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Demonstration of maternal effects of Atlantic cod: Combining the use of unique mesocosm and novel molecular techniques – A new EU-project

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One of the effects of fishing is a reduction in average age and size in exploited fish stocks, leading to an increasing proportion of recruit spawners in the stock. Current management practice assumes equal viability of offspring from first time spawners and from repeat spawners, despite the fact that that first time spawners often produce smaller eggs than older spawners. The aim of this EU-project is to follow offspring from families of first time spawning and older cod, reared under identical and semi-natural conditions in marine enclosures (mesocosms). The parental origin of the larvae is identified using microsatellite DNA methodology. The advantage of this approach, compared to traditional laboratory rearing, is that rearing conditions are close to natural conditions, and all larvae are reared in the same environment. This eliminates the tank-to-tank variability often observed in traditional rearing experiments. The fish are reared from hatching, through the larval and juvenile stages, until sexual maturity. Growth rates, survival and nutritional condition will be measured using methods such as RNA/DNA ratio and otolith micro increment analysis. The results will be related to parental origin and quality measures of the eggs. It is intended to incorporate the results into management models for improvement of fishery management strategies. In this paper we will focus on a description of the project.

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BACKGROUND

The Atlantic cod is one of the major target species for the European fishery fleet. All European cod stocks are under heavy fishing pressure, or are even overexploited (ICES 2000). One effect of overexploitation is a reduction in the average age and size of the fish, with a growing proportion of first-time spawners in spawning stocks, a situation that may influence their recruitment potential.

Differences among offspring produced by a spawning stock have been a relatively neglected field in fisheries science, and such differences have rarely been incorporated into predictive models of fish recruitment. Nevertheless, large larvae are believed to have a competitive advantage over smaller larvae under otherwise similar environmental conditions (e.g. Hunter 1981). Large larvae are better developed in their sensory and swimming abilities, making them more efficient at capturing food and avoiding predators (Bailey and Houde 1989). Fast-growing larvae will also grow faster through stages during which predation risks are high, and will thereby experience lower cumulative mortality (Cushing 1975). In addition to these general relationships, factors such as the size distribution of prey, prey preferences, and predator-prey contact ratios are also significant (Leggett and Deblois 1994). Despite the apparent advantages of large size of eggs and larvae, most marine fish appear to have maintained a reproductive strategy that invests in high fecundity instead of large egg size.

Size differences among larvae of equal age that have experienced the same environmental conditions may be due to:

- differences in growth characteristics – a genetic component
- a different start – different egg size and quality
- the stochastics of encountering food particles

or, indeed, a combination of any of these factors.

The characteristics of the mother (the maternal effect) will largely determine the size and quality of the eggs. These characteristics are partly inherited, but feeding and other environmental conditions that the mother has experienced before spawning are also important. In addition, the mother's age, earlier spawning experience, and the time in the spawning period (female cod spawn in 15-20 batches per year) will influence egg quality. One might also expect similar paternal effects, but these are probably confined to the genetic component, and are likely to be of little importance for the egg and early larval stage, although such effects have not been investigated in any detail (Chambers and Leggett 1992).

Maternal effects on fish were first investigated in the Soviet Union in the 1930s (Nikol´skii 1962), when differences in egg size, spawning period, and fecundity could be traced back to the size and condition of the mother. Solemdal (1997) reviewed the literature on maternal effects on fish. Several maternal factors, including size and earlier spawning experience, have been thoroughly investigated in cod during the past decade (Kjesbu *et al*. 1991; Solemdal *et al*. 1992; Solemdal *et al.* 1995 Kjesbu *et al*. 1996), and are shown to be of significant influence on egg parameters (egg diameter, specific gravity, mortality). Similar results have been obtained from studies of other species, including herring, capelin and turbot (Chambers and Leggett 1996). It is also well established that small eggs develop into small larvae (e.g. Knutsen and Tilseth 1985; Kjesbu *et al*. 1992). Furthermore, mesocosm studies (Rosenberg and Haugen 1982) have shown that the largest turbot larvae at hatching were still largest 12 days later, and experienced lower mortality than the smallest at hatching. Similar results have been reported for Atlantic menhaden (*Brevoortia tyrannus*) (Chambers and Miller 1995). The results of studies conducted in large marine enclosures suggest that limited food resources in

the early juvenile period caused size-specific mortality in cod that could be traced back to small initial differences in egg size (Blom *et al*. 1994).

