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SIZE OF SPAWNING ARCTO-NORWEGIAN COD (*Gadus morhua* L.) AND THE EFFECTS ON THEIR EGGS AND EARLY LARVAE

by

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Abstract

Using a new method for determining the stage of spawning in batch spawners, a positive correlation between the length of Arcto-Norwegian cod females, having spawned 25-75 % of their eggs, and the egg diameter was established. This was not found in 1992 when egg groups from cod females in all stages of spawning were used. The maternal effect of cod female size was compared to the effect of stage of spawning. This comparison revealed the following tentative conclusions: 1) The egg diameter is less affected by the length of the cod female than by its stage of spawning. 2) The fertilization percentage and larval activity are correlated with the stage of spawning of the cod female, but not to its length.

The present results indicate that the maternal effect of individual changes in egg characteristics during spawning is larger than the maternal effect of cod female size. In addition investigations on egg mortality, larval feeding ability, genetics, bacteriology, biochemistry and rearing of selected larvae groups to metamorphosis were carried out. The project will be terminated in 1994.

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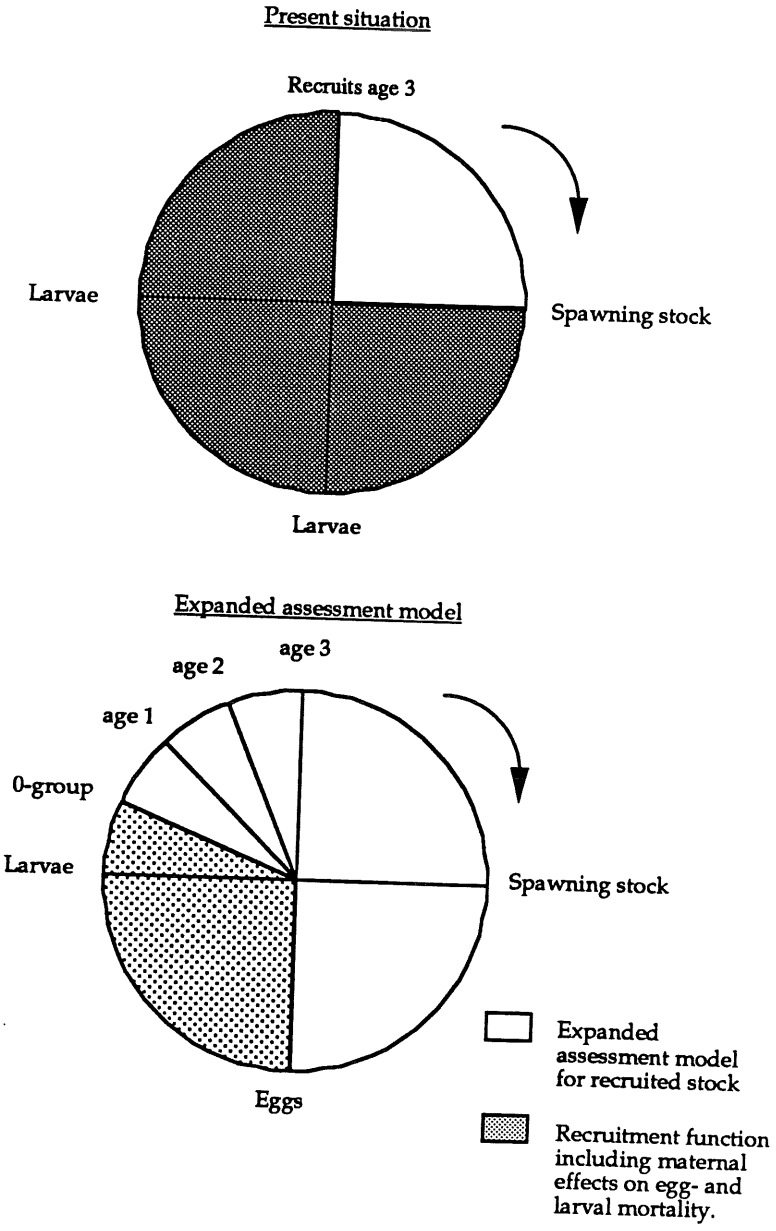
I. Introduction

Information on the reproductive biology of fish and the early stages has grown enormously during the last decades. However, this information has not been incorporated into assessment models. Ulltang (1993) suggests how to reduce the risk in assessment by introducing, among other things, population fecundity and maternal effects. Referring to Rotschild (1986), Ulltang (op.cit.) discusses these issues in relation to a coordinate system developed by Paulik (1973). Data used in the present work fall into first unshadowed quadrant in the stock-recruitment circle, shown below. Taking into account the population fecundity according to age, size and condition and the maturity oogives, the second quadrant will also be unshadowed.



Factors determining the mortality of eggs, larvae and juvenile stages are a combination of the maternal (from mater=mother) and environmental effects. The former is the combined effect of age/size and the condition of the mother fish. When the maternal effect can be given quantitatively for different age/size groups and adjusted for variation in condition, the quadrants 3 and 4 will be partly shaded reducing the variation in this highly variable part of the life cycle. Ulltang (op.cit.) exemplifies his ideas with data from the Arcto-Norwegian cod. Similar ideas have been put forward by Marteinsdottir et al. (1993).

