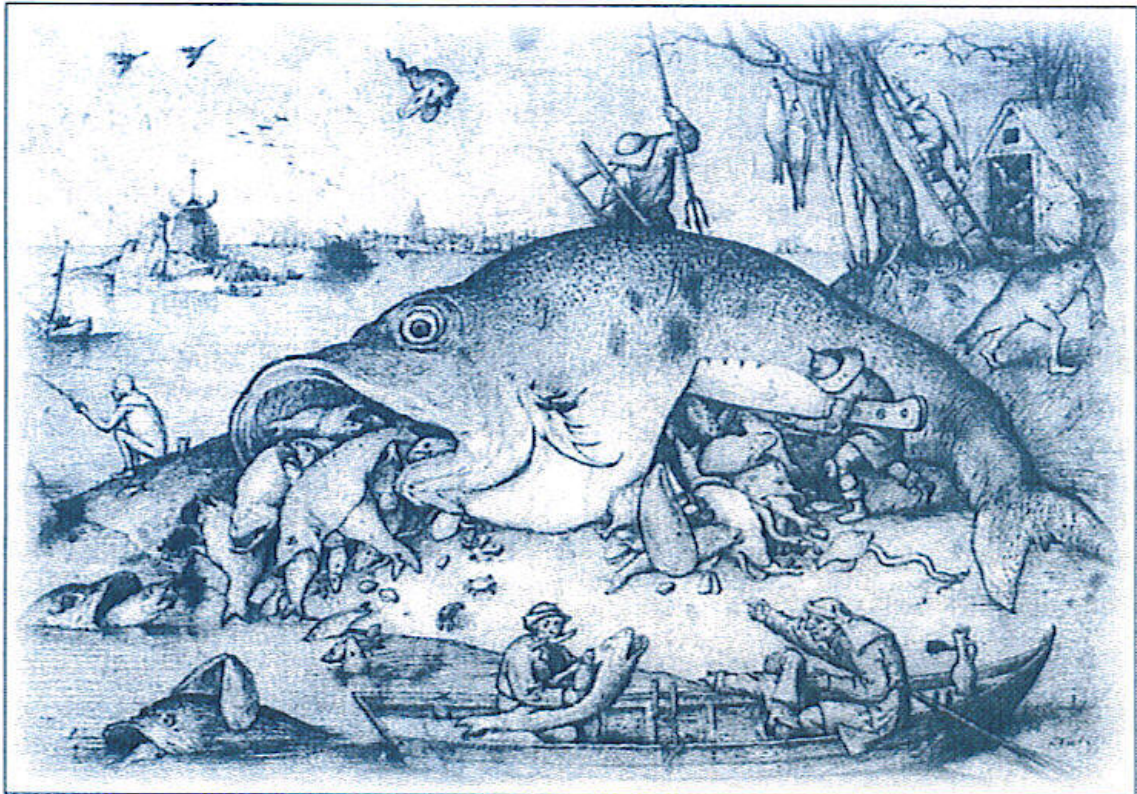


Aus dem Leibniz Institut für Meereswissenschaften
An der Christian-Albrechts-Universität zu Kiel

Influence of maternal effects and environmental conditions on
growth and survival of Atlantic cod (*Gadus morhua* L.)



"Big Fish Eat Little Fish," Pieter Bruegel the Elder (1557)

Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian Albrechts-Universität zu Kiel

vorgelegt von
Vivian Christiane Buehler

Kiel 2004

Influence of maternal effects and environmental conditions on growth and survival of Atlantic cod (*Gadus morhua* L.)

Table of Contents:

Acknowledgements	1
1. Introduction	1
1.1 Importance of cod fisheries.	3
1.2 The Northeast Arctic cod	4
1.2.1 Characteristics of the NEAC Stock	5
1.2.2 Sex ratio and spawning behaviour of NEAC	6
1.2.3 The offspring of NEAC	7
1.3 Factors that may influence stock management	8
1.4 What is maternal effect?	9
1.5 The MACOM Project	12
2. Materials and Methods	12
2.1 Parental Stock	12
2.1.1 Collecting Fish	12
2.1.2 Breeding	13
2.1.3 Incubation	14
2.1.4 Egg quality measurements	14
2.2 Mesocosm Experiment	14
2.2.1 Characteristics of the mesocosms	15
2.2.2 Monitoring of biotic and abiotic parameters	16
2.2.3 Transport and arrival of the larvae in Flødevigen	17
2.2.4 Releasing of larvae in the mesocosms	18
2.2.5 Sampling of cod larvae in the mesocosms	18
2.2.6 Termination of the Mesocosm Experiment	18
2.2.7 Mortality Calculation	19
2.3 Rearing of Juveniles in Indoor Tanks	20
2.3.1 Fish Tagging and fin clipping	20

2.3.2 Fish Measurements	21
2.3.3 Termination of the tanks Experiment	21
2.3.3.1 Estimation of oocytes size and Potential Fecundity	22
2.4 Laboratory Examinations	23
2.4.1 Otolith microstructure analysis	23
2.4.1.1 Otolith Preparation	23
2.4.1.2 Otolith reading	24
2.4.2 RNA/DNA ratio and glycolytic enzymes	24
2.4.3 DNA Fingerprinting	26
2.5 Statistical Analysis	27
3. Results	27
3.1 Description of the broodstock	29
3.2 Spawning and Egg quality parameters	35
3.3 Laboratory control	35
3.3.1 Mortality test on starving groups	35
3.3.2 Relations among egg size, larval size and condition	36
3.3.2.1 Newly-hatched otoliths	39
3.4 Mesocosm Experiment	39
3.4.1 Monitoring of the mesocosms	43
3.4.2 Larvae sampling and survival	53
3.4.3 Analysis of Growth and Condition	76
3.5 Tanks Period	84
4. Discussion	84
4.1 Exploitation and changes in demographic structure	85
4.1.2 Evolutionary Consequences of the shift in demographic structure	87
4.2 The influence of maternal effects	88
4.2.1 Detecting maternal effects in this study	89
4.2.1.1 Egg quality parameters	90

4.2.1.2 The offspring	90
4.2.1.3 Effect on otoliths	91
4.2.1.4 Biochemical methods	91
4.2.1.5 Tanks data	92
4.3 The connection between maternal effects and environment	92
4.4 Environmental effects	95
4.5 Analysis on the approach followed by this study	95
4.5.1 Differences between repeat and first spawners	95
4.5.2 Mating selection	96
4.5.3 Egg parameters	97
4.6 Evolutionary consequences of maternal effects	98
4.7 Conclusions	99
5. Summary	100
6. Zusammenfassung	101
7. References	
List of Figures	
List of Tables	

There is no end, no beginning. There is only the infinite passion of life...
(Frederico Fellini)

*This work is dedicated to the maternal effects of
all mothers that made of this world a better
place to be...*

Acknowledgements

I am very grateful to Prof. Dr. Dietrich Schnack for the kindly supervision of this work, for his comprehension and patience; and to Prof. Dr. Erlend Moksness for giving me the opportunity to work in MACOM, thrusting me for so much responsibility in this project. Dr. Catriona Clemmesen, I thank for all the nice moments and fun we had in this project.

This work could not be performed without the valuable contribution of all MACOM participants: Dr. Gary Carvalho, Dr. William Hutchinson, Dr. Lorenz Hauser and Richard Case were responsible for the genetic fingerprints. Dr. Olav Kjesbu, Dr. Håkon Otterå, Dr. Anders Thorsen were responsible for the broodstock and fecundity data. Dr. Catriona Clemmesen was responsible for all biochemical analyses. Dr. Helge Paulsen was responsible for the egg quality measurements and partially for the 10 week otoliths data. In addition, I would like to thank the coordinator of the project Dr. Terje Svåsand, Dr. Per Solemdal, and Dr. Tore Jakobsen. My best thanks to Jan Pedersen and Jon Kåre Stordal from Parisvatnet and all the staff from Flødevigen.

Kjersti Eline Larsen, I thank for the nice teamwork in Norway. I specially thank Else Torstensen and Bente Lundin for friendship and support. Dr. Halvor Knutsen taught me the PCR and genetic fingerprinting methods. I learned a lot about Phytoplankton from Einar Dahl. Øystein Paulsen took many of the pictures in this project. Prof. Dr. Torfinn Lindem, Dr. Birgit Dannevig, Kitty and Christopher Ottersland, Christine Elmenhorst, Alf Dannevig, Nikolai Dannevig, Petter Baardsen, Tor Birkeland, Helen Nilsen and Inger Henriksen thanks for a great time in Norway.

Dr. Odd Aksel Bergstad knows the most amazing stories about cod fisheries in Norway. Jan Pedersen, and the people from the University of Bergen helped me to identify a bacterial contamination and to control the outbreak of *vibriosis* in juvenile cod during tank rearing. Prof. Dr. H. Rosenthal, Dr. Uwe Waller, Dr. Arild Folkvord and Dr. Ed trippel gave me great advices on aquaculture and fisheries. Dr. Hans-J. Krambeck, Herbert Kiesewetter and Werner Wegner from the Max-Planck-Institute for Limnology in Plön (MPIL), saved my work from many viruses and computer crashes. All my love to Prof. Dr. Wolfgang J. Junk, Prof. Dr. Ulrich Sommer and Prof. Dr. Joachim Adis for all the good advices, friendship and support.

I thank Dr. Frank J. Jochem (Florida International University, US) for helping me out in many questions concerning phyto-zooplankton interactions and environmental factors. Prof. Dr. Joachim Gröger (University of Massachusetts, US) developed a nice model to test the complex MACOM data. Bene Willert and Helgi Mempel for the nice teamwork.

Martin Lembke and Barbara Schmidt transformed every visit to the IfM library in a happy event. Daniel Stepputtis and Jörn Schmitt were a great help with software and graphic design. Bastian Huwer has been always a great friend and indispensable help during the final months of this thesis.

Brigitte Rohloff and her husband Kalle are the most phantastic people, who are able to charm a smile on your face even in the cloudiest days. Isabella Galvao was a nice company in the early hours at the IfM. Rudi Lüthje and Dr. Rudi Voss reminded me that there is always a "lighthouse" somewhere out there. From Muklis Kamal I've learned that friendship can sprout in the most different soils. Su-Kyoung Kim seasoned our lives with the most fragrant Asian flavors. Mary Stradas believe that everyone can be the alchemist of his own life...

Finally, I thank my family, friends and colleagues for their comprehension, love and support.

1. Introduction

Gadus morhua, the codfish (fig.1) belongs to the family Gadidae, order Gadiformes, class Actinopterygii (ray-finned fishes). The maximum historical size reported was 200 cm of total length, 96 Kg, and 25 years of age (Cohen et al. 1990). Cod live in a benthopelagic, oceanodromous, marine or brackish environment with depths ranging from 1 to 600 m. The geographic distribution of the species is the North Atlantic ranging from 35° to 78° N of latitude, i.e. from Cape Hatteras to Ungava Bay along the North American coast, east and west of Greenland, around Iceland; coasts of Europe from the Bay of Biscay to the Barents Sea. Additionally, two stocks are recognized in the brackish Baltic Sea. The distinguishing features are a stout body with a long chin's barbel, relatively small eyes, and three dorsal fins all close together at the base and rounded in outline, and two anal fins. The upper jaw overhangs the lower. The lateral line is continuous, with a smooth curve about the pectoral fin. The color varies according to the local habitat and ranges from light to brown leopard spots on an olive to gray- greenish or sometimes even reddish back. They are white ventrally and the lateral line is conspicuous light. Cod are omnivorous and feeds at dusk and at dawn on invertebrates and fish, including young cod.

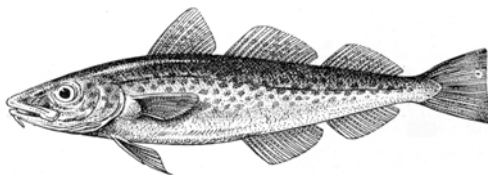


Figure 1. *Gadus morhua* L.

Cod are prized for their white flesh, which has only 0.3% fat and 18% protein per gram. Besides, there is almost no waste to a cod. The head is cooked in soups, while the throat (known as cod tongue) and cheeks are very appreciated in Norway. Additionally, gonads, stomachs, and livers are all eaten or used in many other products. The liver oil is highly valued for its vitamins D and E.

1.1 Importance of cod fisheries

The story of cod fisheries loses itself in the history of times. Cod have been already called "Beef of the sea" and their history goes further back to the first registers during Viking times, or probably even earlier. Through the centuries, cod fisheries sustained different societies in both sides of the North Atlantic, and fisheries became more intense every year. The nineteenth century was marked by the optimism that natural resources would never end up. Although many fishermen started to complain about decreases in catches, Thomas Henry Huxley (1825-1895), a respected British biologist (also known as "Darwin's Bulldog" for his defense on the theory of

evolution) argued in 1883 at the International Fisheries Exhibition in London that “overfishing” was an unscientific and erroneous fear.

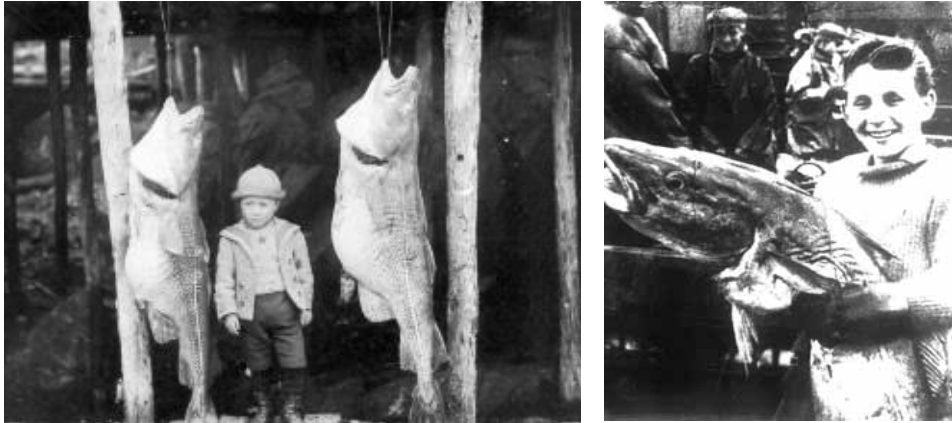


Figure 2. Typical pictures from old times: Boys posing with huge codfishes (left, copyright Ryan Shannon DFO, Canada and right, picture from the Greenpeace archive.)

However, in the beginning of the twenty-first century the fisheries forecasts are frightening, not only for cod, “overfishing” became a global issue. Actually, more than 75 percent of the species tracked by the Food and Agriculture Organization of the United Nations (FAO) are categorized as fully exploited, overexploited, or depleted. With 90 percent of the world’s fishing grounds in the Atlantic closed off by the 200-mile exclusion zones, the fishing industry started to search in greater depths for new species or switching to the Pacific. Looking at the FAO catch statistics from 1950 to 1994, Daniel Pauly and his collaborators (1998) found that the world’s fishing fleets have been steadily fishing down the food web towards lower trophic levels. Populations of fish in the world’s ocean have fallen by 90 percent in the past 50 years (Myers & Worm 2003). The status of the cod stocks worldwide is preoccupant. Even with a complete ban of fishing, after ten years, the Newfoundland cod stocks are still not recovering. Despite of the Federal limits on fishing, stocks have stayed at low levels and in some cases the fish stocks have continued to go down (Rice et al. 2003).

Stocks in the North Sea, Irish Sea, Skagerrak, and off the West coast of Scotland are in even worse state than it was so far believed. North Sea cod are below the recommended level of 70,000 ton. If the pressure continues at the present high levels, the Spawning Stock Biomass (SSB) in 2004 is predicted to 28,000 ton. The spawning stock in Kattegat fell by 71 percent from the 1970s to the 1990s, to about 10,000 ton. In October 2002, estimates suggested that the spawning stock would be 6,700 ton. The present estimation is 2,500 ton (ICES, 2003).

In the Baltic, the situation is not much better. Despite of the long-term management for the Baltic stocks proposed by the EU in 1999, the stocks worsened. In 2001, a recovering plan that included summer ban on cod fishing, restrictions on the design and size of fishing gears, minimum net mesh and landing sizes was established. However, all those measures proved to be ineffective. Consequently, the European Commission has extended the summer ban (from June 1 to August 31), prohibiting

cod and flat fish catches by use of trawls, bottom seines, and similar gears since April 2003.

Experts say that cod are almost commercially extinct in the North Sea and that the cod fishing grounds should be shut down. Nevertheless, they recommend that not only the cod fishing grounds should be shut, but also whiting, haddock and prawn fisheries, in which cod are normally by-catch. EU Fisheries Commissioner Franz Fischler said: "There is a need for urgent measures because cod is threatened with extinction" (Associated Press -May, 2003).

1.2 The Northeast Arctic Cod

The Northeast Arctic cod (NEAC) is one major productive cod stocks in the world. After the large fisheries reduction in 1989, this stock was considered within the safe biological limits in 1992 and as a healthy stock in 1993. Unfortunately, the numbers have declined in the last years. After 1998, the spawning stock has been far below the minimum demand for a precautionary management. According to the International Council for Exploration of the Sea (ICES), the Northeast Arctic cod is outside save biological limits. Fishing mortality among the last four years has been among the highest observed. Economically speaking, the cod in the Barents Sea, i.e. the Northeast Arctic cod is Norway's key fisheries stock, which is divided fifty-fifty percent with Russia. When the 200 miles limit-zone was introduced, most of the Barents Sea was split between Norway and Russia. In spite of Svalbard been under Norwegian jurisdiction, both countries have equal opportunities to exploit the natural resources in the area. However, there is no final agreement on the sharing principles of the Barents Sea. The result was the establishment of a "gray zone" with shared jurisdiction. Additionally, a patch of international waters is left (Fig.3), in which some countries had used to fish cod outside of the total allowable catch (TAC) set by the Commission (Jakobsen 1999). In November 2002, these two countries agreed upon a long-term harvesting strategy for cod. In other words, Norway and Russia laid down a framework that should maximize yield at long terms, keeping a high degree of stability in the total allowable catch (TAC) from year to year. Besides, all data available regarding stock development should be fully utilized in the management. For 2003, Norway and Russia agreed upon a TAC of 395,000 ton, with an additional quota of 40,000 ton of coastal cod from the Norwegian fjords. This is a prolongation from the quotas of 2001 and 2002. Thus, the spawning stock is expected to show an increase in 2004.

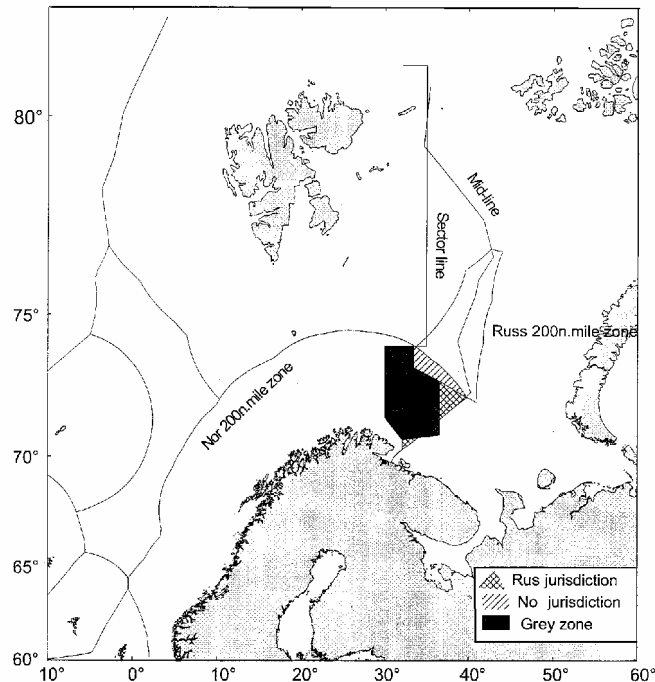


Figure 3. Distribution of the economical zones relevant to the NEAC fishery management in the Barents Sea (from Jakobsen 1999).

1.2.1 Characteristics of the NEAC Stock

The Norwegian Arctic cod spend most of their life in the Barents Sea, but migrate both as juvenile and as mature cod. The migrations occur from the wintering grounds to the spawning, feeding grounds, and back (Fig.4. left and right). The large and mature cod is called "skrei" in Norwegian. The spawning grounds of "skrei" stretch all the way down the northern part of the Norwegian coast from Finmark to Stad. However, the most important spawning grounds are off the Lofoten archipelago. In August and September, mature and immature fish are located northern of Spitsbergen and northeasterly parts of the Barents Sea. In October, they turn south and west towards the Bear Island and the Norwegian coast. Cod are concentrated in a much smaller area during winter than in summer. They are either found off the Bear Island along the line of the front between the warm Atlantic and cold polar waters (Lee 1952, Jakobsen & Ajiad 1999), or in the eastern part of the Barents Sea, close to Goose Bank (Maslov 1960). In cold winters fish are more concentrated on the west, while in milder winters cod overwinter in the east of the Barents Sea (Jones 1968). However, mature and ripening fish do not remain long in the wintering grounds, but migrate to the spawning areas.

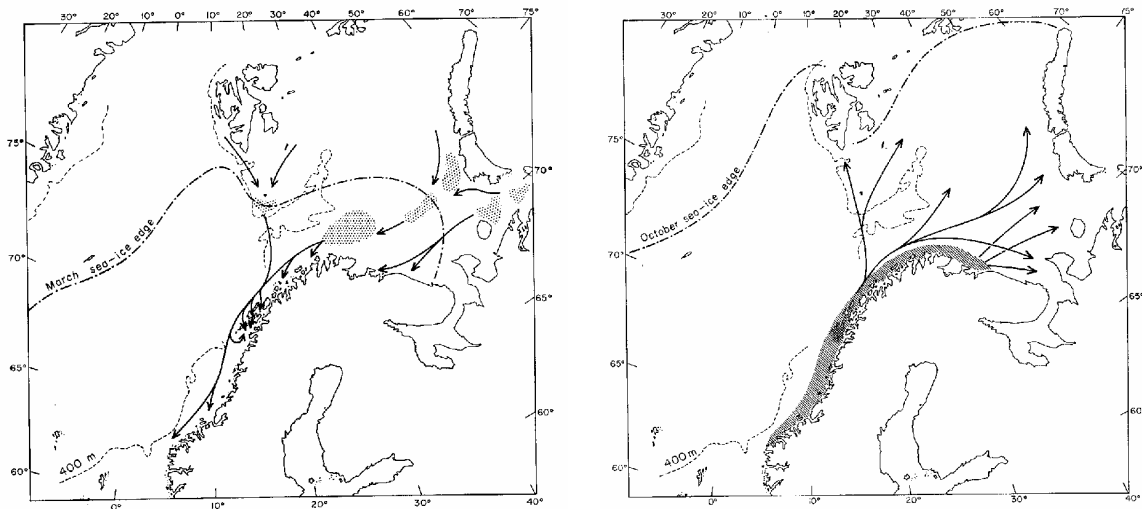


Figure 4. Distribution areas and major migrations of NEAC. On the left, main migrations to the spawning grounds. Right, return of the spent fish from the spawning to the feeding areas (After Jones, 1968).

The spawning season takes place from February to May. The peak spawning is in March/April with temperatures of 4-6 °C (Pedersen 1984, Sundby & Bratland 1987). After spawning, they migrate back towards the north (Jones 1968). Although cod are also caught by trawlers in the Barents Sea, the migrations of "skrei" still form the basis for the most important seasonal fishing activity in Norway, the Lofoten fishery. Additionally, about 25 percent of the cod caught in the Lofoten area belongs to the local non-migrating stock, the coastal cod. Both stocks can be differentiated from each other by the otolith structure, growth differences, age at first maturity, and vertebral count (Rollefsen 1930, Jones 1968).

1.2.2 Sex ratio and spawning behaviour of NEAC

Males become mature in average one year earlier than females, and they tend also to reach the spawning grounds earlier (Ajiad et al. 1999). However, the sex ratio in immature fish is close to 1:1. The dominance of females in older age groups might be caused by a higher spawning mortality in males or by a combination of getting earlier mature and suffering higher exploitation rates (Jakobsen & Ajiad 1999).

Cod are highly fecund, iteroparous, i.e. they spawn over many successive seasons, and are multiple batch spawners. One single female can release from several to ten million eggs, up to nineteen batches in one single season. The eggs are pelagic with sizes ranging from 1.2 to 1.8 mm (Kjesbu et al. 1992, Chambers & Leggett 1996). Furthermore, they are determinate spawners, i.e. the amount of eggs spawned is equivalent to the number of vitellogenic oocytes present before spawning (West

1970). There is no evidence of new vitellogenesis during spawning although some of the oocytes may become atretic (Kjesbu et al. 1991).

Although some authors called them promiscuous (Kjesbu 1989, Morgan & Trippel 1996, Morgan et al. 1999), cod are actually considered lekking spawners (Nordeide & Folstad 2000). A lek is a special kind of polygynious mating system, where aggregated males display and females attend primarily for the purpose of fertilization (Höglund & Atalo 1995). Additionally, this mating behaviour seems to be important to avoid the interbreeding between the migratory Northeast Arctic cod stock and the non-migratory Coastal cod stock (Nordeide & Folstad 2000).

Spawning cod show an aggregated distribution near the sea floor of the spawning grounds. Mature males aggregate at the spawning grounds while the females are distributed peripherally or adjacent to the male concentration. When ready to spawn, which occurs at intervals from two to six days, the female enters in the aggregation. Pairs of male and female cod reveal extensive courtship behavior, in which the females do perform mate selection. Thus, males exhibit intense flaunting displays, in which the courting male swims alongside and in front of the female, making many circles and sharp turns with the back fins fully erected. During the courtship, the male emits grunting sounds in a frequency that ranges from 80 to 400 Hz (Hawkins & Rasmussen 1978). The sounds are produced by the drumming muscles that surround the swimbladder wall (Hawkins 1986), and are believed to excite the female (Brawn 1961b). Subsequently, male and female swim together (Fig.5) performing dorsal, lateral, and ventral mounts (Engen & Folstad 1999) before the gametes are released in the water.

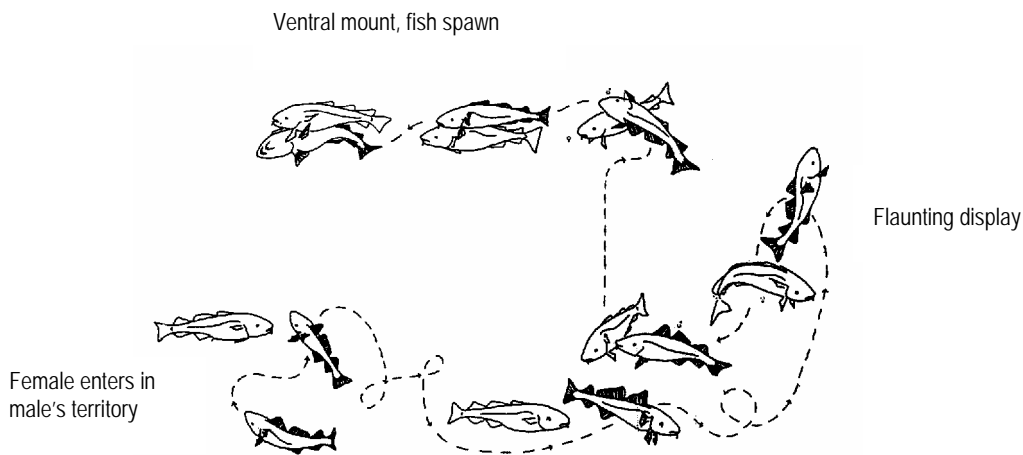


Figure 5. The courtship behaviour of cod. Sequence shows the subsequent moments when the female enters the male territory, the male flaunting display to ventral mount and spawning. (From Brawn 1961).

