International Counicil for the
Exploration of the Sea

CM 1998/DD:8

Longterm studies on spawning in Arcto - Norwegian cod - mortality pattern of eggs and early larvae.

by

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Abstract

The ongoing studies are efforts to document the ideas of Soviet scientists that first time spawners produced eggs and early larvae of lower viability than multiple spawners. This phenomenon will be of importance both in management of fish populations and in a fish farming.

Earlier investigations on the small sized Norwegian coastal cod had confirmed this idea. It was decided to repeat these experiments on Arcto-Norwegian cod, maturing later and at a larger size.

Arcto-Norwegian cod were transferred from the Bear Island to Institute of Marine Research, Bergen, Norway in May 1996. First time spawners were selected and the spawning monitored for the same individuals in the spring of 1997 and 1998.
The quality of each egg batch was investigated during the early cleavage stages, observing the degree of asynchronic development, unfertilized eggs, etc.
In addition to the frequency of malformations and mortality in eggs and early larvae, the following parameters were investigated: Fecundity, egg diameter, dry weight and neutral buoyancy of eggs and early larvae.
It was assumed that the malformations mainly were the results of internal developmental problems. Each type of malformation corresponds to certain morphogenetic events in the embryogenesis.

The results of the experiments in Arcto-Norwegian cod showed a similar trend as for Norwegian coastal cod, with a decreased mortality in egg and early larvae from second spawning compared to first spawning in the same individuals. The reduction in egg and early larval mortality was somewhat smaller in Arcto-Norwegian cod. This may be the result of the size difference in the two tribes of cod, the Arcto-Norwegian cod being of significantly larger size at maturation.

Introduction

Since the documentation of yearclass variation as an important factor of the fluctuation in fish stocks (Hjort 1914), various schools have emerged to explain this phenomenon. Most scientists have focused on the early stages. Synchronization problems between exogenous larval feeding and the production of food organisms, predation on eggs and larvae, location of the spawning sites and varying passive drift patterns, effects of microturbulence etc. are some of the recruitment mechanisms studied.

Focusing on the variation in size of the spawning population is another way of analyzing recruitment variation. Large efforts have been carried out to establish the relation between spawning stock size and recruitment, with varying results. Improvements have been obtained by using egg production (fecundity etc) to calculate the total reproductive potential (Marshall et al. in press).

However, calculating the reproductive potential includes some phenomena which have to be taken into account. The main factors are the atresia and the varying frequency of different types of malformation in the development of eggs and early larvae (before starvation) related to age/ size and condition of the mother fish, called the maternal effects (Nikolski 1962,a,b). Pioneer investigations of the effect of agecomposition of the spawning population on yearclass strength on Arcto-Norwegian cod is given by Ponomarenko (1973), and an overview of the effect of growth rate of spawners on the survival of progeny is found in Vladimirov (1973).

The maternal effect is supposed to be the result of development problems through through the early stages, combined with adverse environmental conditions, mainly studied by Soviet researchers, e.g. Svetlov (1960) and Vladimirov (1975).

Maternal effects based on growth will vary according to the age/size composition and the condition of the spawning population. First time spawners have significantly different egg characteristics and higher mortality than multiple spawners in the Norwegian coastal cod (Solemdal et al. 1995, Kjesbu et al. 1996). Age/size composition of the spawning population therefore will be one of the factors regulating yearclass strength in a systematic way.
Condition variations of the spawners will influence on the general growth pattern and hence influence on the maternal effect and the reproductive potential.

To our knowledge no combined, experimental growth/condition studies on the maternal effect are

found in the literature.

The idea of a high, natural occurring frequency of malformation and mortality in eggs and early larvae from highly fecund fish with pelagic eggs, has little impact in Western literature, see Rothschild (1986).

Investigations of the frequency of malformation in pelagic eggs from the North Sea during the last 20 years have explained the results as effects of pollution and extreme environmental factors, (Rosenthal & Alderdice,1976: Cameron & Westernhagen,1997).

Malformed cod and haddock eggs from the Barents Sea is supposed to be the result of pollution from oil drilling (Mukhina et al.1996, Mukhina pers.com.).

Kjørsvik et al. (1984) investigated early development of cod eggs at the spawning sites in Balsfjord, Northern Norway, and found 20 % with genetic aberrations.

The maternal effect on egg viability as a recruitment mechanism may not be of significant importance. However, it is the only mechanism which can be manipulated by man, through fishing intensity, gear and geographical pattern (Chambers & Leggett, 1996, Solemdal, 1997).

The present paper deals with spawning and egg mortality of Arcto-Norwegian cod during the seasons of 1997 and 1998, using the same individuals as first and second spawners. The study is a continuation of a similar study on the smaller sized Norwegian coastal cod (Solemdal et al. 1995, Kjesbu et al. 1996). In addition mortality experiments were carried out on eggs from Arcto Norwegian cod of first and second spawners in 1995.

Materials and methods

Broodstock of Arcto-Norwegian cod was trawled near the Bear Island in May 1996 by R/V " Johan Hjort". About 500 specimens, length of 50-70 cm, were transported in four tanks of 1m3 and one tank of 2 m3. About 350 cod survived the transport to the Institute of marine Research, Bergen.

No food was given during spawning, as they do not normally feed at this time (Kjesbu et al.1991). The cod was stocked in outdoor tanks of 30m3, and fed to satiation on a commercial special cod dry pellets (Felleskjøpet). The feeding was manipulated to give the same condition factor both spawning seasons. The condition factor and the total weight of the cod females in 1994 and 1995 is shown in table 1, and the weight,Fulton's condition factor and % weight increase of the cod in 1997 and 1998 are found in table 2.

The sex and maturity stage were investigated on a large number of the prespawning cod, by cateterization and metomidate anaestization (Kjesbu 1989, Mattson & Riple 1991). The mean diameter of the most advanced vitellogenic oocytes were measured to forecast the calender day of first spawning (Kjesbu 1994). In this way a group of immature cod and a group of the smallest maturing individuals could be identified. These were supposed to be first time spawners. Later analysis of spawning checks in the otoliths (Rollefsen 1934) will give the final answer to this

question.
Immediately before start of spawning, 10 couples were moved to an indoor circular tank of 200 m3, divided into 10 chambers, with a natural light cycle. In most cases the same male and female were put into the same spawning chamber during the two spawning seasons.
Two females,nos. 9 and 10, died between the spawning periods in 1997 and 1998.
The water temperatures in the outdoor tanks during the six months prior to spawning varied by ± 0.5° C. The temperature differed by 1° C between the spawning seasons 1997 and 1998, being higher in 1998.
During the experiments each egg batch was treated separately. In addition to morphological and mortality studies a series of other parameters were investigated: Egg diameter, egg dry weight, neutral buoyancy, fecundity, larval otoliths etc. (Kjesbu et al. unpubl.)

Early egg development investigations.
Morphological studies were performed on each egg batch at the time of sampling from the spawning tank. The frequency of unfertilized, dead, "good", "medium" and "bad" eggs were calculated. The definitions are :
"Good eggs": Normal development
"Medium eggs": Irregular and asynchronic development
"Bad eggs": Eggs with destroyed membranes
During these investigations the egg samples were icechilled. This procedure was standardized, and the additional mortality during the inspection of the egg samples were comparable between the years 1997 and 1998. Hence a rough estimate of the mechanical strength of the eggs were obtained by calculating the egg mortality during this early observation.

Egg mortality

Eggs sampled from the spawning chambers, temperature 8-9 °C, were kept in a refrigerator at 5 ° C until the eggs reached development stage 1a according to Westernhagen (1970).
Eggs were then sterilized in 1% Buffodin for 10 minutes and rinsed in seawater. Buffodin is a iodine-based fish farming disinfectant, and used against major fish viruses (Evans Vanodine International LTD).
Seawater used for the experiments was passed through 10 and 30 micron filters and an UV-irradiation system.
The following compounds were added to the seawater used for the experiments: streptomycin sulphate (0.05 gram/l) and doktacillin (0.2 gram/l).

Two hundred eggs were chosen for each egg mortality experiment. All experiments were performed in parallel. The egg pipette and the vial to count the eggs were rinsed in 4 % formaldehyde.
30-40 eggs were inspected at the time under a low power stereomicroscope at normal laboratory temperature conditions. Eggs were rejected on the basis of the following criteria: Unfertilized, activated, dead, irregular cleavages and eggs from previous egg batches.

The selected eggs were transferred to one litre glass jars with prepared sea water, and placed in a refrigerator at 5° C.The temperature was recorded daily and average temperatures were 5.04 (59) ± 0.76 and 4.76 (76) ± 0.67 in 1997 and 1998, respectively.
Dead eggs were removed each day with a pipette specific for each jar, counted and staged. Developmental stage was checked on live eggs every third day. In a few cases bacterial infection developed. These experiments were excluded from the material.

The number of egg mortality experiments in 1 l glass, 200 eggs, antibiotics added in 1997 and 1998 were 25 and 37, respectively.

Eggs were put into a NUNC tray (Roskilde, Denmark), containing 24 compartments of 2 ml of 70 % autoclaved seawater each. The NUNC-tray and the eggs were kept on ice during selection of the eggs under the stereomicroscope. Only "good eggs" were selected. During incubation they were kept in a refrigator at 5° C.
Two NUNC-trays of 24 eggs each were used for each egg batch. Numbers of experiments in 1997 and 1998 were 82 and 96, respectively.

In 1994 and 1995 different individuals of first and second spawners of Arcto-Norwegian cod were used in egg mortality experiments.
In 1994 NUNC-tray experiments from different females were performed,
Twenty-one egg mortality experiments in 1995 with eggs from Arcto-Norwegian cod in NUNC-trays had a mean difference between replicates of 2.5 % in two NUNC-trays of 24 eggs each.

Results

Number of days between successive egg batches in 1997 and 1998 are shown in table 2.
Egg batches from the spawning tanks were in different development stages when sampled. Fig.1 shows the average percent of "good eggs" at different development stages at sampling, from 2 blastomeres to the morula stage, with a maximum number of samples at development stage of 64 blastomeres.
The mean frequency of unfertilized eggs from the newly sampled batches are given in fig.2. Females 5 and 7 showed a reduced regularity in their batch spawning compared to the other females, see table 2. Female 8 had a regular timing of the first 11 batches, and irregular intervals between the last batches, resulting in high frequency of unfertilized eggs.
"Medium eggs" are eggs with asynchronic development, resulting in a large frequency of malformed and dead eggs during development. In fig.3 the mean frequency of "medium eggs" from newly sampled egg batches are showing high frequencies in females 5, 7 and 8, as also found in unfertilized eggs from female 8, see fig.2, is also found in female 8 in fig.3.
Eggs defined as "good" at the moment of sampling are shown in fig.4. Females 5,7 and 8 showed a reduced frequency of "good" eggs, specially the last, irregular, batches from female 8.
The results of the standard method to evaluate the mechanical quality of eggs are shown in fig.5.