Investigations on the maternal effects started in the Soviet Union in the 1930's and have been conducted mostly on freshwater fish (Zhukinskiy and Gosh 1988). This recruitment mechanism (Nikolskii 1962) is linked to the idea of the critical period by Hjort (1914) in the following way: "The better the supply of yolk and of external food for the fry at this stage, the better the survival." The maternal effect is manifested in the eggs, larvae and juvenile stage and hence in the year class strength in the following ways: 1) Spawning period. The assumption that the larger fish spawn earlier than first-time spawners (Jonsson 1961, Ruud 1939, Sund,1938) has, however, recently been disputed (Kjesbu 1993, Hutchings and Myers 1993). 2) Effect of batch spawning. A general reduction in egg size throughout the spawning season for batch spawners has been found experimentally in haddock (Hislop et al. 1978) and cod (Kjesbu 1989), and in the field by Hiemstra (1962) and Solemdal and Sundby 1981, the latter paper for cod in Lofoten. 3) Effect of female size/age. The classical maternal effect has been demonstrated, *inter alia*, on egg size between first-time spawners and older fish in herring (Blaxter and Hempel 1963) and Norwegian Coastal cod (Kjesbu et al., in prep.). 4) Variations in female condition. Experiments on different feeding rations in Norwegian Coastal cod revealed large differences in both fecundity and egg size (Kjesbu et al., 1992).

Field studies on eggs and larvae of marine species in light of the maternal effect have recently been initiated (Buckley et al. 1991a,b, Chambers et al. 1989, Marteinsdottir et al.1993). The latter study on the Icelandic cod gives interesting data on seasonal changes in both egg and larval quality. Annual variations in the liver index of Arcto-Norwegian cod are given by Yaragina (in press).

Significant correlations between HbI^2 frequency and Arcto-Norwegian cod otolith characteristics (Dahle and Jørstad 1993) indicate that haemoglobin analysis may be used for reliable classification of Arcto-Norwegian cod. DNA fingerprints analysis with multilocus probes introduces a powerful tool for discriminating between different pedigree groups in a comparative study of growth and survival.

The present paper presents data from experiments on eggs and larvae from

Arcto-Norwegian cod in 1993. These data will be compared with data from similar experiments in 1991 and 1992 (Solemdal et al. 1991, Solemdal et al. 1992a,b), and with data on Icelandic cod (Marteinsdottir et al. 1993

II. Material and Methods

Collection of fish and eggs

The cod were caught at Henningsværstraumen in Lofoten, Northern Norway, 29 March - 5 April 1993, close to the peak spawning period (Pedersen, 1984). The material was collected with Danish seine. The hauls were finished during approximately 1 h. The catches were investigated on deck in portions, keeping the rest of the fish alive in the codend. The seine hauls were carried out from noon to about 4-5 p.m. It is known that only 5-10 % of the cod females have running eggs during daytime (first author, unpublished data). In the present material of 1422 cod females a number of 81 or 5.6 % were running. Blood samples were taken from both males and females for genetic studies, and all cod were tagged for further biological investigations. Running eggs were obtained by a blow to the head. Ripe egg were observed to flow out about one minute later. A few drops of milt from one male were stirred in 0.5 l of sea water (temperature 4 ° C and salinity of ≈32 ‰) and then added to the eggs, also suspended in sea water. After 1-2 h the floating eggs were washed through a sieve with mesh size of 0.5 mm and kept in 1 l jars. The eggs were transferred to a field laboratory in Svolvær, Lofoten as soon as possible after collection, usually 4-8 hours after fertilization, where they were held in 1 l plastic beakers with sea water of ≈32 ‰ salinity. The temperature in the field laboratory was 6-7 °C.

Early characteristics of eggs

Fertilization rate and symmetry of the earliest cells were used to characterize the eggs from different females (Kjørsvik et al. 1991, Kjørsvik in press). Fertilization rate was calculated from the floating eggs of each egg batch, 8-12 h after fertilization. The rate of normally developing embryos calculated from the fertilized eggs of each fish was observed 1-2 days after fertilization.

Selection of egg groups on the basis of female characteristics

The stage of spawning was based on studies of the vitellogenic oocytes. A small piece of fresh ovarian tissue was placed in a dish with grooves, iso-osmotic saline water (1.17 %) was added and a number of 50 oocytes measured for diameter. Information on mean oocyte diameter and standard deviation were used to find the stage of spawning following the principles given in Kjesbu *et al.* (1991; 1992). The present method was designated "the field method" to be held separate from the previous method which relied upon a prolonged digestion of the ovaries in Gilson's fluid prior to examination by an advanced fish egg counter measuring many thousands of oocytes. The field scale was divided into four stages: 1 - early spawning (about 0-25 % of all eggs spawned); 2 - midway (25-50 %); 3 - approaching the end (50-75 %); 4 - end of spawning (75-100%). In 1993, contrary to last year, only eggs from cod females in spawning stage 2 - 3 were used for further experiments on maternal effects.

From the cod females included the following additional data were collected: total length, whole body weight, sample of white muscle myotome for observations on dry weight content and lipids, otoliths for ageing, number of spawning zones and cod type, ovary weight, liver weight and weight of viscera. The selected egg groups were divided into 4 subgroups and sent by air to laboratories in Tromsø, Trondheim and Bergen. An overview of the biological characteristics of the 23 Arcto - Norwegian cod females is given in Table 1. The Fulton's and Clark's condition factors (Fulton 1902, Clark 1928) were calculated on the basis of total and net weight (total - liver, gonads and intestines), respectively.

Genetic analysis

For genetic analysis blood and muscle tissues were sampled from each of the individuals used as parents in the production of offspring. Blood samples were analysed by agar electrophoresis according to Sick (1961) and modified by Jørstad (1984). Chromosomal DNA was extracted from muscle tissue, digested with restrictionenzym Pal I and prepared for DNA fingerprinting according to Wells (1988). Jeffreys multilocus probes 33.6 and 33.15 (supplied by Diagnostica) were used in the hybridization reactions. Fingerprint analyses were based on visual inspection of banding patterns directly from autoradiograms.