In summary, there is substantial information on maternal effects on egg and early larval stages in marine fish in general, and in cod in particular. However, the importance of these maternal effects on later stages has been little investigated, because it is nearly impossible to follow individuals from egg to recruitment in a natural environment, even though otolith microstructure analysis can provide valuable information about selective mortality in the field (Meekan and Fortier 1996). Differences in food availability, temperature, etc. in nature also result in growth variations that make the identification of maternal effects difficult or impossible.

Different approaches using laboratory facilities have been tried to study these mechanisms. However, laboratory experiments on the rearing of marine fish larvae often suffer from high and unexplained variations in growth rates and mortality – even between replica tanks. It has therefore been difficult to perform experiments that compare growth rates and survival.
In a new EU-project "Demonstration of maternal effects of Atlantic cod: combining the use of unique mesocosm and novel molecular techniques" these problems are overcome by the combined use of "mesocosms" (large basins) and "DNA fingerprinting". This type of mesocosm rearing should be regarded as quite different from plastic bags of fish eggs and larvae, floating on the surface of seawater enclosures. In the mesocosm both larval densities and prey conditions are much more similar to the field situation than in laboratory tanks (Blom *et al*. 1994) and it is possible to produce large numbers of juveniles (>10,000). As all the fish experience the same environmental conditions, mesocosm rearing is very suitable for making comparisons of groups of fish that have been hatched and released at the same time. The powerful technique of DNA microsatellite analysis (O´Reilly and Wright 1995; Estoup *et al.* 1998; Estoup and Angers 1998) makes it possible to identify the maternal and paternal origin of each individual produced in the mesocosms. It is the combination of mesocosm rearing with DNA fingerprinting which makes it possible to compare the viability of offspring of different parental origins over an extended period of time without bias from unknown variations in laboratory systems or natural environments.

The results will be relevant for stock assessment. The low correlation between spawning stock size and recruitment level can largely be explained by the high and to a large extent unpredictable mortality during all stages from egg to recruitment size. However, stock recruitment models in current use are usually based on stock biomass, and ignore the actual fecundity of the stock (Marshall *et al.* 1998; Marteinsdottir and Thorarinsson 1998). If the viability of the offspring depends on the age/size of the mother, her spawning status, etc., it may be possible to improve the stock-recruitment relationship significantly by incorporating demographic factors into models. Fishing pressure is a factor of major importance for the age and size distributions and probably also sex ratios of all European cod stocks. Knowledge of the impacts of changes in these factors on the recruitment level will therefore be an important tool for improving the management of these stocks. If first-time spawners have a significantly lower reproductive potential than their fecundity indicates, it might prove necessary to introduce management measures to ensure an optimal age structure within the population.

The project "Demonstration of maternal effects of Atlantic cod: combining the use of unique mesocosm and novel molecular techniques" was developed by the Institute of Marine Research (IMR), Norway, University of Hull, Molecular Ecology and Fisheries Genetics Laboratory (UHULL), UK, Institut für Meereskunde an der Universität Kiel (IfM Kiel),

Germany, and Danish Institute of Fisheries Research (DIFRES), Denmark. EU funds the project for the period 2000-2002. In this paper we will focus on a description of the project. This description is based on the technical annex of the project (Anon. 1999) and currently available results.

OBJECTIVES AND EXPECTED ACHIEVEMENTS

The main objectives are:

1. To examine the viability of offspring from recruit (first-time) female spawners compared to offspring from repeat (elder) female spawners of Atlantic cod.
2. To examine variations in viability between offspring of individual pairs of cod.
3. To analyse the demographic structure (age, sex- and maturity structure) of selected European cod stocks by means of historical data sets.
4. On the basis of the results of objectives 1, 2 and 3, to evaluate possible effects on cod recruitment and their implications for fishery management.

The first two points will be examined by employing rearing experiments in two large marine enclosures (mesocosms: 2500 & 4400 m^3), and comparing measures of viability between individual offspring originating from recruit and repeat spawning females. Such differences will be compared with the variation that occurs between parents within each group.

Our null hypotheses are:

1. *H_{0A}: There is no difference in viability between the offspring of recruit and repeat spawners*
2. *H_{0B}: There is no difference in viability between the offspring of different individual parents within each group.*

The major measures of viability are:

- growth (length, weight, otolith microstructure)
- condition (RNA/DNA of larvae, liver size (index), glycolytic enzymes of juveniles and adults)
- survival (relative – between groups and individual mother fish, and absolute)

These measures will be made during the larval and juvenile stages, and until sexual maturation has been reached. In addition, the fecundity and egg quality (dry weight, fertilisation rate, energy content, fatty acid content and amino acid content) of each female will be studied. The parental identity of offspring will be ascertained using DNA microsatellite markers, which make it possible to relate individual fish, sampled at any stage in their lifespan, to their parents.

