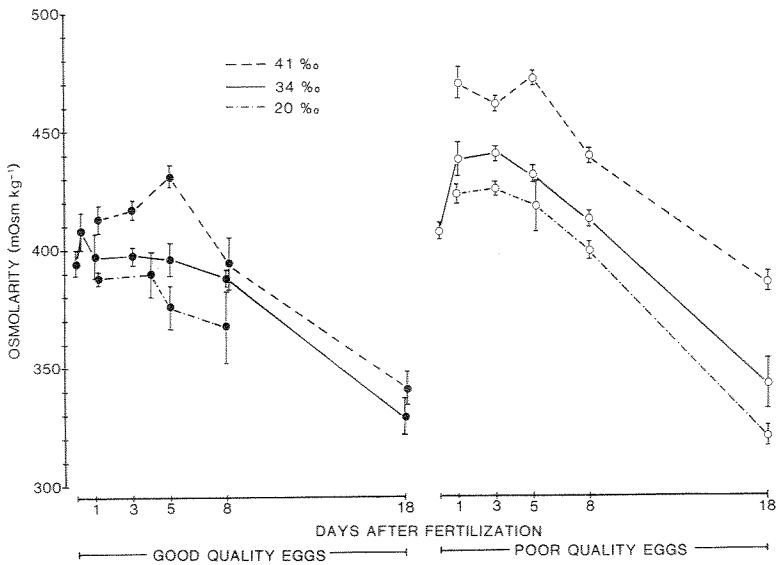


Fig. 6. Yolk osmolarity of eggs fertilized and reared in 20, 34 and 41 o/oo.

Egg diameter and chorion strength

Diameter varied between the cultures (Fig. 7), but egg diameters are known to vary, both between different females and also during the spawning season (Solemdal, 1970). Good quality eggs however, showed an increase in diameter following fertilization, whereas poor quality eggs did not.

TABLE 1

Mean yolk osmolarity of cod eggs from good and poor quality cultures, measured 10 days after fertilization. The eggs were fertilized in 34 o/oo, and transferred to 20, 34 and 41 o/oo 24 h after fertilization.

Salinity (o/oo)	Good quality (mOsm)	Poor quality
20	350	382±14
34	354± 4	388± 7
41	358±10	393± 2

Furthermore, the chorion strength of all eggs increased after fertilization, but the increase in the poor quality culture was only about 65 % of that in the good quality culture (Fig. 7). Strength decreased somewhat during development for both cultures. We did not measure chorion strength for more than 5 days, as very few eggs from the poor culture survived, and the eggs underwent an increasing bacterial contamination.

Bacteria weaken the mechanical strength of the chorion, which is illustrated for one egg culture in Fig. 8. During early development, the eggs were clean and had a mean strength of 142g. Later, some of the eggs got an increasing bacterial contamination, and this resulted in a weakening of the infected eggs compared with the clean eggs.

In some poor quality cultures, particularly at the beginning and end of the spawning season, the chorion of many eggs remained soft and became rather swollen in contact with seawater. The resistance of these eggs was near zero. Most of these eggs could not be fertilized, and those that did developed abnormally.

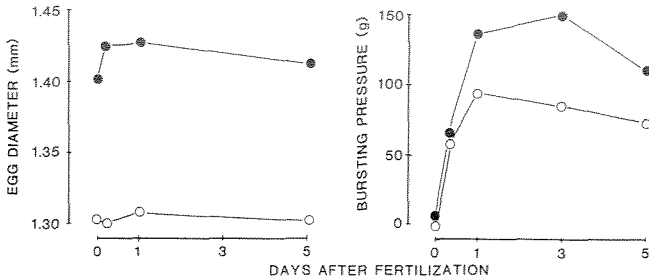


Fig. 7. Egg diameter and chorion strength during development of eggs from good (●) and poor (○) quality cultures.

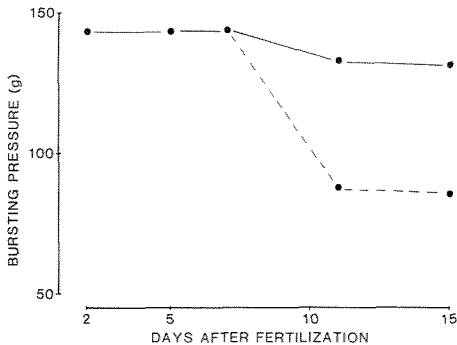


Fig. 8. Chorion strength of cod eggs during development. Some of the eggs from this culture became contaminated by bacteria, with a subsequent decrease in mechanical resistance (----).