Egg parameters

Egg diameter 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 °C. Weighing was performed with a Cahn microbalance.

Biochemical studies

Eggs 48-54 h postfertilization (5-6 °C, blastodisc stage) were studied. Groups of 50 eggs were wiped dry for surface water, weighed on a top balance (precision ± 1 mg), and protein precipitated in 1 ml of 6 % trichloro-acetic acid (TCA). Free amino acids (FAA) were determined in the TCA extract using an autoanalyzer as previously described (Fyhn and Serigstad 1987).

Larval parameters

Dry weight

Before drying 10 larvae were rinsed twice in distilled water, further drying and weighing procedure being as for the eggs.

Developmental stages

The early larvae were staged according to Fossum(1986).

Behaviour studies

At Day 1 after hatching, 50 % hatching being defined as Day 0, 20 larvae from each experimental group were transferred to observation chambers holding 100 ml filtered (0.2 µm) 34 ‰ seawater. The temperature was 5 °C and the larvae were kept in darkness. The activity level was measured by means of a ultrasound system (Scanner 450, linear array scanner). A 7.5 MHz transducer was placed at the top of the observation chamber in such a way that the plane of registration made a section of the water column from top to bottom. The ultrasonic registrations were video taped. The number of swimming larvae through the ultrasonic plane per unit time was defined as the activity level. For each larval group, measuring of larval activity was done for 1h in darkness and 1 h in light (13 lux) at Day 3 and 6.

Bacteriological studies

The eggs were washed three times in autoclaved 25 ‰ seawater (SSW) and homogenized in an autoclaved homogenizer. From a dilution series in SSW, the homogenate was plated onto petri dishes with Difco 2216 Marine Broth (Difco, Detroit, USA) with 15 % agar (MBA) and Tryptone Citrate Bile Salt agar (TCBS) (Oxoid, Basingstoke, U.K.). After an incubation period of 14 days at 10 °C, the number of colony-forming units (CFU) were assessed on both media. 32 isolates from the MBA plates of each group were randomly chosen as representatives for the composition of the adherent microflora, and were subjects to phenotypical characterization.

The tests performed were: Gram staining (Bacto Gran stain set, Difco, Detroit, U.S.A.) oxidase (API 7046, Bio Merieux,France), catalase (measured with 3 % H_2O_2) and growth on TCBS agar. In addition, each strain was morphologically examined by a Nikon microscope operated with phase contrast at 600 or 1200x. The strains were regarded rods or cocci. Further studies are in progress.

Fatty acid studies

Ten eggs from 9 cod and 4 replicate samples of about 10 mg from white muscle were analysed separately by methanolysis followed by gas chromatography of the resulting methyl esters according to the method described by Ulvund & Grahl-Nielsen (1988). The fatty acid 21:0 was added in known concentration as an internal standard.

Rearing experiments

Yolk sac larvae were transferred at an age of 4-6 days after 50 % hatching to black plastic bags of 1.5 m^3. The water in the rearing bags were left stagnant throughout the rearing period. The larvae were start-fed on nauplii (mainly *Eurytemora affinis*) filtered from an 37. 000 m^3 volume basin. Larvae larger than 6 mm were offered *E. affinis* copepod nauplii throughout the larval and early juvenile period. The juveniles were caught and the bags emptied when the juveniles had reached a length of approximately 25 mm.

III. Results

In Fig. 1a,b fertilization percentage of the egg groups in 1993 is plotted against length and Fulton´s condition factor of the cod females in spawning stage 2 -3, respectively . No significant correlation was found. Egg diameter against weight, length, Fulton´s and Clark´s condition factor are shown in Fig. 2a - d. Eggs from three 1992 cod females in the correct spawning stage are also included. All correlations were significant at the 5 % level.

In 1992 egg groups from all spawning stages were collected from different size Arcto-Norwegian cod females. Fig. 3a,b show the relation between egg diameter and length and Fulton´s condition factor, respectively: the correlations were not significant.

The results of the activity studies on cod larval groups from spawning stage 2 - 3 cod females are shown in Fig. 4a,b. No systematic trends according to cod female size and egg diameter were found.

The relative fatty acid composition in cod eggs from Lofoten in 1992 and 1993 were similar (Table 2) and the fatty acid composition in white muscle were similar to that in the eggs. No correlation was found between fatty acid composition and age/size of the mother fish. There is, however, a covariation in the relative composition of fatty acid in eggs and white muscle. Due to improvement in the quantification procedure possible differences in total lipid in the eggs between 1992 and 1993 can not be evaluated.

All strains of bacteria from the eggs were Gram negative eods, and with exception of one strain, all were catalase positive. Most strains were oxidase positive, however, in eggs from 602, 25% of the strains were oxidase negative. With respect to assessments of colony-forming units present on the eggs, variations were large on both the media used (Fig.5). The number of CFU/egg on TCBS agar ranged between 0 and $3x10^2$, whereas on MBA agar, the number ranged between $8.5x10^2$ and $1.5x10^4$.

All haemoglobin analysis during the sampling period (Table 3) indicate total dominance of Arcto-Norwegian cod on the spawning site. DNA samples hybridized with multilocus probes provides a baseline of the broodstock for later analysis of the pedigree relationship in different comparative studies.

The content of free amino acids (FAA) in eggs stripped from spawning cod in the Lofoten area correlated strongly withe size (wet weight) of the eggs. Egg wet weight (range: 1.3-2.1 mg/egg) varied by more than 60 % between individual batches of the sampled cod. The correlation applied to the total content of FAA in the eggs (Fig. 6) as well as to the essential amino acids leucine, isoleucine and lysine.