The mortality during a standard procedure of the early investigation is obviously much larger in 1997 compared to 1998.

The results of egg mortality experiments from the 1 liter glass-method are shown for individual females in fig.6. The average egg mortality from 1997 and 1998 using this method is 45.4 and 58.6 %, respectively. The average egg mortality of the parallels are 54.8 and 55.1 %,respectively.
Comparing mortality during egg development for the "1 liter"-method in 1997 and 1998, a very great difference occured at the blastula stage, fig. 7. Later only minor relative changes in egg mortality occurs.
Individual results from mortality experiments from NUNC-trays are found in fig.8. The cod females excluded from the material on the basis of irregular spawning and data upon sampling, fig.2-4, females 5,7 and 8 ,showed tendency of incresed mortality in 1998, compared to 1997, while the other females showed reduced egg mortality. Average egg mortality in 1997 and 1998 is 56 and 36, 6 %, respectively, fig.9.
The disagreement between the two egg mortality methods is illustrated in fig.10 for the 1998-experiments. The average egg mortality is higher in the 1 liter.method for eggs from all the females. The discrepancy in egg mortality occurs at the blastula stage,fig.11.
Embryonic mortality occurs at the egg stage or early larvae stage, before the effect of starvation starts. The early larval mortality are caused by: mortality at hatching, longlivingmalformed larvae and eggs not hatching. Fig 12. shows the relation of mortality on the egg stage and early larval stage. This relation seems to be similar in offspring from the same female from year to year, resulting in a very similar proportion between 1997 and 1998, 48.2 and 48.5 % during the egg stage, respectively
In fig.13. the egg mortality experiments from the NUNC-trays in 1997 and 1998 are presented on the batch level.
Egg mortality experiments 1994 and 1995, using Arcto-Norwegian cod females being either first or second time spawners are shown in fig.14.

Each type of malformation corresponds to certain morphogenetic events in the embryogenesis:
1. Zygote forming; defective oocytes, unfertilized eggs.
2.Cleavage division; irregular first cleavages, asynchronic cleavages, pseudocleavages.
3.Blastulation; the most critical stage in development; embryogenotype not started, no morphogenetic competence for further development, inner cells not differentiated to hypoblast and epiblast layers.
4. Gastrulation; hypoblast forming disorder cell aggregations with abnormal germ ring shape.
5. Organogenesis-axial structures in embryonic body form; abnormal correlation between development of epiboly periblast and periderm and development of its axial convergation process, cell aggregations forming twisting of the body and blisters.
6. Differentiation of tissue and embryo organs; malformations in older embryos: some epiblast cells aggregate but not included in the axial structures, different faults in the differentiation of the head region, crippled notochord deforming body, tail and finfold.
7. Definite function of embryo organs and structures; dysfunction of the hatching glands prevent

hatching of embryo, dysfunction of chloride cells result in water loss of embryo, or hyperhydration of head hydrosinus, pericardium and finfold.

Discussion

Egg mortality experiments have been criticized for being sensitive to microbial infection as well as to antibiotics to reduce the microbial growth, and also for being the result of artificial fertilization The present egg mortality experiments were performed on naturally spawned eggs, and no systematic infections occurred during the two years of investigation. In the NUNC-trays each egg developed isolated from each other.

The methods used to investigate the maternal effects on the viability of eggs and early larvae in Arcto-Norwegian cod, followed the same lines as the study in Norwegian coastal cod (Solemdal et al. 1995, Kjesbu et al. 1996).
To decrease the variation in the material the same indivduals were used in the successive spawning seasons of 1997 and 1998. In 1994 and 1995 different individuals were used as first and second spawners.
In addition to the systematic mortality experiments morphological investigations on the egg batches was carried out at the time of sampling from the spawning tanks in 1997 and 1998.
The stage of development at the start of the experiment did not seem to influence on the morphological fitness of the eggs, fig.1.
On the basis of irregular spawning rythm, as seen in table 3, and the irregularities in the morphological parameters of females 5,7 and 8, figs.2 - 4,the eggs from these cods were excluded from the material.

The investigation of egg mortality on Norwegian coastal cod, was carried on for three successive spawning seasons. Five out of ten of the the females were excluded from the material on the basis of irregular spawning and irregularities in the development of the fecundity during the later spawning seasons.
In the present material the frequency of irregular spawning incresed from first to second spawning, table 3, and it is most likely that this is an artefact due to the long-term captive situation.The reason why only part of the females develope normal reproduction during longterm experiments is unknown.

The method of calculating egg quality by studying the frequency of dead eggs before and after the standard morphological procedure, showed a significant increase in resitance to handling for second time spawners, fig.5.This study was performed by the same person both years,dr.Makhotin,Institute of Ichthyology, University of Moscow with long experience in similar studies of early egg development.

The methods of measuring the egg mortality during development, the "1 liter glass" and the

NUNC-tray method, show conflicting results. Fig.11. showing the cumulative egg mortality during development from pooled NUNC and "1 l glass"- experiments, clearly indicate a large difference in mortality from fertilization to the blastula stage between the two methods.
It was observed that during the preparing of some of the "1 liter glass" experiments eggs started to sink to the bottom immediately after treatment of Bufodin.
In general, "1 liter glass"-experiments involve more environmental stress than the NUNC-experiments. The "1 l"-method is depending to a greater extent on subjective factors.
The lower egg mortality in NUNC-trays compared to the 1 liter glass-method in 1998 is consistant for all females,fig.10.

On the basis of a total evaluation of the methods it was found correct to exclude the results from the "1l"-method instead of pooling the results from both methods.

Morphological studies on the eggs revealed that mortality always was the result of some kind of malformation. Comparing the results of the egg mortality experiments from the 1997 and 1998 spawning season, identical trends are found in the "good eggs" from the experiments at sampling, fig.4, and the total egg and early larvae mortality, fig.8. According to the Willcoxen non-parametric test,the reduced egg mortality in 1998 is statistical significant
It should also be noted that the increase of of "good eggs" from the early study and the reduction of total egg mortality is proportional to the results from 1997. The same phenomenon is shown for the relation between egg and early larvae mortality.These results indicate the genetic influence on the main level of the individual egg mortality, while the additional reduction in egg mortality in the second spawners is the maternal contribution. The same was found in egg mortality and egg-size changes in similar experiments in Norwegian coastal cod (Solemdal et al.1995,Kjesbu et al.1996).
Hislop(1988) performed longterm studies on the reproduction of individual haddock, and documented the maternal effect.
The large individual difference in the level of eggmortality will mask the maternal effect when different individuals are used as first and second spawners, as shown in fig.14.
Marteinsdottir(1998) studied the effect of egg and larvae from a large number of Icelandic cod females from catches in 1994, and found that maternal effects influenced the viability of the larvae.

The average reduction in egg mortality from first to second spawners in Norwegian coastal cod (Solemdal et al. 1998), and Arcto-Norwegian cod is similar, 23 % and 18% , respectively. It is necessary to look closer into the biological status of the cod females , both the Arcto-Norwegian and the Norwegian coastal cod.
The average weight increase of the eight Arcto-Norwegian females during one year was 45 %,range 19 to 84 %, table 2. Similar value for coastal cod was 160 %, range from106 - 224 %.
The feeding of the Arcto-Norwegian cod was managed to keep the condition as constant as possible during the two spawning cycles. Average Fulton condition factor at the start of first and second spawning cycle was 0.97 (8),range 0.86 - 1.05 and 1.04 (8), range 0.85 - 1.15, respectively.

For coastal cod the condition for first and second spawners were 1.08 (6), range 1.00-1.15 and 1.18(6),range 1.03-1.30, respectively (Solemdal et al. 1998).
Total weight of first and second spawning Arcto-Norwegian cod was 3216(8) gr,range 1872-4372 and 4810 (8)gr,range 2754-7213, respectively.
Similar total weights for coastal cod: 571(6)gr, range 428-733 and 1485(6) gr,
range 1177-1804.
Within the Arcto-Norwegian females, table 2, it is seen that no.5 has lowest condition and growth , together with irregular spawning,table 3 and also high frequency of low quality eggs,figs.2-4. Fish no. 2, has the best condition, but an intermediate weight increase and no reduced egg mortality as a seconf time spawner,fig.8.
Comparing the females of Norwegian coastal cod (Solemdal et al.1998) and Arcto-Norwegian cod used in the experiments, the size at maturation is marked larger than in coastal cod. On the other hand the relative weight increase is faster and the condition is better in the coastal cod. As mentioned at the top of this paragraph, the reduction in egg mortality for the secondtime spawnere are similar in both tribes of cod, coastal cod showing a somewhat larger effect.It is possible that the larger changes in size of coastal cod could be the reason for this differnce in egg mortality.
As seen from fig.7 and fig.11 mortality occurs most frequent during the early stages untill the blastula stage. We know that only the female genetics is active,this period being highly sensitive. The results therefore is a strong indication that the improved egg quality in second time spawners is the result of reduced sensitivity during the early stages. Since the maternal genetic constitution is the same during successive spawning periods in each indivdual, the improvement of egg quality must be associated with the growth process.
There are different opinions of the reasons for the lethal and sublethal malformation in fish eggs. In experimental work artificial fertilization is supposed to be negative for normal development.
The egg mortality pattern from Gotland cod in the Baltic, using artificial fertilization, were similar to the present results, reaching about 50 % during the first 5 days of incubation (Nissling & Westin 1991).
Danielssen & Iversen (1974), studying egg development and mortality from artificial fertilization in plaice, Norwegian coastal cod and herring in different temperatures, showed a pronounced egg mortality during the first days of incubation.
Using artificial fertilization and selecting eggs for the experiments as the present authors, de Braak (1994) also demonstrated the same egg mortality pattern during the first days of incubation in eggs from Arcto-Norwegian cod.

Different views exist to explain malformation in naturally spawned eggs.
Kjørsvik et al. 1984 found large variations in quality from artificially fertilized cod eggs, and also a relatively large frequency of abnormal mitoses from planctonic cod eggs.
Stene (1987) performed a more comprehensive study on the frequency of cod eggs with abnormal mitoses in, optimal, unpollutant areas of Northern Norway and found 6 -12 % of mitotic abberations, sometimes up to 30 %. She refers to A.C.Longwell,pers.comm, who put forward the idea that the abnormal eggs in optimal environment is the consequence of the natural rate of mutation. In humans, more than 15 5 of the embryos die before birth and 7% of children are borne

with abnormalities, mostly due to chromosome errors (Freese 1971).