The size and age composition of cod stocks are influenced by the fishery. If H_{0A} turns out to be false, that is, if the maternal effect is of significant importance; this information will probably have significant implications for fishery management. We will examine the demographic structure of the spawning stock, in particular how the sex, size and age composition is influenced by sexual differences in maturation, by analysing data sets that extend back several decades in history.

This information will be compared with our experimental results, to investigate how the composition of the spawning stock affects recruitment. The results will be incorporated into

management models in order to study how they can be used to improve fishery management strategies.

PROJECT WORKPLAN 2000-2002

Characterisation and selection of broodstock

Wild cod intended for use as broodstock for the experiment in 2000 were collected from the Barents Sea in August 1998. More than 200 fish were collected and transferred to cages. Their sexual maturity was determined by biopsy during the 1999 spawning season. All fish were individually tagged by internal PIT tags and small pieces of fin tissue were collected for genotype determination.

The fish that were still immature in the 1999 spawning season were regarded as recruit spawners if they matured in the 2000 spawning season. By February 2000 the broodstock had been reduced to about 80 individuals, due to mortality in the course of storage. From the survivors we selected 15 recruit spawning females and 15 repeat spawning females, and placed one in each of 30 spawning compartments (Fig. 1). Each female was accompanied by one randomly selected male (but of similar size).

The broodstock for the 2001 experiment was collected in the Barents Sea during August 1999, and an identical methodology will be used as in the 2000 spawning season.

Figure 1. The Spawning system consists of 10 tanks (3 m diameter) divided into three compartments each. One compartment contains about 2 m^3 water, and one male and one female were placed in each.

Egg measurements, incubation and hatching
The amount, egg size and fertilisation rate of each egg batch will be monitored throughout the spawning season. Batches that are sufficiently large will be incubated, and samples for potential egg quality analysis frozen.

Differences in juvenile viability between the offspring of recruit spawners and those of repeat spawners are expected to be mediated through differences in the eggs. Egg quality parameters will therefore be investigated and related to maternal origin and juvenile viability.
Quality measures of eggs used in the mesocosm experiments include:

- percent fertilisation
- egg size
- egg lipids (total lipids, HUFA)
- egg dry weight and energy content
- egg free amino acid content

At hatching:

- Mean length
- Mean dry weight
- Percent malformations
- RNA/DNA ratio

In addition to providing comparisons of eggs from recruit spawners with eggs from repeat spawners, monitoring will also ensure the use of normally developing larvae in the mesocosm rearing. The eggs will be transferred to incubators in the laboratory and will hatch after approximately 16 days at about 5 ºC. For release in the mesocosm, larval groups of representative quality will be selected at a time when a maximum number of families from each group is represented simultaneously in the larval material.

Mesocosm description, monitoring and analysis
Shortly after hatching the selected larval groups will be transferred to the predator-free mesocosms (Figure 2), where the larvae will start feeding on live, natural plankton, and will be kept until they reach a size of approximately 1 g in June/July. The larvae will be released into two mesocosms, one of 2500 m^3 (600 m^2 surface area, maximum depth 5.0 m) and one of 4400 m^3 (1700 m^2 surface area, maximum depth 4.5 m). The larvae will be sampled twice a week by hauling a two-chambered plankton net (500-µm mesh) diagonally across the mesocosms. After metamorphosis, light attraction or food attraction is used to catch larvae. The captured larvae will be immediately frozen at -70°C, and sub-samples will be analysed later.

Figure 2. The two mesocosms at the Institute of Marine Research, Flødevigen Research Station, Arendal (left basin: 2500 m^3 right basin: 4400 m^3).

The basis of mesocosm experiments with fish larvae is the removal of predators by emptying the basins and refilling them with filtered seawater 16 weeks before the experiment starts. A natural phyto- and zooplankton community then develops, on which the larvae prey. Formulated feeds will be supplied when the cod have reached a size of approximately 0.2 g.

The environmental conditions in the mesocosm will be monitored regularly, measuring:

- temperature profile
- zooplankton density, species composition and size distribution
- phytoplankton species composition and total chlorophyll.

Weekly estimates of zooplankton density will be obtained from pump samples taken from depths of 0, 0.5, 1, 2, 3, 4 and 4.5 (5) m. Water is pumped from each depth for a short period prior to filtering a 100 l sample through a 90 µm plankton net. Samples will be preserved in formalin and later examined using a binocular microscope and counting chamber. A similar procedure will be followed for phytoplankton, using a 10-µm plankton net. Temperature will be measured every day at each depth specified above and the mean temperature of the water column will be calculated. Salinity and oxygen will be measured at the same depths once a week.