IV. Discussion

In order to quantify the maternal effects on the viability of eggs and early larval stages, it is crucial to eliminate or control the effects of other factors (i.e. spawning stage, genetics, bacteria etc.). A large step towards a more realistic comparison of eggs and larvae from different fish was the development of a method to determine the stage of spawning of batch spawners (Kjesbu et al. 1991). A field version of this method makes it possible to determine the stage of spawning at sea (Kjesbu, unpublished). For practical reasons cod females in the middle of the spawning were used, omitting eggs from the beginning and at the end of spawning with the most pronounced quality changes (Kjesbu 1989, Solemdal et al. 1991, 1992,a,b.). Thus, contrary to 1992, when the investigations focused on the individual maternal effect of batch spawning, the egg material in 1993 was selected from cod females in approximately the same stage of spawning. Comparing the results from the two years show interesting differences.

The fertilization percentage was significantly correlated to stage of spawning in the 1992 material: at the end of spawning the fertilization percentage was significantly lower than at the beginning (Solemdal et al. 1992a.). No such trend in fertilization frequency in relation to cod female size was found in 1993. This indicates that the maternal factor from batch spawning is stronger than the maternal size-effect.

A significant positive correlation between cod female size (length and weight) and egg size was established in the 1993-material. Significant correlations were also found between egg size and Fulton's and Clark's condition factors. In 1992, when the egg groups came from cod females in all stages of spawning (Solemdal et al. 1992b), no significant correlation existed between egg diameter and cod female size (Fig. 3 a,b). The same result has been previously reported (Solemdal 1970). It must, however, be stressed that the present length interval of the spawning cod females was shorter compared to 1992 ranging from about 80 - 117 cm, only two specimens being first-time spawners. Experiments have demonstrated that the most marked increase in egg diameter occurs from first to second year of spawning (Kjesbu et al. in prep). This fact indicates that the present field method is rather sensitive. The classification of the spawning stage is intended to be further refined to select an even more homogeneous egg material.

The relatively large individual variation in condition in cod females in spawning stage 2-3 within the same spawning season, Fig. 2 c-d, is found to significantly affect egg diameter. Usually, annually variations in the "parent"

environment are considered to make influence upon the reproductive biology (Ulltang 1993). Time series in fatness of Arcto-Norwegian cod have demonstrated significant fluctuations (Yaragina in press).

Larval activity showed to be significantly affected by the stage of spawning as previously shown for the 1992-material (Solemdal et al. 1992b). Since the size reduction in egg and larvae are significant throughout spawning the larval size *per se* is of large survival value, as discussed by Buckley et al. (1991a,b) and Ellertsen and Solemdal (1990). Larval activity from the 1993-material shows, however, no correlation to size or condition of mother fish in spawning stage 2-3. This result adds to the suspicion that the "maternal size effect" is smaller than the "maternal stage of spawning effect" on larval activity, analogous to the maternal effects on fertilization frequency and egg diameter.

The DNA sampled and extracted during the spawning period will be used for analysis like REPP (Random Amplified Polymorphic DNA) and microsatellite DNA for more into depth analyses of possible differences among and within groups of individuals.

The differences found in the number of colony forming units present on the eggs indicate that the degree of bacteria ongrowth on the eggs varied. It is not known to what extent this variation may influence results regarding survival, behaviour and feeding ability. Since this investigation at least will include three spawning seasons, special investigations like the genetical and bacteriological ones are necessary to control the egg and larvae experiments.

The survival advantage of hatching from a large egg is obvious according to the citation by Nikolskii (1962) in the Introduction; the larvae being supplied with a larger packet of essential chemical compounds. Since FAA's are found to be an important energy substrate during embryogenesis in marine fishes in general (Rønnestad and Fyhn 1993), as well as for the Arcto-Norwegian cod specifically (Fyhn et al. 1987, Fyhn and Serigstad 1987), the correlation implies that larger eggs are better supplied with energy substrates to support growth during the egg and yolk-sac stages. Larger yolk-sac larvae should therefore be expected to result from eggs of a larger size and of a higher content of FAA at the time of spawning. Actually, this was found in 1992 (Solemdal et al. 1992b). However, the activity of the larvae are not correlated to egg diameter variations in the maternal size effect material, and the problem of good and poor survivors can probably not be solved at these early stages, though the new genetic techniques described seem promising. However, we believe that the ultimate answer of this question will be systematic rearing of selected larvae groups, based on tests exemplified in this project. This

means that rearing of larvae to metamorphosis in small mesocosms, similar to those reported from the Cod and Climate Change Program (van der Meeren et al. 1993). Selected rearing experiments including growth, mortality and morphology are in progress in Tromsø, and the preliminary results are promising.

Comparing the reproductive biology of Icelandic cod (Marteinsdottir et al. 1993) and Arcto-Norwegian cod, one difference is striking. While the frequency of running cod females in Icelandic waters increases from 20 to 50 % during six weeks, in Lofoten this frequency never exceeds 5-10 %. This discrepancy might be the result of different sampling strategies in the two areas: the Icelanders sampling with bottom trawl during the night while the material in Lofoten was collected with Danish seine by commercial fishing vessels during daytime. It has been shown experimentally that spawning occurs more frequently during the night (Kjesbu 1989), similar behaviour is observed in Lofoten (Solemdal, unpublished). The higher temperatures at the Icelandic spawning sites is a factor speeding up the spawning, but closer investigations on the temperature differences are needed to give exact values on the spawning frequency.

The decrease in egg size throughout the spawning season from field investigations is a general phenomenon (Hiemstra 1962) shown for Icelandic cod by Marteinsdottir et al. (1993) and for Arcto-Norwegian cod by Solemdal and Sundby (1981).This phenomenon could be the double effect of batch spawning and the larger fish, with the larger eggs, commencing spawning first. The last effect has, however, been disputed (Kjesbu 1993, Hutchings and Myers 1993) and needs closer field investigations.