Yannopoulos & Yannopoulos(1981) report about 10% malformation in Sardina pilchardus and Engraulis enchrasicolus eggs from the Aegean Sea, which is consicered to be unpollutant.
Kjørsvik (1994) states that though egg mortality usually is considered to be a laboratory phenomenon, specially caused by the artificial fertilization, field investigations have revealed that genetic and other biological factors contribute significantly to natural egg mortality.

Vladimirov (1973,1975) reviewed the Soviet literature on the effects of growth rate of spawners and the critical stages during development on the survival of progeny. High growth rate is shown to have positive effect on the viability of the progeny, as shown for cultured carp (Vladimrov 1973) High fat content also effect the production of offspring positively, as demonstrated by Taranenko (1964) on Azov anchovy.
Vladimirov(1975) discuss the term critical stages in early development, and use another definition than Hjort (1914). In highly fecund animals, as fish, a certain frequency of gamets with morphological and physiological deficiencies will occur. During sensitive developmental stages, critical stages, and specially during unfavourable environmental conditions, malformation and mortality will result.
Closer morphogenetic studies have revealed that the development during sensitive stages in itself will produce a certain amount of malformed embryos, depending on the quality of the females, as shown by Svetlov 1960.

The view of the Soviet researchers on the cause of malformation and deaths during the embryonic period have not got much support in western literature. Blaxter (1988) cites Vladimirov(1975), and Ballard (1981)
has a similar interpretation on the dynamics of morphogenetic development as the Soviet scientists.
Rothchild (1986) seems to be open for their idea to a certain extent: " The possible causes of nonpredatory egg death are not well understood, but they might be linked to cytological competence or to unfavourable environmental conditions".

Longterm, experimental, studies on the reproduction and maternal effect at the individual level,similar to the present stydy, is not found in the Soviet literature.

Egg mortality in the present study always started as a characteristic malformation. Different external causes for these morphological deficiencies has been put forward: pollution through the gonads or in the pelagic stage and natural factors, like changes in salinity, temperature and oxygen (Westernhagen et al. 1988).
In the present study the Arcto-Norwegian cod were caught in the very clean area near the Bear Island in 1996. During the following two years they were kept in water pumped from 140 meters depth in the fjord outside Bergen. Investigations of the content of aromatic hydrocarbons and PCB in the seawater supply to the public Aquarium and the Institute of Marine Research,

(Palmork & Wilhelmsen 198The values were low, reaching an average of 2.95 / l and o.82 ng/ l for the aromatic hydrocarbons and PCB, respectively.

Temperature variations are very limited throughout the year, and annual only 1 ° C. On this background it seems reasonable to conclude that external varibles can not be the cause of the relatively high egg mortality and the change in egg characteristics from first to second spawning.

During the last 20 years a huge literature have appeared correlating malformations in planktonic fish eggs to the increasing detrimental effect of pollution.

An overview of the different types of sublethal characteristics are given by Rosenthal & Alderdice (1976) Of more general interst is Cameron et al.(1992) referring that 85 % of the malformed eggs died within 5 days. Vallin & Nissling (unpublished) and de Braak(1994) demonstrated that aberrations on early stages in artificial fertilized eggs recovered during further development in many cases.

Analysis of old egg material from earlier periods with reduced level of pollutants could give valuable comparative information of the present state of the frquency. However, formaldehyde conserved egg material is difficult to analyse and also damaged from too high speed of the net (Cameron et al.1988).

Mukhina et al. (1996) reported frequency of malformation of max. 9 %, in water supposed to be polluted by oil drilling.

The interesting problem of of the longevity of malformed embryos is discussed by Westernhagen et al. (1988). Lethal failure will lead to immediate death and sinking out of the pelacic system, while sublethal effected egg will develope and float for a period.

Dethlefsen et al. 1996 give a precaution in interpreting the earlier data on the high frequency of malformation, specially in coastal waters. Introducing the experimental data from Westernhagen (1970) on the effect of suboptimal temperatures and the occurrence of malformed eggs, the authors conclude:

"From these findings it is concluded that temperatures possibly predispose developing fish embryo to the impact of pollutants".

Cameron and Westernhagen (1997) conclude that the average frequency of malformation was only 10 % in 1992, similar to the values in 1984,when the studies started, probably the result of the reduction of pollutants released into the North Sea.

Acknowledgements.

We thanks the crew on R/V "Johan Hjort" for carefull treatment of the Arcto - Norwegian cod during catching and transportation.

During the laboratory work we had the benefit of the daily communication with the collegues dr.O.S.Kjesbu and dr. Anders Thorsen, and specially we thank Merete Fonn and Laura Rey for their skillfull cooperation.

One of us, dr.Valeri Makhotin, Institute of Ichthyology,Moscow State University, thanks the Norwegian Research Council, projectno.116601/100, and Norwegian ministry of foreign affairs for economic support.

Kirsten Arnesen has been a valuable consultant for necessary English corrections.

References

Ballard,W.W.,1981. Morphogenetic movements and fate maps of vertebrates. Am.Zool.,18:119 - 135.

Blaxter, J.H.S. (1969) 1988 ?. Development: eggs and larvae. In Fish Physiology, 3: 177-52. Eds.: W.S. Hoar and D.J. Randall. Academic press , NY. 485 pp.

de Braak, K.v. 1994. Morphological characteristics of early embryos in relation to later egg and larval development of cod (Gadus morhua). Thesis, Wageningen Agricultural University, 1994. 40 pp.

Cameron,P. and Westernhagen, H.v., 1997. Malformation rates in Embryos of North Sea Fishes in 1991 and 1992. Marine Pollution Bulletin,34(2): 129 - 134.

Cameron,P., Berg,J., Dethlefsen, V. and Westernhagen,H.v., 1992. Developmental defects in pelagic embryos of several flatfish species in the southern North Sea. Netherlands Journal of Sea Research 29(1-3):239-256.

Chambers, R.C., Legget, W.C. 1996. Maternal influence on variation in egg sizes in temperate marine fishes. Am. Zool. 36,18o - 196.

Danielssen, D.S. & Iversen, S.A. 1974. Egg og larveutvikling hos rødspette (Pleuronectes platessa L.), torsk (Gadus morhua L.) og vårgytende sild (Clupea harengus L.) ved konstante temperaturer. Fisken og Havet, Ser. B, nr. 22: 29 pp. (In Norwegian).

Hislop,J.R.G.,1988. The influence of maternal length and age on the size and weight of the eggs and the relative fecundity of the haddock, Melanogrammus aeglefinus in captivity. J.Fish Biology 13:923-9320

Hjort, J. 1914. Fluctuations in the great fisheries of northern Europe. Viewed in the light of biological research. Rapp. P.- v. Reun. Cons. perm. int. Explor. Mer., 20: 1-228.

Kjesbu, O.S. 1989. The spawning activity of cod (Gadus morhua L.). J. Fish. Biol. 34: 195 206.

Kjesbu,O.S., 1994. Time of start of spawning in Atlantic cod (Gadus morhua) females in relation to vitellogenig oocyte diameter, temperature, fish length and condition. J. Fish. Biol. 45: 719-735.

Kjesbu, O.S., Solemdal, P., Bratland, P. and Fonn, M. 1996. Variation in Annual Egg Production in Individual captive Atlantic cod (Gadus morhua). Can. J. Fish Aquat. Sci. 53: 610-620.

Kjørsvik,E., Stene, A., Lønning,S.1984. Morphological,physiological and genetical studies of egg quality in cod (Gadus morhua L.). Flødevigen rapportserie nr.1,part 1: 67-87.

Kjørsvik, E. 1994. Egg Quality in Wild and Broodstock Cod Gadus morhua L. J. World Aquaculture society, 25 (1): 22-29.

Marshall,C.T., Kjesbu,O.S., Yaragina,N., Solemdal,P., Ulltang. Ø., in press. Is spawner biomass a sensitive measure of the reproductive potential of marine fish stocks ?

Marteinsdottir, G. & Steinarsson,A., 1998. Maternal influence on the size and

viability of Iceland cod Gadus morhua eggs and larvae. J.Fish Biology 2: 241 - 1258.

Mattson,N.S. and Riple,T.H. 1989. Metomidate, a better anaesthetic for cod (Gadus morhua) in comparison with benzocaine, MS-222. Chlorobutanol, and phenoxyethanol. Aquaculture, 83: 89-94.

Mukhina,N., Plotitsyna,N.F., Golubeva,T.A. 1996. Disturbamces in embryogenesis of cod from the Lofoten - Barents sea stock. ICES, CM, 1996/ Q:6. 13 pp.

Nikolskii, G.V. 1962a. On some adaptations to the regulation of population density in fish species with different types of stock structure. In: :Le Cren and Holdgate, eds. The exploitation of natural animal populations: :265-282.

Nikolskii, G.V. 1962b. Concerning the causes of fluctuations in the abundance of fishes. Voprosy Ikhtiologii, 1 (4): 659-665. Translation series No. 389, Fisheries Research Board of Canada.

Nissling, A. and Westin, L. 1991. Egg mortality and hatching rate of Baltic cod (Gadus morhua) in different salinities. Marine Biology 111: 29-32.

Ponomarenko, V.P.,1973. On a probable relation between age composition of spawning stock and abundance of the year class of cod in the Barents Sea. Rapp.P.-V. Cons perm Explor. Mer.,164: 69 - 72.

Palmork,K. & Wilhelmsen,S. 1988. Kjemisk analyse av utvalgte aromatiske oljehydrokarbiner (NPD) og polyklorerte bifenfyler (PCB) i sjøvannsinnstaket til Akvariet i Bergen. IMR, BKO 8804.(In Norwegian)

Rollefsen, G. 1934. The cod otolith as a guide to race, sexual development and mortality. Rapp. P.-v. Explor int Mer, 88(2)

Rothschild, B.J. 1986. Dynamics of marine fish populations. Harvard University Press, Cambridge. 277 pp.

Rosenthal,H. & Alderdice,D.F.,1976. Sublethal Effects of Environmental Stressors, Natural and Pollutional, on Marine Fish eggs and Larvae. J.Fish.Res.Board Can. 33: 2047 - 2065.

Solemdal,P.,1997. Maternal effects - a link between the past and the future. Journal of Sea Research,37: 213 - 227.

Solemdal,P.,Kjesbu,O.S. & Fonn,M.,1995. Egg mortality in recruit- and repeat spawning cod - an experimental study. ICES,CM. 1995 /G:35, 10 pp.