Survival during the larval period is estimated from the number of larvae in the net samples, and on the precise number of juvenile fish remaining at the end of the enclosure experiment. The relative survival rates of the main spawning groups will be estimated from their fraction of the sampled material based on the parental identification.

Larval sampling and analysis
Sub-samples of the frozen larvae will be analysed for growth (length and dry weight) and nutritional status (RNA/DNA ratio). Samples of 600 larvae from each mesocosm every second week will be analyzed during the larval and early juvenile periods (Table 1).

RNA/DNA ratios have proven useful as indicators of nutritional condition and growth in larval fish up to metamorphosis (Buckley 1984; Clemmesen 1988, 1994). They are capable of reflecting differences in metabolic activity and protein metabolism and are able to differentiate between well-growing, intermediate and poorly growing larvae. A sub-sample of the homogenate prepared for the RNA/DNA analysis will be taken from every cod larva and used for parental identification by microsatellite markers. We will thus then not only know from which main group each larva comes, but also who its individual parents are. In addition, otoliths will be dissected out, and after we know their parental origin a sub-sample in which all major family groups are represented will be analysed for growth history by otolith microstructure analysis.

Otolith increment studies allow a retrospective analysis of the growth history of the individual as recorded in the microstructure of the otoliths. To be able to read and analyse this record, knowledge of the mechanisms that cause coupling and de-coupling of somatic and otolith growth is essential. Reaction scales need to be known and differences in latency time determined for qualitative growth history analysis. Otoliths obtained will be mounted on slides, ground to reveal the core and the otolith microstructure will be read through a light microscope at magnifications of up to 1000x. Measurements of the otolith microstructure will be made directly through the microscope and by the use of a specially designed software program combined with video output of images on a monitor. Growth rates based on nucleic acid determinations and otolith microstructure will be compared and evaluated in relation to food availability, temperature, larval age and size.

Microsatellite markers
A wide selection of hypervariable cod microsatellite loci are currently available (Brooker *et al.* 1994, Bentzen *et al.* 1996, Miller *et al.* 2000), and are highly suited for the purpose of parental identification. Initial simulations illustrated that three loci are sufficient to unequivocally identify any larvae arising from a random crossing of the broodstock. Complete larval identification may also be possible with only two loci if fish with the rare alleles are selected for the broodstock.

Preliminary experiments have already indicated that for the younger larvae tissue homogenates from RNA/DNA analysis can be directly PCR-amplified if heat-treated first, thus reducing workload. However, DNA samples from older larvae and earaly juveniles require further processing and hence will be extracted using a standard sodium chloride salting-out method (Bruford *et al.* 1992). The extracted DNA will be amplified using a selection of two to three published loci, and allele sizes determined using a Pharmacia ALFexpress™ automatic sequencer (Pharmacia –Amersham). Parental identities will then be revealed using the program PROBMAX (Danzmann 1997), which assigns individual multi-locus genotypes to specific parental crosses.

Tank experiment and analysis of juveniles and adults
The mesocosm part of the experiment will come to an end when the fish have reached an age of nearly three months. At this time all juveniles will be collected and counted, and a sample will be taken for parental identification and growth and condition estimates (Table

1). The juveniles will then be size-graded and transferred to on-growing tanks (2.5 m^3 and 5 m^3) in the laboratory. At a size of about 20 g (September 2000) a random sample of 600 fish originating from each of the two mesocosms will be individually tagged (PIT tags) and their parental origin will be determined by taking a small piece of a fin for microsatellite marker analysis. These fish will be weighed and their length measured every second month until the age of 24 months. By that time, the majority of farmed cod will be sexually mature, and possible differences in fecundity and spawning time will be determined (Svåsand *et al*. 1996).

During transfer from the mesocosms to the tanks a sample will be taken for growth and condition estimates. In addition to length-weight relationships, liver index values and lipid content, RNA/DNA ratios will be determined and enzyme activity tests using glycolytic enzymes (pyruvate kinase (PK) and lactate dehydrogenase (LDH)) will be performed. The use of RNA/DNA measurements in juvenile fish is debatable, since liver metabolism is more important than protein metabolism at that stage and ribosomal RNA activity instead of RNA amount may be the controlling factor. Since only few results are available, a comparison of RNA/DNA ratios and other indicators for growth and condition will be made. A positive correlation between growth rates and activity of the glycolytic enzymes LDH and PK in white muscle of Atlantic cod has already been demonstrated (Pelletier *et al.* 1993, 1995).