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Table 1. Data on the Arcto-Norwegian cod females collected in Lofoten 1993.

Fish nr.	Length (cm)	Weight (g)	Ovary (g)	Liver (g)	Intestine (g)	Age (years)	Spawn. Zones	Spawn. stage	Egg diam. (mm)
54	118	17 534	3054	2077	625	10		2.5	1.47
55	96	8 684	1488	1226	304	10		3.0	1.45
58	99	9 103	1536	534	367	10		3.0	1.44
68	101	8 136	1122	673	344			2.5	1.42
69	95	7 392	850	861	450			2.5	1.46
630	96	9 000	1166	998	356	10	2	2.5	1.51
631	95	8 000	1352	594	260	10	2	2.5	1.42
634	86	6 000	738	700	236	10	2	2.5	1.52
635	96	10 000	2230	1042	376	10	1	2.5	1.49
639	98	8 000	500	492	372	10	1	2.5	1.45
641	116	12 000	1312	1758	408	11	2	2.5	1.46
72	107	14 434	2887	1388	612			2.5	1.47
602	111	14 950	2280	770	652	10	1	2.5	1.54
606	90	5 750	454	466	264	11	2	3.0	1.47
607	89	5 300	704	420	170	9	1	2.5	1.42
617	84	5 100	490	376	168	8	1	2.5	1.47
619	111	13 200	2190	1208	502	13	3	2.5	1.50
620	84	5 700	896	444	176	9	1	3.5	1.42
901	102	11 000	2030	725	395	9	0	3.0	1.56
903	83	4 700	335	355	205	10	1	4.0	1.44
907	84	5 550	500	410	195	8	1	4.0	1.43
908	83	5 450	755	475	200	8	2	1.0	1.42
911	97	8 600	1455	515	400	10	2	2.5	1.47
918	96	6 500	730	405	180	9	0	2.5	1.38
924	90	7 450	1120	430	300	10	1	3.0	1.45
933	105	14 950	3025	2445	565	11	2	2.5	1.50

Table 2. Relative composition of fatty acids (with standard deviations) in cod eggs from the samplings in Lofoten in 1992 and 1993, and in muscular tissue from the samplings in 1993.

Fatty acids	Eggs 1992	Eggs 1993	Muscle 1993
14:0	3.4 (0.7)	2.6 (0.3)	1.7 (0.4)
15:0	0.6 (0.1)	0.6 (0.1)	0.4 (0.1)
16:0	22.4 (1.9)	20.5 (1.4)	23.6 (2.5)
16:1 n9	1.4 (0.2)	1.2 (0.2)	-
16:1 n7	2.9 (0.6)	2.4 (0.3)	1.2 (0.3)
16:1 n5	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)
18:0	3.5 (1.1)	6.6 (1.1)	7.1 (1.0)
18:1 n11	-	-	2.4 (0.7)
18:1 n9	8.4 (1.1)	8.2 (0.8)	6.5 (0.9)
18:1 n7	3.4 (0.9)	2.9 (0.3)	2.8 (0.4)
18:1 n5	0.4 (0.1)	0.5 (0.1)	0.4 (0.1)
18:2 n6	1.1 (0.4)	0.9 (0.1)	1.3 (0.3)
18:3 n3	0.6 (0.1)	0.2 (0.1)	-
18:4 n3	0.7 (0.3)	0.6 (0.1)	-
20:0	-	0.2 (0.1)	-
20:1 n9	2.5 (0.7)	3.5 (0.5)	3.6 (1.0)
20:4 n6	2.0 (1.0)	1.2 (0.1)	1.3 (0.4)
Unknown 1	-	-	2.0 (0.3)
20:5 n3	15.5 (1.9)	13.6 (0.9)	9.7 (1.9)
Unknown 2	-	-	3.6 (0.6)
22:1 n11	-	0.8 (0.1)	0.6 (0.3)
Unknown 3	-	-	3.4 (0.7)
22:5 n3	1.1 (0.2)	1.0 (0.2)	0.9 (0.2)
22:6 n3	28.0 (1.8)	27.8 (2.8)	7.2 (4.5)
Unknown 4	-	-	2.7 (0.7)
24:1 n9	1.1 (0.3)	-	3.0 (0.4)
Unknown 5	-	-	4.2 (1.0)
Unknown 6	-	1.0 (0.2)	-
Unknown 7	-	3.3 (0.7)	-

Table 3. Haemoglobin allele frequencies of cod used as broodstock sampled at different times at the spawning site in the Lofoten area.

Vessel/date	N	*Hb*^{1}	*Hbl*^{2}
F/F J. Ruud, 29.03.93	9	0.00	1.00
F/S Osan, 30.03.93	21	0.14	0.96
F/F J. Ruud, 30.03.93	11	0.09	0.91
F/S Osan, 01.04.93	23	0.09	0.91
F/S Osan, 02.04.93	24	0.08	0.92