Solemdal,P., Kjesbu,O.S. & Fonn, M,1998. Nafo-Symposium, Lisboa 8 - 11 1998.

Stene,A.,1987. Light microscopical studies of chromosomes in embryos of cod, Gadus morhua l.. J.Fish Biology, 31: 445 - 450.

Svetlov,P.G.,1960. The theory of the critical periods of development and its ignificance for an understanding of the principles of environmental effects on ontogeny. In: Vopr. tsitologii i obshchey fiziologii (Questions of cytology and general physiolgy. Moscow, Leningrad, Acad. Sci USSR press (in Russian).

Vladimirov, V.I,1973. The effect of the growth rate of spawners on the survival and abundance of the progeny. J. Ichthyology 13: 801 - 812.

Vladimirov, V.I. 1975. Critical periods of fish development. J. Ichthyology, 1975, 15 : 851 - 867.

Westernhagen,H,v.,1970. Erbrütung der Eier von Dorsch (Gadus morhua), Flunder (Pleuronectes flesus) und Scholle (Pleuronectes platessa) unter kombinerten Temperatur- und Salzgehaltsbedingungen. Helgoländer wiss. Meeresunters. 21 : 21 - 102.

Westernhagen,H.v., Dethlefsen,V., Cameron,P., Berg,J. & Fürstenberg,G., 1988. Developmental defects in pelagic fish embryos from the western Baltic. Helgoländer wiss. Meeresuntersuchungen, 42: 13 - 36.

Yannopoulos,A. & Yannopoulos,C.,1981. Fish egg mortality and abnormal embryogenesis. Rapp P.-v. Réun.int.Explor.scient.Mer. Mediterr. 27(5): 143-146.

Female no.	Spawning seasons	Whole body weight (g)	Fulton's condition factor
5	1	6010	1,03
6	1	3574	1,00
7	2	6164	1,12
8	2	4238	1,07
9	1	5893	1,01
10	1	4938	1,02

Table 1. Weight and Fulton's condition factor in Arcto-Norwegian cod. A mixture of first and second time spawners where used in 1995.

	Whole body weight (g)		Fulton's condition factor		% increased body weight
Female no.	1997	1998	1997	1998	1997/1998
1	3906	7213	1,05	1,10	84,66
2	4372	5865	1,08	1,15	34,15
3	1872	2754	0,87	1,03	47,12
4	2768	4465	1,01	1,10	61,31
5	3440	4108	1,00	0,85	19,42
6	2701	4372	0,86	0,98	61,87
7	3054	4543	0,93	0,98	48,76
8	3618	5160	0,93	1,05	42,62

Table.2.Weight, Fulton's condition factor and % weight increase in the eight female Arcto-Norwegian cod, used in mortality experiments in 1997 and 1998.

Female		Batch no.																			
no.	Year	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	1997	0	1	3	1	2	2	2	2	3	2	2	2	2	4						
	1998	0	2	2	2	2	2	1	2	2	2	2	2								
2	1997	0	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2				
	1998	0	2	1	2	2	2	2	1	2	2	2	1	2	2	2	1	2	2		
3	1997	0	2	2	2	2	2	2	3	2	2	2	2								
	1998	0	2	2	2	2	2	2	2	2	2	2	2	1	2	2					
4	1997	0	2	3	2	2	2	2	2	2	2	2	2								
	1998	0	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2		
5	1997	0	2	2	3	2	2	2	2	2	3	2	2	2	2						
	1998	0	2	4	2	1	6	5	2	2											
6	1997	0	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2			
	1998	0	2	2	2	1	2	2	1	2	2	2	1	2	2	2	2	2	2	2	1
7	1997	0	4	2	2	3	2	2	2	2	3	2	2	2	2	2	2	2	3	2	
	1998	0	3	2	2	1	4	4	6	4	2	5	2	5							
8	1997	0	2	2	2	2	1	2	2	2	2	2	2	2	2	2	5				
	1998	0	1	2	2	2	2	2	1	2	2	1	10	5	3	2					

Table.3. Days between egg batches in Arcto-Norwegian cod during the spawning seasons 1997 and 1998.The grey areas show the batches used in the NUNC-tray experiments.

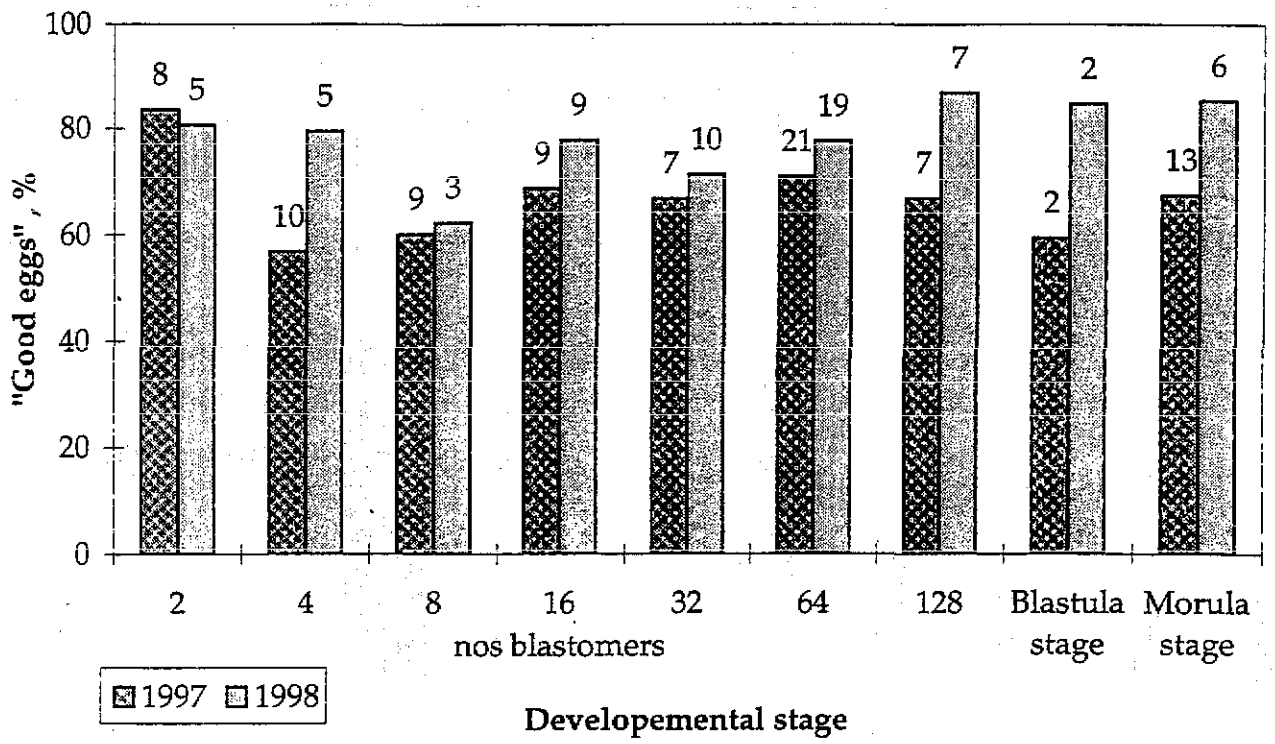

Fig.1 Percentage of "good eggs", at the time of collection, from egg batches at different stages of development, during the 1997 and 1998 spawning seasons. Numbers of cases are given at the top of the histograms. Data from female 1,2,3,4,6,7 and 8.

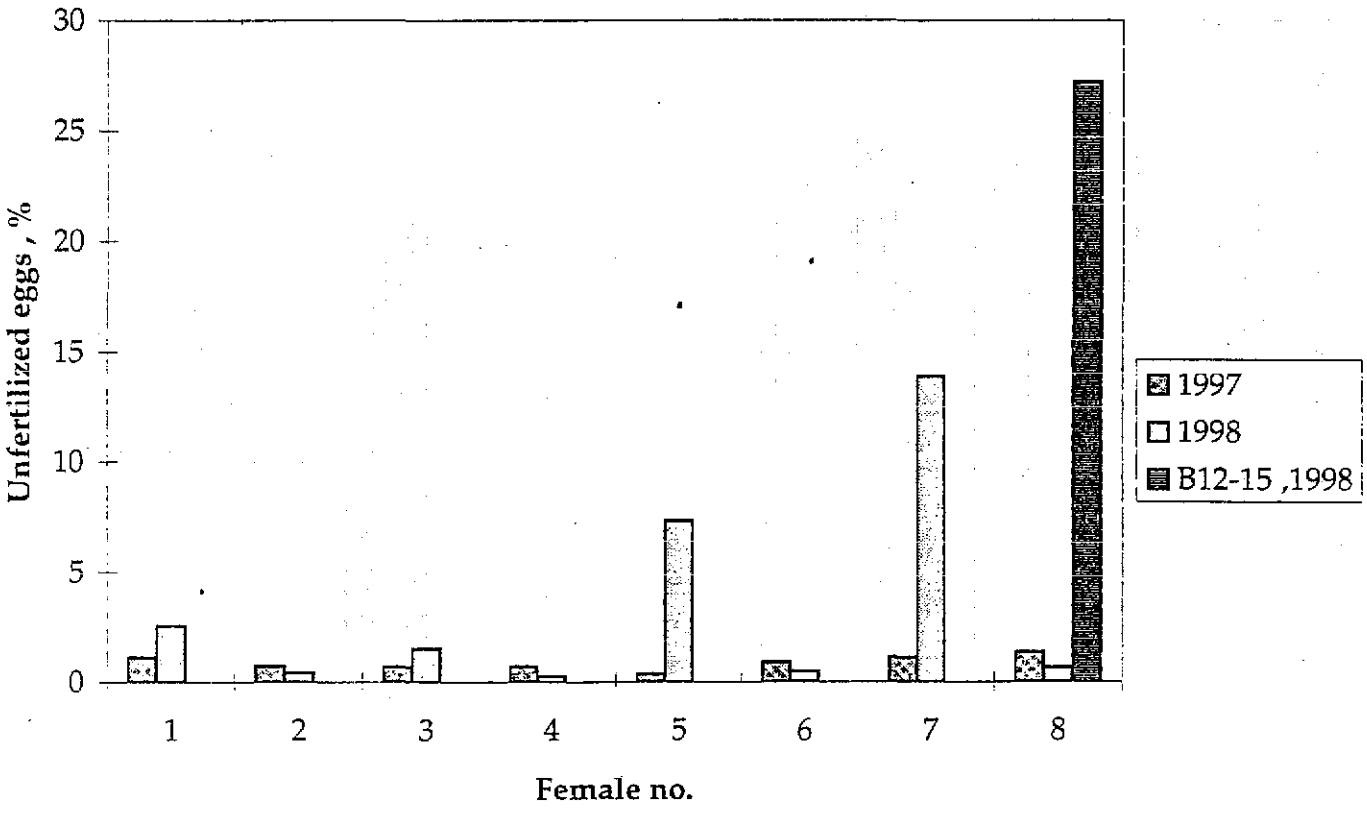

Fig.2. Percentage of unfertilized eggs, at the time of collection, from Arcto-Norwegian cod, during the 1997 and 1998 spawning seasons. Egg batches 12-15 from female eight are given separately.

