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The effects of maternal status of Arcto-Norwegian cod on egg quality and vitality of early larvae. I. The collection and characteristics of the cod females, a pilot study

by

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Abstract
On the basis of a new field method to determine the stage of spawning, egg groups from Arcto-Norwegian cod females in different stage of spawning were selected for further studies on egg quality and vitality of early larvae. The fertilization rate decreased with increasing stage of spawning, while the frequency of normal cell division seems unaffected by this variable. A high incidence of normally developing embryos was always correlated to a high fertilization rate.

I. Introduction

Investigations on recruitment mechanisms in fish have partly concentrated on the early larval stages. During this short period two processes operate simultaneously:

1. *The synchronization of fish larvae to its food*
A variable degree of synchronization of the peak density of fish larvae to the peak density of food organisms was proposed to initiate year-class variations of fish in Norwegian waters (Hjort, 1914). Field investigations to test this hypothesis have usually failed (May, 1974), though Cushing (1988) gives examples of the correlation between the feeding situation during early stages and the subsequent year-class strength. Ellertsen *et al.* (1989) demonstrate a positive relation between high temperature, early peak density of *Calanus* nauplii and large year-classes of the Arcto-Norwegian cod.

2. *Maternal effects*

Eggs are supplied with different amount of energy which is of great importance for larval survival during the first days of life. The variation in energy content in the egg is partly the result of age/size differences in the spawning female and partly the result of the condition of the female caused by the feeding regime during the preceding year (Nikolskii, 1962a). In addition, a reduced egg size is introduced during batch spawning (Kjesbu, 1989).

Nikolskii (1962b) puts the two mechanisms together and states: "The better the supply of yolk, and of external food to the fry at this stage, the higher the survival rate."

A mechanism of an adaptive larval production, based on these assumptions, was demonstrated by Ellertsen & Solemdal (1990). The maternal factor as a recruitment factor was originally a Soviet idea and for many years such investigations were performed exclusively by scientists from the former Soviet-Union, mostly on fresh water fish, but also on herring (Nikolajev, 1958). During recent years, several scientists have initiated similar investigations on wild marine species and demonstrated significant effects on egg quality and larval vitality (Solemdal, 1970; Chambers *et al.*, 1989; Zastrow *et al.*, 1989; Buckley *et al.*, 1991a,b; Kjesbu *et al.*, 1991;1992).

One of the independent maternal effects, the reduction of the egg size due to batch spawning will easily mask the effects of the two others. To study the maternal effects caused by varying age/size or condition of the female in field conditions, Kjesbu *et al.* (1990) developed a method to measure the stage of spawning of the female during batch spawning. In this way egg material of similar stage of spawning can be selected during field collection.

Little is known about the causes of natural mortality of fish eggs (Blaxter 1989). Both biological and environmental parameters will influence the survival potential of fish eggs (Rosenthal & Alderdice, 1976), which is clearly demonstrated in recent field studies in the Baltic and the North Sea (Grauman, 1986; Cameron *et al.*, 1989) as well as in the New York Bight (Longwell & Hughes, 1981; Longwell *et al.* 1984). Possible egg characteristics to indicate viability of eggs from stripped fish are fertilization rate and rate of cellular malformation in the early stages of development (as reviewed by Kjørsvik *et al.*, 1990).

A field study was initiated during spring 1992 with the aim to investigate if maternal status of the spawning Arcto-Norwegian cod (*Gadus morhua* L.) would influence the egg and larval viability. The egg groups were selected on the basis of biological parameters and the stage of spawning of the females and sent to different laboratories for various experiments on egg and larval viability. The present paper is a characterization of the spawning fish and the early stages of their eggs.