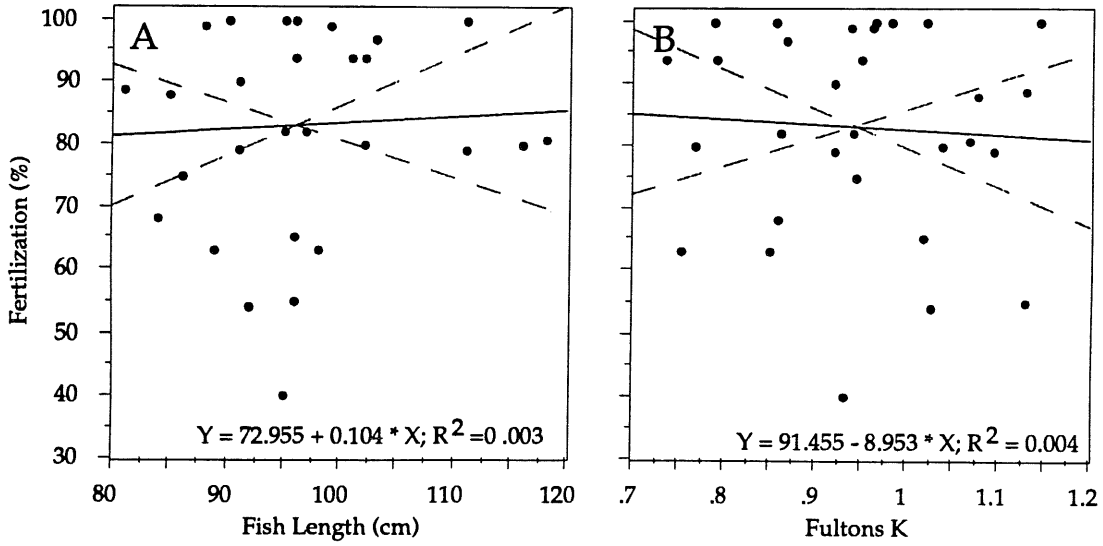

Figure 1 (A-B). Maternal effects on fertilization success. All fishes were captured during the 1993 season and characterized as Arcto-Norwegian cod in spawning stage 2-3. Dashed lines represent the 95 % confidential interval for the slope of the regression line.

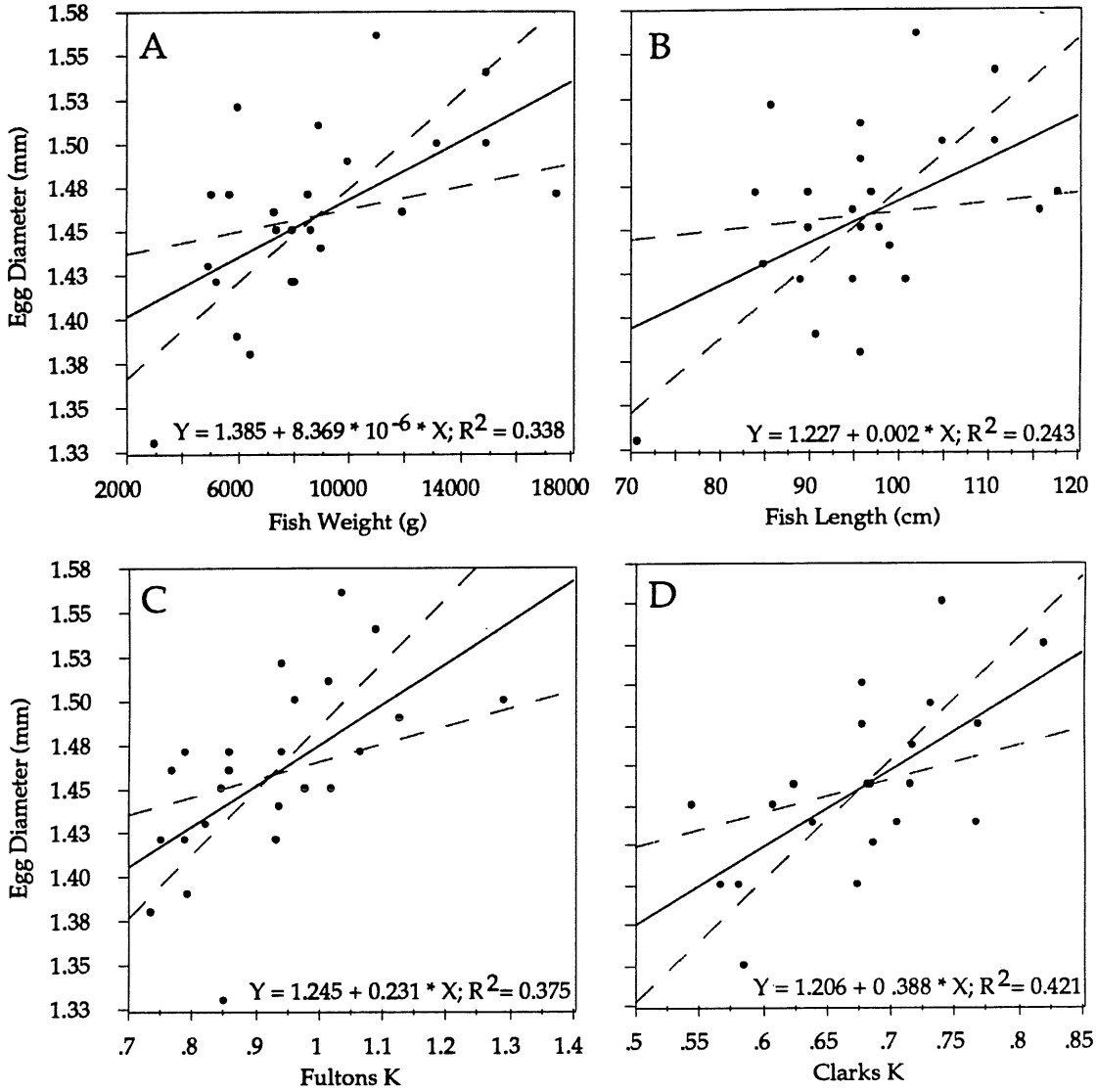

Figure 2 (A-D). Maternal effects on egg diameter in the 1993 material. All females were characterized as Arcto-Norwegian cod in spawning stage 2-3. Dashed lines represent the 95 % confidential interval for the slope of the regression line.