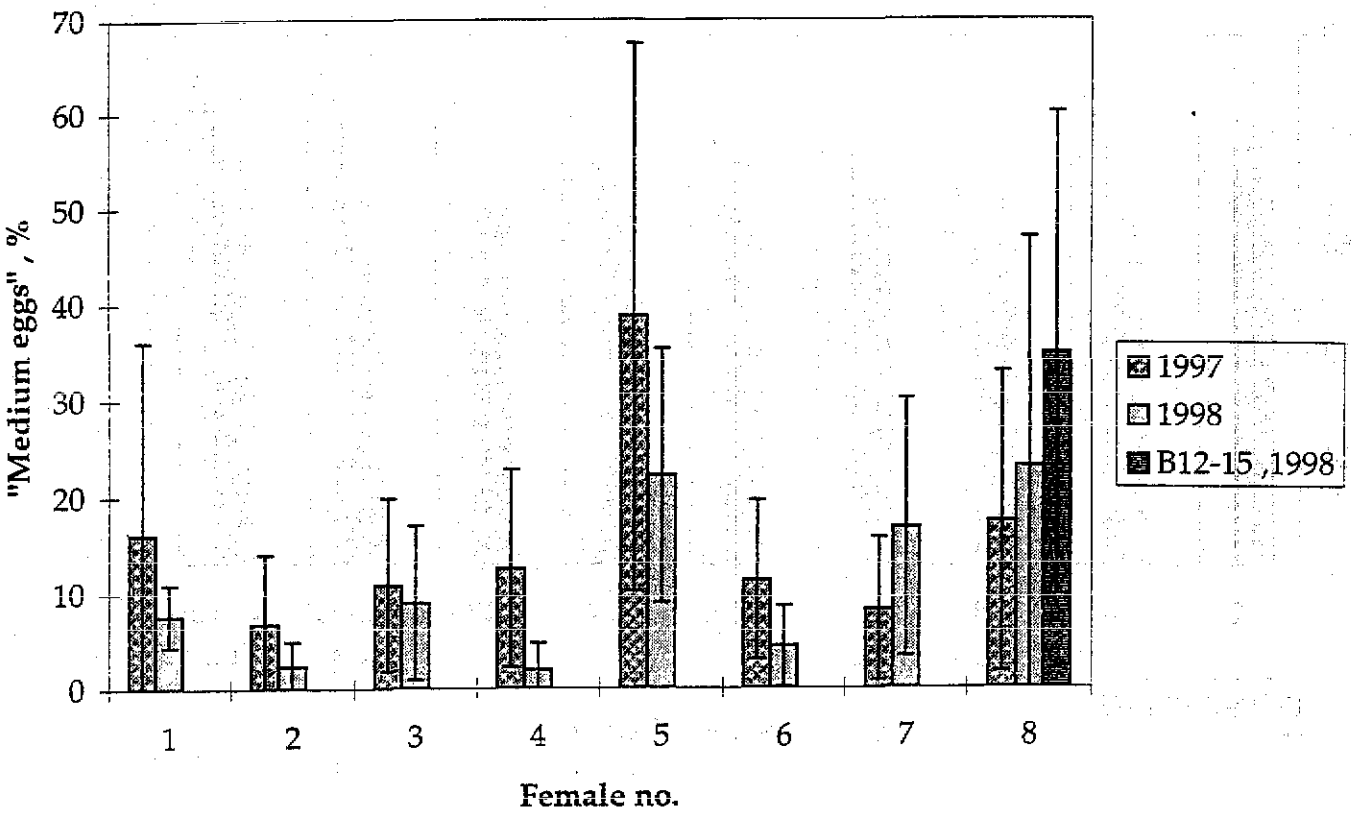

Fig.3 Percentage of "medium eggs", at the time of collection, from Arcto-Norwegian cod, during the 1997 and 1998 spawning season. Egg batches 12-15 from female eight are given seperately.

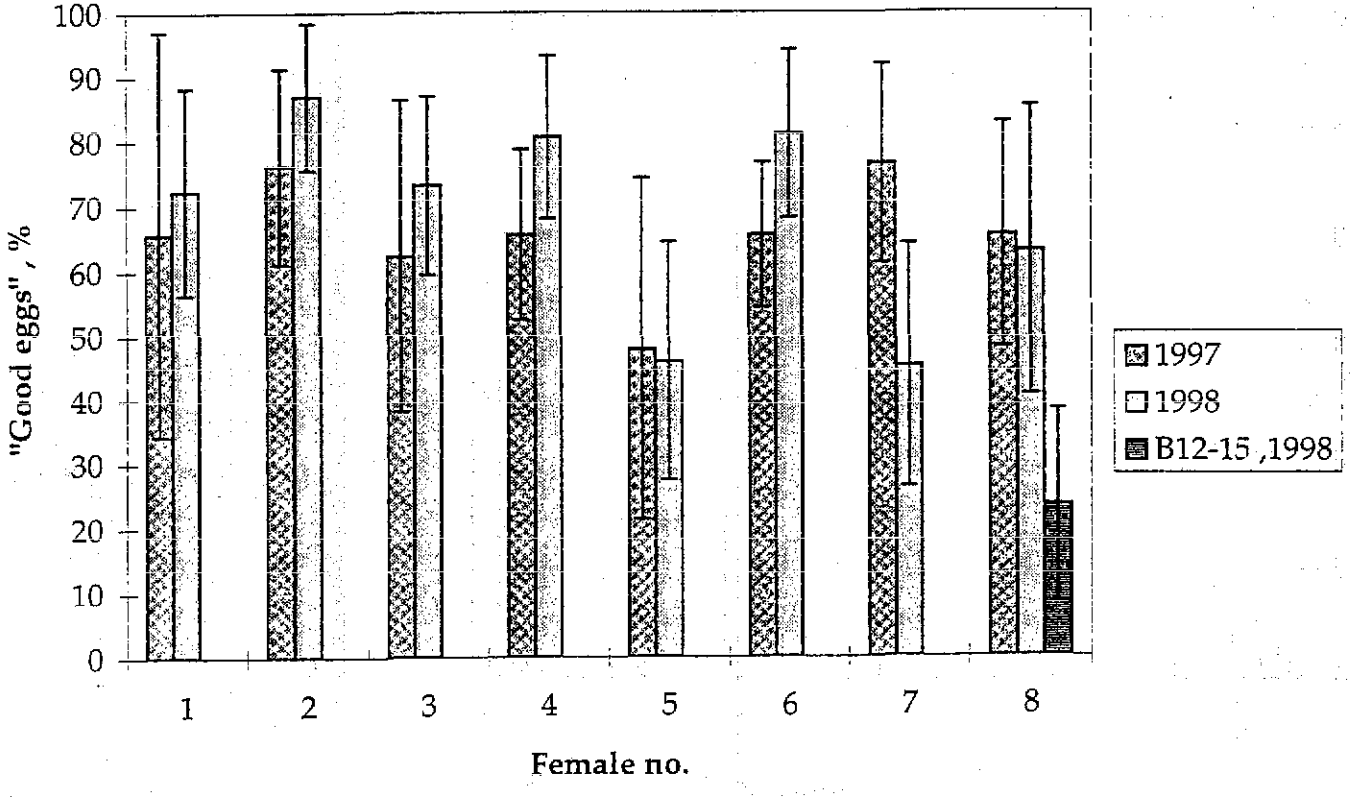

Fig.4. Percentage of "good eggs", at the time of collection, from Arcto-Norwegian cod, during the 1997 and 1998 spawning seasons. Egg batches 12-15 from female eight are given seperately.

Fig.5. Increased egg mortality, in %, during experimental procedure of egg batches at time of sampling, 1997 and 1998.

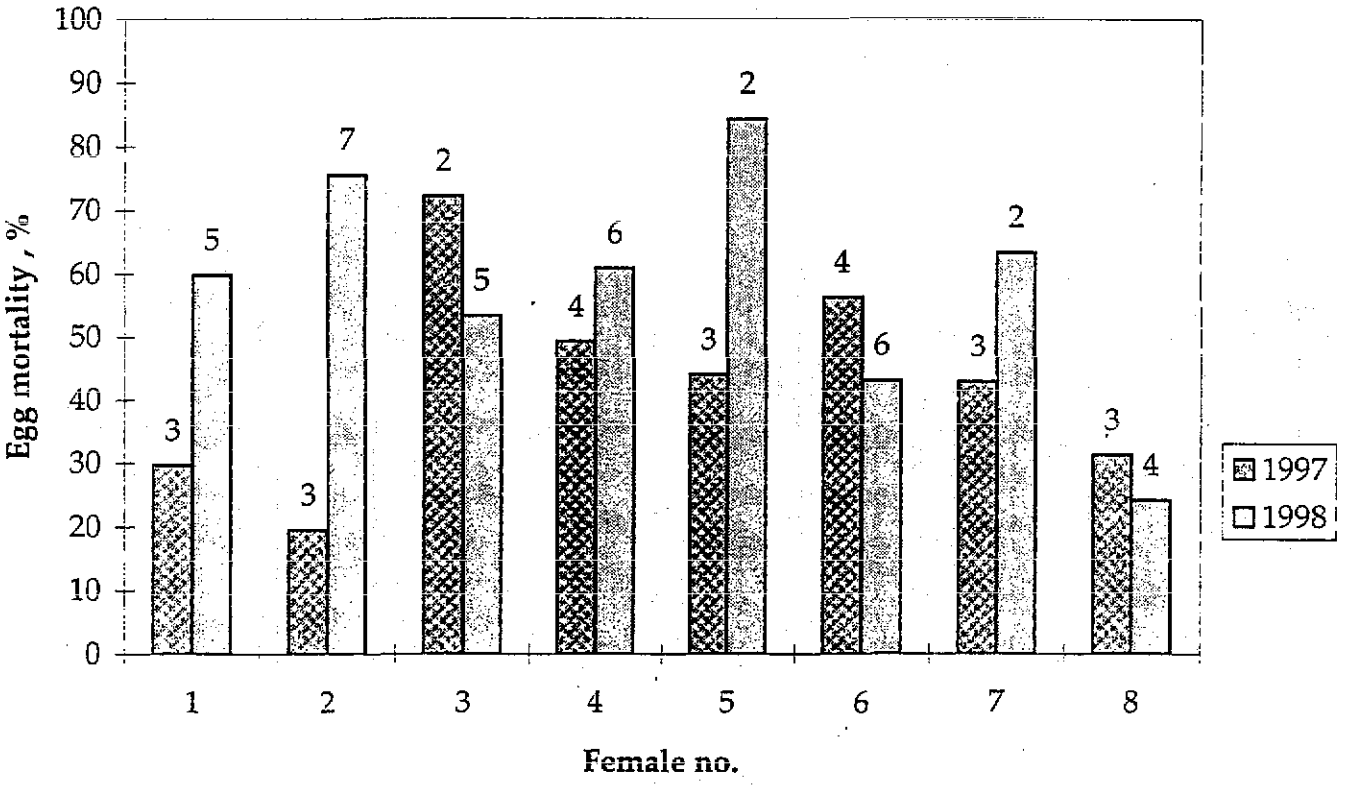

Fig.6. Average egg and early larval mortality (before starvation) from eight Arcto-Norwegian cod females, during the 1997 and 1998 spawning seasons. Method: 1 l glass jar, 200 eggs with antibiotics added. Numbers of experiments are indicated at the top of the histograms.