1.2.3 The offspring of NEAC

After fertilization, the eggs just float in the seawater, distributed along the first 100m-water column (Rollesen 1930, Jones 1968). They are carried by the currents and, at a temperature of 5° C, they will hatch in about two weeks. The larvae swim in the middle water for the first three to five months. As a juvenile, cod start migrating through the water column, crossing the thermocline and, going down to the bottom and up again, many times until they finally settle to near bottom life (Jones 1968, Hüsey et al. 2003). In the Norwegian Rinne, pre-settled cod migrate down to 200 and 400m in the water column (Corlet 1958). Cod larvae and early juveniles feed mainly on *Calanus finmarchicus* of different stages (Gjøsæter et al. 1996).

Individuals are transported by the currents to more than 600 miles away from the spawning grounds, before they settle. Young cod are mostly stationary during that period, until they get big enough to start feeding on small pelagic fish, like capelin (Sundby et al. 1989). The immature cod of three and four years, move around in the Barents Sea. The Norwegians know them as "Loddetorsk", when they migrate to the Norwegian coast after capelin, which spawn in March and April. Those immature cod form the basis for the early Finmark fishery, in the summer they are dispersed, feeding on herring and capelin over the Barents Sea.

1.3 Factors that may influence stock management

Stock-recruitment relationships are normally used for assessment of many fish stocks as a biological reference point to conduct medium to long-term forecasts. Many models were produced (Ricker 1954, Beverton & Holt 1957, Shepherd 1982) attempting to relate the number of recruits to the size of their spawning stock. Instinctively, one would expect a reasonable correlation. However, this correlation is rather poor, suggesting that recruitment is related also to other factors than only spawning stock biomass (SSB) (King 1995, Chambers & Trippel 1997, Jennings et al. 2001). Many authors argued that the time lag between spawning and recruitment is very long and offspring survival might be influenced by many factors. One of the crucial goals in fisheries biology is to identify and understand the dynamic of those factors in order to improve predictions in fisheries management.

Recruitment in marine fishes is characterized by high fecundity (Mertz & Myers 1995), followed by subsequently high mortality during early life stages (Bailey & Houde 1989). Variability in growth and mortality are frequently appointed as the cause for fluctuations in recruitment (Houde 1989, Pepin & Myers 1991). Hence, such variability is often assumed to be caused by environmental conditions as temperature (Jobling 1988, Bailey & Houde 1989, Blaxter 1992), spawning time and oceanographic conditions (Sinclair 1988), food availability (Hjort 1914, Cushing 1975, Lasker 1981, Mertz & Myers 1994), density-dependent effects (Folkvord 1991, Otterå & Folkvord 1993, Bogstad & Mehl 1997, Godø & Haug 1999). Moreover, climatic fluctuations in general speaking may play an important role (Stenseth et al. 2002).

The question that might arise is whether mortality is random or not. In other words, do all the individuals have the same chance of being removed from the population, or may some traits reduce their relative risk to mortality? Studies have shown that mortality in early life stages is not random. Actually, it is currently assumed that

mortality is inversely proportional to body size (Miller et al. 1988), which is known as “bigger is better hypothesis” (Houde 1987). Additionally, survival of a cohort is directly related to growth rates during the pre-recruitment period (Anderson 1988), since larger individuals enhance their abilities to feed and to avoid predators (Rice et al. 1993).

One of the consequences of overexploitation is the reduction of mean size and age within the stock, by removing the larger individuals. The Northeast Arctic cod stock is showing the same tendencies as the Newfoundland cod stocks did, just before they collapsed. The studies conducted over many years indicate that within a stock, large individuals have different reproductive characteristics than small ones (Hislop 1988, Solemdal et al. 1995, Chambers & Leggett 1996). Although many evidences indicate that “overfishing” changes the demographic structure of the surviving population, and as a consequence alters important aspects of reproduction (Trippel et al. 1997, Marteinsdottir & Thorarinsson 1998), the present fisheries management, considers fishes of different ages to contribute equally to recruitment. The qualitative differences among offspring produced by a spawning stock have been a relatively neglected field in fisheries science, and have been seldom included in forecasting recruitment models (Jakobsen & Ajiad 1999).

Although the literature on maternal effects is abundant and diverse, maternal effects in fishes have received little attention, except as confounding factors in quantitative genetics experiments. However, due to the commercial interest of certain fish species, considerable research has provided evidence for large and ubiquitous maternal effects in fishes (Heath & Blouw 1998). A brief review of the historical background of maternal effects in fishes is giving by Solemdal (1997).

1.4 What is maternal effect?

Some authors divide maternal effects in “genotypic” and “environmental”, where “maternal genotypic effects” represent genes expressed in the mother with phenotypic consequences in the offspring, while “maternal environmental effects” represent environmental factors experienced by the mother that will influence the phenotype of the offspring (Reznick 1982). However, a more concise and general definition of maternal effect is “that effect which occurs when a mother’s phenotype directly affects the phenotype of her offspring” (Arnold 1994). In other words, the maternal effect is part of the offspring that does not result from the action of its own genes. Those contributions to the offspring may happen in general, through cytoplasmic inheritance (Bernardo 1996).

The maternal effects most known are on egg and offspring size. Large females produce larger eggs (Sargent et al. 1987, Solemdal 1997). Age and size of the mother are important maternal effects, because they are not attributable to genetic variations of the mother, but will affect the offspring that the female produces, once egg size increases from one spawning season to the next. For example, when reproductive performance among two and three years old rainbow trout females was compared, the older females produced significantly larger eggs (Gall 1974, Springate & Bromage 1985). Larger eggs produce larger hatchlings (Reznick 1991, Roff 1992, Chambers & Leggett 1996). The offspring from larger eggs were able to survive for

longer periods under starving conditions (Bagenal 1969, Blaxter & Hempel 1963, Marsh 1986). Magnusson (1977) found out that fry from larger eggs are more aggressive and competitive. Larger fry are able to utilize a wider range of particle sizes (Blaxter & Hempel 1963, pers. obs.) Hatchlings from larger eggs have better swimming performance and are less susceptible to predation (Palumbi 2004).

Moreover, the mechanism that allows phenotypic response of the offspring to an environmental cue like food availability, salinity and temperature conditions perceived by the mother during egg formation is also considered a maternal effect. For example, Reznick et al. (1996) found that in *Poecilia reticulata* (Guppies), females in bad feeding conditions produced few and larger eggs.

1.5 The MACOM Project

The main objective of MACOM “Demonstration of maternal effects of Atlantic cod: combining the use of unique mesocosm and novel molecular techniques”, a EU-funding project (QLK5-OT1999-01617), was to examine the viability of offspring from first-time female spawners compared to offspring from repeat female spawners of Atlantic cod. MACOM was joint by research institutes of four European nations, sharing tasks according to their specific know-how, facilities and expertise: Institute for Marine Research, IMR, Norway (as a EU-associate), Danish Fisheries Research Institute, Denmark, University of Hull, England, and Institute for Marine Sciences in Kiel, Germany.

The idea was to create an empirical set-up in which offspring from experienced (repeat spawners) and inexperienced (first or recruit spawners) mothers would grow experiencing simultaneously the same environment. Large mesocosms offered the perfect site for this experiment, because they present similar conditions to those found in nature (Moksness 1982, Pedersen et al. 1989, van der Meer & Næs 1993), with the advantage that the monitoring of the experimental system is easier (Folkvord et al. 1996). Offspring's parental identity was assessed by DNA microsatellites markers. The major viabilities measured were growth (length, weight and otolith microstructure), condition (RNA/DNA ratio for larvae, Fulton condition, liver index), and relative survival between groups and families. These measurements were performed on larval and juvenile stages until the individuals have reached the sexual maturity (two years after hatching). In addition, fecundity and egg quality of each female was investigated. Being employed by the IMR in Norway and as a PhD student at the Leibniz Institute for Marine Science in Kiel, the author has actively worked in all activities described by the present study, with exception of following procedures:

- a) Collecting fish and monitoring of broodstock, done by the Norwegian staff in Parisvatnet under the coordination of O. Kjesbu.
- b) Analyses of egg quality, coordinated by H. Paulsen (DIFRES, Denmark).
- c) Larval biochemical condition factors, coordinated by C. Clemmesen (IfM, Kiel).
- d) DNA-fingerprinting from parental and offspring genotypes, performed by W. F. Hutchinson and R. Case (University of Hull, UK).

- e) Oocyte size and Potential Fecundity, performed by A. Thorsen (IMR, Norway).

The methodology and data from the above listed procedures was kindly provided by those researchers. In addition, the data on otolith microstructure analysis of larvae and juveniles was produced and delivered by the author, with exception of otoliths from week 10, which the Danish partner was responsible for.

Otoliths are calcified structures in the inner ear of teleost fishes. The inner ear of fishes (fig. 6a) is characterized by having three semicircular canals and three otolithic organs, saccule, utricle and lagena. Inside of each otolithic organ, there is an otolith rounded by endolymph, close to the macula. The macula is an epithelial tissue, rich in clusters of different bundles of sensory hair cells, which are oriented in different directions to allow fish to perceive frequency, amplitude, and direction of sound, as well as static and dynamic position (Popper & Lu 2000). To function as an acoustic detector in aquatic environments, the otolith has to be denser than the fish body (Fay & Popper 1974), and the density increases with age and therefore, otoliths constantly grow. Although otoliths have a biological function in fishes, they are now the preferred tool for fishery studies, since they can provide valuable insights into age determination, growth estimates, mortality, migratory and environmental history, competition, abundance, condition and taxonomy among others (Campana & Neilson 1985, Jones 1986, Ré & Gonçalves 1993).

Otoliths appear very early in fish life. In cod, sagitta and lapillus can be seen on the sixth day after fertilization (M'Intosh & Price 1890). The embryonic otolith matrix is dominated by a non-collagenous protein known as "otolin", while some calcium must come from the yolk. After hatching, the inorganic elements that build the otolith come from the water. They enter into the blood plasma via gills or intestine, then into the endolymph, and finally into the crystallizing otolith.

The otolith growth is very dependent on the endolymph's chemical composition (Takagi 2002). However, factors influencing endolymph composition remain poorly understood (Campana 1999). Protein in the soluble matrix appears to play important roles for supporting and controlling CaCO_3 crystallization. The most common crystall form is aragonite, but also vaterite and calcite may occur in some otoliths. In larvae and juvenile fishes, the diurnal, nocturnal, and small-scale variations in the endolymph chemical composition, promote the deposition of alternated concentric zones (increments), with higher or lower organic content, respectively. The variations in the optical properties of those zones provide the basis for the otolith microstructure analysis.

The cyclic variations in endolymph composition influence the otolith accretion rate, producing different growth rates. Although the overall formation rate is under metabolic control, environmental influences may play an important role (Campana & Neilson 1985, Mosegaard & Morales-Nin 2000, Otterlei et al. 2002, Hüsey et al. 2003).

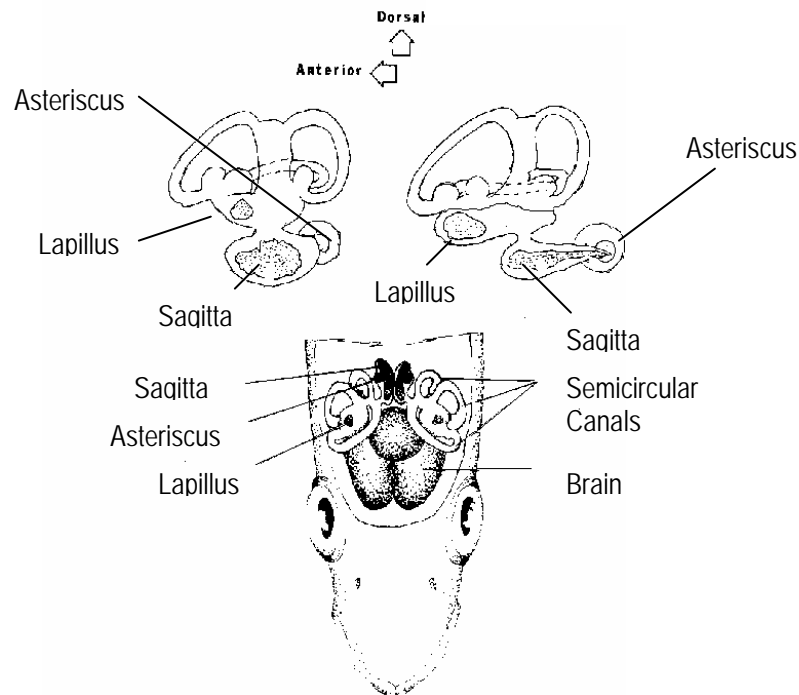


Figure 6. Inner ear of a teleost fish with respective otoliths within the labyrinth systems (after Secor et al. 1991).

The short background given in the previous pages on Atlantic cod is supposed to situate the reader in the actual context of cod fisheries, stressing the importance of cod fishery as source of white meat worldwide. Additionally, the characteristics of the Northeast Arctic cod stock and its biology will help the reader to understand the experimental set-up followed in this study. Since the overall aim was to analyze the influence of maternal effects on growth and survival of cod offspring, a short foreword was giving about maternal effects. Finally, the main objectives of this study were:

- a) To examine the viability of offspring from first time female spawners comparing to those from repeat spawner females of Atlantic cod.
- b) To examine variations in viability between offspring from individual spawning pairs of cod.
- c) To evaluate the possible effects of these experimental findings on cod recruitment and the implications for the fishery management.

The null hypotheses were:

H_{0A} : *There is no difference in viability between offspring concerning female spawning experience (first and repeat spawners).*

H_{0B} : *There is no difference in variability between offspring concerning female size (large and small females) of the spawning pairs.*

H_{0C} : *Maternal size does not influence offspring size, growth or survival in mesocosm reared cod.*

H_{0D} : *Maternal effect does not persist beyond the juvenile stage.*

2. Materials and Methods

2.1 Parental Stock

2.1.1 Collecting Fish

Northeast Arctic cod was caught by bottom trawling in August of 1998 in the Barents Sea around the Bear Island (lat. 74°30'10"N and long. 19°00'05'E) at 200m depth. In spite of all efforts of trawling and pulling the net very slowly to avoid the danger of bursting swimbladders, 80% of the fish caught died right after the capture (Jan Pedersen, IMR- Bergen, pers. Comm.). About 200 fish were transferred into cages in Parisvatnet, a Station in the Western coast of Norway, located at 60 km from Bergen. The sexual maturity was determined by biopsy (Kjesbu 1991) during the spawning season in January 1999. Immature fish that did not spawn during that season were classified as recruit spawners while fish that had spawned were classified as repeat spawners, respectively in the following year of 2000. Fish selected for this experiment were individually tagged with passive integrate transponder (PIT) tags, and fin-clips from the caudal fin were collected for posterior genotyping. Cod were fed on dry-pellets during the captivity, and the temperature ranged from 5°C to 16°C in winter and summertime, respectively.

2.1.2 Breeding

Ten circular tanks with a diameter of 3m were individually divided in three equal parts with a volume of 2.3 m³, respectively. Subsequently, some months before the spawning season started, 15 recruit and 15 repeat female spawners were selected as broodstock and placed in each one of the compartments. In addition, one male of unknown spawning experience was randomly selected and placed to a female in each compartment, establishing a total of 30 spawning pairs (fig.7). Tanks were labelled from 1 to 10 and compartments were designated by a, b and c. Each couple was named after its tank number and compartment, e.g. 1a, 1b, 1c, 2a, 2b and so on, until 10c, and therefore will be later on always referred as family 1a, 1b and so on. Length and weight were determined in January 1999, January 2000 and April 2000. From the measurements recorded in January 2000, Fulton's condition Index was calculated (*see formula in section 2.3.2*). Specific Growth Rate (SGR) was determined for the females based on the measurements done in January 1999 and 2000, using the following formula:

$$SGR = [LN(W_2) - LN(W_1)] \times (t_2 - t_1)^{-1} \times 100$$

Where $\text{LN}(W_2)$ = natural logarithm of final weight, $\text{LN}(W_1)$ = natural logarithm of initial weight, and $t_2 - t_1$, the time variation, i.e. one year.

Fish were not fed during the spawning period, as cod do not feed during that time (Kjesbu et al. 1996). Each pair was supposed to spawn inside of its own compartment. Natural spawning has proven to produce better quality eggs and a higher percentage of fertilization than the currently employed stripping method (Jon Kåre Stordal, IMR- Bergen, pers.comm).



Figure 7. External tanks used for spawning during MACOM at the facilities of Parisvatnet. On the right, detailed view of a compartment with a spawning couple inside (photos by V. Buehler).

2.1.3 Incubation

From February 8 to April 23, 2000, the tanks were inspected daily for egg batches. Eggs were collected from the surface by using a catcher, because fertilized eggs float (fig.8a). Afterwards, batch volume was estimated by using a measuring cylinder. Subsequently, the eggs were transferred to 180 liters conical tanks inside of a hatchery, where single family batches were incubated per tank. The incubators (fig.8b) were supplied with filtered sea water ($20\mu\text{m}$) pumped from a location at 20m depth in the sea, outside the station. Temperature was not controlled by a thermostat, and oscillated according to the ambient sea temperature (at 20m).

A total of 62 egg batches from the experimental spawning pairs were incubated between March 10 and 14. The temperature during the incubation was 5°C ($\pm 1.5^\circ\text{C}$). To compensate the lag of four days between the first and last batch incubation, the hatching date of some batches had to be delayed. That was achieved by placing ice bags in some of the incubators, reducing the temperature by 1°C (Håkon Otterå, pers.comm.). At the end, newly hatched larvae were obtained from 26 of the 30 experimental pairs.



Figure 8. a) Jon Kåre (IMR Bergen) collecting eggs from the compartments. b) the incubators at the facilities of Parisvatnet (photos by V. Buehler).

2.1.4 Egg quality measurements:

Egg quality tests were performed based on numerical, morphological and biochemical parameters (E.g. Barnung & Grahl-Nielsen 1987, Fyhn et al. 1987, Fraser et al. 1988, Ulvund & Grahl-Nielsen 1988, Vallin & Nissling 2000, Riis-Vestergaard 2002). The egg size from the different batches was measured to the nearest 0.01 mm by an ocular ruler under a stereomicroscope. Additionally, the amount of dead eggs, the percentage of fertilized eggs, cleavage patterns and stage of development were recorded. To perform egg analysis, individual samples were taken from the incubators. The water was removed from each sample by filtering the content through a 0.5mm mesh screen. 10 ml eggs were frozen at -18°C for egg wet / dry weight relation, and energy content determination. 1 ml egg samples were frozen in liquid nitrogen for free amino acid (FAA), and lipid content analysis. Video images were taken of all egg batches for later control of egg size, appearance, etc. Egg dry weight was measured after drying 10 g wet weight egg at 58°C for 24h. After the egg samples were dried, the energy content was estimated by using a bomb-calorimeter (IKA type 7000C). Free Amino Acid content was measured using a standard Ninhydrine assay, based on samples containing 1g WW and recalculated to single egg content. Fatty Acids were measured as percentual distribution of fatty acid methylesters (FAME) using a gas chromatographic method, after Bligh & Dyer (1959). The following groups of lipids were measured: Wax esters (WE), triglycerids (TAG), free fatty acids (FFA), cholesterol (CHL), and phospholipids (PC).

2.2 Mesocosm Experiment

2.2.1 Characteristics of the mesocosms

The mesocosm experiment was performed at the facilities of Flødevigen Marine Station (fig.9). The Station is situated on the south coast of Norway in Skagerrak (58°25'585"N and 08°45'280"E), and possesses two large basins – 2500m³ and 4400m³, also called small and large mesocosm, respectively. The small one has a surface area of 600m² while the large one has a surface area of 1700m². Both mesocosms have an uneven bottom topography, and an average depth of 4.5 meters. They were characterized by being an environment free of predators, in which a natural community of phyto- and zooplankton developed. That was achieved by emptying the basins in late summer and refilling them again in December 1999, with seawater pumped from a location at 75m depth in the nearby fjord, 16 weeks before the experiment has started. The organisms developed their natural cycles inside of the mesocosm, building an ecotope that could be considered as very close to the conditions found in the sea. Both environments had a constant turnover; the water-exchange rate was 109 l/min in the small mesocosm and 38.4 l/min in the large mesocosm. Thus, the small mesocosm was characterized by a higher water exchange.



Figure 9. Air photograph of Flødevigen Marine Station in southern Norway showing the mesocosms location (photo from Flødevigen archive).

2.2.2 Monitoring of biotic and abiotic parameters

The mesocosms were continuously monitored from March 5 until the end of the experiment on June 5. All measurements have taken place around 10 am. Following parameters were measured for the successive 0-0.5-1-2-3-4m depths: Temperature was measured daily, salinity was weekly determined by the standard method of aerometer (Knutson 1901), and oxygen content was verified weekly by an oximeter model Oxi 330 WTW.

Chlorophyll-a and phaeo-pigments were determined weekly by spectrophotometric method (Lorenzen 1967). For this purpose, a single water sample was collected from through the entire water column by filling a hose vertically from the surface to the

bottom. Right after, sample was placed in a dark bottle and transferred to the lab. In the lab, 100 ml were immediately filtered, in subdued light, through a Whatman GF/F filter under suction pressure of 0.5 atm. The filters were placed in 15ml centrifuge tubes and stored at -20°C for later analysis. Later, 2-3 ml of 90% acetone were added to the tubes, and chlorophyll was extracted in total darkness for 60 minutes at room temperature. Samples were centrifuged at 4000 rpm for 10 minutes and measured by a spectrophotometer (Shimadzu UV-1201) at 750, 663-665 (at the peak), 647 and 630 nm. Subsequently, for the measurement of the phaeo-pigments, the extract was acidified with 1 M HCl (0.06ml to 5ml of extract). The absorbance was measured at 750 and 663-665 nm, 3 minutes after acidification. The calculations for estimation of chlorophyll-a content followed the equations of Jeffrey & Humphrey (1975).

The species composition of phytoplankton was recorded weekly by a 10µm net, which was towed by oscillatory movements in the whole water column for about 10 minutes. The samples were analyzed according to the 100 cells-method (Mateuci & Colma 1982).

Estimation of zooplankton density was done twice a week by pumping 80 litres of water from each depth given above, filtered through a 80 µm plankton net at 2.67 liter/s with an electric water pump MEZ IMB35 4AP-80-2, 60Hz, 1.8HP. The samples were preserved in formaldehyde 5%. Species composition, and density per liter were determined with help of a stereomicroscope (Wild), with measuring ocular and counting chamber.

2.2.3 Transport and arrival of the larvae in Flødevigen

Immediately after hatching, the larvae were placed in 20 l plastic bags, filled with seawater, put into individual styrofoam boxes, and sent by airplane from Bergen to Flødevigen. This procedure took about six hours. Distinct batches of yolk-sac larvae from the many families arrived successively on March 28, 29 and 30.

Right after the larvae arrived in Flødevigen, the boxes were opened, and temperature and oxygen content were recorded. Subsequently, sub-samples of larvae were taken to check malformation. Twenty larvae from each family were individually placed in 1.5 ml Eppendorf-vials filled with seawater and frozen at -70°C, for posterior biochemical analysis, while another group of thirty larvae per family was preserved in ethanol 96% for otolith studies. The ethanol was changed after 24h to avoid otolith damage (Butler 1992).

To test mortality rates over time, about 150 larvae from each family were kept in 200ml cups, at 4°C, as control (starving) group. The the seawater was changed on a daily basis, and the number of dead larvae per family was recorded until the mortality had reached 50%. Due to the small number of larvae, it was not possible to keep a starving group for families 2c, 3c,4b, 4c, 5a and 10c.

2.2.4 Releasing of larvae in the mesocosms

A total of 4000 larvae per family/batch were counted, placed in buckets with seawater and released at different places of the 2500m³ mesocosm, while another 8000 larvae from the same family/batch were released into the 4400 m³ mesocosm. Some families had a lower number of larvae than expected, and in this case, priority was given to release them in the small basin, with the aim to obtain a better proportion among families, since a lower number of larvae was required for the small mesocosm. The first batch from the family 9C received on March 30 showed too many mal-formations and almost 50% were still eggs. Therefore, those larvae have not been released. Another sample from the same family was released on April 1. Additionally, 200 larvae from family 2C were released in the small mesocosm without laboratory sample. In total, 82,285 larvae were released in the small mesocosm and 134,314 were released in the large mesocosm (see table 1).

Table 1. Number of larvae from the different families released in the mesocosms: Family, Group (concerning spawning experience), volume of eggs incubated, incubation date, hatching date, releasing date, fertilization rate, percent of larvae mal-formation, amount of larvae released in the 2500m³ mesocosm, amount of larvae released in the 4400m³ mesocosm and total of larvae released.

Family	Group	volume [ml]	Incubation date	Hatching date	released	fertiliz. rate %	mal-formation%	2500m ³	4400m ³	total
1b	repeat	780	12/3	28/3	29/3	95	3	4000	8000	12000
1c	recruit	340	13/3	28/3	29/3	96	2	4000	8000	12000
2a	repeat	1000	10/3	28/3	28/3	97	10	4000	2800	6800
2c	recruit	30	13/3	29/3	29/3			239	0	239
3a	recruit	700	12/3	28/3	29/3	85	4	4000	8000	12000
3b	recruit	360	11/3	27/3	28/3	96	10	4000	8000	12000
3c	repeat	380	10/3	27/3	28/3	92	43	700	0	700
4a	recruit	790	12/3	28/3	29/3	100	1	4000	8000	12000
4b	recruit	360	13/3	29/3	29/3	98	8	475	0	475
4c	recruit	60	15/3	30/3	30/3		11	2314	0	2314
5c	recruit	970	11/3	28/3	29/3	98	4	5975	8000	13975
6a	repeat	1100	12/3	27/3	28/3	92	7	4000	8000	12000
6b	repeat	1080	11/3	28/3	29/3	95	4	4000	7550	11550
6c	recruit	1100	11/3	28/3	29/3	93	2	4000	8000	12000
7a	recruit	220	10/3	27/3	28/3	89	8	4000	8000	12000
7b	repeat	700	11/3	28/3	28/3	96	7	4000	8000	12000
7c	recruit	280	10/3	27/3	28/3	94	6	4000	8000	12000
8a	repeat	1520	13/3	28/3	29/3	83	11	1900	0	1900
8b	recruit	130	11/3	28/3	29/3	80	1	4000	764	4764
8c	repeat	400	13/3	28/3	29/3	86	3	4000	7200	11200
9b	repeat	360	11/3	28/3	29/3	99	7	4000	8000	12000
9c	repeat	2100	14/3	30/3	1/4	99	45	4000	8000	12000
10a	recruit	120	10/3	28/3	28/3	99	8	2100	4000	6100
10b	repeat	120	14/3	30/3	30/3		2	4000	8000	12000
10c	repeat	400	11/3	28/3	29/3	49	15	582	0	582
						Total:		82285	134314	216599

2.2.5 Sampling of cod larvae in the mesocosms

Sampling started on April 3, 2000, and was carried out within the two mesocosms, twice a week, always at night. A two-chambered net with a 0.3m² opening and initially with a mesh of 180 µm, was manually towed at constant speed of 1m/s along the longest track of the basins, covering a distance of 35m and 50m in the 2500m³ and 4400m³ mesocosms, respectively. In order to supply the statistical needs for reasonable conclusions 600 larvae were caught per sampling on weeks 1, 3, 4 and 5. Additionally, one haul a week was performed for mortality estimations. After each haul, the net was rinsed with sea water and the larvae collected in net cups were transferred to buckets, also filled with sea water. In the laboratory, larvae still alive were individually placed in 1.5ml Eppendorf-vials filled with seawater, and immediately frozen at -70°C for biochemical analysis. The total number per cup and per haul was recorded and the larvae that were not used for biochemical analysis, were placed in ethanol 96%.

On April 19, when the larvae had an average length of 8mm, the mesh of the net was increased to 350 µm. Even though, after the fifth week, the catch-ability decreased substantially. The dark interval was very short and the fish started to reach the post-metamorphosis stage, resulting in a large increase of the net avoidance. On May 16, the mesocosm sampling was suspended.