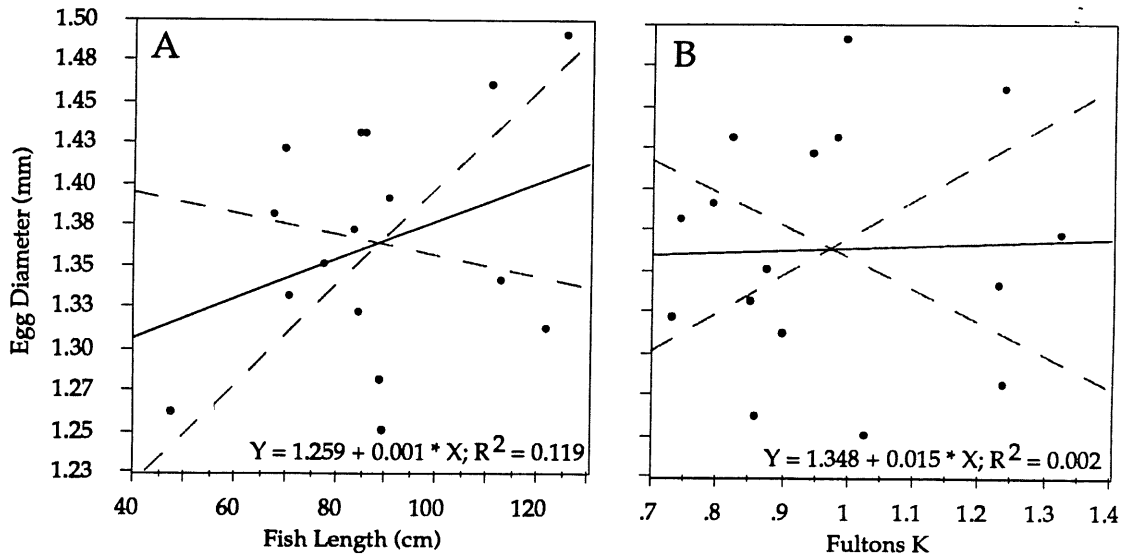

Figure 3 (A-B). Maternal effects on egg diameter in the 1992 material of Arcto-Norwegian cod. Broken lines represent the 95 % confidential interval for the slope of the regression line.

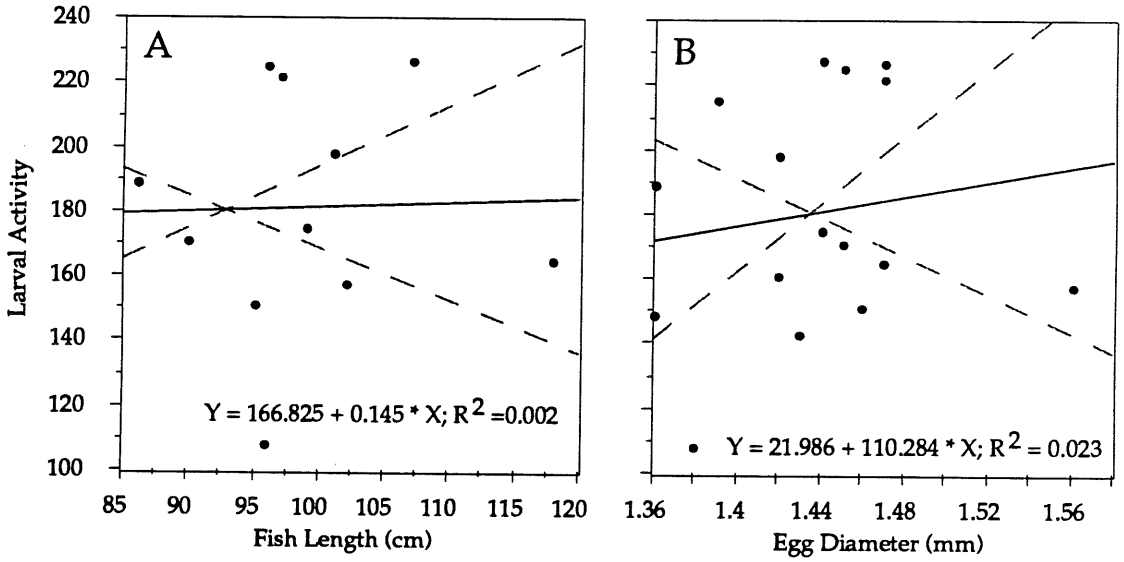

Figure 4 (A-B). Larval activity on day six after hatching in light. Egg material from the 1993 season. The activity was measured by an ultrasound system, and the activity was measured as nos. of larvae swimming through the ultrasonic plane.