Further growth and condition analysis will include length and weight determinations every second month until the age of two years.

Random samples of the sexually mature cod that have been produced by the end of the experiment will be taken to measure fecundity (number of developing oocytes) and stage of maturity. A number of 20 females will be included from each family group, to provide a total of about 300 ovaries. The females will be killed shortly before their first spawning season, i.e. at an age of about two years, and their body size and organ sizes (ovary, liver and viscera) recorded. Fecundity will be estimated from the ovary size, mean oocyte diameter and oocyte standard deviation (Kjesbu *et al.* 1998) and stage of maturity from oocyte diameter of the leading cohort (Kjesbu 1994), using whole mount preparations fixed in phosphate-buffered formaldehyde and a semi-automatic image analyser. Following these analyses, the fecundity of each offspring (family member) will be related to the fecundity of the mother fish, using in the latter respect the total number of egg spawned as the variable. Family groups will be contrasted for fecundity and oocyte size differences.

Synthesis and evaluation

Collation and analysis of historical field data

For Northeast Arctic cod, sexual differences in maturity have been investigated from recent data, establishing that they are stable and significant, with females on the average maturing one-year later than males (Ajiad *et al.* 1999). However, long historical time series of spawning stock and recruitment are fundamental to the understanding of recruitment mechanisms in fish stocks, particularly those that are strongly affected by environmental conditions. Historical data from Northeast Arctic cod with information on maturation relevant for this project extend back to 1932, and from Eastern Baltic cod back to 1980. The data for Northeast Arctic cod have not been processed in a way that makes them directly usable, and a substantial amount of work, including quality control, will be needed before they can be used for the purpose of this project. The data for the Eastern Baltic cod

cover a shorter period and are, except for the most recent years, of poorer quality. However, the data, which are available from ICES Working Group reports, are sufficient to establish a time series of male and female spawning stock sizes and compositions and the work required to do this is not very extensive.

Application in stock assessment and management advice

It has been shown for Northeast Arctic cod that observed sex differences in maturity of Northeast Arctic cod are large enough to have an effect on biological reference points used in management advice (Jakobsen and Ajiad 1999). The effects on viability and growth will be analysed with respect to existing stock assessment models in order to reveal whether these models should include information on age-related reproductive success, i.e. if spawning stock biomass as a reference point for management advice should be replaced by another unit or index more representative of the reproductive potential of the stock. The composition of the spawning stock by size, age, spawning class (first-time, second-time, etc.) and sex is influenced by the exploitation rate. Therefore, our concept of the sustainable exploitation rate for the stock might change. Hence, taking these factors into account in stock assessment and management advice could have implications for the choice of management strategy.

Table 1. Overview of the planed larval sampling and analysis; first-year experiment. The second-year experiment will be similar, but will end after 20 weeks (From EU, 1999).

			No of analyses performed					
	Weeks after release	**Size of cod**	**Length/ weight**	**RNA/ DNA**	**Microsat. markers**	**Otolith microstr.**	**Other**	**Comm.**
Mesocosm	0	4 mm/40µg DW	600	600				[1]
	1		2x600	2x600	2x600	2x200		
	3		2x600	2x600	2x600	2x200		
	5	12 mm/1.2 mg DW	2x600	2x600	2x600	2x200		
	Met.							
	10	1 gram	2x600	2x350	2x600		2x350	[2]
Tanks	20	20 gram			2x600			[3]
	..		1200					[4]
	104	Maturation (2.5 kg)	1200				1200/300	[5]

[1] Samples from every family released (2x30x10).
[2] Transfer to tanks, additional analysis of glycolytic activity
[3] A random sample of 2x600 fish are individually tagged and kept together for the rest of the experiment
[4] 1200 individual tagged fish will be kept in one cage or tank and measured every second month
[5] Liver index, fecundity (300 fish)

STATUS OF FIRST-YEAR EXPERIMENT - SUMMARY

Production of egg groups

Most of the cod pairs spawned easily in the spawning compartments. Egg volumes over one litre per batch were registered, but more normal values were around 1.5 l per batch during the top of the spawning curve (Fig. 3). A total of 216 litres of eggs were collected during the spawning season. The fertilisation rate was usually close to 100 %, although unfertilised groups occurred. Eggs from each family were daily incubated in separate incubators.