2.2.6 Termination of the Mesocosm Experiment

The last measurements and collections of phyto- and zooplankton were done on June 5 and right after that basins were drained. On June 8, the remaining fish of the 2500m³ mesocosm were carefully captured by small catchers, placed in buckets, and transferred to indoor tanks. 1200 individuals were randomly sampled and individually frozen at -70°C for biochemical analysis and genetic fingerprinting. About 150 juveniles from each mesocosm were immediately standard length (SL) measured and weighted for Fulton's Index calculations. Note that SL, for all fish measurements, was considered to be the distance between the tip of the snout to end of the notochord. Subsequently, the fish were counted, size graded and divided in tanks. On the next day, the same procedure was followed in the 4400m³ mesocosm. At the end, a total 2,927 fish from the 2500m³ and 11,400 fish from the 4400m³ mesocosm were recovered.

2.2.7 Mortality Calculation

The mortality was estimated by the amount of larvae sampled at different days during the mesocosm period. However, to be able to use those records as indication on the number of larvae that were still alive in the basins, several assumptions had to be made:

- Larvae were all along, homogeneously distributed within the mesocosms.
- There was no net avoidance.
- Volume of water filtered during the hauls was invariable.

The amount of larvae present in the mesocosm (Y) in a certain time was calculated by:

$$Y = N \times \frac{M}{d \times n}$$

Where N was the number of larvae caught per sampling date. In case that more than one haul in a particular night was made, the average number of larvae caught in all hauls was used. M was the volume of the mesocosm, i. e. 2500 m³ or 4400 m³, according to the basin. d was the distance covered by the net in the mesocosm during the haul, which is 30.5m and 48.8m, for the small and large mesocosm, respectively. Finally, n was the total opening area from both net chambers, which was equal to 0.3m². The initial number of larvae, plus the calculated number from samplings and the final number obtained by the mesocosm termination, plotted against time, provided the mortality estimation for the entire period. After larvae genotyping for parental assignment, offspring contributions from each family were estimated to each sampling date. This number was corrected by the amount of larvae initially released, by the number of individuals caught in the sample and finally, converted to percentage.

2.3 Rearing of Juveniles in Indoor Tanks

After the mesocosm period, the cod juveniles were placed in indoor tanks of 0.8m³ (1.40 L x 1.40 W x 0.4 H m). Temperature and natural mortality were recorded daily, from June 8, 2000 until the end of the experiment on April 11, 2002, when fish were slaughtered. From June 2000 until June 2001, fish were exposed to a light regime of 24h light. In the first ten days, fish were hand fed in intervals of 30 minutes, 24 hours a day (fish need to learn how to feed on pellets). Subsequently, automatic feeders with a feeding interval of 15 minutes were installed. To avoid diseases, the water temperature was maintained below 10°C and suspicious fish were removed. Vibriosis, caused by the bacteria *Vibrio anguillarum*, is a very frequent disease in intensively reared cod cultures, and it is known to cause clinical mortality in Atlantic cod (Vet. Magne Hansen, pers comm). Therefore, on July 2, 2000 all fish were vaccinated against vibriosis by immersion bath (Alpharma's R&D) and in addition, they received medicinal feed beside the normal pellets.

On August 17, 2000 the fish were transferred to 3.2m³ tanks and were kept there until the end of the experiment. From July 2001 onwards a new light regime was adopted, following the natural day-length in southern Norway. Summer days in Norway are characterized by many hours of daylight, but when Autumn and Winter times take place, the daylight period becomes proportionally shorter, while the dark period increases. The dark period is necessary to stimulate cod maturation, since Melatonin, the major hormone involved in timing reproduction of fish, is mainly produced by pineal photo-receptor cells of teleost fish during the dark hours (Bromage et al. 1995; Mayer et al. 1997; Porter et al. 2000). Although in the wild, NEAC need around 8 years to mature, farmed cod mature when two years old

(Jobling 1988; Svasånd et al. 1996). Since fast growth is related to maturation at early life stages, early maturity in farmed cod might be associated to the optimised growth conditions offered by aquaculture (Thorpe 1994).

2.3.1 Fish tagging and fin clipping

In February 2001, when fish reached sizes of 160-180 mm, 1200 individuals, i.e. 600 fish from each mesocosm, were randomly chosen from the tanks. They were standard length (SL) measured, weighted and subsequently tagged with soft-alpha VI tags (Northwest Marine Technology, Inc. USA).



Figure 10. Small juvenile cod being tagged with soft -alpha tags. On the left: position where the injector's needle is placed. On the right: how it looks like after tagging (photo Ø. Paulsen).

Soft-alpha tags VI have been successfully used in Salmonids (Dr. David Solomon pers. comm.). They consist of a three-digit code labels made of a biocompatible elastomer fluorescent material, which are implanted into suitable transparent tissue with an injector, and become visible by using a UV light torch.

In Salmonids those tags are placed under their thick eyelid. However, this method is not feasible for cod. Therefore, several tests were performed with the aim to develop a methodology for the use of soft-alpha VI tags in codfish. The best area to place the tag was found to be the basis of the pre-operculum (Buehler et al. in prep.). After being tagged (fig.10), a small fin-clip from the caudal fin tissue (0.5cm²) of each individual was cut out and placed in ethanol 96% for DNA finger-printing.

2.3.2 Fish Measurements

From the tagging day onwards, fish were standard length (SL) measured, weighted, and the tags were checked, every second month. Based on this information, growth and Fulton Condition could be estimated.

In 1902, Fulton proposed the use of a mathematical formula that would quantify the condition of fish, assuming that growth is isometric:

$$K = \frac{W}{L^3} \times 100$$

Where: **K** is the coefficient of condition; **W** is the weight of the fish in grams (g) and **L** is the length of the fish in centimeters (cm). The cube of the length was used because growth in weight is proportional to growth in volume. **K** varies from species to species and within species; a **K**= 1 indicates that the fish is in isometric "normal" condition.

2.3.3 Termination of the tanks Experiment

Fish were slaughtered in the beginning of April, 2002. They were standard length (SL) measured and weighted. Afterwards, sex, maturity stage, liver weight and gonads weight were recorded. Furthermore, the otoliths *sagittae* were removed and placed in Eppendorf-vials for later analysis. All the ovaries were weighted to the nearest gram and preserved in 3.6% buffered formaldehyde (Thorsen & Kjesbu 2001). The gonadosomatic Index (GSI) was calculated as the ratio of the gonad weight to the fishes' total weight.

$$GSI = \frac{GW}{BW} \times 100$$

Where **GW** is gonad weight in grams (g) and **BW** is body weight in grams (g).

Subsequently, the liver was removed and weighted for calculation of the hepatosomatic index. The hepatosomatic index (HSI) is the proportion of liver weight to total body weight.

$$HSI = \frac{LW}{BW} \times 100$$

Where **LW** is the wet weight from the liver and **BW** the body weight.

2.3.3.1 Estimation of oocyte size and Potential Fecundity

After a storage of at least 14 days in buffered formaldehyde, sub-samples of ovary tissue were weighted, and the oocytes within the cortical alveolus or in vitellogenic stage were counted (Kjesbu & Holm 1994). Fecundity was calculated based on the sphere-volume method (Kjesbu & Holm 1994; Svåsand et al. 1996, Thorsen & Kjesbu

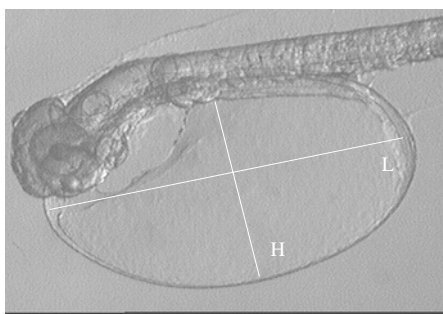
2001). The vitellogenic oocyte specific gravity was set to 1.046, the weight fraction of connective tissue and immature oocytes was set to 5% and the vitellogenic oocyte swelling in buffered formaldehyde was set by the linear formula:

$$\text{Fresh diameter } (\mu\text{m}) = 19 + 0.947 \times \text{preserved diameter}$$

Each ovary sub-sample was placed in a petri-dish with buffered formaldehyde and one drop of detergent. Subsequently, the oocytes measurements were performed using a freeware program NIH-image v1.62 on a Power McIntosh 7600/120 connected to a video camera JVC TK-1070E on a stereomicroscope Olympus SZX12 with a SZX-ILLB200 light foot at 7x magnitude. A Macros program written for NIH-image automated the measured data into an Excel table. The measurements of oocytes area, and perimeter were performed in density slice mode using the particle analysis function in NIH- image (Thorsen & Kjesbu 2001). About 150 particles were measured. About 20-30% of the records were discarded by a Macro program written for Excel. Subsequently, the oocyte diameter (defined as the average of ellipse major and minor axis) was calculated for each oocyte. Based on those measurements a mean oocyte diameter, and the standard deviation (SD) were calculated for each sample.

2.4 Laboratory Examinations

The standard length (SL) of newly-hatched larvae preserved in ethanol was recorded and the yolk-sac was measured in length and height (Fig. 11a). To calculate the yolk-sac volume, the ellipsoid volume formula was used (Fig. 11b). The radius of the yolk-sac width was assumed to be the same as the radius of the height, and therefore $r^3 = (H/2 * L/2 * W/2)$.



a)

$$YS = \frac{4}{3} \pi \times r^3$$

b)

Figure 11a) Yolk-sac measurements done on newly-hatched larvae (larva from family 7c, photo by V. Buehler) and b) formula applied to calculate yolk-sac volume.

After that, both pair newly-hatched larvae otoliths were mounted on glass slides and read, according to the method described in 2.4.1.1 and 2.4.1.2.

In Kiel, frozen samples were thawed in groups of 30 –50 larvae. The standard length (SL) was measured, and the gut content was investigated for presence or absence of food particles. After that, larvae were placed in Eppendorf-vials and freeze-dried, overnight, with a freeze dryer Christ, alpha 1-4. Dry weight of larvae was taken using an electronic microbalance Sartorius SC-2 accurate to 0.1 µg. For individuals older than 5 weeks, the freeze-drying process took two or three days; and individuals were weighted with a balance Sartorius R- 660 D accurate to 0.001 mg. Subsequently, RNA/DNA ratio analysis was performed. From a sub-sample of 200 larvae of the 600 larvae sampled in week 3, 4, 5 and 10, per each mesocosm, both pair of otoliths (*sagittae* and *lapilli*) were extracted before the biochemical analysis was completed.

2.4.1 Otolith microstructure analysis:

2.4.1.1 Otolith Preparation

After the larval dry weight was recorded, the larvae from the sub-samples in which the otolith microstructure and RNA/DNA ratio analysis would be combined, were replaced in the Eppendorf-vials and kept on ice in groups of 10-30 individuals. When otolith microstructure and RNA/DNA ratio analysis needed to be combined for the same larva, the otolith extraction had to be done very quickly to avoid RNA losses caused by RNase activity. Therefore, glass slides were previously prepared: two small circles were drawn on the inferior side of the glass slides, to help the otolith localization for later reading. A small label with the fish code was placed in one of the slide corners. The larva was positioned with its head in the middle of the two circles, and a drop of water was added, to re-hydrate the tissue. After that, both otoliths were localized inside the *capsula otica*, using a stereomicroscope Wild (50x magnification), with polarized light filter. The *capsula otica* was carefully opened with help of dissection needles, avoiding tissue losses, as the entire larva was required as homogenate for the RNA/DNA analyses. The otolith pairs (*sagitta* and *lapillus*) from each head side were gently removed and glided into the right and left circles. Subsequently, the larva was replaced in an Eppendorf-vial to rest on ice until a sufficient number of samples (about 30 individuals) were available to perform the biochemical analysis. After the dissection, if there were still some organic residues, the otoliths were rinsed with EDTA 0.01% (Dr. Audrey Geffen, pers.comm.) and left for ten minutes drying at room temperature. Subsequently, the otoliths were fixed with the convex side up on the slide with common colourless nail polish. As for the 10 weeks old fish, only part of the dorsal muscle is needed to perform the biochemical analysis, fish heads could be preserved in ethanol 96% for posterior otolith extraction. The criteria for selection of the week 10 otoliths was based on parental genotyping and condition (RNA/DNA ratio).

2.4.1.2 Otolith reading

Otolith reading was performed at the Leibniz Institut for Marine Sciences in Kiel, using a digitized computer video system (Leutron) with a CCD camera (XC-77CE) connected to a Leitz Labor Lux S microscope at 1000x magnification by immersion oil. The readings were analysed by an adaptation of the "Oto program" written by Eng. Herwig Heilmann at the IfM Kiel. More than 1800 otoliths (*sagitta* and *lapillus*) from newly- hatched larvae, and week 3, 4 and 5 for each mesocosm were analysed.

In addition to the otolith readings, the otolith sagitta from week 10, juveniles and adults were weighted. Finally, pictures and otolith (*Lapilli*) radius measurements from week 10 fish were kindly provided by Dr. Helge Paulsen (DIFRES- Denmark).

2.4.2 RNA/DNA ratio and glycolytic enzymes

RNA/DNA content was determined based on a fluorimetric method using ethidium bromide as nucleic acid specific dye (Belchier et al. 2004). The RNA/DNA analyses were performed using the whole larvae until week 5, and only a weighted portion from the dorsal muscle for individuals from week 10. The freeze-dried tissue was rehydrated in Tris-SDS-buffer (Tris 0.05M, NaCl 0.01M, EDTA 0.01M, SDS 0.01%) for 10 minutes, in ice-bath. Glass beads (diameter 2mm, 0.17-0.34 mm) were added to the homogenate. Subsequently, the samples were shaken in a shaking-mill Retsch MM2 for 15 minutes. The homogenates were centrifuged for 8 minutes at 6000 rpm at -2°C. The supernatant was divided in two portions. To one 50 µl Ethidium Bromide (EB) was added and the amount of nucleic acids was fluorimetric determined in a microtiter fluorescence reader (360 nm exc. and 590nm emission, wavelengths) at 25°C. In the other aliquot, RNA was enzymatically digested in a water bath at 37°C after adding 50 µl Rnase. After cooling to room temperature, the samples were centrifuged at 2000 rpm for two minutes. DNA fluorescence was measured. The RNA content was determined from the difference between the total fluorescence and DNA fluorescence. The fluorescence measurements for DNA and RNA were directly used to calculate the RNA/DNA ratio of the larvae or tissue. Linear calibrations were obtained using known amounts of DNA (Boehringer DNA) and RNA (Boehringer 16S,23S) for each series of measurements, and these were used to calculate absolute DNA and RNA contents.

Analysis of glycolytic enzymes, lactate dehydrogenase (LDH), and pyruvate kinase (PK) were performed on white muscle samples obtained from the back of cod juveniles (week 10). Assays were performed by using modified conditions of Bücher & Pfeleiderer (1950), Bergmeyer & Bernt (1970), Pelletier et al. (1993, 1994), Saborowski (pers. comm.) and Vetter & Buchholz (1997). The enzyme activity was measured at 25°C using a UV/Vis spectrophotometer. LDH and PK were measured by following the disappearance of NADH at 340nm. Activities were expressed in International Units ($\mu\text{mol substrate transformed to product min}^{-1} \text{g}^{-1}$ tissue wet mass).

2.4.3 DNA Fingerprinting

Microsatellite analysis was used to characterize the broodstock families and identify their offspring. DNA was extracted from fin-clippings taken from the broodstock and their tagged offspring from the tanks experiment, using a standard phenol-chloroform method (Taggart et al. 1992).

Conditions were developed to PCR- amplify the broodstock DNA using three microsatellite loci: (Gmo2, Brooker et al. 1994; Gmo8 and Gmo 19, Miller et al.2000). The amplified segments were analyzed on an automated DNA sequencer (ALFexpress, Pharmacia) for parental assignment of larvae. Simulations (PROBMAX, Danzmann 1997) indicated that the use of these three loci permitted 99.3% of 4500 simulated larvae to correctly assigned parentage.

The inclusion of a fourth locus, Gmo 132 (Brooker et al. 1994) allowed full identification of all simulated combinations. Tissue homogenates obtained from the RNA/DNA analysis from 1- to 10-week-old offspring sampled in the mesocosms could be directly PCR-amplified without any need to clean or concentrate the DNA. As the samples contain natural DNAses that could disintegrate the DNA molecule during transport between Kiel (RNA/DNA analysis) and Hull (microsatellite analysis), the homogenate was first heat-treated (95° C for 10 minutes) to denature those enzymes. The heat-treatment proved to successfully destroy the DNAses and samples treated in this way were stable at room temperature for at least five days.

The samples were homogenised and the treated homogenate diluted between 4 to 16 times, depending on the size of the fish prior to being used for PCR amplification. DNA was recovered from fin tissue using a Phenol/Chloroform method (Taggart *et al.* 1992) for the broodstock (the extracted DNA was diluted around 100x prior to PCR-amplification). According to this method, the ethanol was removed from the tissue, prior to the DNA extraction by squeezing the excess ethanol from the sample and air drying on clean tissue paper. Samples of fin, weighing approximately 0.1g, were placed in 375 µl of extraction buffer (0.1 M Tris, 0.01M EDTA, 0.1M NaCl, 1% SDS, pH8) and 25 µl of 10 mg/ml proteinase K (Boehringer), and incubated for 3 hours at 55 °C with occasional shaking to assist the digestion. Subsequently, 800 µl of phenol/chloroform/isoamylalcohol (24:24:1, pH 8.0) was added, and the mixture shaken vigorously for 30 seconds, before being gently mixed on a rotary mixer for 10 minutes. Centrifugation at 14,000g for 3 minutes, separated the organic phenol/chloroform phase, containing the proteins and cellular debris, from the aqueous phase containing the DNA. The aqueous phase was pipetted into a clean microfuge tube, taking care to avoid the interface of coagulated proteins, and an equal volume of chloroform/isoamylalcohol (24:1) was added. The mixture was again shaken vigorously for 30 seconds, mixed on a rotary mixer for 10 minutes, and then centrifuged at 14,000g for 3 minutes. The aqueous phase was transferred to a fresh tube and the DNA precipitated through the addition of 1 ml of chilled absolute ethanol. Generally, the DNA would normally be seen in the form of white cotton wool like strands, which were pelleted by centrifuging at 12 000 g for 3 minutes. The supernatant was discarded, and a further 1 ml of 70% ethanol was added. The tubes were placed on a rotatory mixer for 10 minutes to wash the DNA, and then centrifuged at 12 000 g for 1 minute. The ethanol was then removed and the tubes placed in an oven at 55 °C for 15 minutes to evaporate off the remaining ethanol. Finally, the DNA was resuspended in 50 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8) and stored at 4 °C.

Since the phenol/chloroform method used to extract DNA from the broodstock samples was fairly labour-intensive, the HOT-SHOT method (Truett et al. 2000) was applied for DNA extraction of the tagged juvenile samples. Based upon the HOT-SHOT method, square pieces of 1mm by 1mm dried ethanol-preserved fin tissue were placed in 75µl of alkaline lysis solution composed of 25mM NaOH and 0.2mM disodium EDTA (pH 12), and heated for 15 minutes at 100 °C. The solution was

subsequently cooled to 4° C and 75µl of neutralizing reagent composed of 40 MM Tris-HCl (pH 5) was added. The solution was centrifuged at 12 000 g for 3 minutes to pellet any remaining tissue debris and then added directly to the PCR reaction without any dilution. Subsequently, a range of primer annealing temperatures, combined with varying MgCl₂ concentrations, were used to PCR-amplify the various types of DNA extractions. Reaction conditions for each locus and each type of DNA were done as outlined in Hutchinson et al. (in prep.). The 15µl reactions were overlaid with a drop of mineral oil (Sigma) to prevent evaporation, and PCR-amplified in an Omnigene thermocycler (Hybaid). Alleles were then sized by running the PCR products on a 6% polyacrylamide gel using an ALFexpress™ automatic sequencer (Amersham Pharmacia Biotech). Comparison with internal markers (van Oppen 1997) facilitated sizing of the products using the accompanying Fragment Manager™ software.

2.5 Statistical Analysis

Statistical analysis was performed with help of Sigma-Plot and Statistika Software, with significance level of 95%. The test applied is always mentioned for the data processed in the results. Additionally, in order to identify the main maternal and environmental parameters influencing offspring growth and survival, a multiple regression analysis, with help of a SAS Model was carried out by Dr. Joachim Gröger (University of Massachusetts Dartmouth, USA).

3. Results

3.1 Description of the broodstock

The characteristics of the broodstock selected for this experiment are given in Table 2. Only data collected in January 2000, two months previous to the commencing of the spawning season are shown. Fish aging and distinction between NEAC and coastal cod were both based on otolith macrostructure analysis (Rollefsen 1930). Age for males 4c, 8a, 8b, 8c, 9b was not determined because some of them died before the end of the experiment, and otoliths were not extracted, while others were used for another experiment. The spawning experience of males was unknown and therefore, the term “first or repeat spawners” refers only to the females.

Table 2. Characteristics of the broodstock utilized in the experiment 2000. The families were named after the compartments in which male and female pairs were placed during the spawning season. Note: First spawners were called “recruit spawners”.

Family	Group	females				males			
		age	Length [cm]	W [g]	Fulton Index	age	Length [cm]	W [g]	Fulton Index
1b	repeat	7	76	6377	1.45	8	78	4945	1.04
1c	recruit	6	74	5422	1.34	7	75	4526	1.07
2a	repeat	7	73	5390	1.39	8	75	4943	1.17
2c	recruit	8	81	7400	1.39	6	82	6281	1.14
3a	recruit	6	80	6920	1.35	8	80	5538	1.08
3b	recruit	6	78	6140	1.29	6	76	3513	0.80
3c	repeat	7	74	5740	1.42	9	78	5390	1.14
4a	recruit	7	83	6768	1.18	7	85	7322	1.19
4b	recruit	5	80	6975	1.36		81	6553	1.23
4c	recruit	8	77	5201	1.14	8	84	6628	1.12
5c	recruit	6	81	6014	1.13	8	79	6237	1.27
6a	repeat	9	85	8850	1.44	6	87	6663	1.01
6b	repeat	7	78	7415	1.56	9	78	6692	1.41
6c	recruit	9	93	8502	1.06	7	91	7774	1.03
7a	recruit	7	71	4688	1.31	9	68	4448	1.41
7b	repeat	9	88	7111	1.04	7	85	7918	1.29
7c	recruit	6	76	6206	1.41		80	5522	1.08
8a	repeat	9	81	6144	1.16		79	5964	1.21
8b	recruit	7	77	5693	1.25		75	5230	1.24
8c	repeat	8	86	7450	1.17		84	7286	1.23
9b	repeat	7	80	6262	1.22		77	6111	1.34
9c	repeat	8	81	6383	1.20	6	80	7002	1.37
10a	recruit	9	85	7265	1.18	8	84	8111	1.37
10b	repeat	7	85	7168	1.17	8	85	8020	1.31
10c	repeat	8	77	3172	0.69	8	76	3233	0.74

In average, first spawners were 6.92 years old (SD= 1.25), with mean length of 79.69 cm (SD=5.47), mean weight of 6399.54 Kg (SD= 1040.12), and mean Fulton Condition Index of 1.26 (SD= 0.11).

Repeat spawners were in average 7.75 years old (SD= 0.87), with mean length of 80.33 cm (SD= 4.91), mean weight of 6455.17 Kg (SD= 1386.79), and mean Fulton Condition Index of 1.24 (SD= 0.23). Therefore, first and repeat spawners were not significantly different in age, size or condition. In the beginning of 1999, repeat spawners were in average 8 cm larger and 0.6 Kg heavier than first spawners. However, they were not significantly different from each other either (ANOVA, $p>0.05$). After one year, the first spawners had grown 16.30 cm, while the repeat spawners increased only 8.92 cm. Since for Gadoids, weight gain is a more accurate parameter for growth estimations, specific growth rate (SGR) was calculated for each female. Estimations were based on weight measurements done in beginning of 1999, and in January 2000, i.e., one year before the onset of spawning season 2000 (Fig.12).

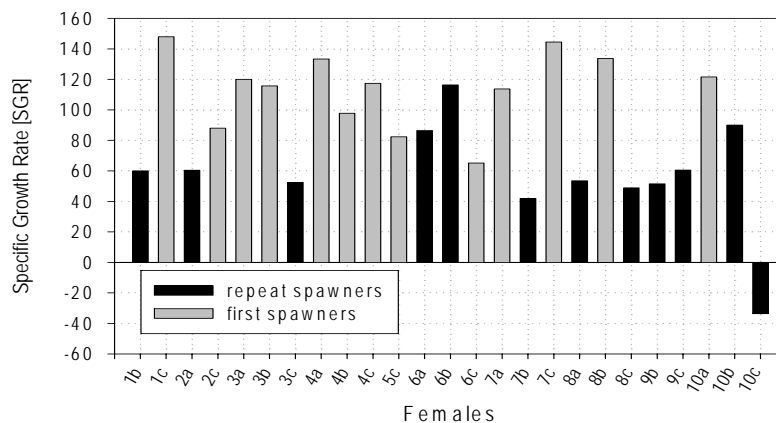


Figure 12. Specific Growth Rate (SGR) of females calculated for the period January 1999 to January 2000.

Female 1c (first spawners) had the highest SGR among families, followed respectively by 7c, 8b, 4a, 10a, 4c (all first spawners), and 6b (repeat spawner), 3b and 7a (first spawners). Female 7b (repeat spawner) had the lowest SGR among females, followed by 8c, 9b, 3c, 8a, 1b, 2a, 9c (all repeat spawners) and 6c (first spawners). Moreover, female 10c lost weight and therefore, had a negative SGR. First spawners had in average a SGR of 115.8, while repeat spawners had an average SGR of 65.7, which means that repeat spawners grew only 56% of the growth achieved by first spawners.

Females 2c, 4b, and 10c did not spawn naturally and were stripped. However, only few larvae hatched. Although some of these larvae were released in the small mesocosm (2c= 239 larvae, 4b=475, and 10c= 582 larvae), these families will not be considered in further results.

3.2 Spawning and Egg quality parameters

Figures 13a and 13b show the main batches produced by the families, and the mean egg size per batch. Most of the females did not spawn regularly. Thus, in order to obtain offspring with close hatching dates, batches were chosen by spawning date and not by batch number. The egg sizes decreased towards the end of the spawning season (Fig.13a, b). The spawning period was not followed until the end, and total mean egg sizes were estimated only among the batches released from the beginning of spawning until April 2000 (Fig. 14). The largest incubated eggs, whose offspring was released in the mesocosms, were produced by females 8b, 10a, 8c, 1c and 6c. They were all first spawners, with exception of female 8c. The smallest eggs were produced by females 6b and 1b, which were repeat spawners. The average egg size produced by the first spawners during all monitored batches was 1.43 mm, while the incubated mean size was 1.47. For repeat spawners the average was 1.42, and the incubated mean size was 1.43. Although incubated eggs from first spawners were slightly larger than the mean, they did not differ significantly (t-test $p > 0.05$) from each other (Fig.14).

Total egg volume or relative fecundity could not be estimated because the females were slaughtered before the end of the spawning season. Egg size and SGR (Fig.15) were slightly positively related for first spawners ($r^2 = 0.09$). For example, female 8b and 1c had a large SGR and produced larger eggs (1.62mm and 1.48mm, respectively). However, egg size and SGR were negatively related for repeat spawners ($r^2 = 0.57$). Female 6b had the largest SGR among repeat spawners and produced the smallest egg (1.34 mm). Family 8c had a low SGR and produced a large egg (1.49 mm).

Female size and age were positively related ($r^2 = 0.42$). Egg size was positively related to female size and age. Nevertheless, for first spawners, female age explained egg size better than female size, while for repeat spawners the opposite occurred. Egg size was not related to female weight, and was negatively related to female condition (Fig.16). Additionally, Fulton Index estimated from eviscerated fish improved the relation to egg size ($r^2 = 0.29$) (Fig. 16).