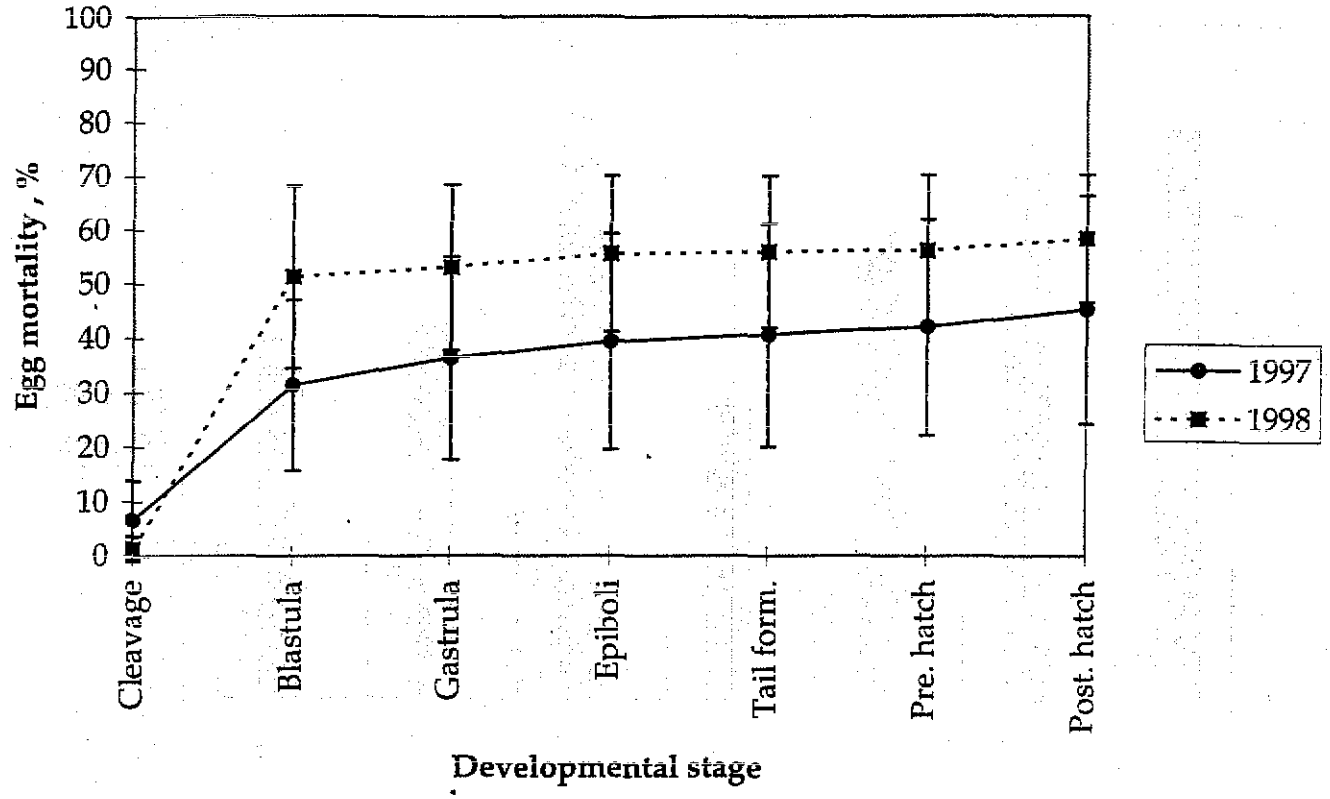

Fig.7. Cumulative mortality in eggs and early larval stages during development, in the 1997 and 1998 spawning seasons. Method: 1 l glass jar, 200 eggs with antibiotics added. Data from female 1,2,3,4 and 6.

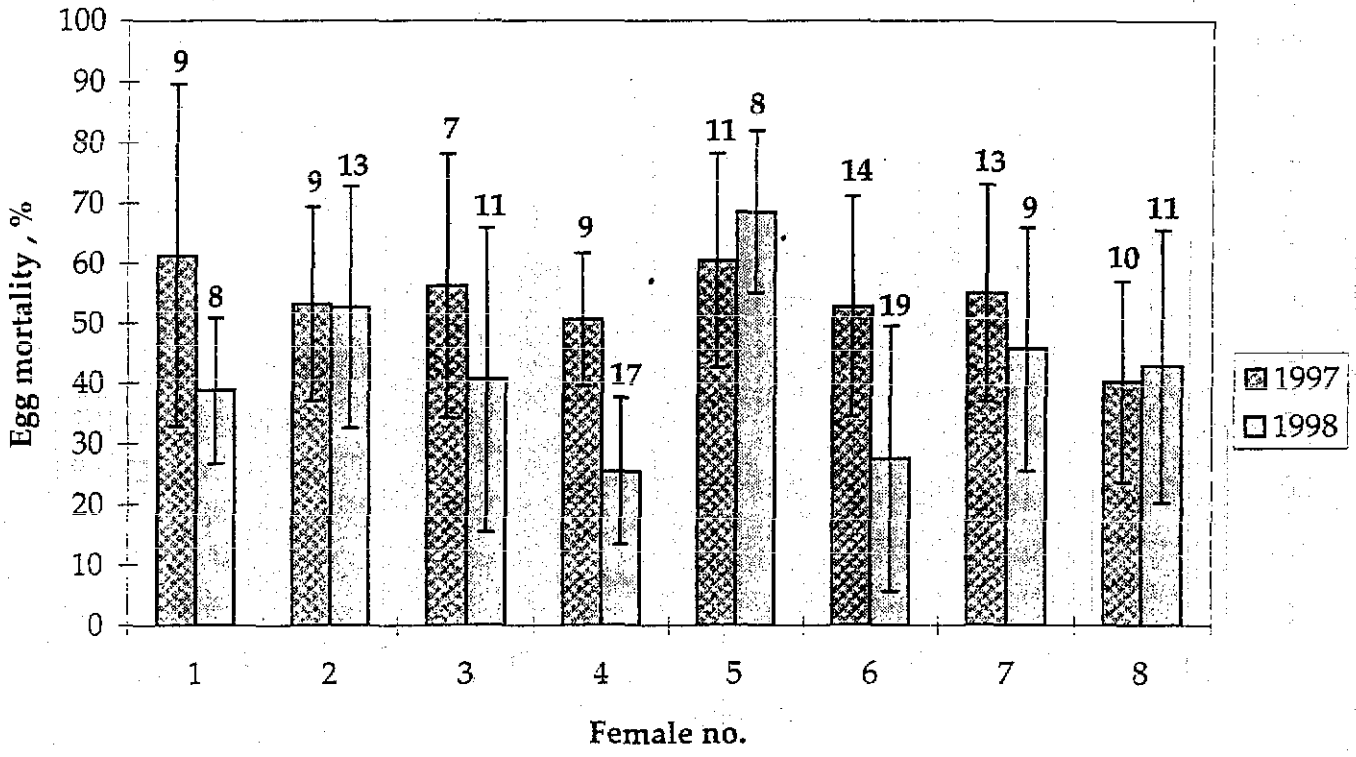

Fig.8. Average egg and early larval mortality from eight Arcto-Norwegian cod females, during the 1997 and 1998 spawning seasons, using the NUNC-tray method. Numbers of experiments are indicated at the top of the histograms.