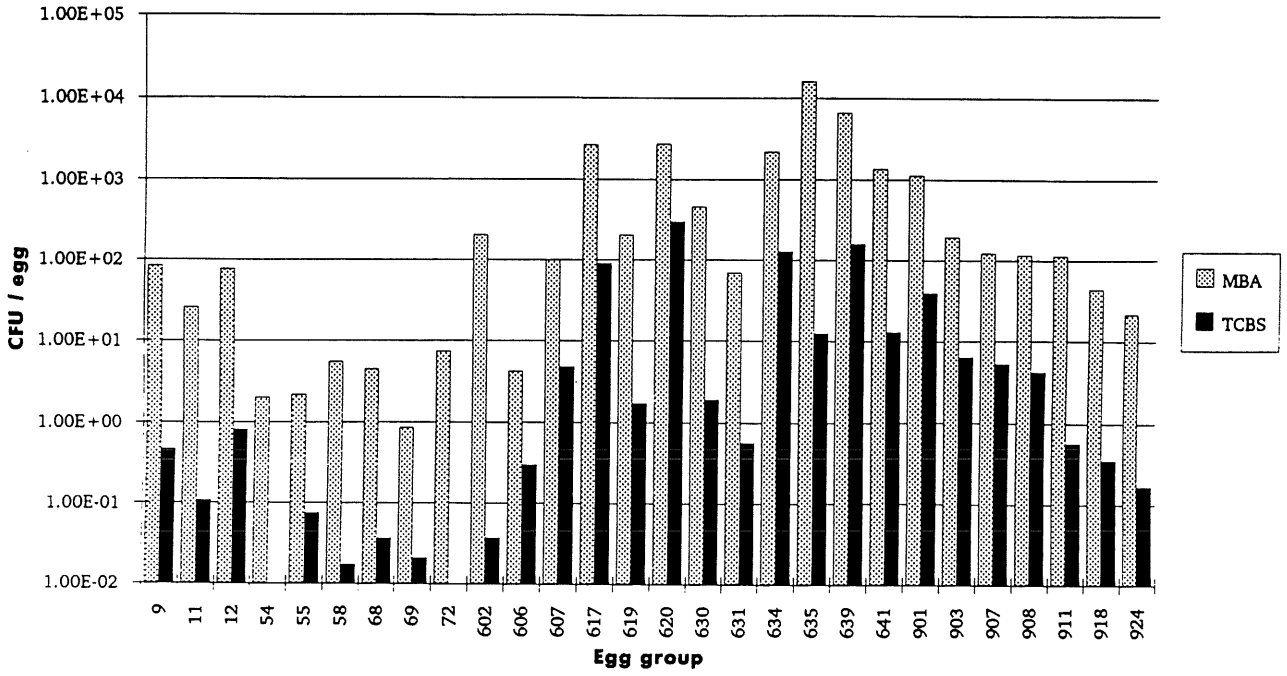

Figure 5. Amounts of bacteria present on eggs of the different groups, measured as CFU (colony-forming units)per egg on the media MBA and TCBS.

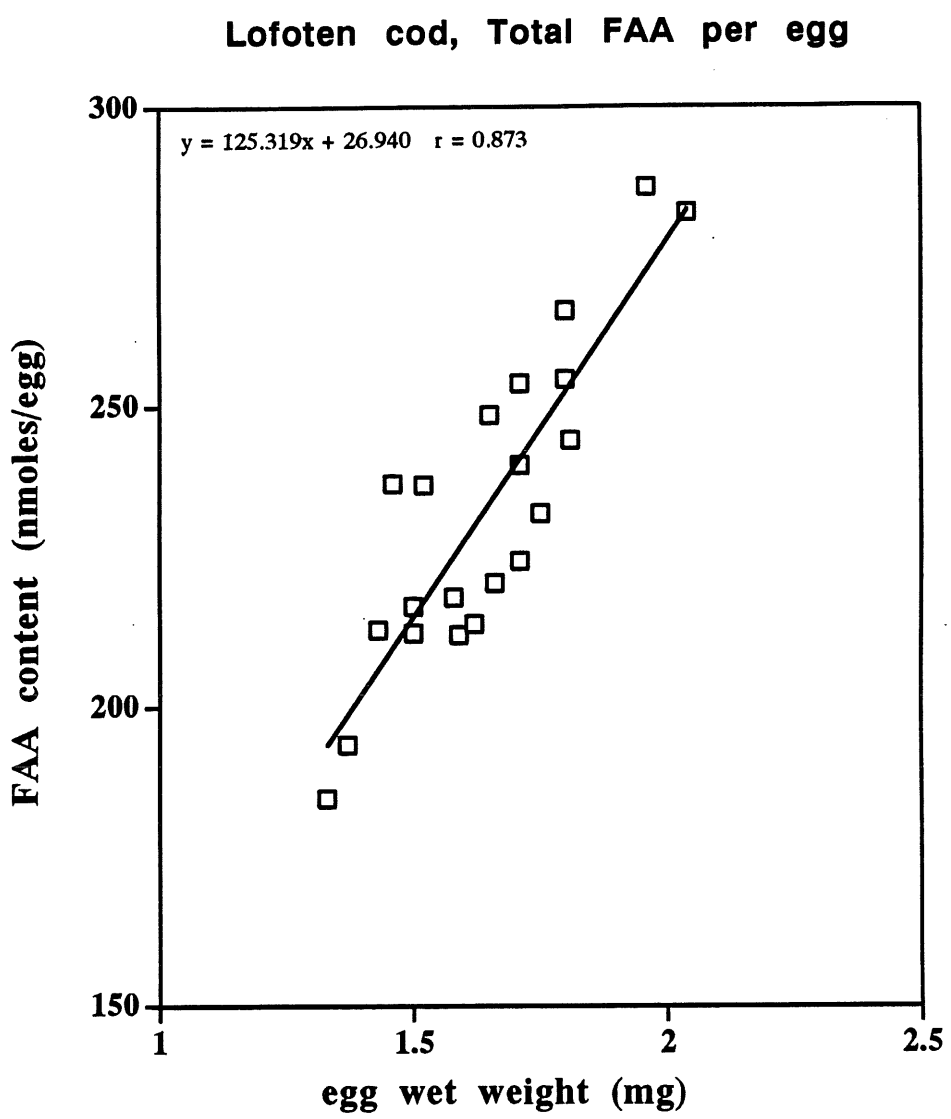

Figure 6. Total content of free amino acids in eggs (48-54 hrs post-fertilization, 5-6 °C of Atlantic cod sampled on the spawning ground. Each point represents the mean value of five groups of 50 pooled eggs.