By the end of the spawning season, first spawners had lost up to 25 percent of their body weight, while repeat spawners lost about 19 percent. There was no slaughtering data for females 1c (first spawners), 3a (first spawners), and 9c (repeat spawner) available. First spawners had a mean liver weight of 418.89 grams (SD=107.50) while repeat spawners had a mean liver weight of 513.3 grams (SD=98.49). However, the weight difference was not statistically significant (t-test $p > 0.05$). In addition, liver weight was not related to any parameters analyzed for egg quality.

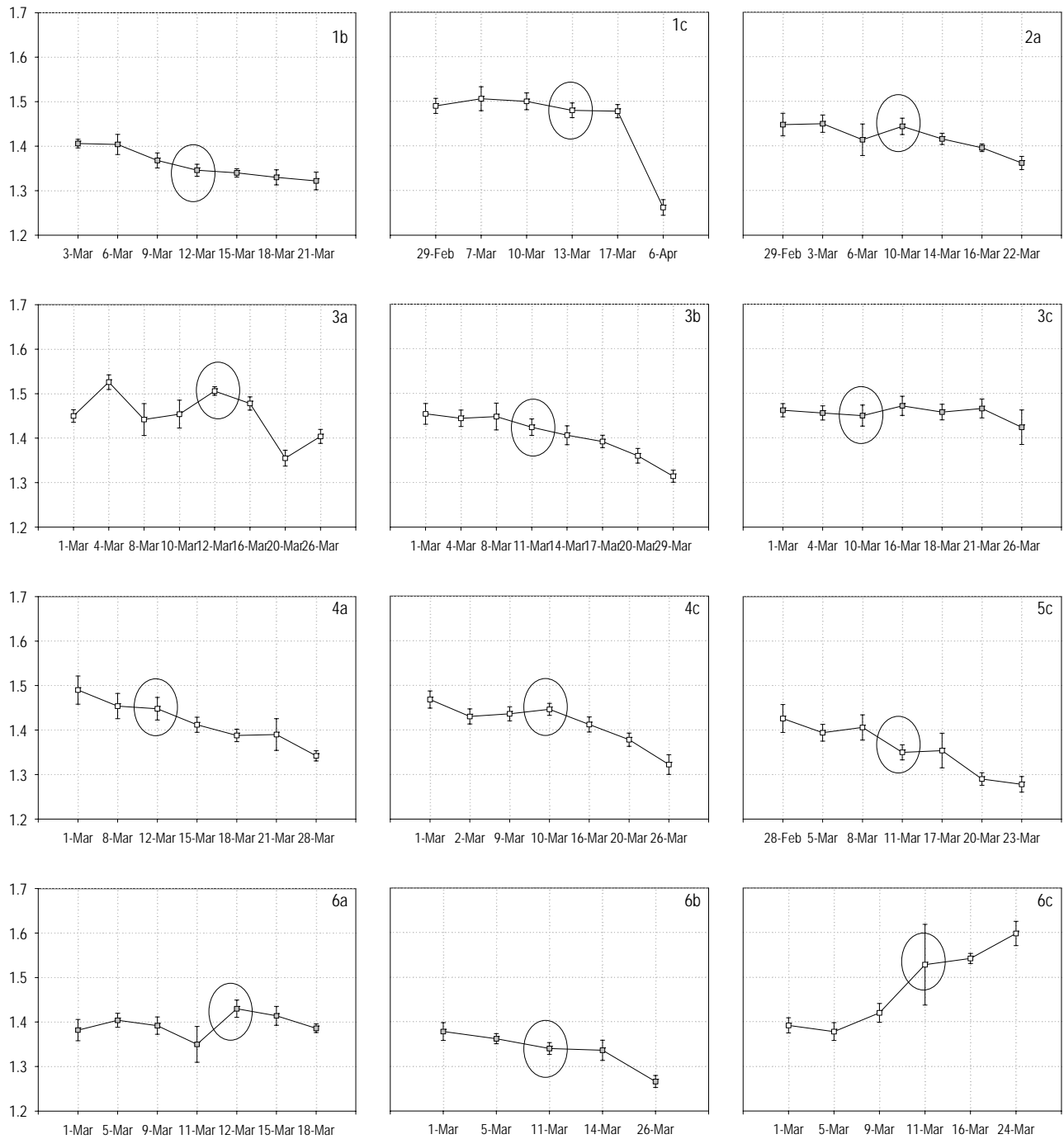


Figure 13a. Monitoring of the spawning season 2000. The families are indicated on the right upper corner of the graphs. Y axis represents the mean egg size in mm, while X axis indicates the spawning date. White squares represent the spawning sequence of first spawners. The squares in gray indicate the spawning sequence of repeat spawners. Bars give the standard errors. The circle indicates which batch was incubated for each family.

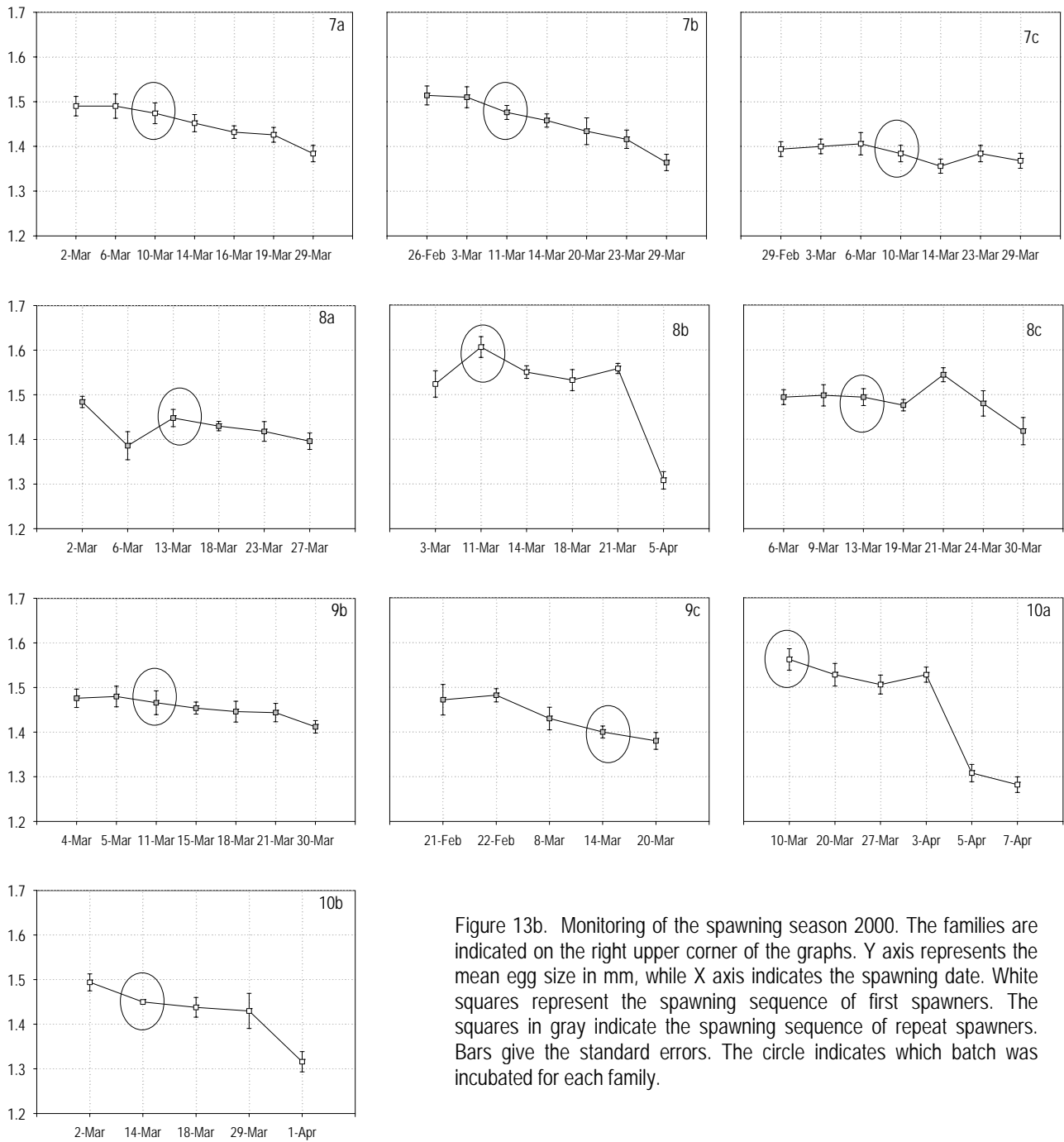


Figure 13b. Monitoring of the spawning season 2000. The families are indicated on the right upper corner of the graphs. Y axis represents the mean egg size in mm, while X axis indicates the spawning date. White squares represent the spawning sequence of first spawners. The squares in gray indicate the spawning sequence of repeat spawners. Bars give the standard errors. The circle indicates which batch was incubated for each family.

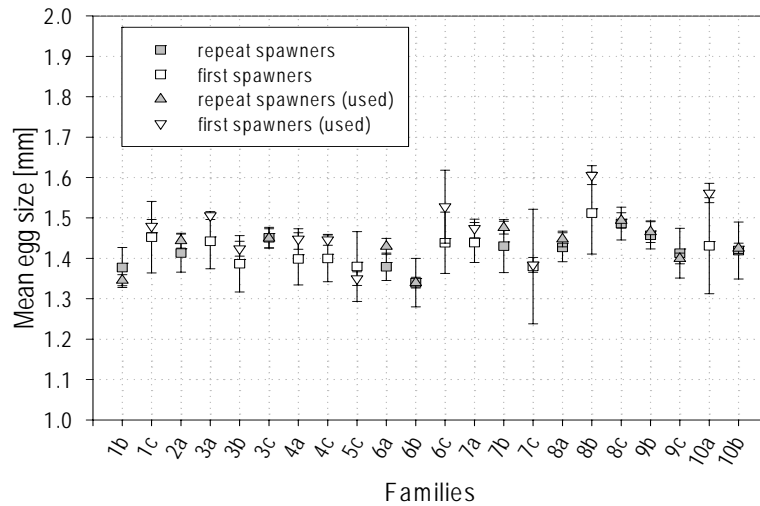


Figure 14. Squares (white= first spawners and gray= repeat spawners) indicate the mean egg size produced by the mothers during the spawning season 2000 (all batches were included). Triangles indicate the mean egg size from the batches incubated for the mesocosm experiment (inverted white= first spawners, triangles in gray= repeat spawners). Error bars give the standard deviation from the mean.

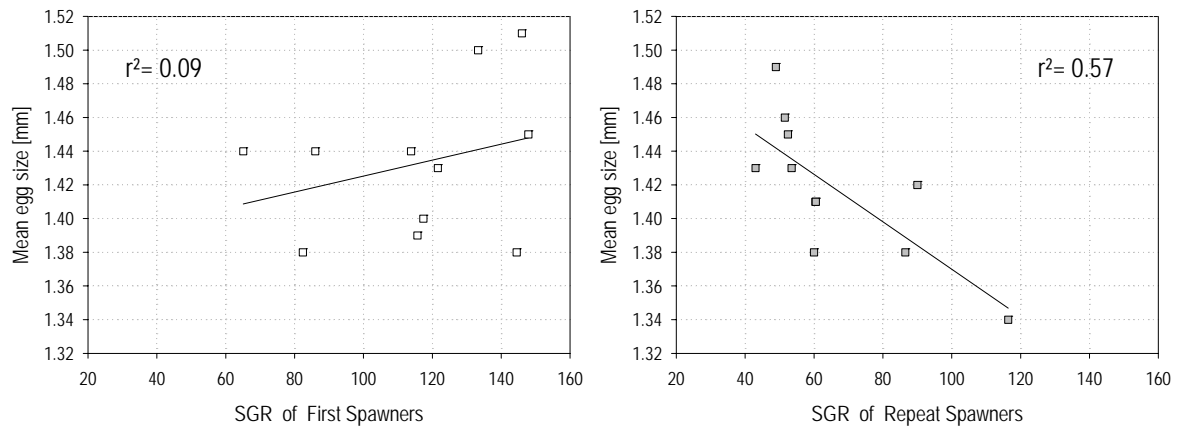


Figure 15. Relation between mean egg sizes produced by the females during the spawning season 2000 and females Specific Growth Rate (SGR). On the left, first spawners (white squares). On the right, repeat spawners (squares in gray).

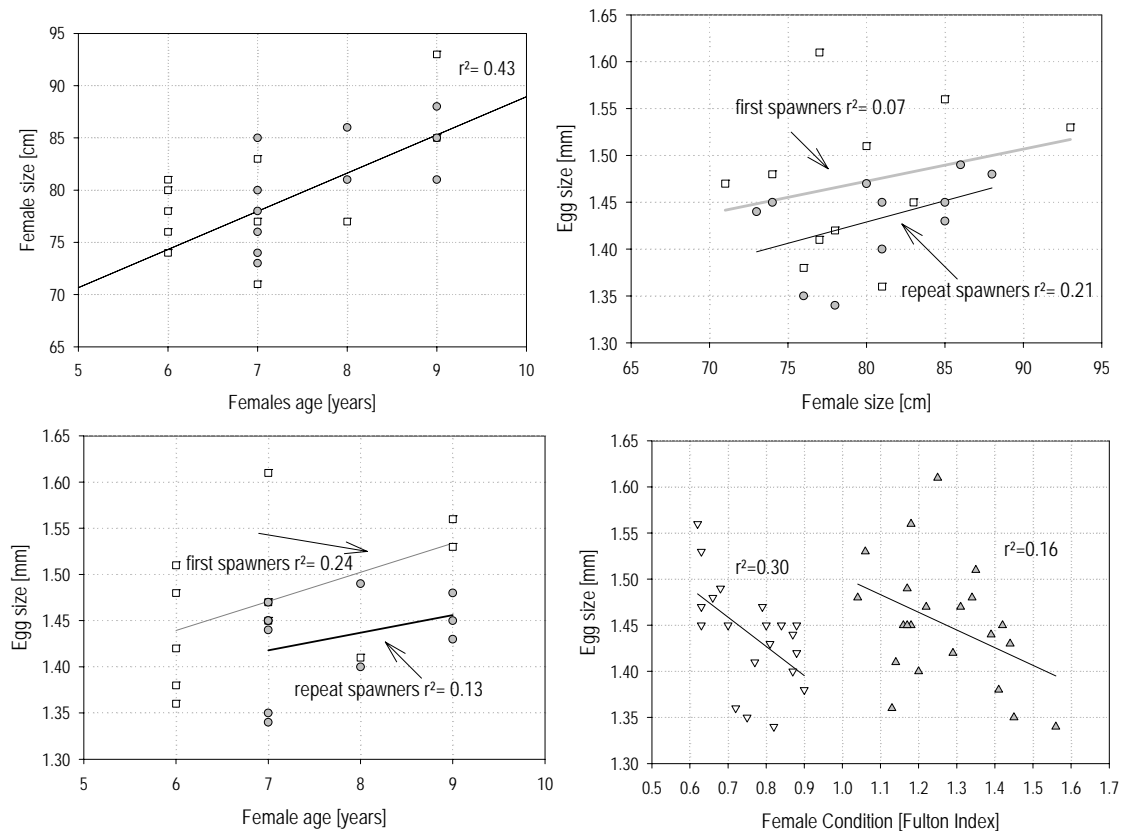


Figure 16. On the upper left: Relation between female size and age. On the upper right: Relation between egg size and female size. On the lower left: Relation between egg size and female age. White squares represent first spawners and gray circles represent repeat spawners. On the lower right: Relation between egg size and female condition for both first and repeat spawners. Triangles in gray indicate Fulton Index before spawning, while inverted white triangles indicate the Fulton Index for females after the spawning season, weighted without gonads.

Analyses of egg quality were performed by the Danish partner (resp. Helge Paulsen). Egg wet-weight, dry weight, energy content, and amount of Free Amino Acids (FAA) were positively related to egg size (Fig.17). Egg size and amount of Wax Esters (WE) were not related ($p > 0.05$). Triglycerids (TAG) and egg size were not significantly related ($p > 0.05$). Egg size and amount of Free Fatty Acids were slightly negatively, but not significantly related ($p > 0.05$). Egg size and cholesterol (CHL) or amount of Phospholipids (PC) were not significantly related ($p > 0.05$) either.

Eggs from females 8b, 6c, 10a, 8a, 8c and 1c had the highest energy content, while eggs from females 1b, 6b and 3b had the lowest energy content. Eggs from females 8c, 8a, 1c, 8a, 6c had the largest amount of triglycerids (TAG), while eggs from females 10a, 10b, 1b, 8b and 3b had the lowest amount of triglycerids (TAG).

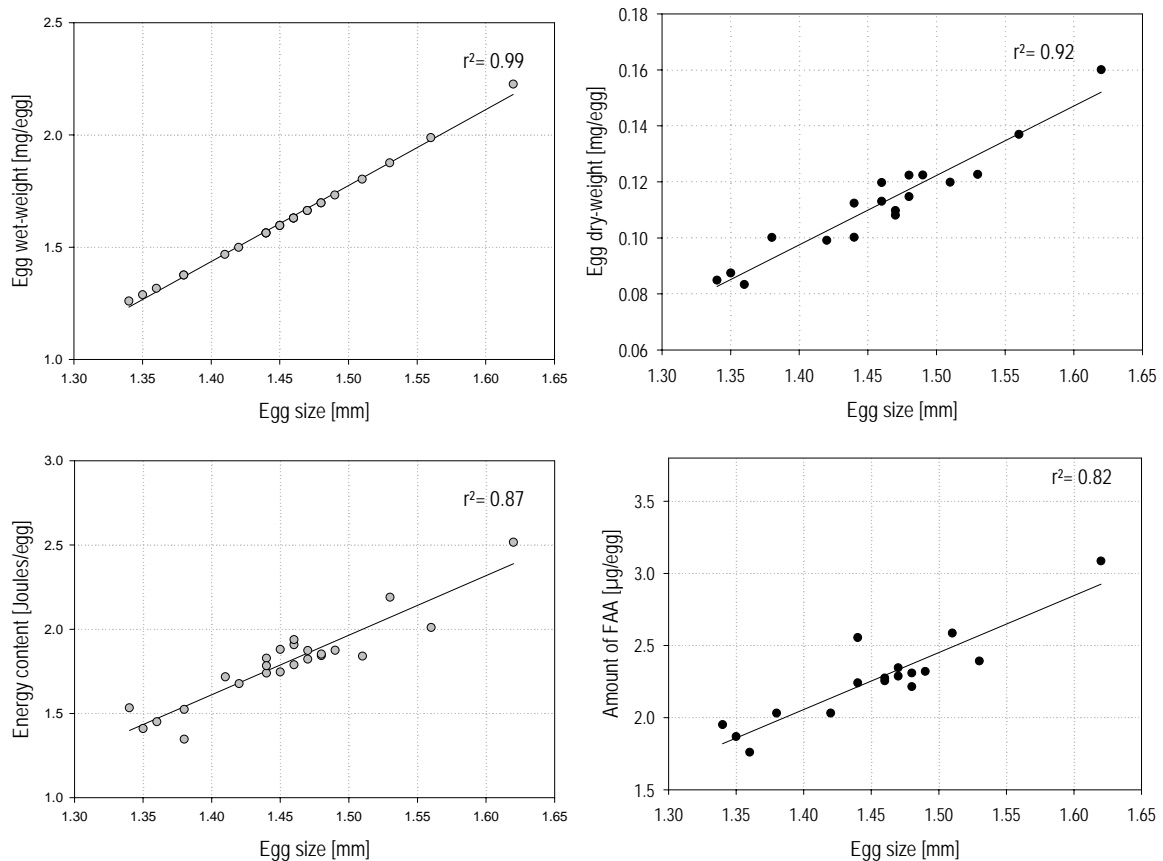


Figure 17. On the upper left: Relation between egg size and egg wet- weight ($r=0.99$). On the upper right: Relation between egg size and egg dry-weight. On the lower left: Relation between egg size and energy content. On the lower right: Relation between egg size and amount of FAA. Equations combine egg measurements from first and repeat spawners.

Summing up, in this study, first (recruit) and repeat spawners had similar age, size and weight. First spawners had higher growth previous to spawning, were slightly better conditioned than repeat spawners, and contrary to the expected, produced larger eggs than repeat spawners. In other words, exactly the opposite situation found in nature.

Maternal size was positively related to egg size and female age. Larger eggs had a larger amount of dry-matter, energy content and FAA. However, best conditioned females previous to spawning had produced the smallest eggs. For example female 6b and 1b had the highest pre-spawning condition (Fulton Index of 1.56 and 1.45) and had produced the smallest eggs. Females with the lowest eviscerated post-spawning condition (Fulton Index of 0.62 and 0.63) had produced the largest eggs. However, comparison between pre- and post spawning Fulton Index, indicated a larger loss in condition for those females that had produced smaller eggs.

3.3 Laboratory control

3.3.1 Mortality test on starving groups

Based on the pre-release mortality test performed at the laboratory in Flødevigen, families 3a, 4a, and 9c had a mortality of 95 percent on day 1. By the end of week 1, families 1b, 2a, 5c, 6b, 7a, 7c, 9b and 10a had a mortality of 50-60 percent, while for 1c, 6a, 6c, 7b, 8a, 8b, 8c mortality reached 30-40 percent. By the end of week 2, mortality was close to 100 percent for all families. Nevertheless, families 6a, 6c and 8b have shown higher survival. Additionally, survival was not related to newly-hatched size or yolk-sac volume, indicating that another factor, as for example the transport might have influenced the mortality in some groups.

3.3.2 Relations among egg size, larval size and condition

Data obtained from newly-hatched larvae before releasing in the mesocosms have shown that the larvae's SL and egg sizes were positively related ($r^2= 0.25$), (Fig. 18). Family 6c, 10a and 8b produced the largest eggs and consequently the largest larvae, while female 1b produced the smallest eggs and larvae. The yolk-sac volume of newly-hatched larvae was positively related to egg size ($r^2= 0.32$), (Fig.18). However, yolk-sac volume and larval size were poorly related (Fig.19). No relation was found between newly-hatched RNA/DNA ratios and egg size. There was no relation among RNA/DNA ratio, yolk-sac volume, larval size or weight (Fig.19), which implies that larger larvae are not necessarily better conditioned. In addition, RNA/DNA ratios did not significantly differ among families (ANOVA, $p>0.05$).

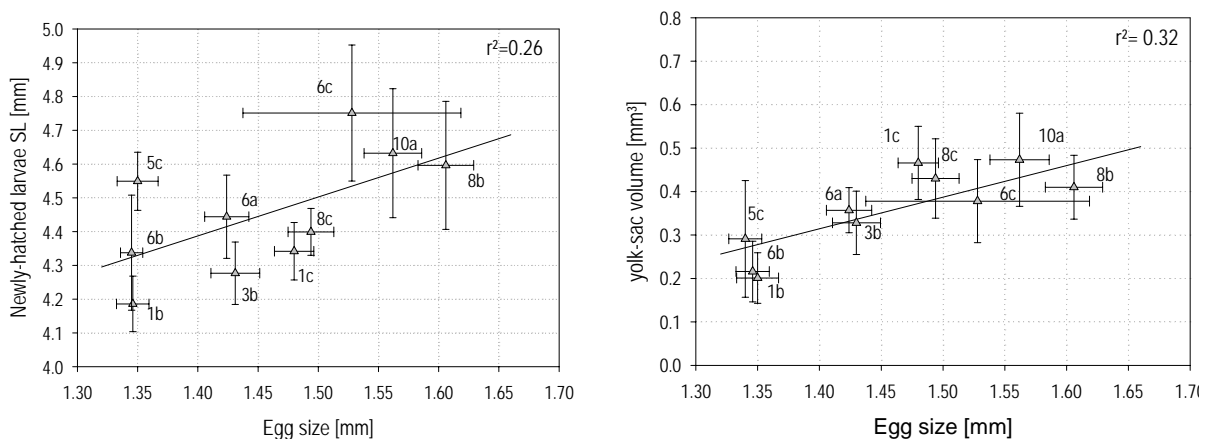


Figure 18. On the left: Relation between egg size and larvae size at hatching. On the right: relation between egg size and yolk-sac volume. Main families are shown in the regression. Bi-directional bars give the standard deviation from the mean for y and x values, respectively.

3.3.2.1 Otoliths from newly-hatched larvae

The otolith *lapillus* was larger than *sagitta* at hatching, and remained so, up to 20 days old larvae. After that, there was a switch, and *sagitta* grew faster than *lapillus*. Close to hatching, cod larvae laid a distinguished clear and bright mark around the otolith nucleus (core), called the “hatch-check”. The hatch-check width varied from 0.7 to 1.7 μm , and was alike for *lapilli* and *sagittae*. By definition, the otolith nucleus was called “core”, while “otolith radius” indicated the longest axis measured (Fig.20). In newly-hatched larvae, *Lapillus* was better related to body size than *Sagitta*. Therefore, only lapillar relations are shown. Even though, those relations were rather weak. Since larvae did not hatch at the same time, otolith radius at hatching (*i.e.* the distance from centre to the outer edge of the hatch-check) was plotted against egg size. Otolith radius at hatching was better related to egg size ($r^2= 0.15$) (Fig. 21, left) than hatch-check width ($r^2=0.06$) (Fig. 22, left).

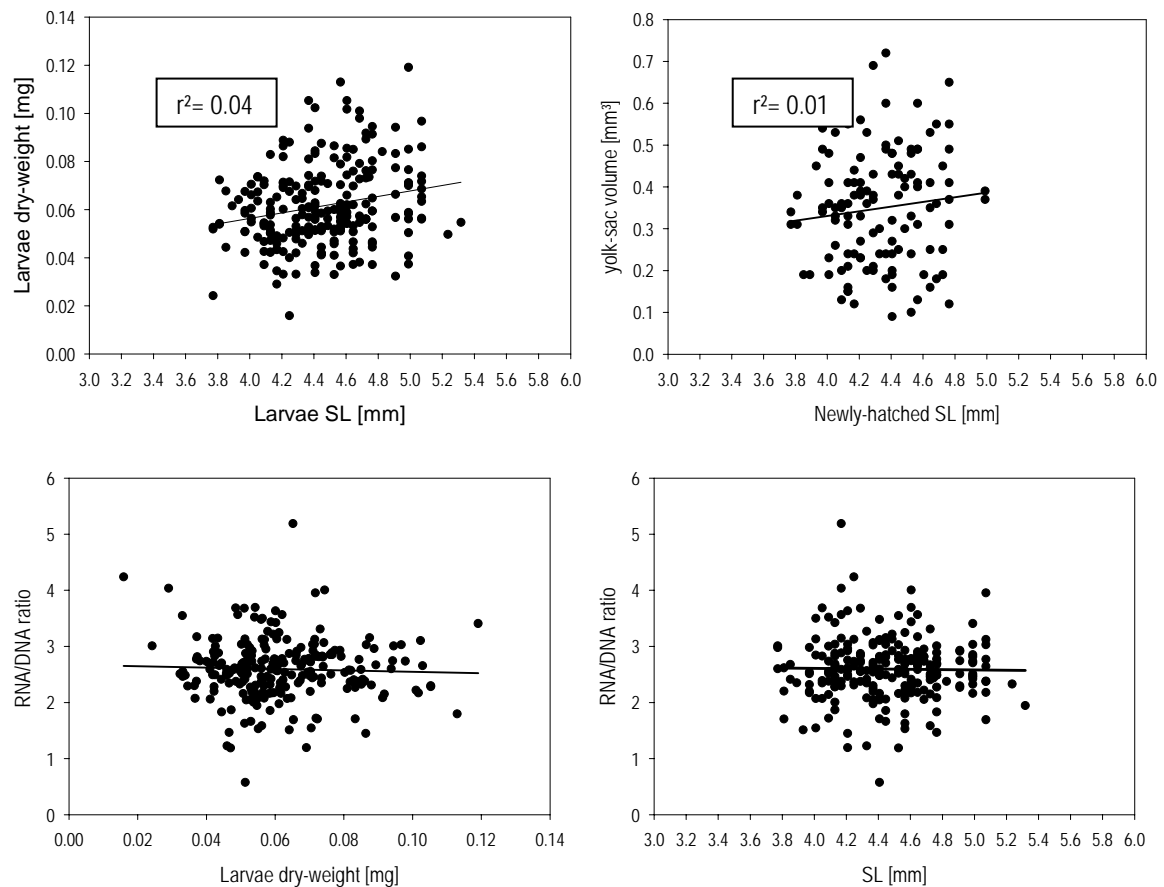


Figure 19. On the upper left: relation between larval size and dry-weight. Linear regression. On the upper right: relation between newly-hatched yolk-sac volume and body size. Linear regression. On the lower left: relation between RNA/DNA ratio and newly-hatched dry-weight. Linear regression. On the lower right: relation between RNA/DNA ratio and newly-hatched size.