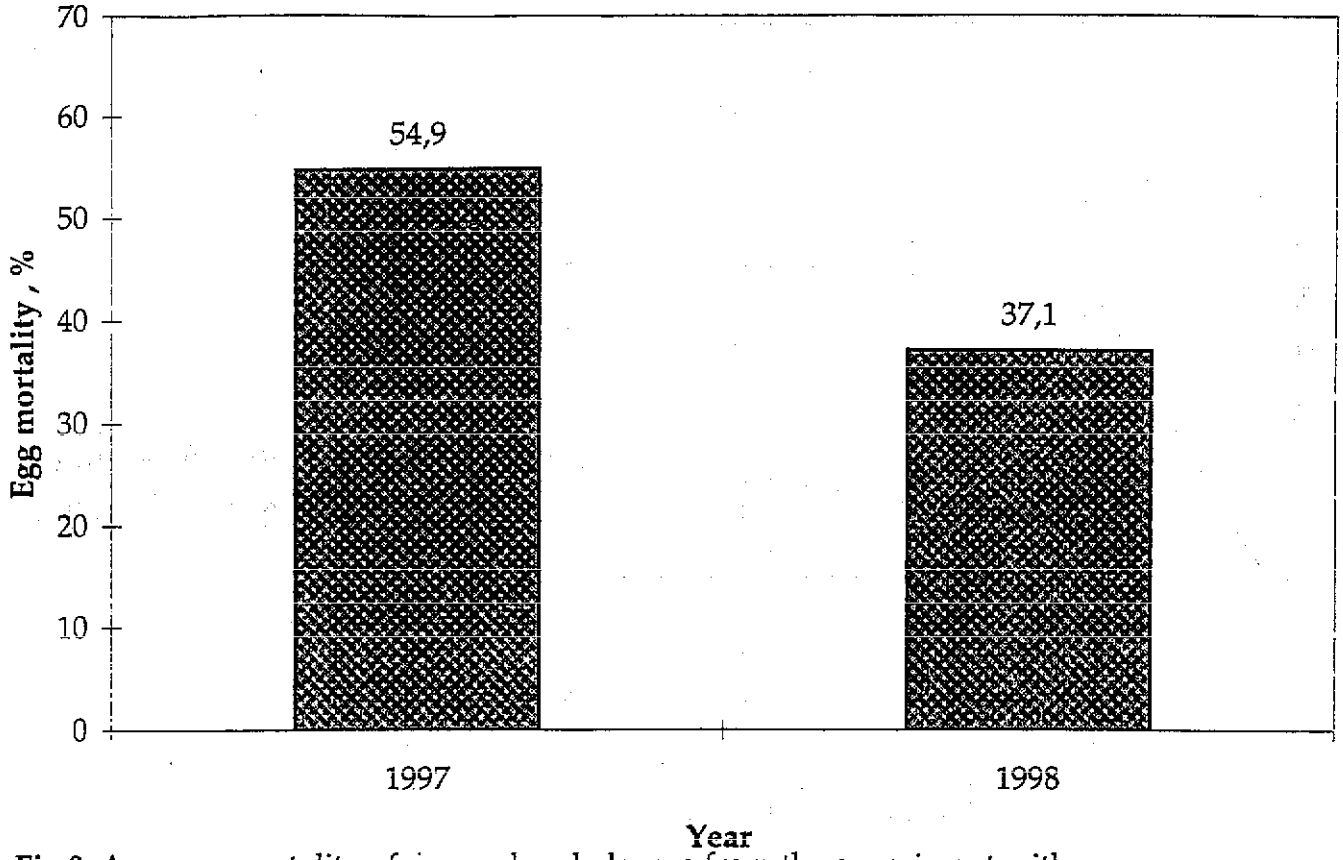

Fig.9. Average mortality of egg and early larvae from the experiment with Arcto-Norwegian cod in 1997 and 1998, using the NUNC-tray method. Data from female 1,2,3,4 and 6.

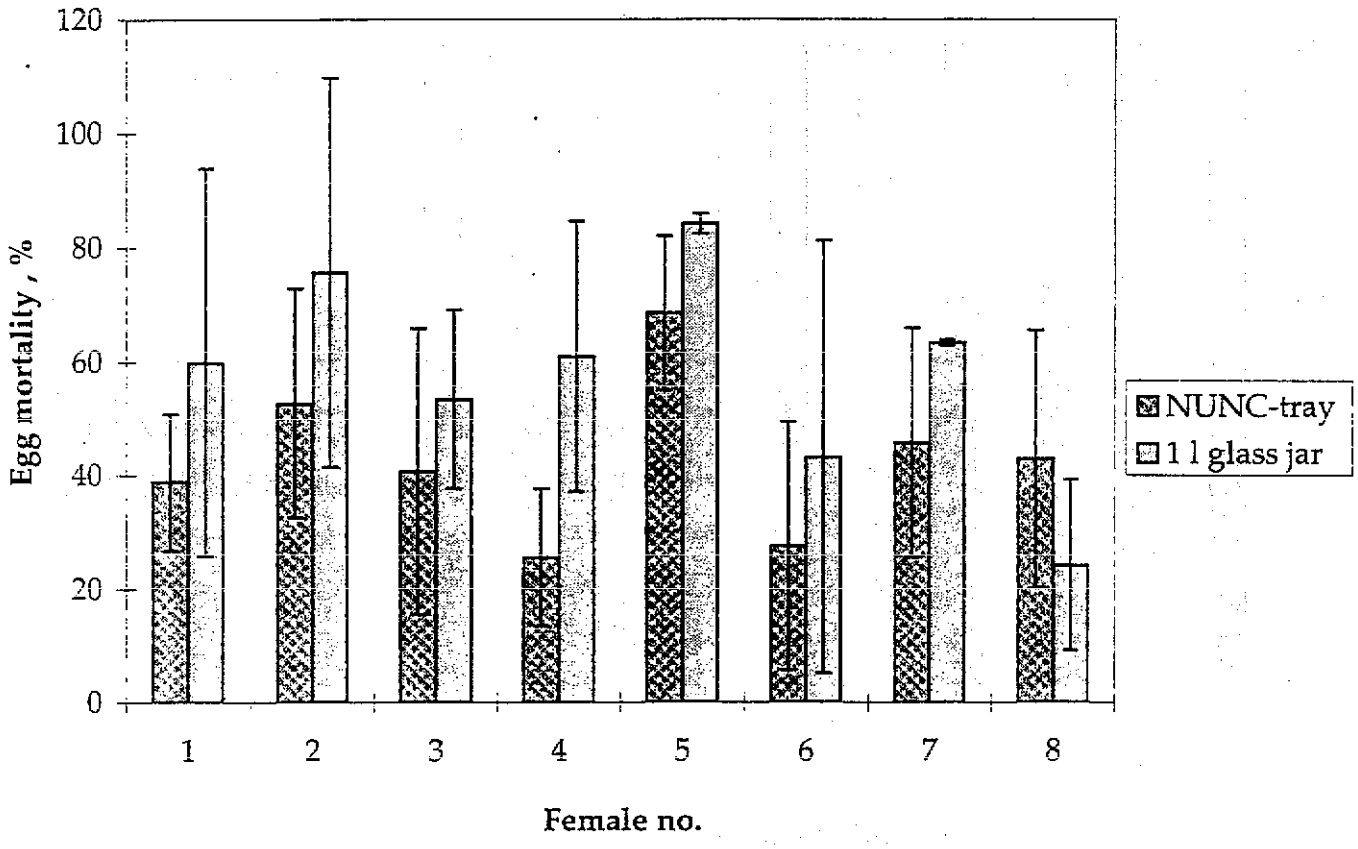

Fig.10. Average egg and early larvae mortality from NUNC-tray experiments and 1 l glass jar experiments, during spawning season 1998.

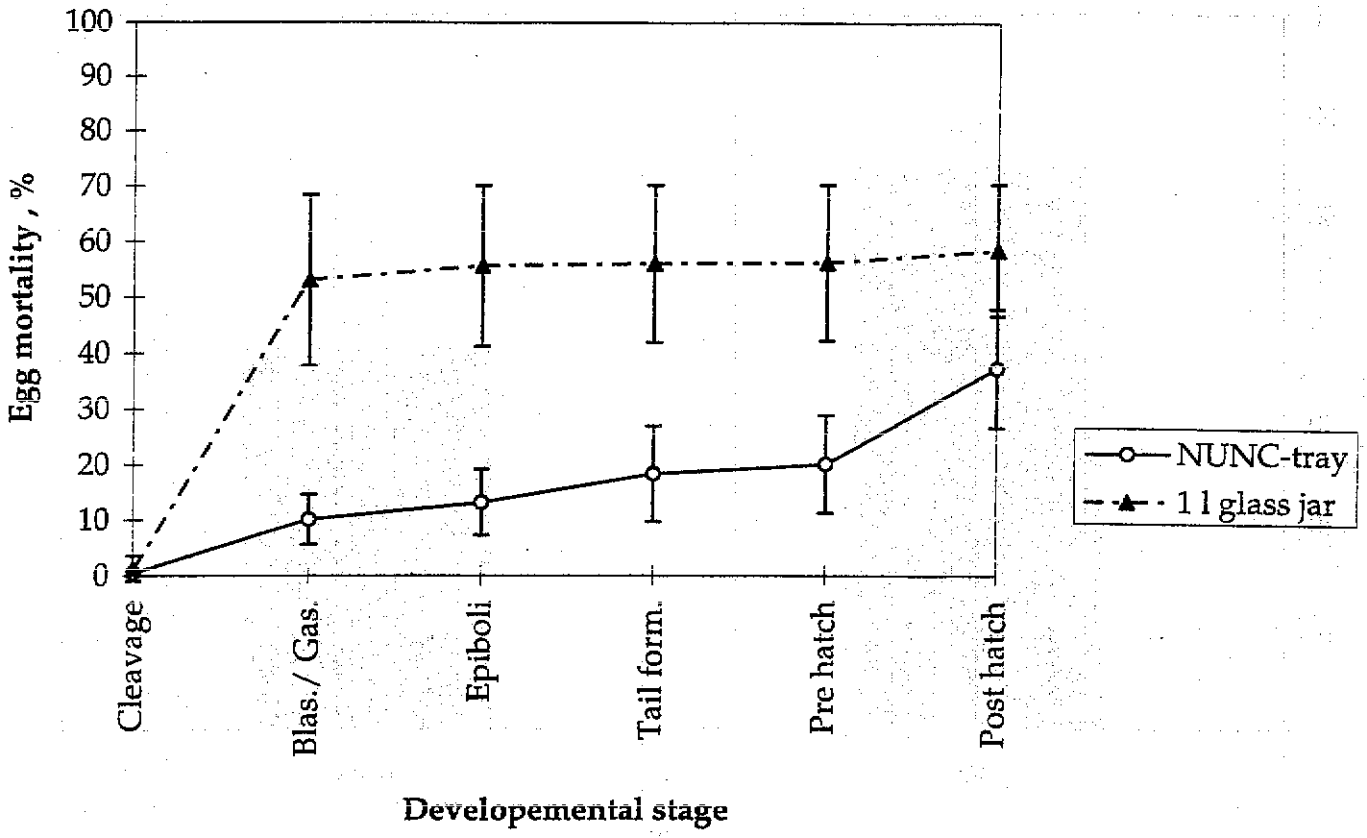

Fig.11. Cumulative, average, egg and early larvae mortality during 1998 spawning season. NUNC-tray experiments and 1 l glass jar experiments. Data from female 1,2,3,4 and 6.