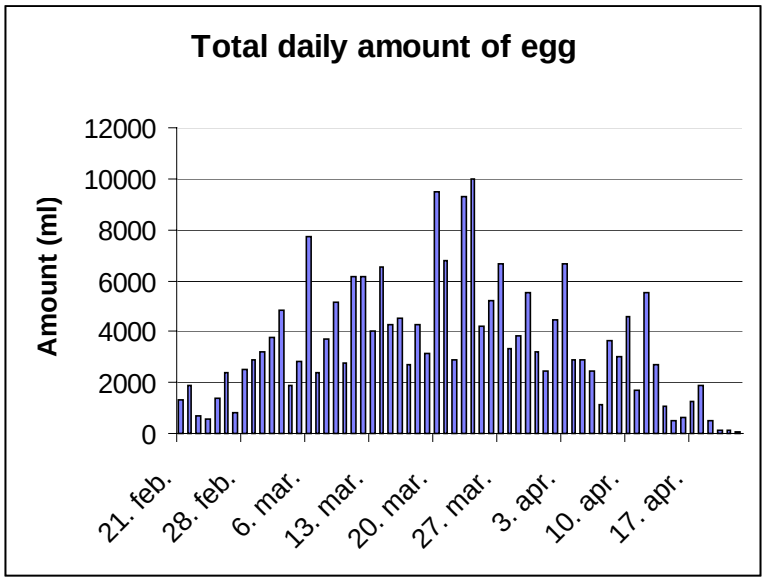

Figure 3. Total daily egg production from the 2x15 family groups in 2000.

Egg quality

Egg quality was assessed by microscopic examination of the eggs collected. Fertilisation rate, no. of unfertilised eggs, no. of dead eggs and cleavage regularity were determined and used for the decision on incubation of the eggs for use in the experiments (Danish Institute of Fisheries Research). Samples of eggs were taken at incubation for determination of energy content, lipid content and free amino acid content. Samples were taken from 62 incubations. Energy content was determined in two samples from each incubation using a bomb calorimeter. The difference between the energy content in the two samples was on average 2.2% (max. 5.3%). The difference between min. and max. energy content was 31.9%. Free amino acid (FAA) content was determined using the CD-ninhydrine method in two samples from each incubation. For each sample, FAA was determined twice. The variation within a sample was on average 1.2% (max. 5.4%), while the variation between the two samples from the same incubation on average was 3.7% (max.14.4%). The difference between min. and max. in the incubations was 263%, with a few samples containing very low amount of FAA. Lipid content is presently being analyzed. The very high lipid content in the eggs has made it necessary to adjust the lipid extraction procedure.

Mesocosms experiments

The newly hatched cod larvae were transferred from the hatchery to the Flødevigen Marine Research Station where they were counted and released in the two mesocosms between 28 and 30 March 2000. In total, 82,285 (32.9 larvae m^{-3}) and 134,314 (30.5 larvae m^{-3}) larvae

were released in the 2500 m^3 and 4400 m^3 mesocosms, respectively (Table 2). The larvae released in the 2500 m^3 mesocosms represented 26 spawning groups and for eight groups the initial numbers varied between 239 and 2314 larvae. The numbers for the rest of the groups were 4000 larvae each in each group. The larvae released in the 4400 m^3 mesocosm represented 19 spawning groups and for four groups the initial numbers varied between 764 and 7550 larvae. The numbers for the rest of the groups were 8000 larvae in each group. The temperature in the two mescosms (Fig. 4) was at the time of the release (28-30 March) around 4 °C in the 2500 mesocosms and 5 °C in the 4400 m^3 mesocosms. For most of the experimental period the temperature in the 4400 m^3 mesocosms was higher compare to the 2500 m^3, and on maximum more then 2 °C in the middle of May. The phytoplankton bloom took place in the beginning of March in the 2500 m^3 basin, and two weeks later in the 4400 m^3 basin(Fig. 5).

Between the period from 3 April to 16 May, 1782 and 3414 cod were sampled in the two mesocosms, respectively. When the two mesocosms were terminated on 5 and 7 June 2000 respectively, 2671 early juveniles (1.1 cod m^{-3}) had survived in the 2500 m^3 mesocosms, equal to 3.3 % of the initial number. In the 4400 m^3 mesocosm, 10,530 early juveniles (2.4 cod m^{-3}) survived, equal to 7,8 % of the initial number. The average standard lengths of the cod were 43.6 ± 6.9 mm (2500 m^3) and 38.8 ± 3.2 mm (4400 m^3) in the two mesocosms respectively.

Table 2. Number of newly hatched cod larvae from each of the spawning groups released in the 2500 m^3 and 4400 m^3 mesocosms respectively in 2000.