Otolith hatch-check width was slightly positively related to egg and larval size at hatch ($r^2=0.06$) (Fig. 22, right). However, it was not related to yolk-sac volume

(Fig.23, lower right) or RNA/DNA ratio. The substitution of hatch-check by otolith radius at hatching did not improve the relation, in opposite, it even decreased. Hatch-check was also poorly related to core size ($r^2=0.05$) (Fig. 23, lower left).

Otolith radius was poorly related to larval size ($r^2=0.08$) (Fig. 24, left). However, the relation improved when data was plotted for individual families (Fig. 24, right). For example, families 1c, 6b, 8c have shown a strong positive relation ($r^2= 0.30$, $r^2= 0.68$ and $r^2= 0.26$). On the other hand, family 6a has shown a strong negative relation ($r^2= 0.60$).

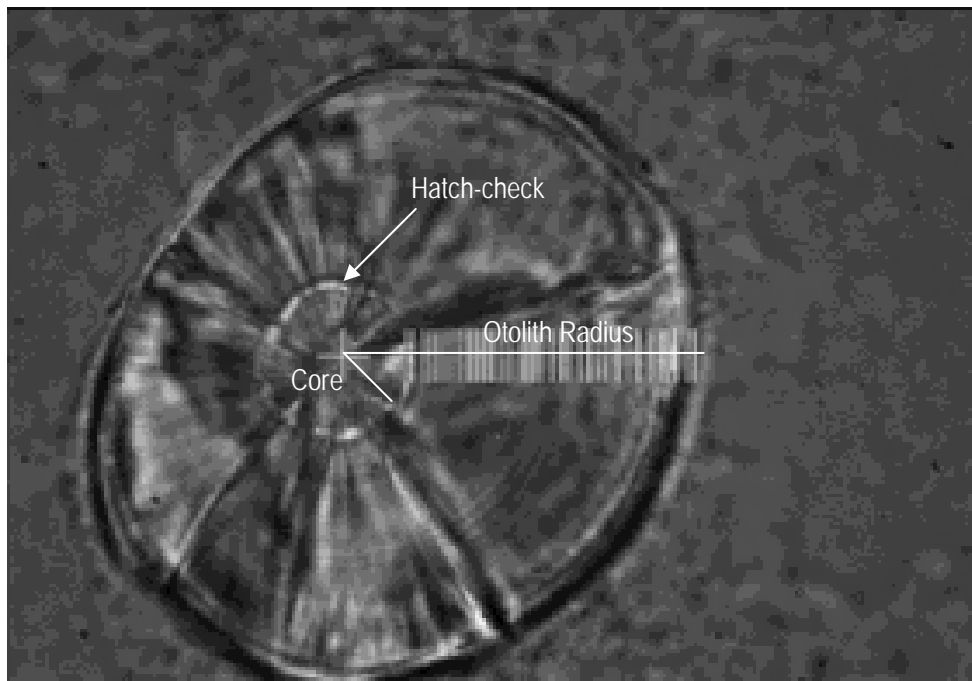


Figure 20. Lapillus of a five weeks old cod larva. Observe the hatch check - a bright circle (ring) around the core. The core is the central area within the hatch-check (diagonal white line). The otolith radius is the distance from the centre to the outer border (white horizontal line), while the small vertical parallel lines indicate the daily increment widths.

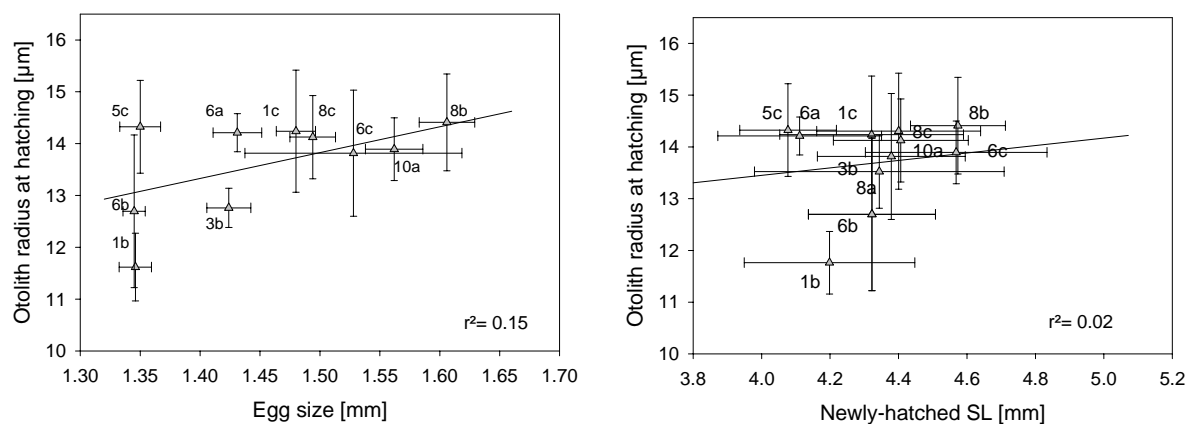


Figure 21. On the left: relation between otolith radius at hatching and egg size. On the right: relation between otolith radius at hatching and newly-hatched standard length. Bi-directional bars give the standard deviation from the mean for y and x values, respectively. Only main families were indicated.

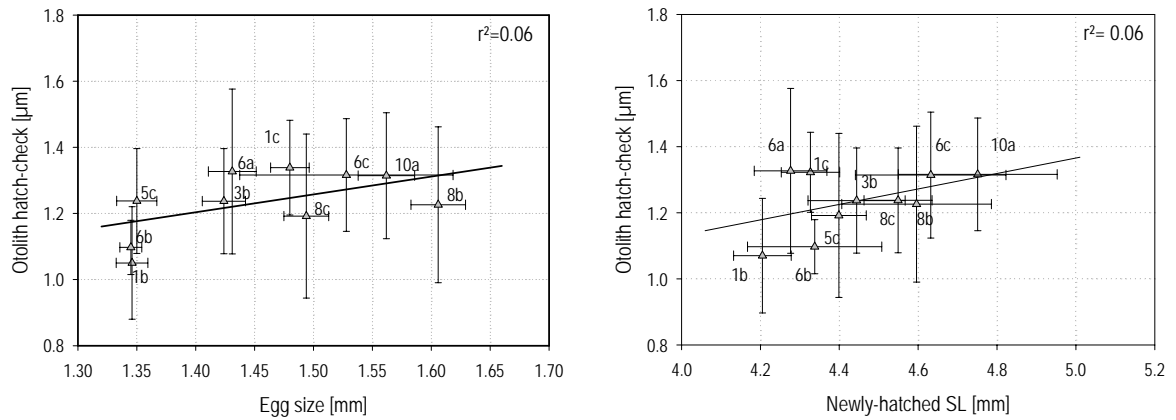


Figure 22. On the left: relation between hatch-check and egg size. On the right: relation between hatch-check and larval size. Bi-directional bars give the standard deviation from the mean for the y and x values, respectively. Only main families were indicated.

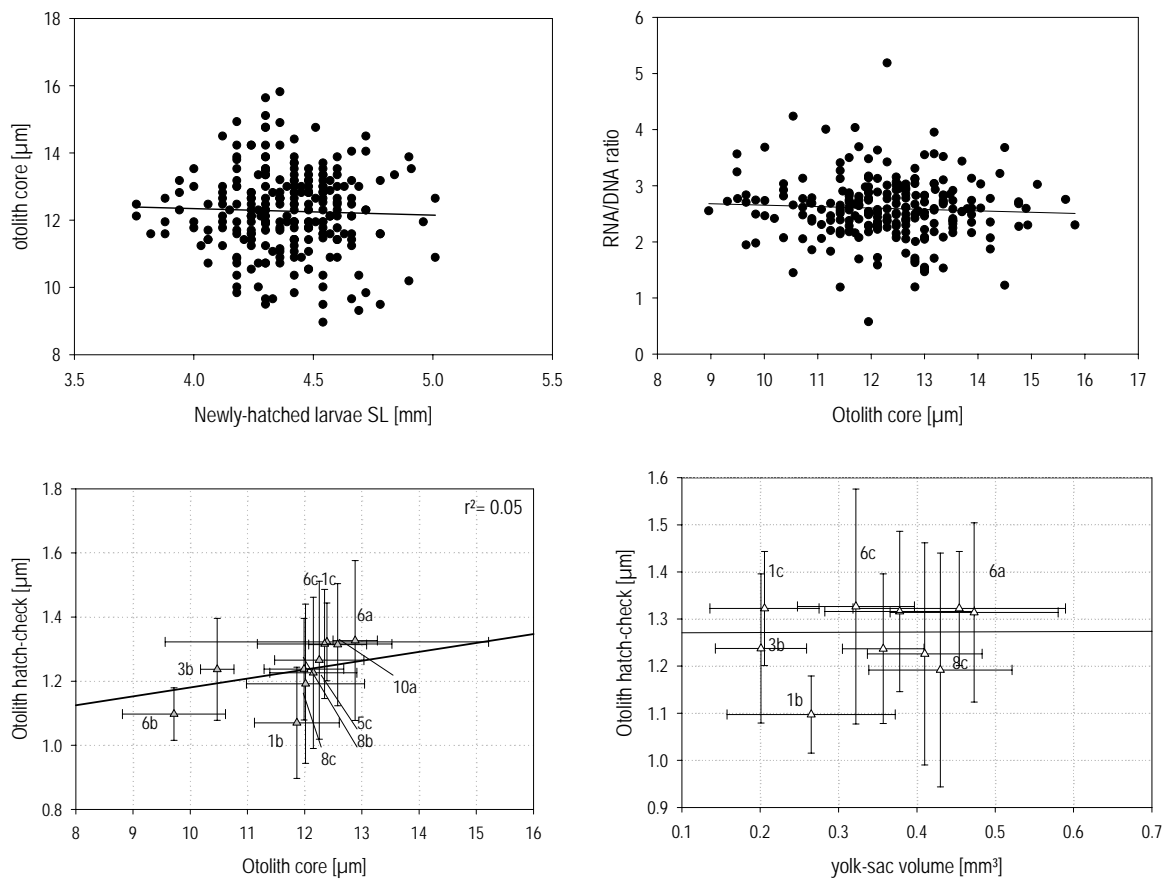


Figure 23. On the upper left: relation between otolith core size and Newly-hatched standard length. On the upper right: relation between RNA/DNA ratio and otolith core. On the lower left: relation between otolith hatch-check width and core size. On the lower right: relation between otolith hatch-check and yolk-sac volume. Only main families were indicated.

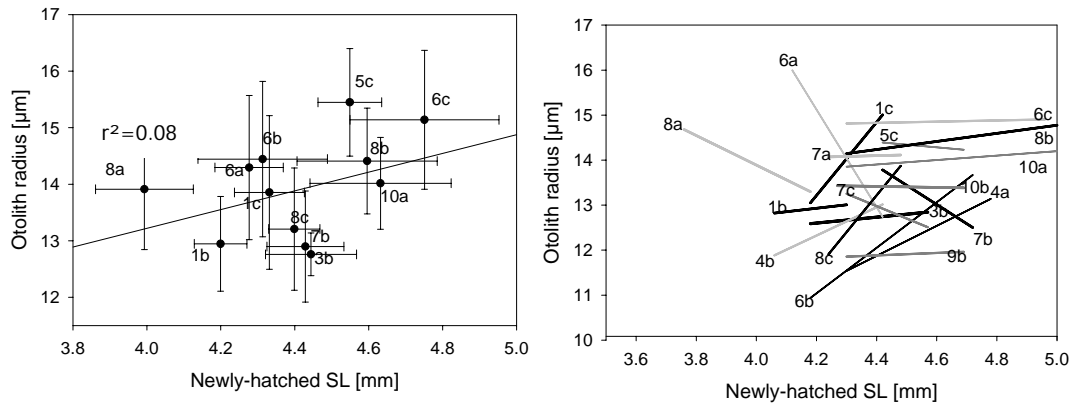


Figure 24. On the left: relation between otolith radius and newly-hatched standard length. Bi-directional bars give the standard deviation from the mean for y and x values, respectively. On the right: linear regressions plotted for individual families.

Resuming, larvae from larger eggs tend to be larger and to have larger yolk-sacs, and therefore, more reserves. The newly-hatched stage is characterized by a large variability in size, weight and condition. RNA/DNA ratios did not differ significantly among families. *Lapillus* was larger and better related to egg and larval sizes than *sagitta*. Even though, those relations were poor, indicating that other mechanisms rather than larval size might be involved.

3.4 Mesocosm Experiment

3.4.1 Monitoring of the mesocosms:

The monitoring of the mesocosms started a month before the newly-hatched larvae were released. The amount of dissolved oxygen was always above saturation and therefore, it has never been a limiting factor in the mesocosms (Fig.25, right). Salinity ranged from 31.5 to 34 and was slightly lower in the small mesocosm (Fig.25, left).

The average temperature in the large mesocosm ranged from 6°C in the beginning of March to 16°C at the end of May, dropping to 13°C, shortly before the end of the experiment (Fig. 26, left). In the small mesocosm the initial average temperature was 4°C, increased to 14.1 °C in May 19, and dropped to 11°C at the end of the experiment. The temperature in the large mesocosm was most of the time about 2°C higher than the small one since the releasing of the larvae, on March 28, 29 and 30 until June 5. The only exception was the first week of May, when the temperature was similar, and around 10°C in both mesocosms (Fig.26, left). The water column in the large mesocosm was stratified, while the small mesocosm it was thoroughly mixed.

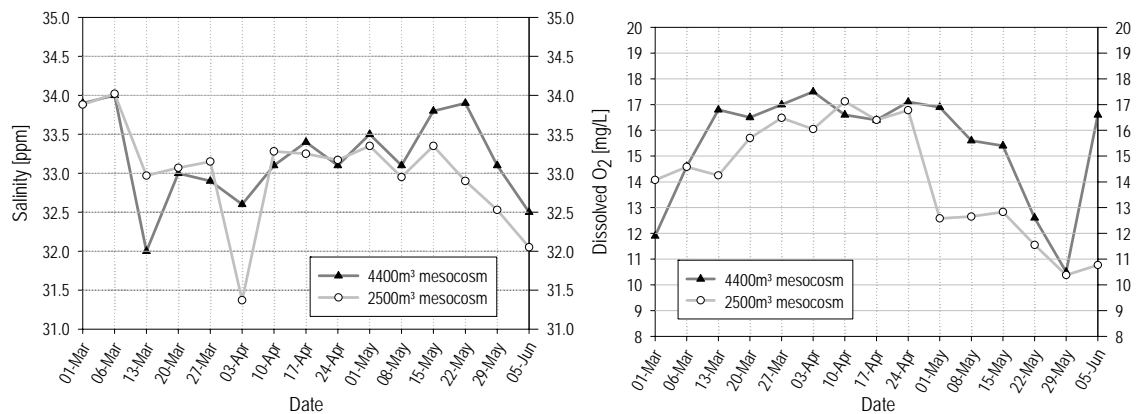


Figure 25. On the left: White circles and black triangles indicate the weekly salinity variation within the 2500m³ and 4400 m³ mesocosms, respectively. On the right: same symbols indicate the oxygen content in the mesocosms.

A useful chemical method for determining the total quantity of phytoplankton in seawater is to estimate the amount of chlorophyll-a (Fig.26, right). However, chlorophyll degradation products sometimes make up a significant fraction of the total plant pigment in the sample. Degradation products resulting from digestion processes of zooplankton convert chlorophyll into phaeo-pigments (phaeophorbide and phaeophytin), while decomposition processes, due to hydrolytic enzymes in the phytoplankton, may convert chlorophyll into chlorophyllide. Thus, analysis of phaeo-pigments (Fig.26, right) was a helpful parameter to determine the amount of chlorophyll available to the photosynthesis.

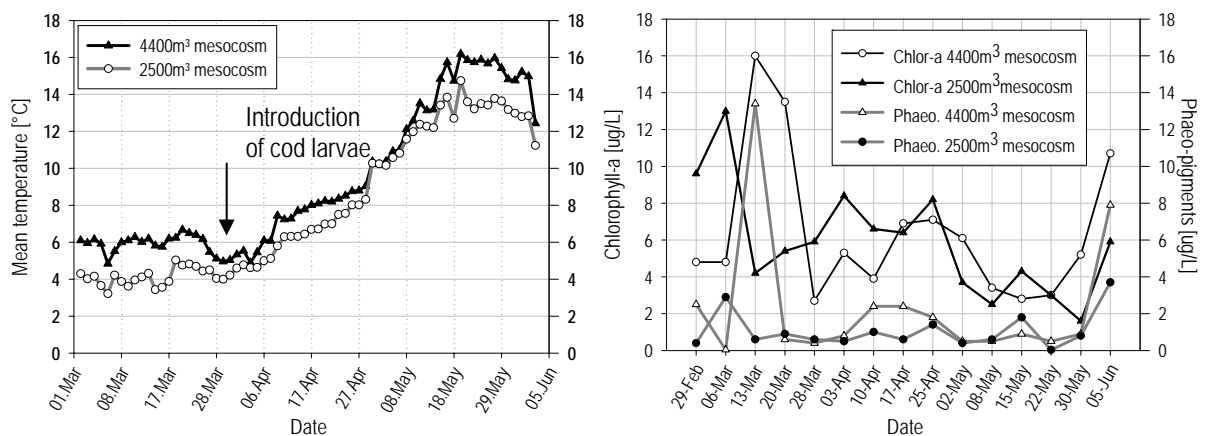


Figure 26. On the left: White circles and black triangles indicate the daily average temperature in the 2500m³ and 4400 m³ mesocosms, respectively. On the right: Black lines, white circles and black triangles indicate the amount of chlorophyll-a in the 2500m³ and 4400 m³ mesocosms, respectively. Grey lines, black circles and white triangles indicate the amount of phaeo-pigments for the same samples, in the 2500m³ and 4400 m³ mesocosms.

On February 28, the amount of chlorophyll-a and phaeo-pigments was lower in the 4400m³ than in the 2500m³, while at the end of the experiment the opposite situation occurred. Since phytoplankton biomass decline could be related to grazing,

the variation in chlorophyll-a might be an indirect indication of the zooplankton density variation.

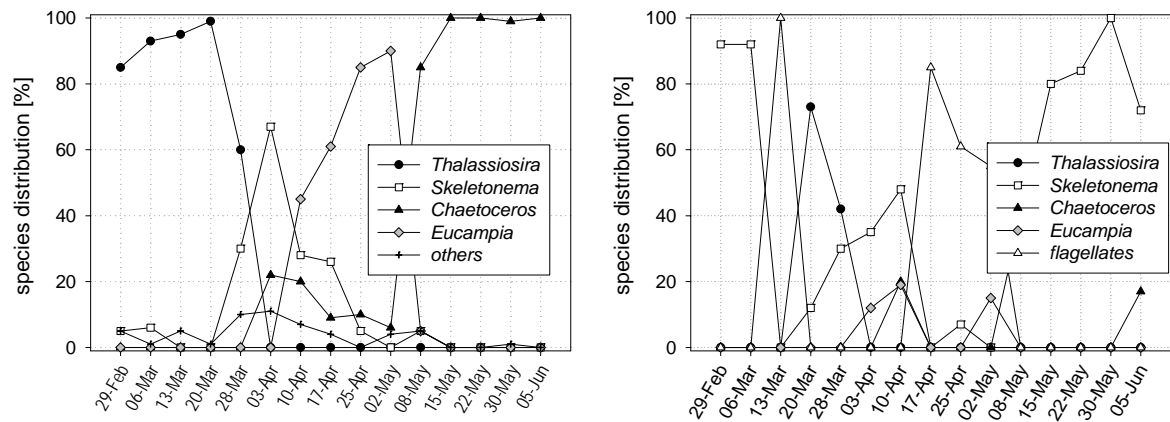


Figure 27. Phytoplankton composition in the mesocosms (based on the 100-cells method). On the left: data from the 2500m³ and on the right, data from the 4400m³ mesocosms. Legend: black circles = *Thalassiosira* sp., white squares = *Skeletonema costatum*, black triangles = *Chaetoceros similis*, gray diamonds = *Eucampia zodiacus*, white triangles = flagellates, cross = others.

Thalassiosira sp., *Chaetoceros similis*, *Skeletonema costatum* and *Eucampia zodiacus* were the most frequent phytoplankton species found in the mesocosms. From February 28 until April 5, *Thalassiosira* dominated in the 2500m³ mesocosm. After that, *Skeletonema* and *Eucampia* followed. From May 15 until June 5, *Chaetoceros* was the most numerous.

Skeletonema costatum was the most frequent species in the 4400m³ (Fig.27), while *Thalassiosira* occurred only from March 23 to April 3, and disappeared in both mesocosms after April 4.

Thalassiosira is a common diatom that frequently occurs as part of the spring bloom, which begins the new growing season for the phytoplankton. Moreover, *Thalassiosira* and *Skeletonema* are considered a good food species for copepods (Koski et al 1998). Therefore, the frequency of such species gave an indication on the zooplankton density. The low abundance of *Thalassiosira* at the beginning of the experiment might be related to the high concentration of zooplankton in the 4400m³ mesocosm (Fig. 27). During the high peak of zooplankton, only flagellates were found in this pond. After that, i.e., in the last two and half weeks of the mesocosm experiment, the zooplankton density became very low and *Skeletonema costatum* developed. In the 2500m³ mesocosm, the reverse situation occurred: there was a higher frequency of *Thalassiosira* at the beginning and low zooplankton density, followed by low *Skeletonema* density at the end of the experiment, when the zooplankton increased. The reason for the higher presence of *Chaetoceros* at the end of the experiment in the 2500m³ mesocosm can be only speculated. Due to its morphology (large spines), *Chaetoceros* might be not appreciated by grazers (E. Dahl pers. comm.).

Calanoida copepods belong to the main group fed on by cod larvae. At the onset of exogenous feeding larvae feed mainly on Calanoida nauplii, and afterwards on Calanoida copepodites. The density and main stages are presented in Figure 28.

Additionally, total numbers of organisms were plotted for all sampling dates. Samples from 3m were chosen for the plot because they were supposed to be representative for average zooplankton composition (Moksness pers. comm.).

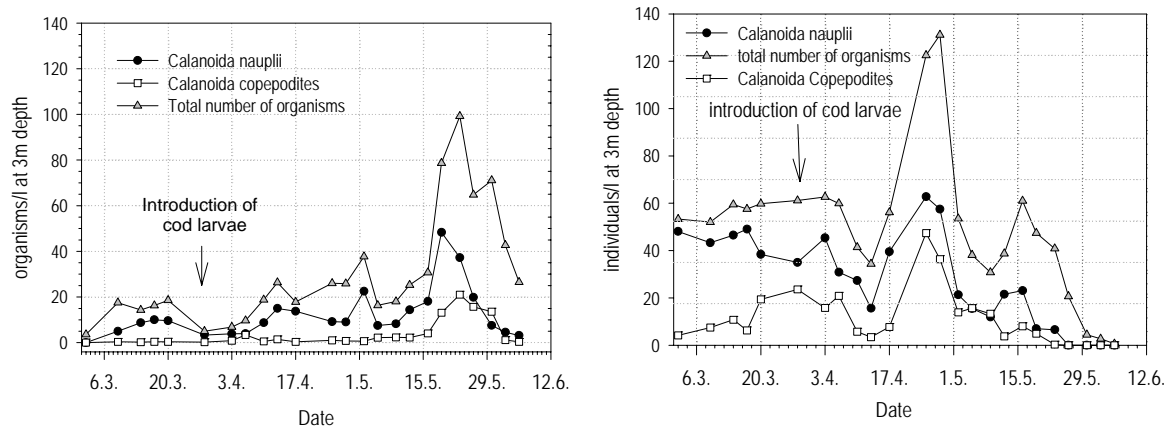


Figure 28. Zooplankton density from samples pumped at 3m depth. On the left: data from the 2500m³ and on the right, data from the 4400m³ mesocosms. Legend: black line and gray triangles = total organisms found in the samples per liter, black line and black circles = calanoida nauplii per liter, black line and white squares = calanoida copepodites per liter.

Although the species composition was similar for both mesocosms, the food availability contrasted quite a lot. In the beginning of March, the large mesocosm was characterized by high densities of *Calanoida nauplii*, *Calanoida copepodites*, adult stages of *Acartia tonsa*, very few *Eurytemora sp.*, and *Temora longicornis*. The small mesocosm was initially characterized by extreme low densities of zooplankton, with a large quantity of copepoda eggs. In the beginning of April, 2000 *Calanoida nauplii* and copepodites dominated in the 4400m³. Right after, adult stages of *Centropages hamatus* were the most frequent *Calanoida*, followed by *Pseudocalanus*, while *Acartia* became rather rare. The small mesocosm was still characterized by low densities of *Calanoida nauplii* and copepodites. In the middle of April, Rotifers were present in low numbers in both mesocosms. At the same time the presence of *Centropages typicus* became more frequent, and this species dominated the system with *Centropages hamatus* until middle of May. From the middle of May onwards, the number of Harpacticoids and Gastropoda moluscs started to increase. About two weeks before the termination, mostly Harpacticoida in different stages were found in the small mesocosm.

To summarize, when the cod larvae were introduced in the mesocosms, the zooplankton density in the large mesocosm was about 10 times higher than in the small one. Zooplankton density was always higher in the large mesocosm, until middle of May, when densities decreased so steeply that by the termination of the mesocosm period only "clear" water remained. When the cod larvae were released, the density in the 4400m³ mesocosm was about 61.4 organisms l⁻¹, it increased to 131 organisms l⁻¹ on April 28 (the highest peak) and declined to 0.6 l⁻¹, on June 5. The small mesocosm was characterized by initial 6.85 organisms l⁻¹ and organism density started to increase from beginning of May onwards. It reached the highest peak in May 23 with 99.19 organisms l⁻¹ and dropped to 26.5 organisms l⁻¹ on June 5.

3.4.2 Larvae sampling and survival

Sampling in both mesocosms started on April 3, 2000. However, larvae from the first sampling were only used for mortality calculations. On April 6, i.e., one week after the releasing of the offspring in the mesocosms, 600 larvae were sampled in each mesocosm, genotyped and used for length and weight and biochemical measurements.

Based on the amount of larvae caught in week one, estimations suggested that mortality was larger in 2500m³ (Fig. 29). Approximately 27% (36,761 larvae) of the 134,314 larvae initially released in the 4400m³ mesocosm have survived on week 1. In the 2500m³ mesocosm, about 9,605 larvae of the 82,285 initially released have survived on week one, which means about only 12% survival.

The initial plan of sampling 600 larvae in weeks 1, 3 and 5 was only achieved for the 4400m³ mesocosm. Nevertheless, sampling in week 5 was rescheduled to week 4, in order to obtain larvae before metamorphosis. In the 2500m³ mesocosms, 600 larvae were caught at week 1, 255 were caught at week 3, and 342 larvae were caught in week 5.

After week 5, the catch-ability decreased. On May 7 and 12, all individuals had reached metamorphosis in the 4400m³ and 2500m³ mesocosms, respectively. After that, the net avoidance tremendously increased. On May 15, after many unsuccessful attempts, the sampling was suspended. On June 8, 2927 fishes were recovered from the 2500m³ mesocosm, meaning that 3.5% offspring had survived. On the following day, 11400 fishes were caught in the 4400m³, making 8.5% survival.