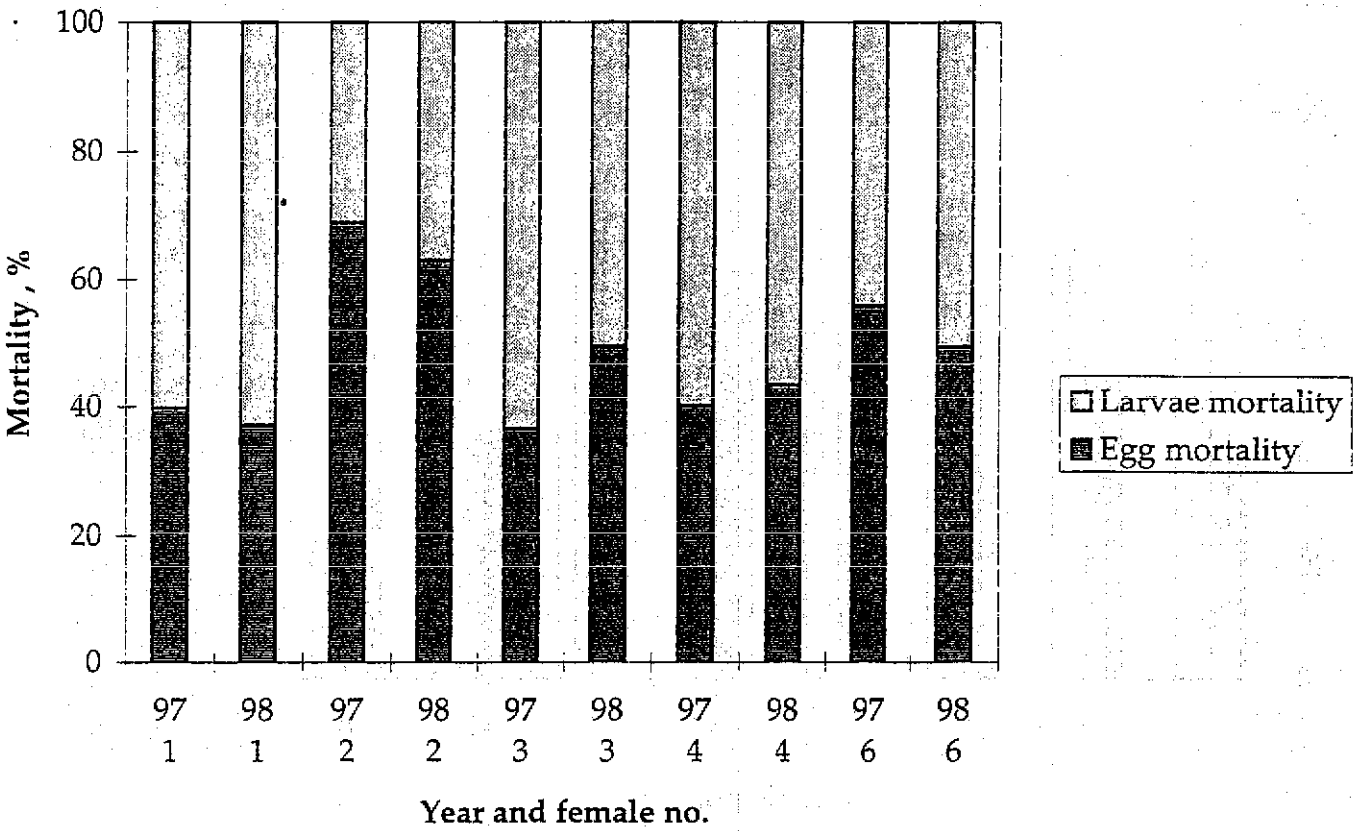

Fig.12. The individual proportion of mortality during the egg stage and early larval stage (before starvation). The relation is given separately for 1997 and 1998. Data from female 1,2,3,4 and 6.

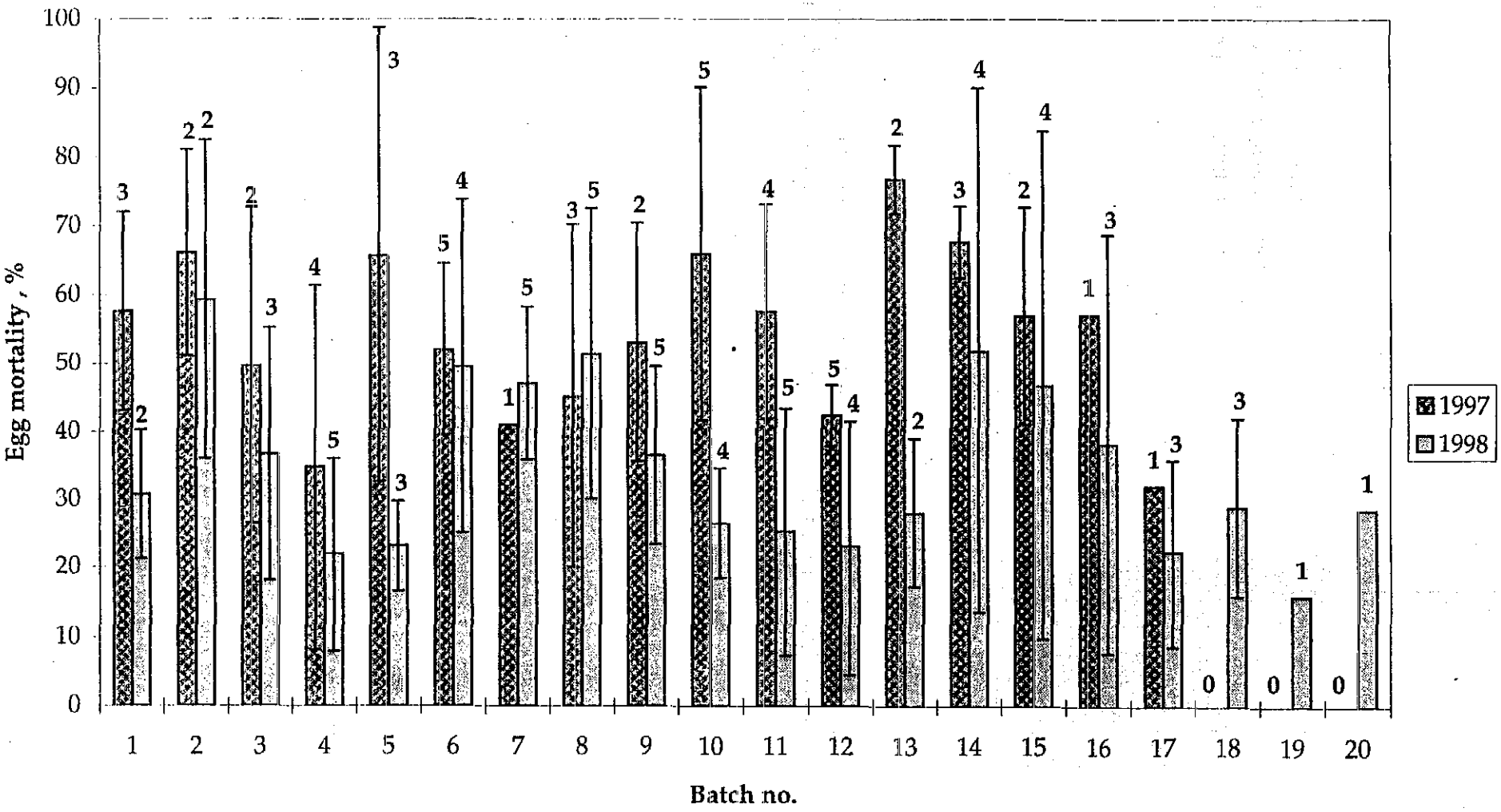

Fig.13. Average mortality on the egg and early larval stages on the batch level, 1997 and 1998. Numbers of experiments are indicated at the top of the histograms. Data from female 1,2,3,4 and 6.

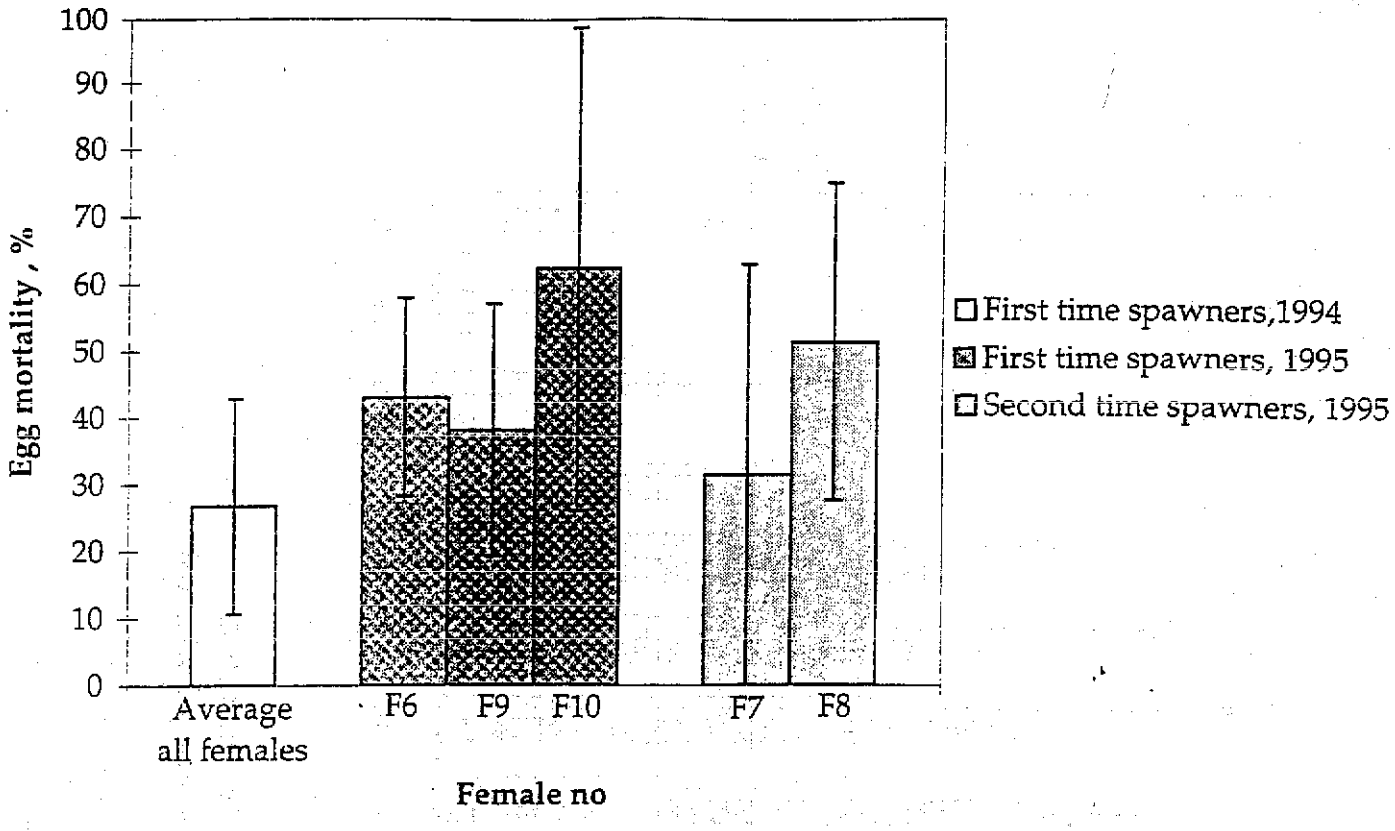

Fig.14. Experiments on egg mortality, NUNC-trays. Data from first time spawners of Arcto-Norwegian cod in 1994. In 1995 different induviduals were studied as first and second time spawners.