Eggs collected from plankton

In the plankton samples, there seemed to be a peak in number of cod eggs at the end of March (Fig. 9), and the percentage of cod eggs younger than 5 days (embryo not visible) decreased just before the number of cod eggs decreased. This indicates that most of the spawning in Balsfjorden took place during March,

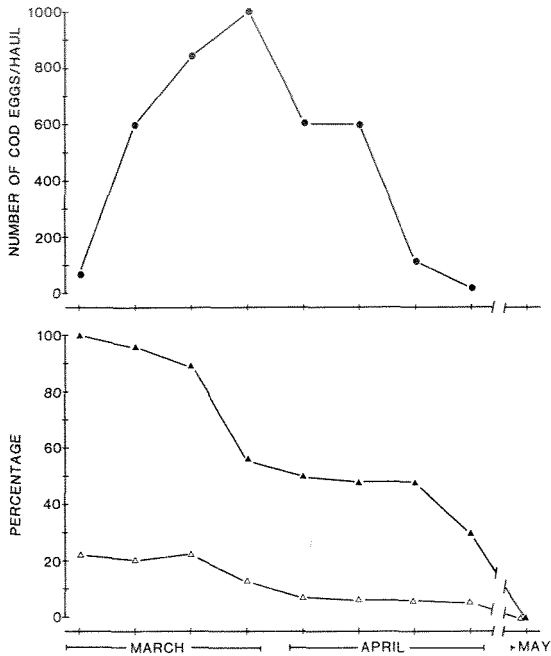


Fig. 9. Number of eggs (●), percentage of cod eggs less than 5 days old (▲), and percentage of morphologically abnormal cod embryos (△) from planktonic samples in Balsfjorden 1983.

which is also in agreement with our catches of mature cod this spring.

The lower curve in Fig. 9 shows the percentage of abnormally developing cod eggs from the plankton samples. When early developmental stages predominate, the percentage is as high as 20, whereas it drops down to about 5 % when embryos at a more advanced stage are more common.

Mechanical strength of planktonic eggs (Table 2) was similar to good laboratory cultures, with mean values ranging between 120 and 140 g. There was no difference between early and late developmental stages.

TABLE 2

Mean values of chorion strength measured from cod eggs found in planktonic samples from Balsfjorden.

	2-16 cell	32-cell to mid-gastrula	Late gastrula to hatching
Mean bursting pressure (g)	120±34	120±41	140±42
N	15	13	18

Chromosome studies

The mitosis of artificially fertilized eggs of different quality were compared. Laboratory eggs were also compared with eggs from the plankton. The results are summarized in Table 3. There was a marked difference between the good and the poor quality cultures. In the good quality culture, the embryos had few abnormal mitoses (range 7-30 %, see also Fig. 10A). The aberrations were delayed anaphases, and no fragments or bridges were observed. Whether such delayed anaphases are normal or repairable by the cells we do not know, but this phenomenon seemed to be present in most eggs, independent of the quality.

In eggs of poor quality, also in the culture with incomplete cortical reaction, the embryos had a high percentage of abnormal mitoses (range 11-60 %). Both fragments and bridges were commonly observed (Fig. 10B). Thus even though the embryos examined appeared morphologically normal, severe abnormalities could be observed from the chromosomes.

Eggs from plankton resembled the good quality eggs to a certain extent, but the embryos had a more variable quality according to the percentage of abnormal mitoses (range 6-60 %). In planktonic eggs, delayed anaphases were predominant, but some eggs had severe chromosome damages such as fragments and bridges.

TABLE 3

Results from chromosome studies on artificially fertilized eggs of good quality, poor quality, and on eggs with incomplete cortical reaction. Eggs collected from the plankton were also included. 1:delayed anaphases, 2:anaphase bridges, 3: chromosome fragments. **: commonly observed, *: observed, -: not observed.

	Embryos examined (N)	Embryos of poor quality (%)	Mitoses counted (N)	Abnormal mitoses (% per embryo)	Mitotic aberrations		
					1	2	3
Good quality	17	6	1300	7-30	**	-	-
Poor quality	25	84	2000	11-60	**	**	**
Incomplete cort.reaction	7	86	600	20-70	**	**	**
Plankton	27	15	2000	6-60	**	*	*

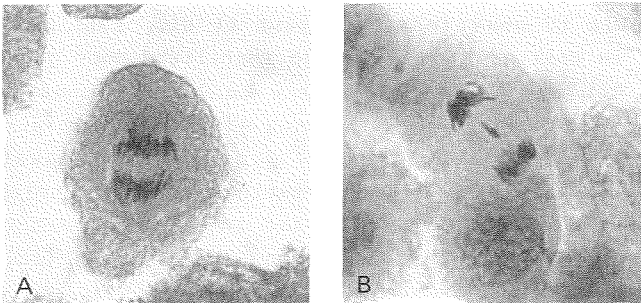


Fig. 10. Mitotic divisions in 3 day old cod embryos (1500 x). A: normal anaphase of an embryo from a good quality culture. B: anaphase with fragments of a normal looking embryo from a poor quality culture.

DISCUSSION

In our previous paper (Kjørsvik and Lønning, 1983), poor quality eggs were found to have a long (c. 30 min) and often incomplete cortical reaction. The present study indicates that a complete cortical reaction is necessary for normal development. Also, in contrast to good quality eggs, poor quality eggs undergo no significant increase in egg diameter, have higher internal osmotic values, and do not attain high mechanical strength. We feel that these phenomena are linked.