II. Material and Methods

Collection of fish and eggs

The cod were caught at Henningsværstraumen in Lofoten 26-29 March 1992, 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 cod-end. Thus the fish were in excellent condition. 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 and peak spawning (Solemdal, unpublished data).The total catch during the four days was about 7 tonnes, amounting to approximately 600 cod of each sex All females were stripped for eggs as soon as possible upon landing on deck. Altogether eggs from 45 cod females were used, and each of the fish were individually tagged. The males were also tagged for a closer investigation in the laboratory. Running eggs were obtained by a blow to the head of the fish. 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 of 4 °C and salinity of 32‰) and then added to the eggs, also suspended in sea water. New males were used for every fertilization and the same biological data were taken as for the females. 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 as soon as possible after collection, usually 4-8 h after fertilization where they were held in one litre plastic beakers with sea water of approximately 32 ‰. The temperature in the field laboratory was 6-7°C.

Early characteristics of the eggs

Fertilization rate and the symmetry of the earliest cells were used to characterize the eggs from the different females (Kjørsvik *et al.*, 1990; Kjørsvik, in press). Fertilization rate was calculated from the floating eggs of each egg batch that were brought to the laboratory, at 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 grous 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 in spawning (about 0-25 % of all eggs spawned); 2 - midway (25-50 %); 3 - approaching the end (50-75 %); 4 - end of spawning (75-100%). A finer scale has to await calibration in the laboratory.

From the cod females the following additional data were collected: total length, whole body weight, sample of white muscle myotome for observations on dry weight content, 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 6 subgroups and sent by air to laboratories in Tromsø, Trondheim and Bergen.

III. Results

Table 1 shows the data on the cod females chosen for the subsequent experiments on eggs and larvae at different laboratories in Tromsø, Trondheim and Bergen. Females 1, 2, 4 and 5 were collected in Lofoten in the beginning of May. This to improve the material on fish in most advanced stage of spawning. The egg groups were chosen according to the following criteria:

1. Stage of spawning. Since this characteristic has been demonstrated in experimental work to be very potent, a systematic collected material from wild fish was highly desirable.

2. Female size. It was considered important to include the size variation found in the population.

3. Dry weight of white muscle myotome was used as a measure of the condition of the fish.

4. A general evaluation of the egg groups.

Totally 38 cod females were investigated for the stage of spawning. Egg groups from 16 females were subsequently selected and divided into subgroups for further studies in the different laboratories. The frequency of the different stages of spawning of the 38 cod females were as follows: stage 1: 14, stage 1/2: 4, stage 2: 5, stage 2/3: 2, stage 3: 5, stage 3/4: 2 and stage 4: 6. The frequencies of the stage of spawning of the 16 selected cod females are given in Table 1.

From the otoliths information on age, spawning zones and type of cod are given. As seen from Table 1 the cod females used in the experiment are between 6 and 10 years old and with from 0 to 2 spawning zones. The majority of the females belong to the Arcto-Norwegian tribe, with a few specimens from the

coastal population.

Fig. 1 illustrates the relationship between the stage of spawning and the percentage fertilization found to be significant and negative ($r = -0.469$, $0.01 < P < 0.05$). The relation between fertilization rate and the rate of early embryos with normal cell morphology is given in Fig.2. Egg groups with a high incidence of normally developing embryos always had a high fertilization rate, whereas a high fertilization rate was not necessarly linked to a high incidence of normal embryos.

IV. Discussion

The maternal factor has very complex effects on the quality of fish eggs and the vitality of early larvae (Ellertsen & Solemdal, 1990; Solemdal & Kjesbu, 1992). The pioneer investigations by the former Soviet scientists were performed on species without multiple batches of eggs (see Nikolskii, 1962a,b). The main problem in comparing egg and larvae quality from different sized females classified among the marine multiple batch spawners is the collection of females in the same stage of spawning. Experiments on individually spawning cod females have demonstrated profound effects of the stage of spawning on egg and larval characteristics (Kjesbu, 1989; Kjesbu, unpublished data; Solemdal, unpublished data). Based on experiments with controlled spawning a method to determine the stage of spawning has recently been developed (Kjesbu *et al.*, 1990). The present field version of this method now makes it possible in principle to choose the egg material from wild cod females in defined stages of spawning.