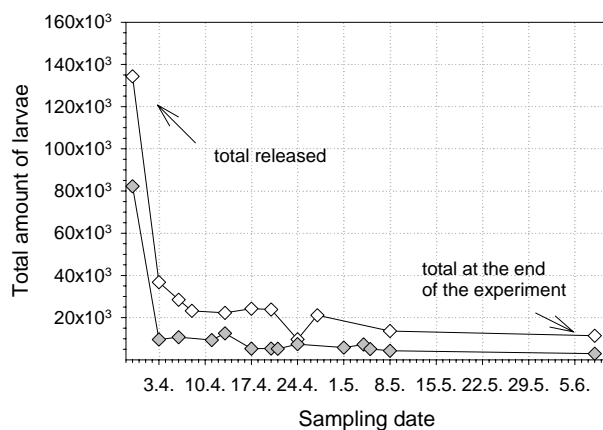


Figure 29. Reduction of estimated abundance of larvae over time. White diamonds indicate the amount of larvae estimated for the 4400m³, while gray diamonds indicate the amount of larvae for the 2500m³ mesocosm. Note that releasing and termination numbers are known.

The relative frequency of offspring per family was estimated based on the genetic fingerprinting performed by W. Hutchinson et al. for weeks 1, 3, 4, 5 and 10. Regarding spawning experience, no significant difference was observed on survival

between repeat and first spawners offspring in the mesocosms. However, there was a tendency for higher presence of repeat spawner's offspring in week 1 and 10 in the 4400 m³ (Fig. 30, left). In the 2500m³ mesocosm, there was a trend for increase of the first spawner's offspring frequency. However, a closer look has shown that family 1c was responsible for that trend. When family 1c was excluded from the analysis, it turned out to be no difference between spawning groups among sampling weeks (Fig. 30, right).

Since net avoidance is considered insignificant in week 1 (A. Folkvord, pers. comm.), data obtained from the first sampling week were used as a baseline to check upon post-release mortality in the mesocosms (Fig.31). Mortality was not random and sampling data fitted quite well to the results obtained by the pre-release mortality test. For example, families 3a, 4a and 9c had a high mortality on day one and were practically not recaptured in the mesocosms. In addition, families 8b, 8c, 7a, 6a, 6b, etc, had a higher survival in the test and higher recovering rate.

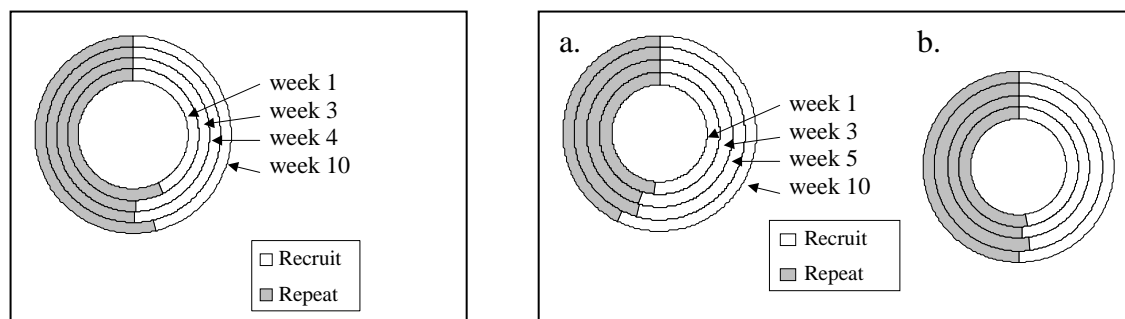


Figure 30. On the left: relative frequencies of recruit and repeat spawner's offspring in the 4400m³ mesocosm. On the right: relative frequencies of recruit and repeat spawner's offspring in the 2500m³ mesocosm before (a) and after (b) removal of recruit family 1c. Figure adapted from L. Hauser, Univ. Hull.

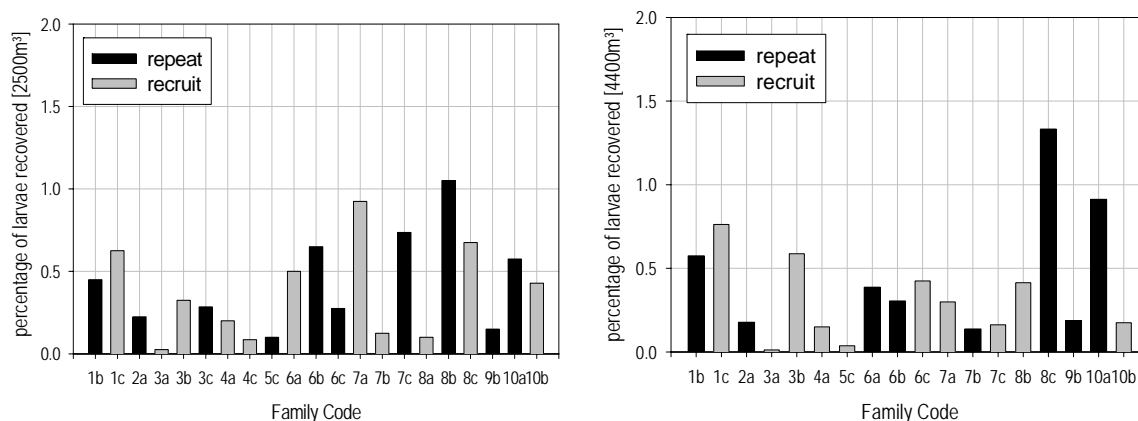


Figure 31. Percentage of larvae recovered in the first sampling week in the 2500m³ mesocosm (left) and 4400m³ mesocosm (right).

A large variation on survival was detected. Additionally, families either decreased or increased in frequency, and therefore, no bias caused by sampling errors could be detected. Moreover, in spite of the different environments, frequencies of families 1c, 6a, 6b and 6c increased while frequencies of families 8c and 10b decreased in both mesocosms (Fig.32, and Fig. 33).

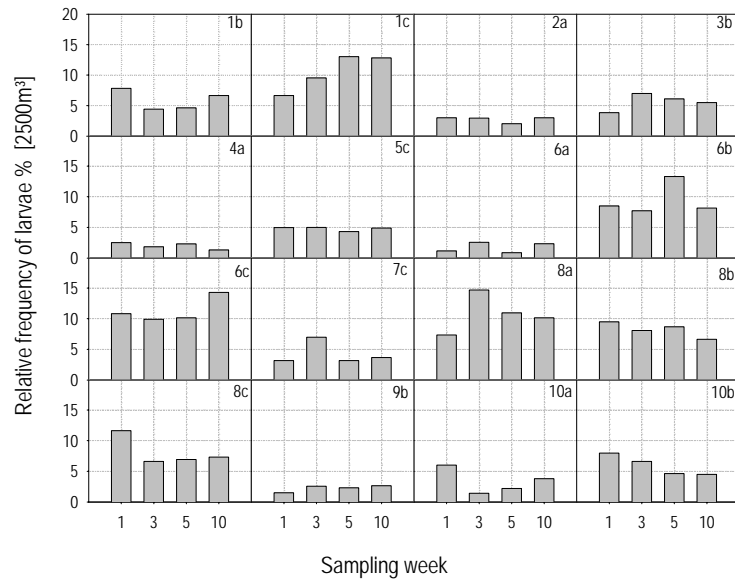


Figure 32. Histograms indicate the relative frequency of the families sampled during the week 1, 3, 5 and 10 in the 2500m³ mesocosm.

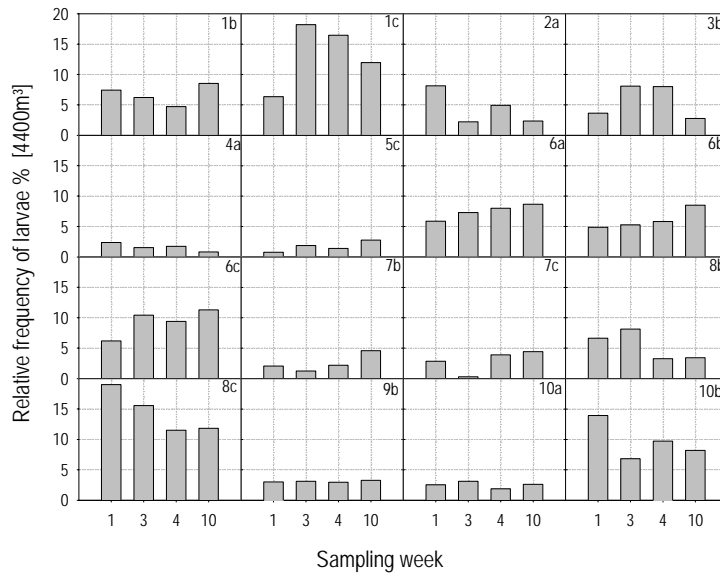


Figure 33. Histograms indicate the relative frequency of the families sampled during the week 1, 3, 4 and 10 in the 4400m³ mesocosm.

Shannon's evenness, which is a measure of equitability, was obtained by dividing the Shannon diversity index through the maximum possible diversity with the number of families present (L. Hauser, pers. comm.). The equitability assumes a value between zero and one being complete evenness. The Shannon's evenness showed an increase in the initial weeks in the 4400 m³ mesocosm while in the 2500 m³, the opposite occurred (Fig. 34). This indicates that in the small mesocosm, an initial selection might have taken place, while the environment in the 4400m³ allowed the survival of small and large sized offspring families.

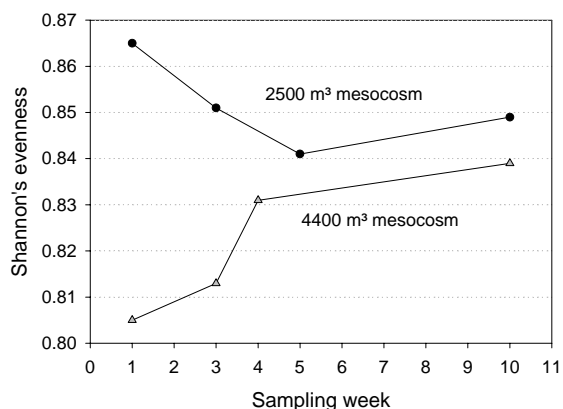


Figure 34. Shannon's evenness calculated for family frequencies in the 2500m³ and 4400m³ mesocosm during sampling weeks. Figure adapted from L. Hauser.

In the 2500m³ mesocosm, family survival was slightly positively related to egg size in week 1 and 10 ($r^2=0.03$ and 0.05), indicating that families with larger eggs might have had a slight advantage during this period. However, there was no relation between egg size and survival in weeks 3 and 5 (Fig. 35).

In the 4400m³ mesocosm, survival was positively related to egg size in weeks 1, 3 and 4. However, this relation was lost in week 10 (Fig.36). In addition, mean family survival within sampling weeks was slightly positively related to egg size in both mesocosms indicating a slight ($r^2= 0.05$ and 0.04), but general tendency for larger eggs to have a higher survival (Fig.37).

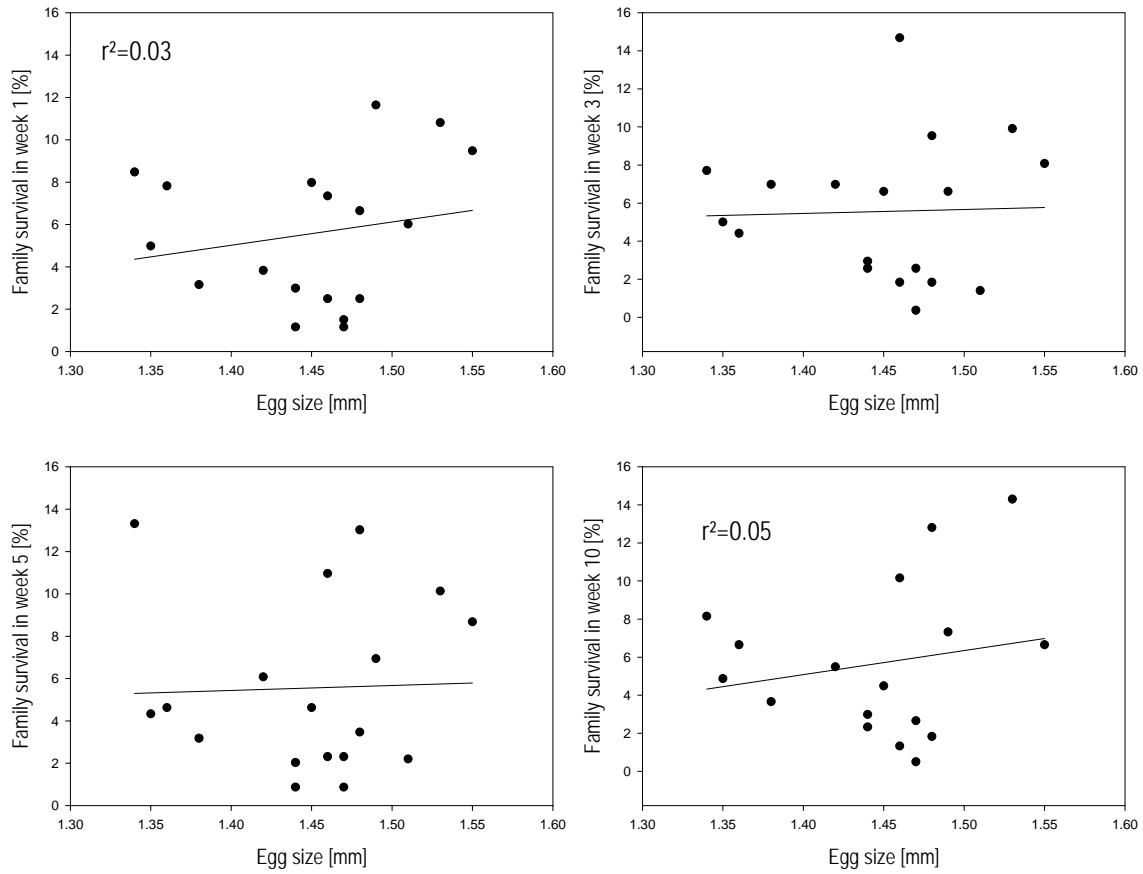


Figure 35. Relation between family survival and egg size at the sampling weeks 1, 3, 5 and 10 in the 2500m³ mesocosm experiment 2000. Note that there was no relation on weeks 3 and 5.

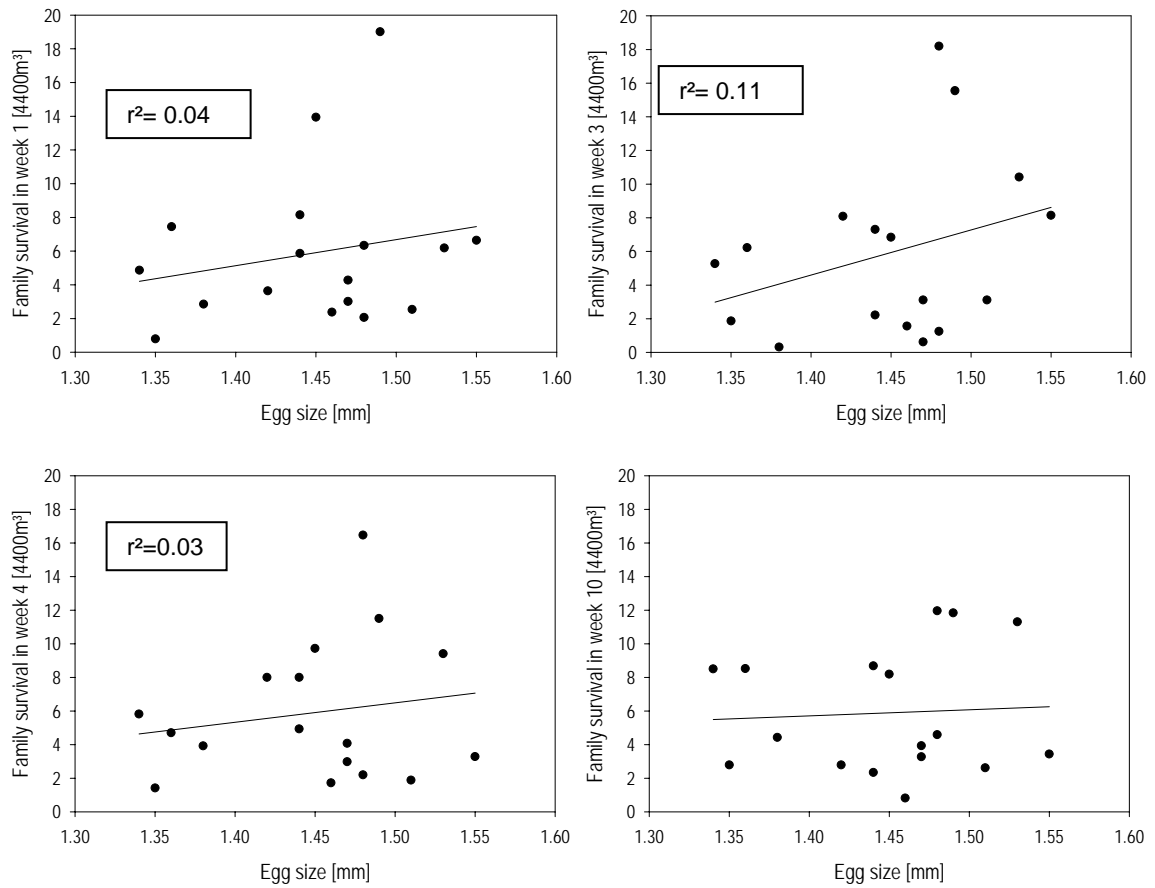


Figure 36. Relation between mean family survival in [%] and egg size at the sampling weeks 1, 3, 4 and 10 (no relation) in the 4400m³ mesocosm experiment 2000.

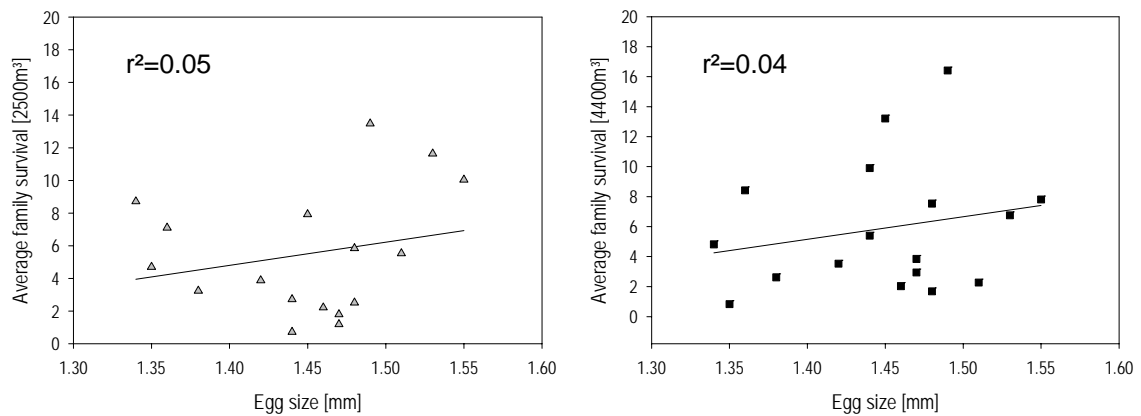


Figure 37. On the left: Triangles in gray represent the relation between egg size and mean survival of families in [%] during sampling weeks 1, 3, 5 and 10 in the 2500 m³ mesocosm . On the right: Black squares represent the same relation for weeks 1, 3, 4 and 10 in the 4400 m³ mesocosm.

The offspring's mean standard length (SL) was significantly positively related to egg size during all mesocosm periods ($p=0.000$), with exception of week 10 in the large mesocosm (Fig. 38). Although egg sizes already gave an indirect indication of the influence of maternal size on offspring size during the mesocosm period, a positive relation between maternal size and offspring size was again obtained for all sampling weeks, with exception of week 10 in the large mesocosm. However, the relation between maternal and offspring size was stronger in the small mesocosm (Fig.39).

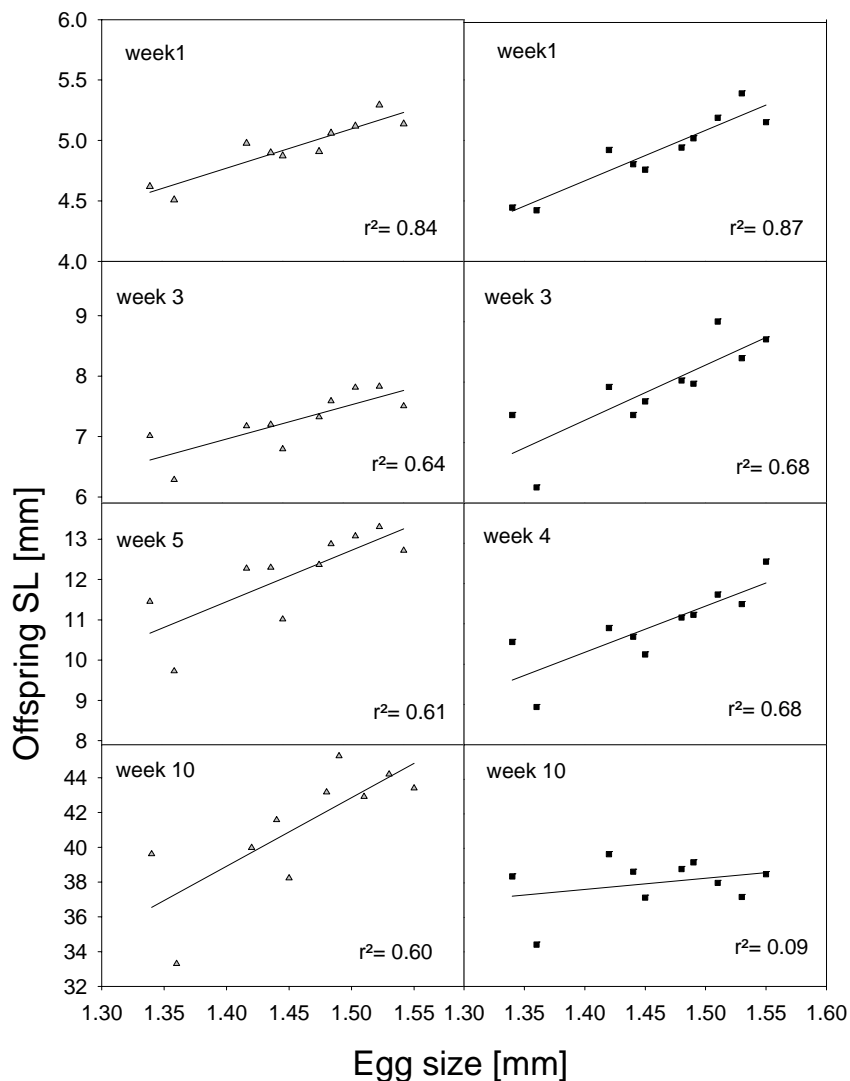


Figure 38. Relation between offspring SL and egg size in the different sampling weeks. Each symbol gives the mean between a certain family offspring size and the respective egg size mean. On the left: triangles in gray show results from the 2500m³ mesocosm. On the right: black squares indicate results from the 4400m³ mesocosm.

Therefore, one can conclude that individuals from large mothers have grown larger in all sampled weeks, with exception of the last period in the 4400m³. Larval RNA/DNA ratios were not related to female size or weight in any of the sampling weeks.

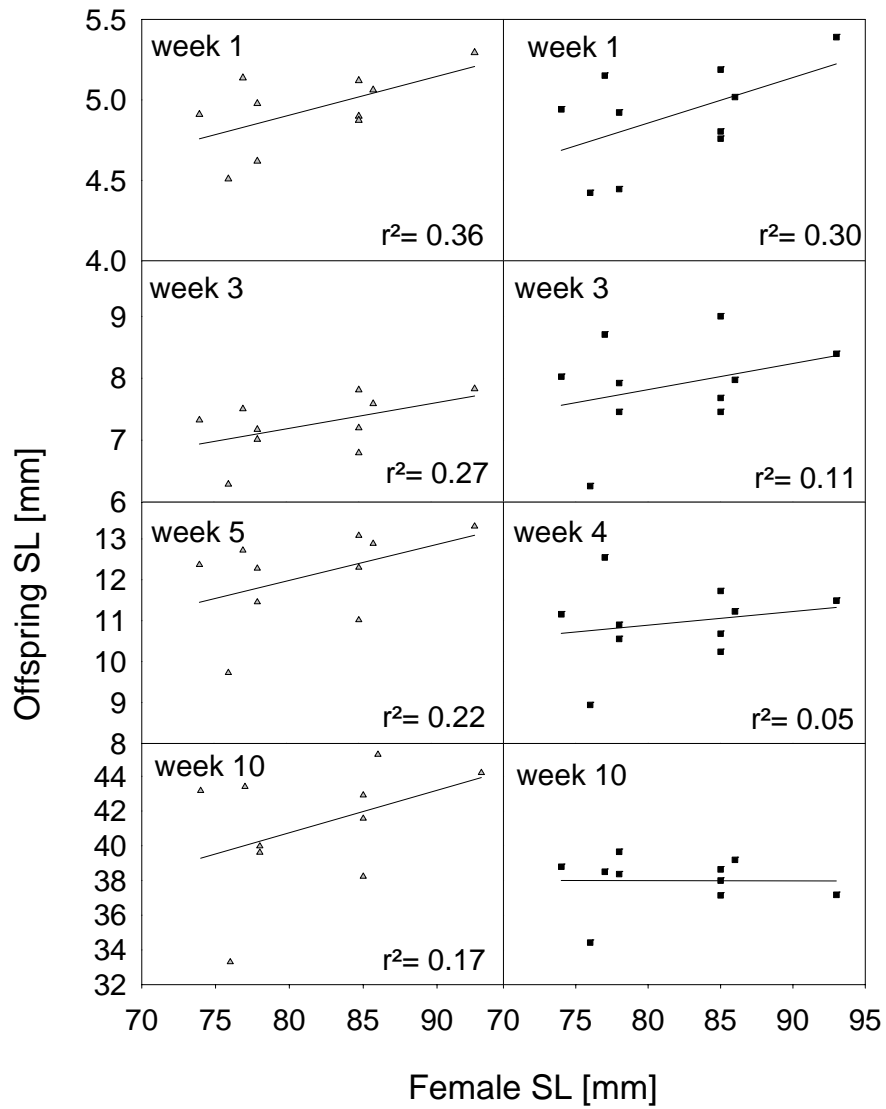


Figure 39. Relation between maternal size and mean offspring SL in the different sampling weeks. On the left: triangles in gray indicate results for the 2500m³ mesocosm. On the right: black squares indicate results for the 4400m³ mesocosm.