The rapid formation of a perivitelline space in good cultures is probably due to the release of colloids from the cortical layer (Yamamoto, 1961). As the cortical reaction is slow or incomplete in poor quality cultures, it might lead to a smaller perivitelline space, and is probably also the cause of the lack of increase in egg diameter.

A prolonged fertilization process may also affect membrane characteristics. The vitelline membrane in teleost eggs changes from high to a very low permeability shortly after fertilization (Potts and Rudy, 1969; Loeffler and Lovtrup, 1970; Potts and Eddy, 1973). Unfertilized cod eggs in the ovary are strongly hypoosmotic to seawater (Davenport et al., 1981). A greater increase in yolk osmotic values in fertilized poor quality eggs than in good eggs may therefore be a result of a longer fertilization process, since an egg with a permeable membrane will tend to lose water to the hyperosmotic seawater. The salinity will affect both fertilization rate and development of teleost eggs (Holliday, 1969), and in this respect cod eggs seemed to respond rather like eggs of plaice (*Pleuronectes platessa*), being quite tolerant to high salinities. As with the eggs of plaice (Holliday and Jones, 1967), fertilization and rearing of cod eggs in various salinities resulted in corresponding differences in yolk osmolarity. However, the poor cultured always had higher value than the good one. Transfer to various salinities 24 h after fertilization did not seem to affect yolk osmotic values much. The vitelline membrane in poor quality eggs must therefore reach low permeability within 24 h.

Small or no changes in yolk osmotic values were also observed when lumpsucker (*Cyclopterus lumpus* L.) eggs were transferred to various salinities 1 h after fertilization (Kjørsvik et al., in press).

In addition, egg cultures of both cod (Kjørsvik, unpublished) and lumpsucker (Kjørsvik et al., in press) even less viable than those presented here, showed increasing yolk osmotic values during development until all eggs were dead. Abnormally developed embryos had values up to 6-700 mOsm, signifying that osmoregulatory mechanisms had broken down.

When cod eggs are transferred to seawater, they harden during the first 24 h (Kjørsvik and Lønning, 1983), and it is typical for poor cultures to harden less than good cultures. The hardening process is probably dependent on enzymes which might be stored in the cortical layer (e.g. Ohtsuka, 1960; Lønning et al., in press), and less chorion strength in poor quality cultures can be due to enzyme reactions not functioning properly. Low chorion strength could make it easier for the larvae to hatch, but will also make the embryo less protected against mechanical damage. Cod eggs sampled from the plankton had high chorion strength, but unlike laboratory reared eggs (see also Kjørsvik and Lønning, 1983), the planktonic eggs did not seem to decrease in strength during development. Also Pommeranz (1974) found that chorion strength in laboratory cultures of plaice (*Pleuronectes platessa*) eggs decreased during development, and the average bursting pressure for plaice eggs was usually much higher in the natural than in the test material. Decreasing chorion resistance might therefore be a laboratory phenomenon, influenced by for example bacterial contamination.

Poor quality eggs were less buoyant than good eggs. It seemed that higher salinities resulted in a better development of such cultures, probably because of better buoyancy. Cod eggs are susceptible to mechanical stress (Rollefsen, 1932), and are also generally more subjected to bacterial contamination if they develop near the bottom.

Problems with variation in egg quality is rather well known

But are poor quality eggs merely a laboratory phenomenon, and are eggs spawned naturally generally of good quality? Little is known about the natural environmental factors that control the mortality of fish eggs and larvae (NOAA Technical Report, 1976). The results from the plankton surveys indicated a surprisingly high percentage of abnormalities among the early egg stages. The percentage of abnormally developing eggs in the plankton decreased as later developmental stages became more abundant, indicating that eggs of poor quality die during the early embryonic development.

Chromosome conditions in normal looking planktonic cod eggs indicated a mixture of eggs of different quality. Not many studies deal with chromosomal work on fish eggs, but early egg stages of the Black Sea Scorpionfish reared in the laboratory have been found to have about 12 % abnormal mitoses (Ivanov, 1979). Severe chromosomal errors occurring in fish eggs before the gastrula stage are supposed to be lethal (Crosby Longwell, 1977, 1983), and chromosome abnormalities at the early embryonic stage may therefore be a sensitive indicator of sublethal damage to the embryo.

In a review, Rosenthal and Alderdice(1976) discuss various responses of environmental stress to marine fish eggs and larvae. They conclude that gonadal tissue and the early embryos are especially susceptible, and that stress induced at an early stage may possibly result in reduced fertility, embryonic malformation and malfunctions. Our results also show that even if many embryos from poor quality cultures seemed to develop normally, they were obviously functioning quite differently.

Laboratory organisms are probably more susceptible to stress than "wild" organisms, but could be easier to observe. Reduced quality of eggs is probably caused in the ovary or near fertilization, and our results from the plankton so far indicate that good quality eggs are not necessarily guaranteed if fishes are allowed to spawn naturally. Finding appropriate methods for handling of fish and gametes must therefore be considered important, as must early assessment of egg quality.

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