Date	Spawning group	Number released		
		Mesocosms 2500m^3	Mesocosms 4400m^3	Total
29.03.00	1b	4000	8000	12000
29.03.00	1c	4000	8000	12000
28.03.00	2a	4000	2800	6800
29.03.00	2c	239	0	239
29.03.00	3a	4000	8000	12000
28.03.00	3b	4000	8000	12000
28.03.00	3c	700	0	700
29.03.00	4a	4000	8000	12000
29.03.00	4b	475	0	475
30.03.00	4c	2314	0	2314
29.03.00	5a	1975	0	1975
29.03.00	5c	4000	8000	12000
28.03.00	6a	4000	8000	12000
29.03.00	6b	4000	7550	11550
29.03.00	6c	4000	8000	12000
28.03.00	7a	4000	8000	12000
28.03.00	7b	4000	8000	12000
28.03.00	7c	4000	8000	12000
29.03.00	8a	1900	0	1900
29.03.00	8b	4000	764	4764
29.03.00	8c	4000	7200	11200
30.03.00	9a	0	0	0
29.03.00	9b	4000	8000	12000
31.03.00	9c	4000	8000	12000
28.03.00	10a	2100	4000	6100
30.03.00	10b	4000	8000	12000
29.03.00	10c	582	0	582
30.03.00	Total released	82285	134314	216599

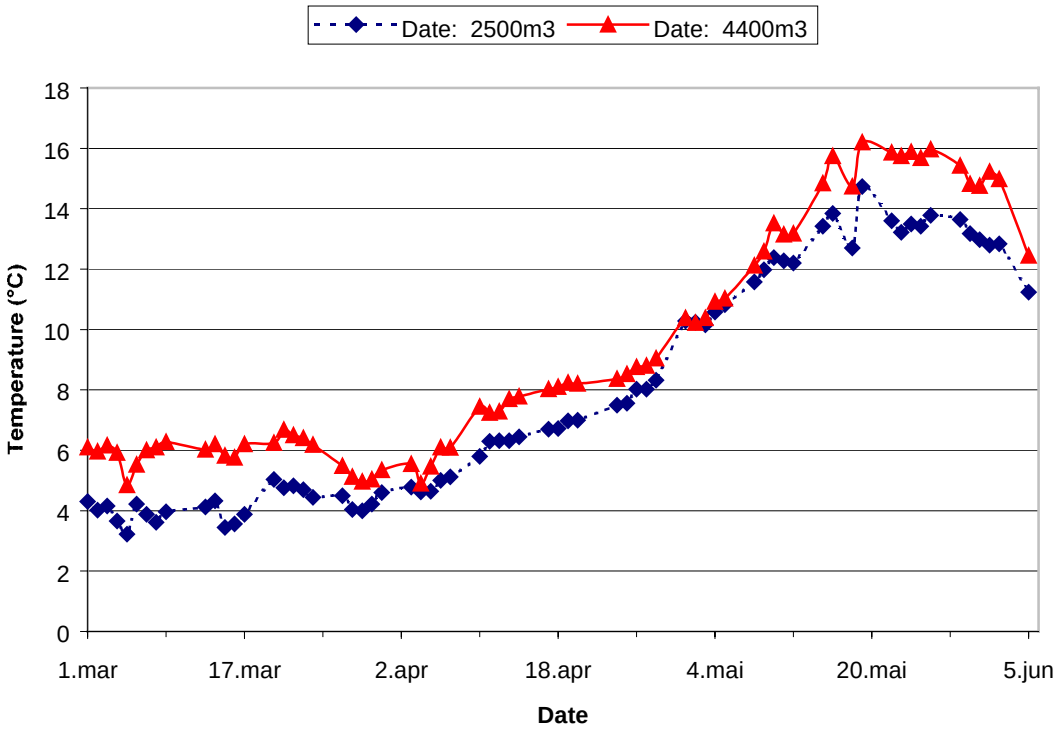

Figure 4. The temperature (average from bottom to depth 0.5 m) in the two mesocosms during the experimental period in 2000