The present material clearly demonstrates that during peak spawning at the end of March (Pedersen, 1984), all stages of spawning are found in the Arcto-Norwegian cod. This is the combined effect of the long spawning period of the individual cod female, spawning up till 20 batches during a period of about 5 weeks (Kjesbu, 1989), and the intra-population variation in the time of start of spawning. Investigations on the effect of female size on the start of spawning is in progress (Kjesbu, unpublished data). It is supposed that larger fish start spawning earlier than the first-time spawners (Sund, 1938).

The stage of spawning seems important for egg fertility, as the variation in fertilization rate was larger in cod females approaching the end of spawning. Lower fertilization rates are frequently observed towards the end of the spawning season for groups of broodstock cod that are spawning naturally in a tank (Kjørsvik, unpublished data). The present results may indicate that a reduced fertility in a group of spawning fishes may be linked to the stage of spawning to a greater extent than to specific fish having low fertility eggs.

Low fertilization rate is a usefull parameter to detect low-viability eggs, but a high fertilization rate is not necessarily correlated with good development and performance of developing eggs and larvae (see review by Kjørsvik *et al.*, 1990). The present results are thus in accordance with earlier observations, as egg

groups with a high percentage of normal embryos also had a very high fertilization rate. In other studies, a significant correlation has been observed between the rate of normally developing fish embryos and the survival potential (Westernhagen *et al.* 1988; Kjørsvik, in press).

Results from the further experiments on the selected egg groups are now in progress (Solemdal *et al.*, 1992).

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Table 1. Data on the cod females selected for different stage of spawning.

Stage of spawning	Fish nr.	Length cm.	Weight g.	Dry muscle %	Age	Type	Spawning zones
1	4842	84	7850	17.59	7	4	0
1	4961	113	17750	18.16	9	5	2
1	4577	86	6250	18.16	9	5	2
1	4973	126	19950	17.27	10	4	1
1/2	4838	70	3250	17.22	9	4	1
1/2	4868	111	16950	17.35	8	4	1
2	4595	85	5050	18.28	7	5	0
2	4855	91	6000	16.6	9	5	1
2	4864	78	4150	17.53	6	1	0
3	4834	71	3050	17.02	9	4	2
3/4	4965	85	4500	15.52			
4	4590				9	2	0
4	1	122	16400	17.63			
4	2	89	8750	17.14			
4	4	90	7500	17.59			
4	5	48	950	16.6			

The scale of stage of spawning is as follows:

1- early in spawning (0-25 % of all eggs spawned)
2- midway (25-50 %)
3- approaching the end (50-75 %)
4- end of spawning (75-100 %)

The code for the cod types are as follows:

1. Coastal cod
2. Uncertain coastal cod
3. Svalbard cod
4. Uncertain Arcto - Norwegian cod
5. Arcto - Norwegian cod.

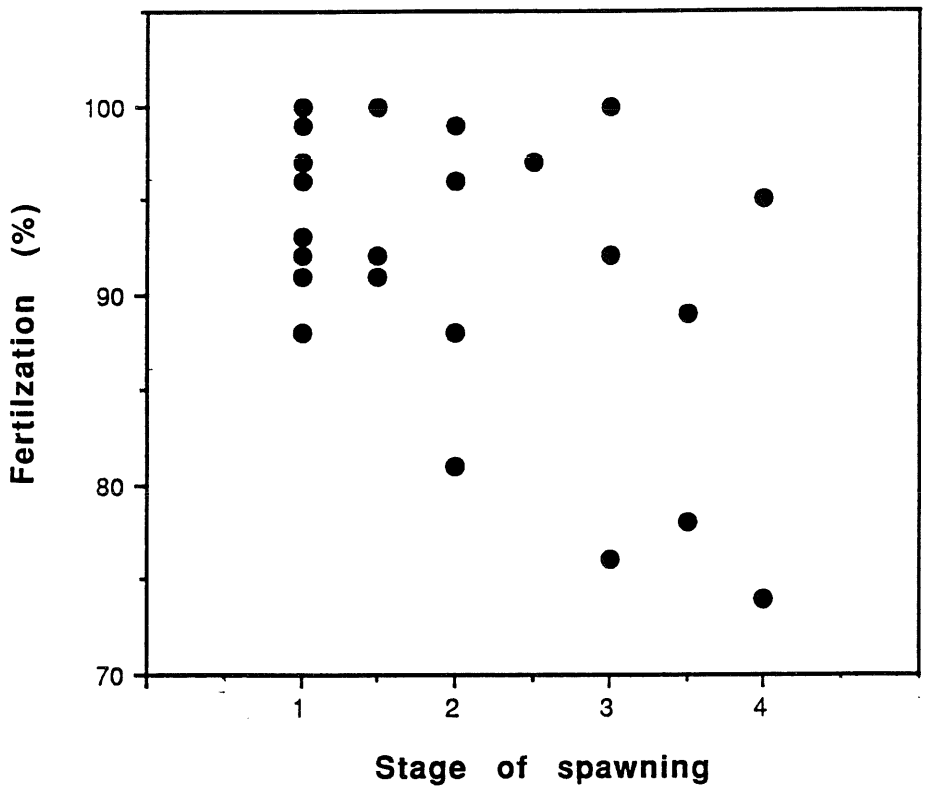

Fig.1. Fertilization percentage as a function of stage of spawning.

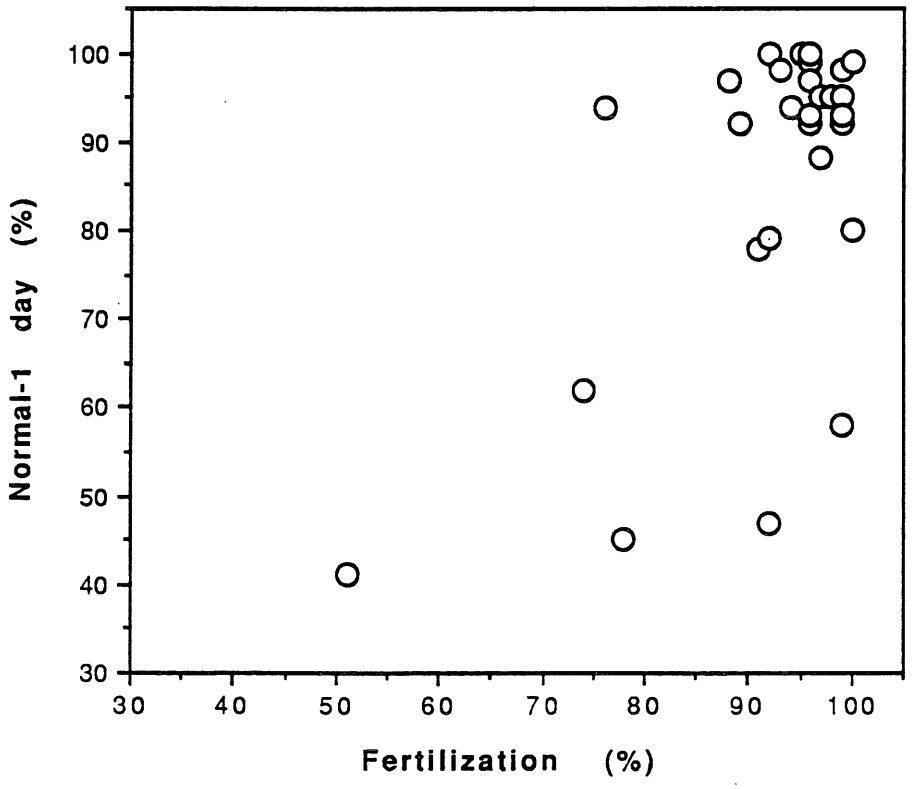

Fig. 2. Fertilization percentage plotted against percentage of normally developing eggs 24 hours after fertilization.