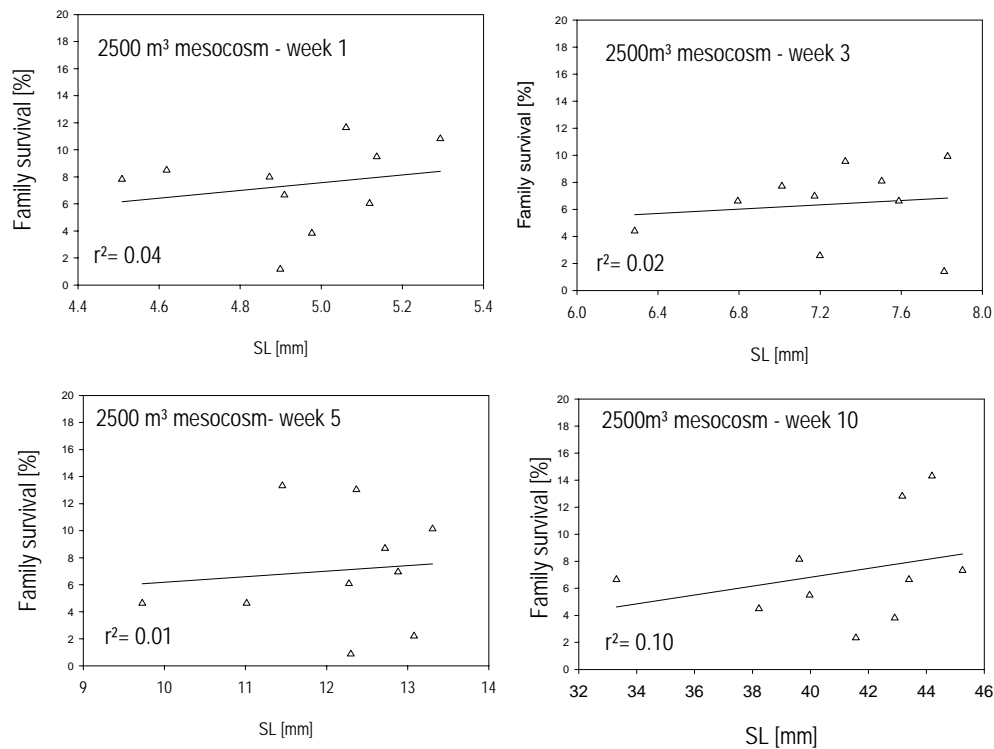


Figure 40. Relation between family survival [%] and mean offspring size during sampling weeks 1, 3, 5, and 10 in the 2500m³ mesocosm.

Concerning the influence of size on survival, it was already observed that large egg sizes had a slightly positive influence on survival. However, the relation between offspring size and survival in the small mesocosm was rather poor (r^2 ranged from 0.01 to 0.10) (Fig.40); and in the large mesocosm there was no relation at all. At week 10 the relation was even slightly negative (Fig.41). This confirmed the trend shown by the Shannon evenness, and indicated that small individuals were present in the large mesocosm all time, while in the small mesocosm a slight size selection might have taken place. Nevertheless, individuals from small eggs (eg. families 1b and 6b) were present in both mesocosms until the end of the mesocosm experiment, on week 10.

A rapid growth is generally accepted as an advantage for Gadoids, since those individuals are able to reach metamorphosis and settle to life near bottom earlier. Analysis of Specific growth rate (SGR) has shown that offspring from larger eggs, and therefore from larger mothers, grew faster than offspring from small eggs during all sampling dates in the small mesocosm (Fig.42) and in week 1, 3 and 4 in the large mesocosm. However, on week 10 the trend inverted and offspring from large eggs had grown slower (Fig.43).

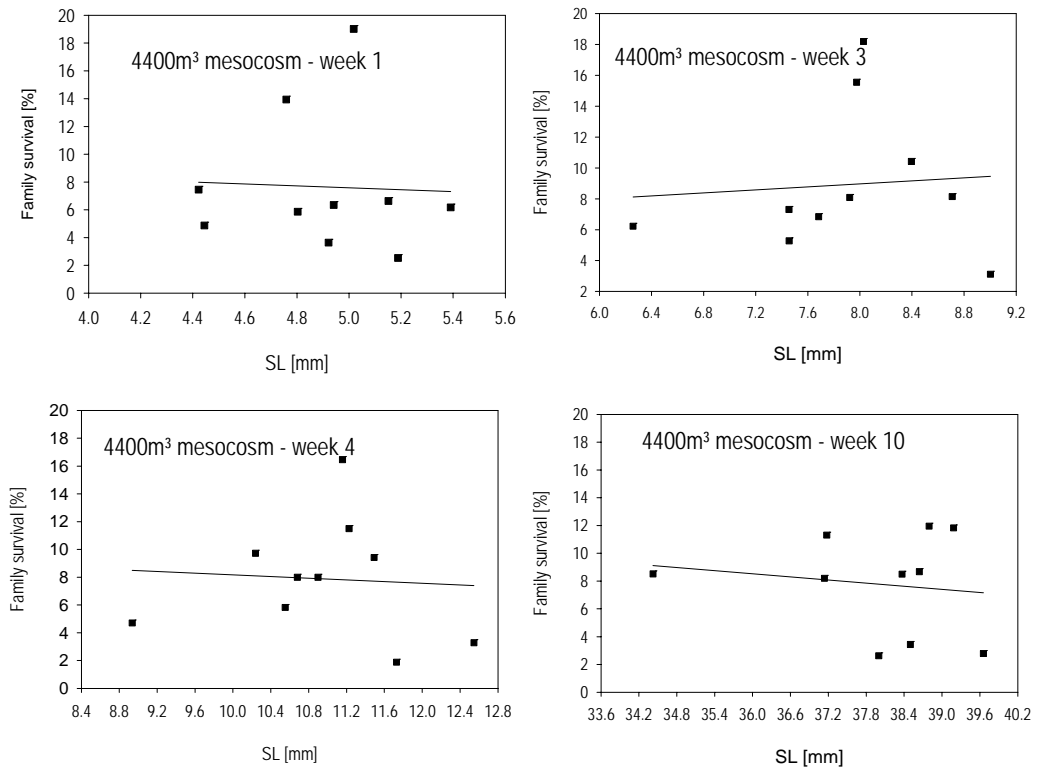


Figure 41. Relation between family survival [%] and mean offspring size during sampling weeks in the 4400m³ mesocosm.

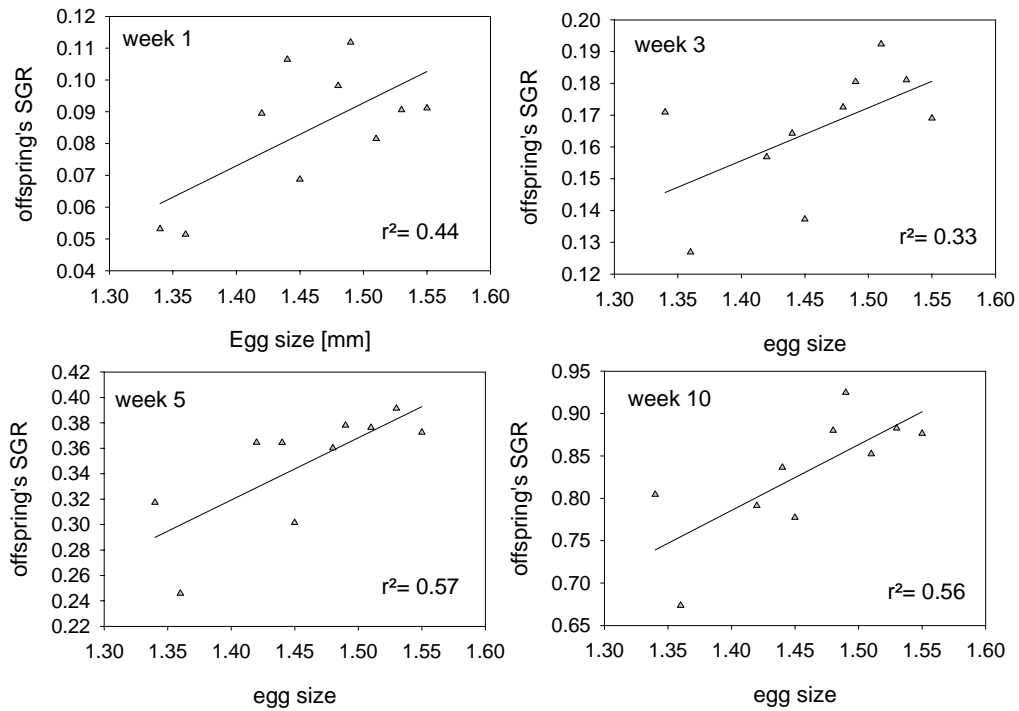


Figure 42. Relationship between egg size (mm) and offspring's Specific Growth Rate (SGR) in the 2500m³ mesocosm.

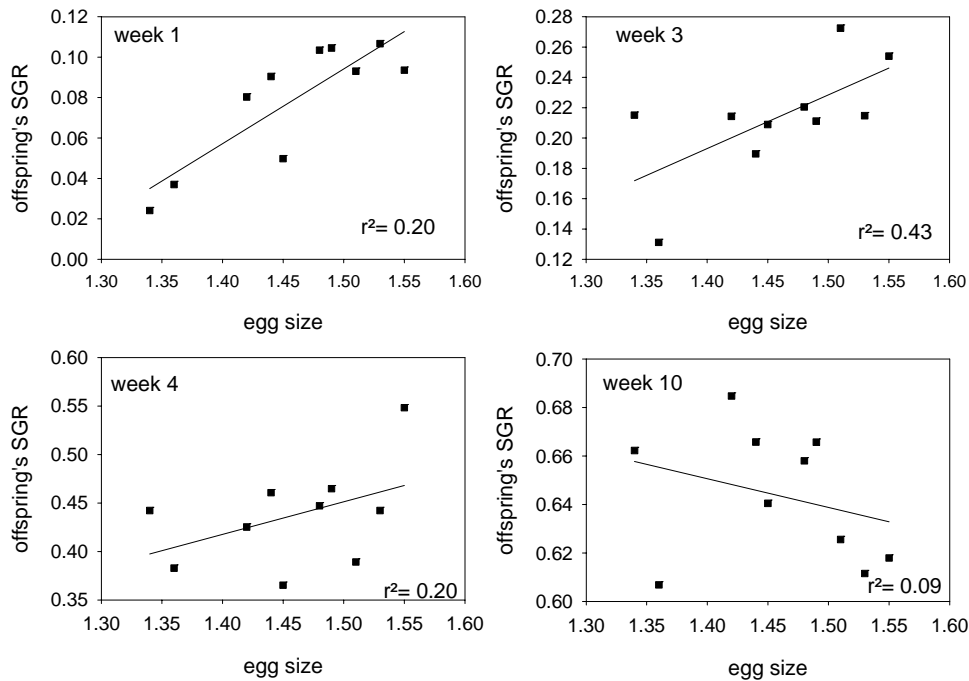


Figure 43. Relationship between egg size (mm) and offspring's Specific Growth Rate (SGR) in the 4400m³ mesocosm.

3.4.3 Analysis of Growth and Condition

Offspring growth, weight and condition were classified according to environment and spawning experience (Fig.44). Newly-hatched larvae from first spawners were significantly larger, heavier and better conditioned than offspring from repeat spawners (Table 3a, 3b and 3c). During week 1, 3 and 4 larvae from the 4400m³ mesocosm were heavier, larger and better conditioned (RNA/DNA ratio). After that, the data suggested that by middle of May the situation reversed and at week 10, individuals from the 2500m³ mesocosm ended up to be larger, heavier and better conditioned than those from the 4400m³ mesocosm. Additionally, offspring from first spawners, independently of environment, was always better conditioned than from repeat spawners. Nevertheless, the difference between mesocosms was larger than between spawning groups (Fig.44).

In addition, there was a significant difference among families. Table 4. indicates the sample sizes (N) used in all plots for biochemical and otolith analysis.

Concerning standard length and weight, offspring from large eggs maintained their trend of being larger and heavier from the beginning to the end of the mesocosm period (e.g family 6c, 8b, 8c). However, in the 4400m³ mesocosm, the differences of size and weight have decreased towards the end of the period, while in the 2500m³ mesocosm, they were rather intensified (Fig. 45a and Fig. 45b). Looking at the mean standard length (SD) of first spawners (recruit) in the 2500m³ mesocosm (Fig.45a, upper left), it was possible to observe that offspring from large eggs (8b and 6c)

ended up larger than that from small eggs (7c and 3b). The same occurred for offspring from repeat spawners (Fig.45a, upper right). Individuals from large eggs (8c and 6a) ended up larger than those from small eggs (1b), with exception of 6b, which has grown quite well, although it came from the smallest eggs. Individuals from large eggs ended up heavier in the 2500m³ mesocosm.

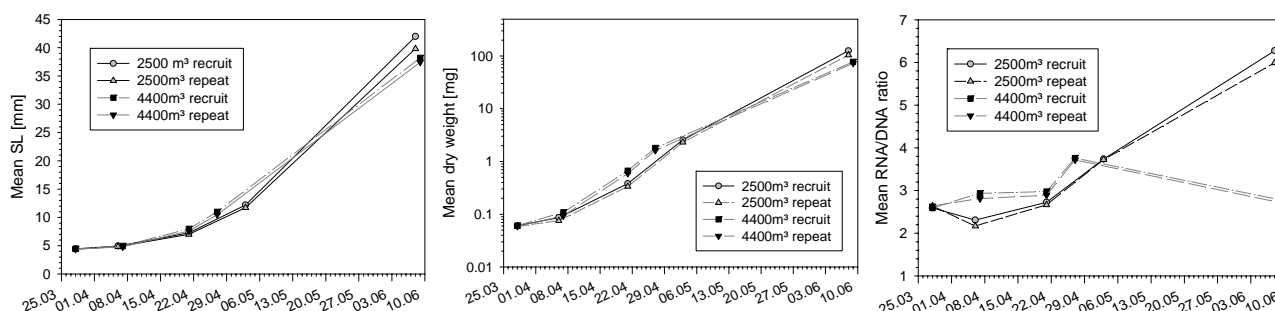


Figure 44: Comparison of standard length, dry weight and RNA/DNA ratio of cod larvae and juveniles from recruit spawners and repeat spawners reared in the mesocosms. Data points indicate mean values. For 2500m³ mesocosm, gray circles and gray triangles represent the offspring from recruit and repeat spawners, respectively. For 4400m³ mesocosm, black squares and black inverted triangles represent the offspring from recruit and repeat spawners, respectively. Figure adapted from C. Clemmesen. Note that first spawners are called "recruit".

Table 3a. Comparison of mean values of standard length (SL) between recruit (first spawners) and repeat spawners offspring. Significance was tested by Student's t-test and significant differences are highlighted in bold.

Sample	spawning group/ mesocosm	Mean SL [mm]	
		2500m ³	4400m ³
newly-hatched	recruit	4.44 (N=252,SD= 0.37)	
	repeat	4.36 (N=229,SD= 0.28)	
		P= 0.002	
week 1	recruit	4.95 (N=352,SD= 0.47)	4.94 (N=273,SD=0.55)
	repeat	4.84 (N=241,SD= 0.41)	4.81 (N=322,SD=0.53)
		P=0.0036	P= 0.0026
week 3	recruit	7.34 (N=123,SD=0.78)	8.06 (N=288,SD= 0.85)
	repeat	7.00 (N=98,SD=0.76)	7.59 (N=274,SD= 0.88)
		P= 0.006	P= 0.0000
week 4	recruit		11.09 (N= 286,SD= 0.11)
	repeat		10.49 (N=290,SD= 1.29)
			P=0.0000
week 5	recruit	12.39 (N=174,SD= 1.61)	
	repeat	11.62 (N=142,SD=1.50)	
		P= 0.0008	
week 10	recruit	42.80 (N=168,SD=5.30)	38.38 (N=138,SD=1.88)
	repeat	41.00 (N=125,SD=5.83)	37.57 (N=159,SD=2.49)
		P= 0.0000	P= 0.0000

Table 3b. Comparison of mean values of dry weight (DW) between recruit (first spawners) and repeat spawners offspring. Significance was tested by Student's t-test and significant differences are highlighted in bold.

Sample	spawning group/ mesocosm	Mean DW [mg]	
		2500m ³	4400m ³
newly-hatched	recruit	0.061 (N=232,SD= 0.01)	
	repeat	0.059 (N=249,SD=0.01)	
		P=0.023	
week 1	recruit	0.097 (N=281,SD=0.024)	0.10 (N=273,SD=0.04)
	repeat	0.076 (N=241,SD=0.020)	0.09 (N=322,SD=0.03)
		P= 0.0000	P= 0.0000
week 3	recruit	0.400 (N=123,SD=0.13)	0.67 (N=338,SD= 0.17)
	repeat	0.336 (N= 102,SD=0.12)	0.59 (N=274,SD=0.17)
		P=0.0001	P= 0.0075
week 4	recruit		1.81 (N=286,SD=0.5)
	repeat		1.62 (N=290,SD=0.6)
			P= 0.0000
week 5	recruit	2.71 (N=174,SD=0.8)	
	repeat	2.22 (N=110,SD=0.8)	
		P= 0.0000	
week 10	recruit	132.46 (N=168,SD=59.7)	76.6 (N=138,SD=14)
	repeat	113.7 (N= 125,SD=48.8)	72.6 (N= 159,SD=13.8)
		P= 0.004	P= 0.013

Table 3c. Comparison of mean values of RNA/DNA ratios between recruit (first spawners) and repeat spawners offspring. Significance was tested by Student's t-test and significant differences are highlighted in bold.

Sample	spawning group/ mesocosm	Mean RNA/DNA ratio	
		2500m ³	4400m ³
newly-hatched	recruit	2.59 (N=232,SD= 0.67)	
	repeat	2.64 (N=249,SD= 0.51)	
		<i>P= 0.67</i>	
week 1	recruit	2.30 (N=252, SD= 0.49)	2.94 (N=273,SD=0.73)
	repeat	2.16 (N=241, SD= 0.50)	2.81 (N=322,SD=0.66)
		P= 0.0009	P=0.0009
week 3	recruit	2.75 (N=123,SD=0.52)	2.97 (N= 236,SD=0.91)
	repeat	2.67 (N=98,SD=0.91)	2.82 (N=274,SD=0.82)
		<i>P= 0.633</i>	<i>P= 0.303</i>
week 4	recruit		3.76 (N=331,SD=0.75)
	repeat		3.72 (N=258,SD=0.84)
			<i>P= 0.122</i>
week 5	recruit	3.74 (N=231,SD= 0.66)	
	repeat	3.73 (N=110,SD= 0.63)	
		<i>P= 0.472</i>	
week 10	recruit	6.08 (N=168,SD=1.71)	2.78 (N=259,SD=0.61)
	repeat	5.79 (N=125,SD=1.49)	2.72 (N=234,SD=0.87)
		P= 0.045	<i>P=0.377</i>

Table 4. Size of the samples (N) used for all plots concerning biochemistry and otolith data. For otoliths symbols represent (*lapillus*) and [*sagitta*] respectively.

Family	week 1		week 3		week 5		week 10	
	Biochem.	Otoliths	Biochem.	Otoliths	Biochem.	Otoliths	Biochem.	Otoliths
2500m ³ mes.								
1b	47	0	12	(8) [6]	16	(8) [3]	40	(8) [14]
1c	40	0	26	(14)[5]	45	(27) [4]	77	(14) [18]
2a	18	0	8	0	7	0	18	0
3b	23	0	19	(6) [8]	21	(8) [3]	33	(18) [18]
4a	15	0	5	0	8	0	8	0
5c	44	0	20	(8) [6]	22	(17) [3]	43	0
6a	7	0	7	0	3	0	14	(8) [8]
6b	51	0	21	(5) [6]	46	(29) [6]	49	(14) [16]
6c	65	0	27	(3)[10]	35	(25) [7]	86	(17) [19]
7a	7	0	1	0	3	0	3	0
7b	15	0	5	0	12	0	11	0
7c	19	0	19	0	11	0	22	0
8a	21	0	19	(6) [8]	18	(13) [3]	29	0 [4]
8b	57	0	22	(8) [8]	30	(25) [4]	40	(17) [17]
8c	70	0	18	(4) [6]	24	(23) [5]	44	0 [11]
9b	9	0	7	0	8	0	16	0
10a	19	0	2	0	4	0	12	0
10b	48	0	18	(7) [3]	16	(8) [9]	27	(11) [10]
Family	week 1		week 3		week 4		week 10	
4400m ³ mes.	Biochem.	Otoliths	Biochem.	Otoliths	Biochem.	Otoliths	Biochem.	Otoliths
1b	47	0	40	(12) [10]	30	(8) [8]	52	(17) [19]
1c	40	0	117	(15) [19]	105	(25) [23]	73	(15) [15]
2a	18	0	5	0	11	0	5	0
3b	23	0	52	(15) [8]	51	(13) [9]	17	(15) [15]
4a	15	0	10	0	11	0	5	0
5c	5	0	12	(5) [3]	9	(0) [2]	17	0
6a	37	0	47	(14) [11]	51	(16) [15]	53	(17) [18]
6b	29	0	32	(11) [5]	35	(21) [13]	49	(13) [16]
6c	39	0	67	(24) [18]	60	(18) [19]	69	(16) [19]
7a	27	0	4	0	26	0	24	0
7b	13	0	8	0	14	0	28	0
7c	18	0	2	0	25	0	27	0
8b	4	0	5	0	2	0	2	(2) [2]
8c	108	0	90	(14) [14]	66	(10) [12]	65	0
9b	19	0	20	0	19	0	20	0
10a	8	0	10	(2) [3]	6	(1) [3]	8	0
10b	88	0	44	(16) [10]	62	(19) [15]	50	(15) [10]

Analysis of nutritional condition (RNA/DNA ratios) has shown that offspring from large eggs were better conditioned than offspring from small eggs in the 2500m³ mesocosm (Fig. 45c, upper left and right). However, at week 10, the opposite occurred in the 4400m³ mesocosm, i.e., small egg's individuals (eg. Families 1b and 6b) were better conditioned than large individuals (eg. families 6c, 8b) (Fig. 45c, lower left and right). This indicated that large individuals were more sensitive or suffered more than the smaller individuals under the precarious feeding conditions and high temperature that characterized the 4400m³ mesocosm in the last two weeks. Hence, it became also clear why RNA/DNA ratios were not related to maternal size: offspring condition was induced by environmental changes.

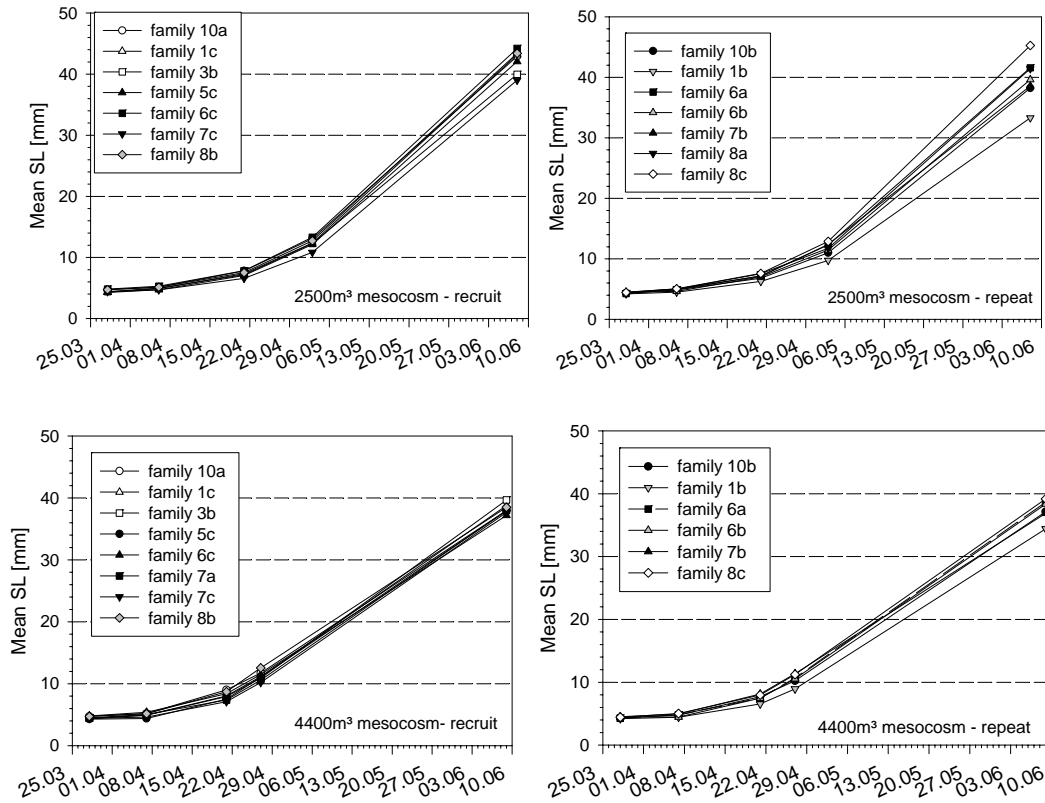


Figure 45a. Comparison of standard length among families. On the upper left: recruit spawner's offspring from the 2500m³ mesocosm. On the upper right: repeat spawner's offspring from the 2500m³ mesocosm. On the lower left: recruit spawner's offspring from the 4400m³ mesocosm. On the lower right: repeat spawner's offspring from the 4400m³ mesocosm.

Analysis of specific growth rate (SGR) has clearly shown that larger individuals (eg. Families 6c, 8b, and 8c) initially have grown faster in the 4400m³ mesocosm. However, increase in growth slowed down growth at the end of the period, while smaller individuals continuously increased growth rate. This aspect had minimized the initial differences between those groups and they have ended up with similar sizes (Fig. 46, right).

After the improvement of feeding conditions within the 2500m³ mesocosm, larger individuals caught up their growth, and ended up visibly larger than individuals from small eggs (Fig. 46, left).

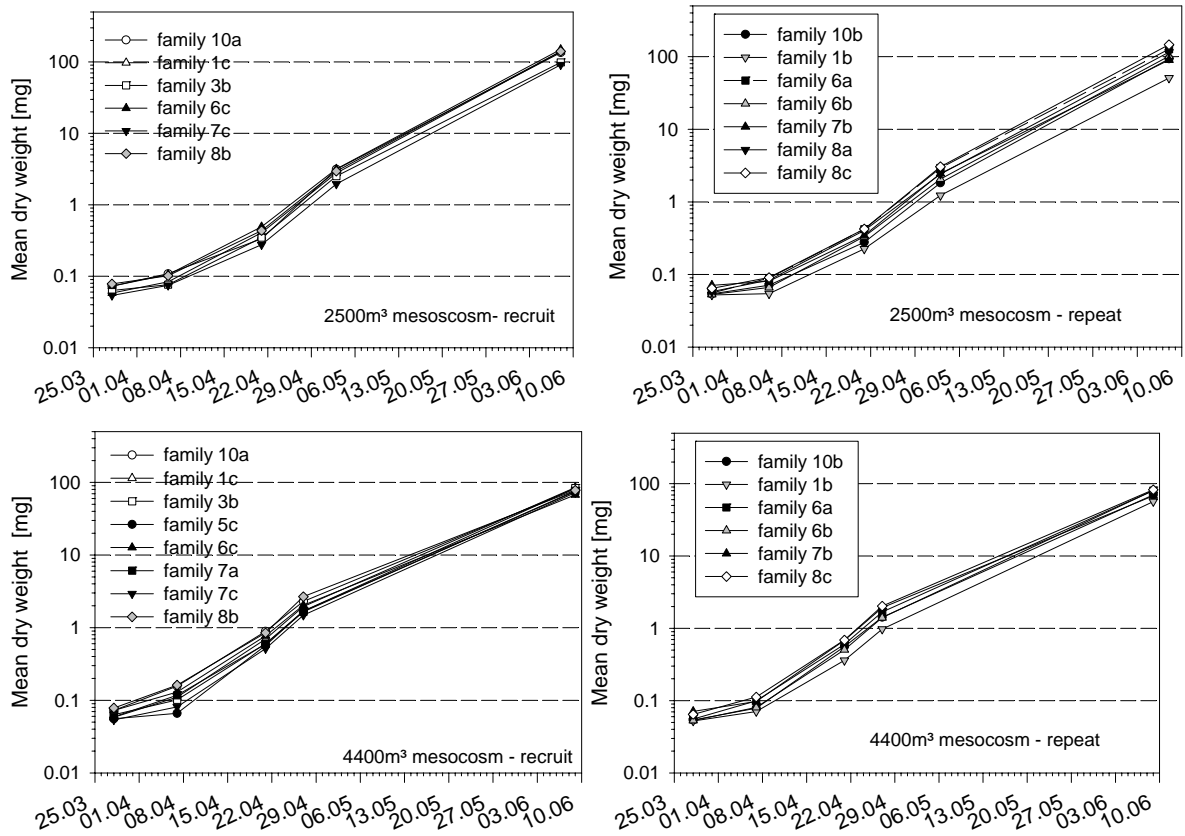


Figure 45b. Comparison of dry weight among families. On the upper left: recruit spawner's offspring from the 2500m³ mesocosm. On the upper right: repeat spawner's offspring from the 2500m³ mesocosm. On the lower left: recruit spawner's offspring from the 4400m³ mesocosm. On the lower right: repeat spawner's offspring from the 4400m³ mesocosm.