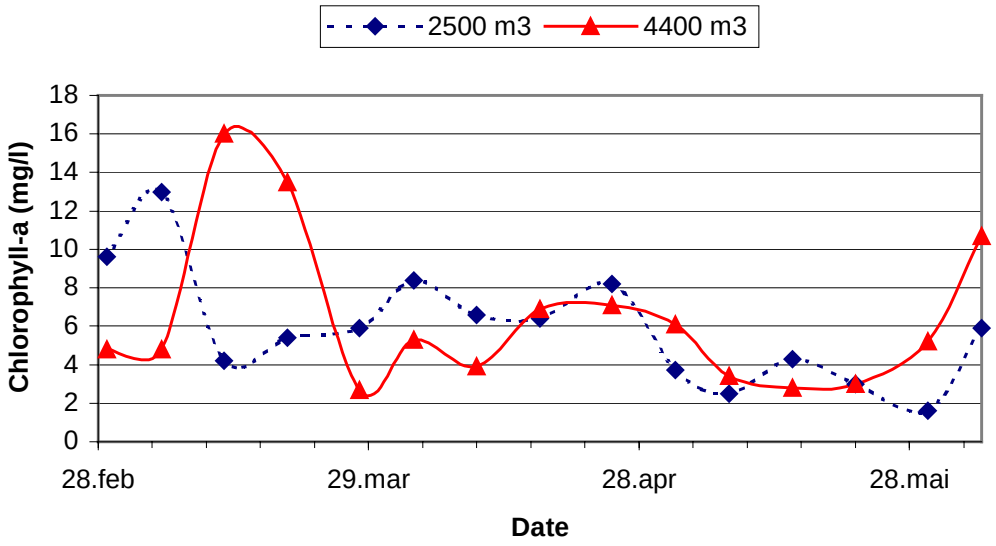

Figure 5. The Chlorophyll-a value in the two mesocosms during the experiments in 2000.

Analysis of cod larvae
Individual cod larvae from the mesocosms have been thawed, standard length taken and dry weight determined after 24 h in a freeze drier (ifm Kiel). Prior to the analysis of RNA and DNA content, otoliths were dissected from a random sub-sample of the larvae and mounted on glass slides. Based on a newly developed protocol (Clemmesen and Hutchinson, unpublished) part of the larval homogenate was taken for later DNA fingerprinting. First samples have been sent to the University of Hull for parental identification. ifm Kiel keeps a second aliquot in case samples are lost or destroyed during shipment. RNA/DNA determinations of cod larvae up to four weeks of age will be performed on whole larvae, meaning that a homogenate of each individual larva will be prepared and the RNA/DNA content of this individual determined. In older larvae/juveniles it will be necessary to determine the RNA/DNA ratio on parts of the larvae. Since muscle tissue best reflects differences in growth and condition, parts of the dorsal muscle will be taken for analysis. Differences in the determination of muscle versus whole body RNA/DNA determinations will be evaluated on sub-samples.

So far no problems have arisen and analysis have been performed as planned.
Length, dry weight and RNA/DNA determinations as well as first dissections of otoliths have been performed on the following samples:

Starting samples: 500 cod larvae measured
1st week :2500m³ basin: 300 cod larvae measured
3rd week : 4500m³ basin: 233 cod larvae measured and otoliths from 200 larvae dissected
4th week : 4500m³ basin: 97 cod larvae measured
5th week: 2500m³ basin: 5 cod larvae measured, 5 otoliths dissected
Analysis of the data in relation to family effect has not been started yet.

Parental assignment of larvae using genetic microsatellite markers
The broodstock was initially screened with eight cod microsatellite loci. Loci, which were difficult to score accurately due to stuttering and the presence of non-amplifying alleles, were discarded, and three, GMO2 (Brooker *et al.* 1994), GMO8 and GMO19 (Miller *et al.* 2000) were finally selected. Conditions for PCR-amplifying the broodstock DNA were developed and all the parental samples were screened at the three loci. The program PROBMAX (Danzmann 1997) was used to create 4500 simulated progeny from the parental genotypes to test how many loci were required to assign parentage to the larvae. Use of the three loci allowed 99.3% of the larvae to be accurately assigned, and where necessary, the addition of a further locus GMO132 (Brooker *et al.* 1994) ensured the full identification of all larvae.

The three loci are currently being re-optimised using larval homogenate and samples of known parentage being screened to fine-tune the genotyping procedure. An Microsoft Excel © macro is also being developed to aid the allelic scoring of the data.

Collation and analysis of historical field data
During the first year of the project, the Institute of Marine Research has identified, checked and organized all available data for Northeast Arctic cod. Individual cod data exist back to 1932. These comprise age, length, sex, age at first-time spawning and maturity stage. Most of the data are from the Lofoten area and consist of approximately 200,000 individual samples. The practical problems with historical data comprise checking the various punching codes (sex, fishing gear, etc) employed over the years. At the moment all data from 1932 - 1999 are

now available in SAS data format and are ready to be used in the project. In addition, an attempt has been made to improve the Gulland approach (Gulland 1964) by including stock numbers and fishing mortalities in order to back calculate the fraction of mature fish by age. A comprehensive SAS program was written to establish a full time-series of fraction of mature fish and of 50% maturity at age, split by spawning classes and sex and based on spawning rings in the otoliths.

Updated information about the project can also be found on the Internet, at: http://macom.imr.no/

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