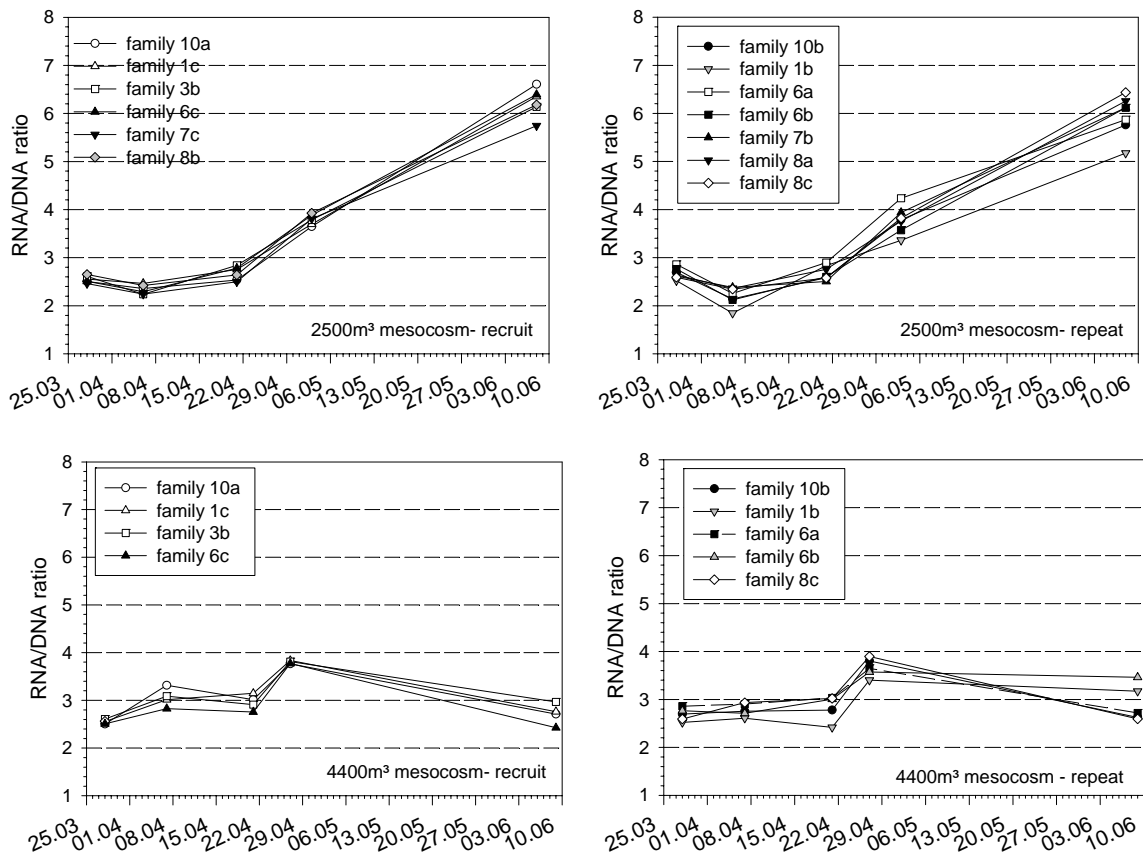


Figure 45c. Comparison of RNA/DNA ratio among families. On the upper left: recruit spawner's offspring from the 2500m³ mesocosm. On the upper right: repeat spawner's offspring from the 2500m³ mesocosm. On the lower left: recruit spawner's offspring from the 4400m³ mesocosm. On the lower right: repeat spawner's offspring from the 4400m³ mesocosm.

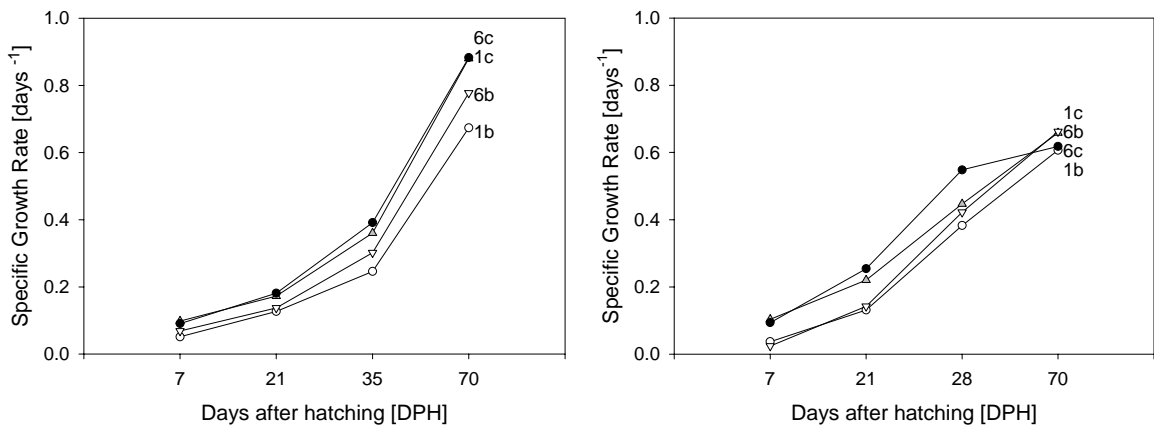


Figure 46. On the left: Mean Specific Growth Rates for the different families offspring in the 2500m³ mesocosm. On the right: Mean Specific Growth Rates for the different families offspring in the 4400m³ mesocosm. Note: to avoid superposition only some families are shown.

Otolith microstructure analysis revealed poor growth, with low variability for all families during the initial three weeks in the 2500m³ mesocosm (Fig.47a, 47b, 47c, 47d). As expected, growth was comparatively higher at week 3 in the 4400m³ mesocosm. However, the standard deviation was also much larger, indicating a large

variability in growth within individuals of this mesocosm. Additionally, slow and fast growing individuals were present within the same family. This was more evident in *sagittae*, because of their larger growth. Thus, in spite of the great feeding conditions observed in the initial weeks within the large mesocosm, the mean increment width was lowered down by the presence of slow growing individuals.

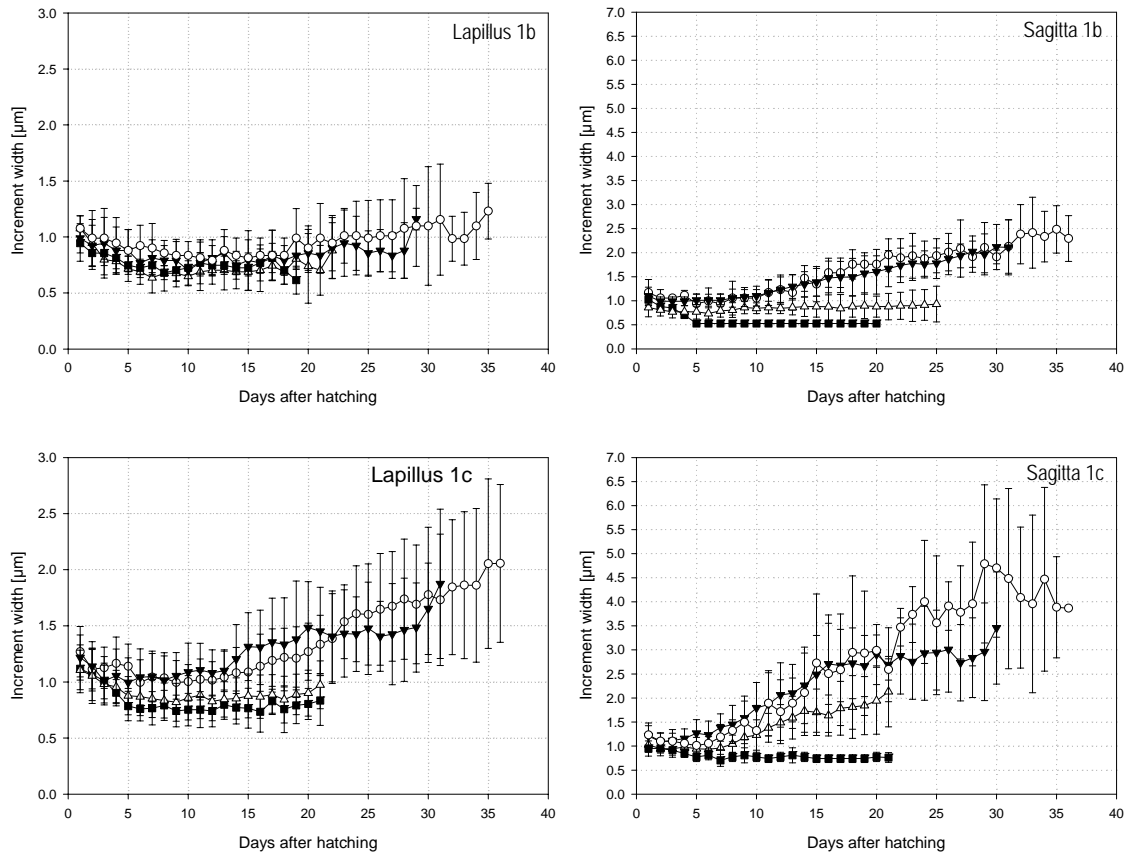


Figure 47a. Mean increment width at age from otoliths obtained from individuals of the same family sampled at weeks 3, 4 and 5 in the mesocosms. On the left: readings obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m³ mesocosm, respectively. White triangles and inverted black triangles indicate increment widths of week 3 and 4 of the 4400m³ mesocosm, respectively. Error bars give the standard deviation (SD) from the mean. Note that sagittal increments are wider than lapillar increments.

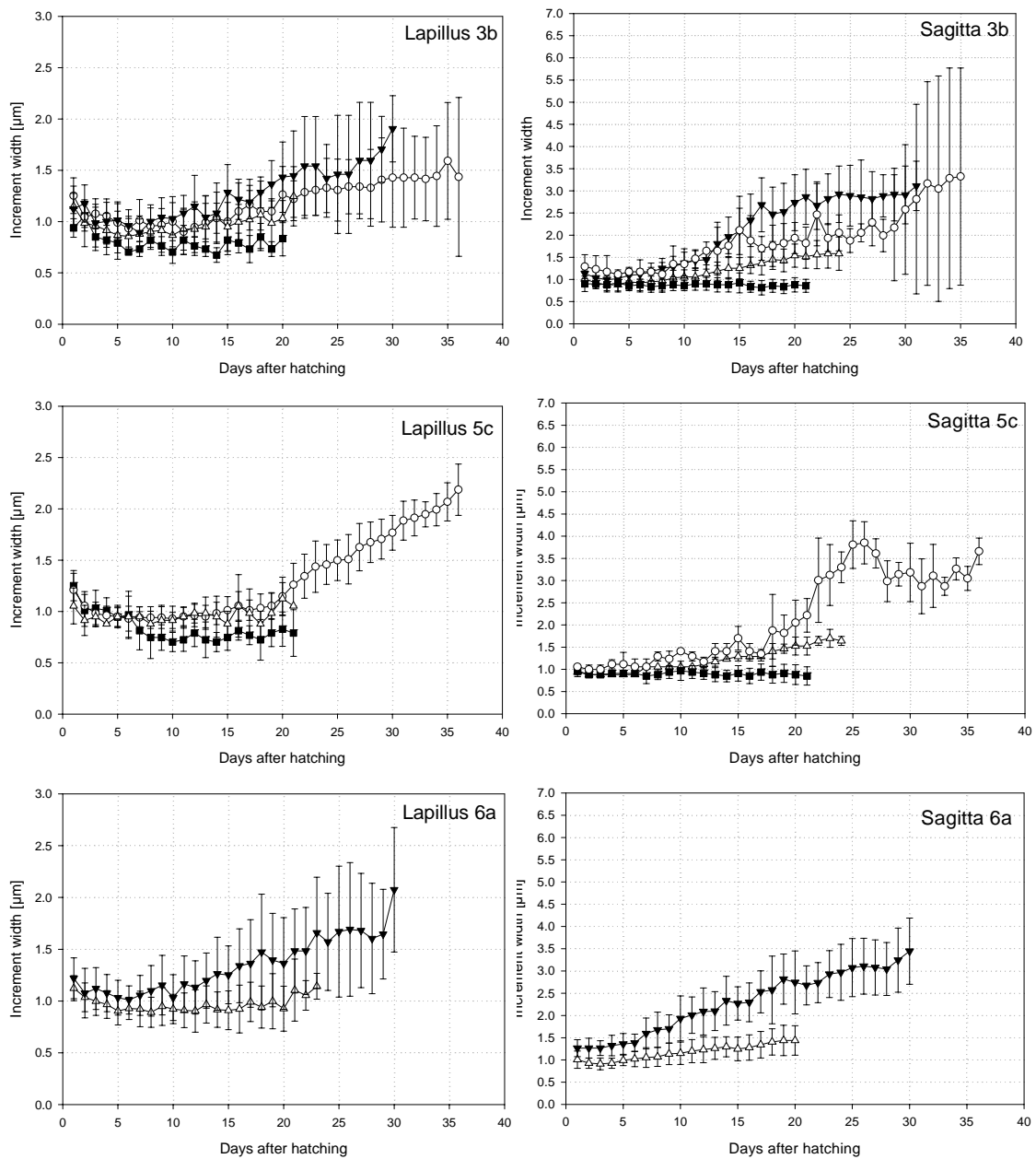


Figure 47b. Mean increment width at age from otoliths obtained from individuals of the same family sampled at weeks 3, 4 and 5 in the mesocosms. On the left: readings obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m³ mesocosm, respectively. White triangles and inverted black triangles indicate increment widths of week 3 and 4 of the 4400m³ mesocosm, respectively. Error bars give the standard deviation (SD) from the mean. Note that sagittal increments are wider than lapillar increments. Some families are missing in some sampling weeks.

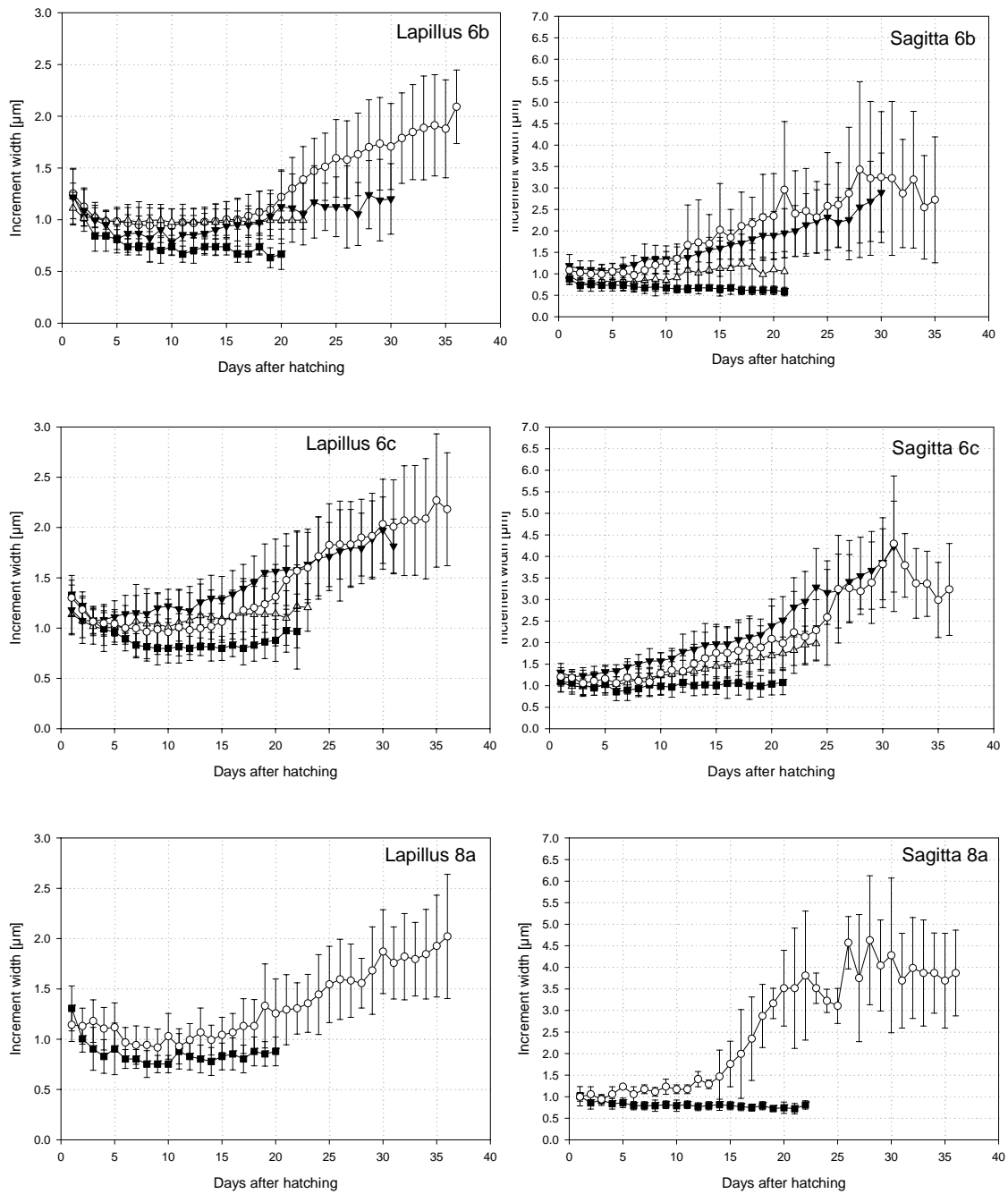


Figure 47c. Mean increment width at age from otoliths obtained from individuals of the same family sampled at weeks 3, 4 and 5 in the mesocosms. On the left: readings obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m³ mesocosm, respectively. White triangles and inverted black triangles indicate increment widths of week 3 and 4 of the 4400m³ mesocosm, respectively. Error bars give the standard deviation (SD) from the mean. Note that sagittal increments are wider than lapillar increments. Some families are missing in some sampling weeks.

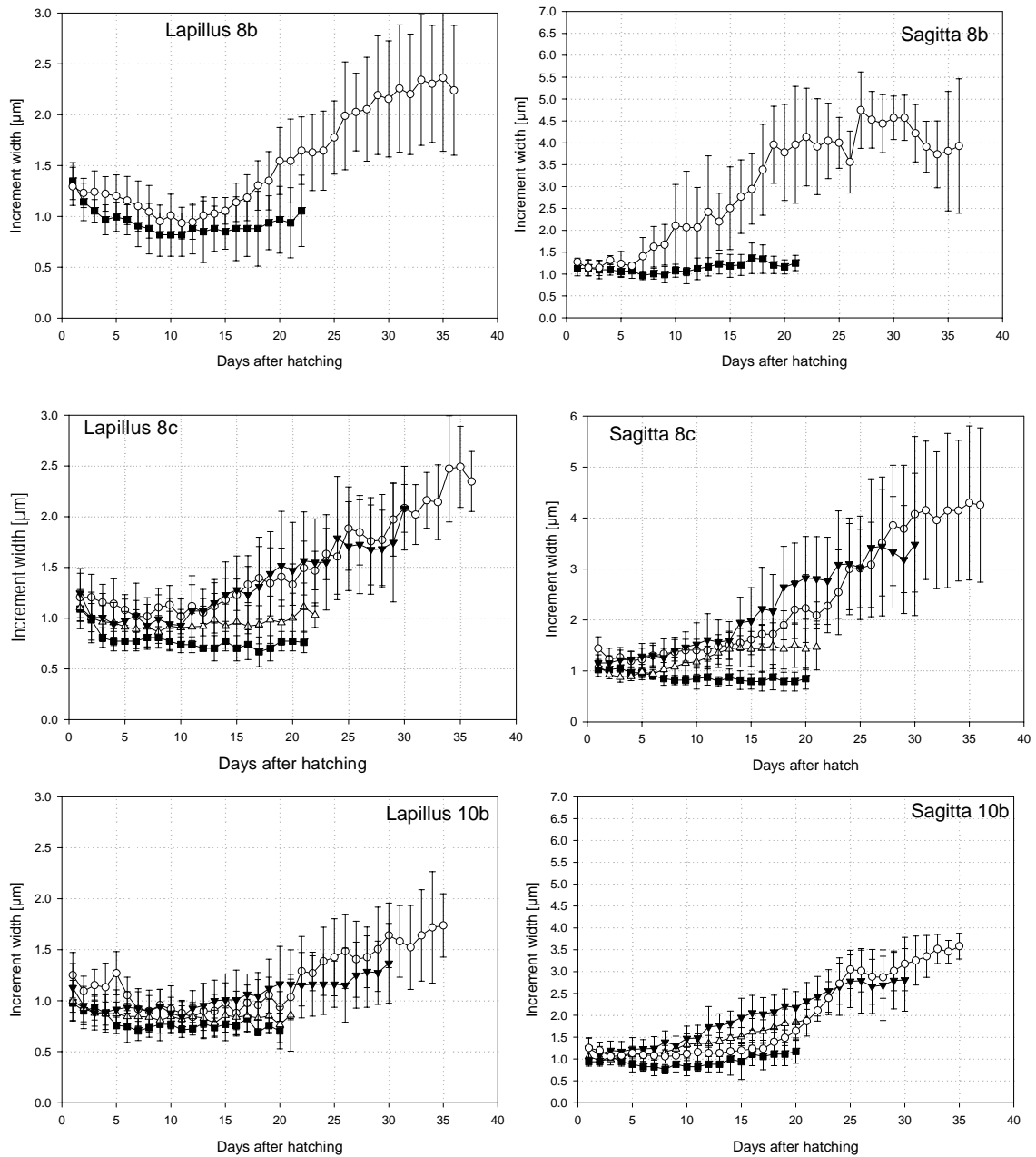


Figure 47d. Mean increment width at age from otoliths obtained from individuals of the same family sampled at weeks 3, 4 and 5 in the mesocosms. On the left: readings obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m³ mesocosm, respectively. White triangles and inverted black triangles indicate increment widths of week 3 and 4 of the 4400m³ mesocosm, respectively. Error bars give the standard deviation (SD) from the mean. Note that sagittal increments are wider than lapillar increments. Some families are missing in some sampling weeks.

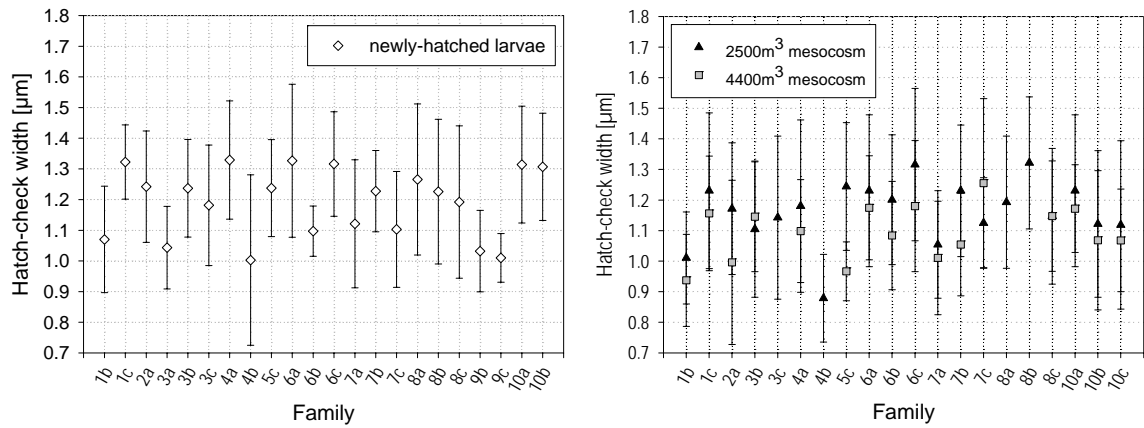


Figure 48. On the left: hatch-check widths observed in otoliths from newly-hatched larvae. On the right: Hatch-check widths observed in otoliths of larvae sampled at week 3. Gray squares represent the family means for the 2500m³ mesocosm, and black circles represent the means for the 4400m³ mesocosm. Error bars give the standard deviation from the mean.

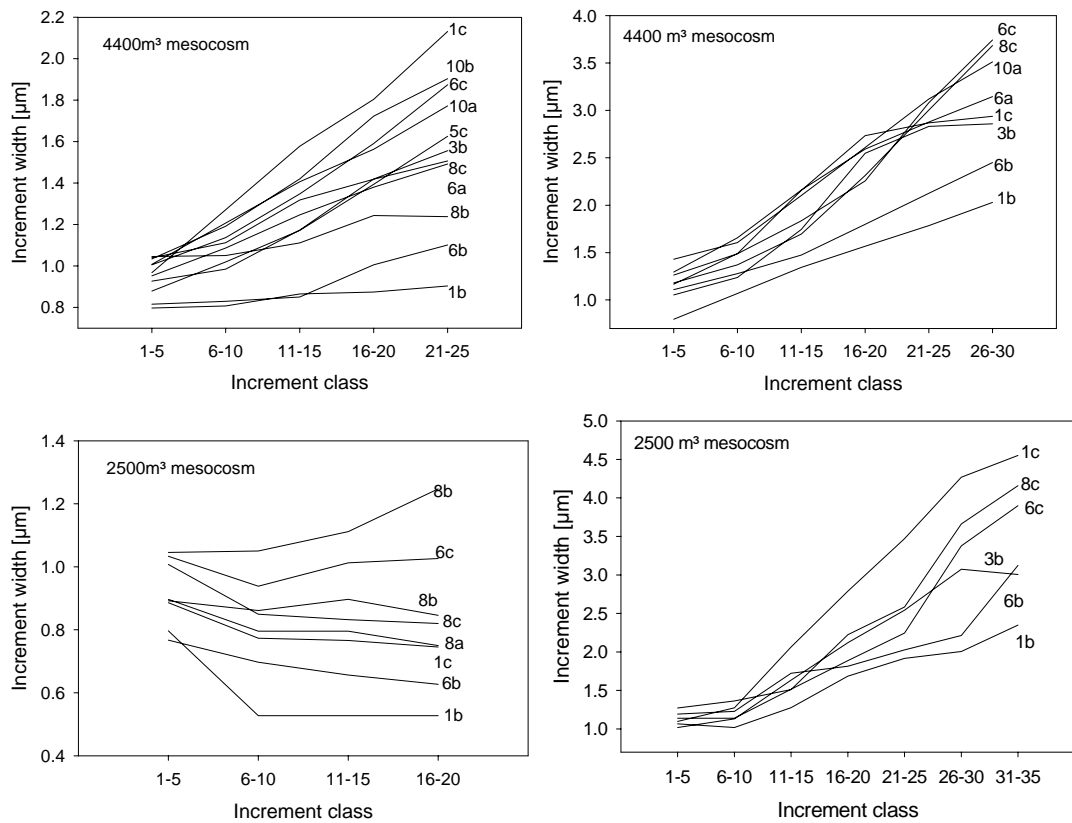


Figure 49. Comparison of mean increment width (in intervals of five increments) among families. On the upper left and right: offspring sampled in the 4400m³ mesocosm, during weeks 3 and 4 respectively. On the lower left and right: offspring sampled in the 2500m³ mesocosm, during weeks 3 and 5 respectively.

Increment width at increment number of otoliths sampled at week 5 were comparatively larger than from week 3. The same tendency was observed on week 4 in the 4400m³ mesocosm, indicating a selection towards wider increment widths, independently of environment, although this was more evident in the 2500 m³ mesocosm (Fig. 47 a,b,c,d).

Comparison of the hatch-check widths at newly-hatched stage and week 3, pointed out a tendency for wider hatch-checks in the 2500m³ mesocosm (Fig. 48), suggesting that individuals with larger hatch-checks might have an advantage under poor feeding conditions. Nevertheless, a large variation in increment width was observed for most of the families, implying that slow growing individuals were still present, even after initial selection.

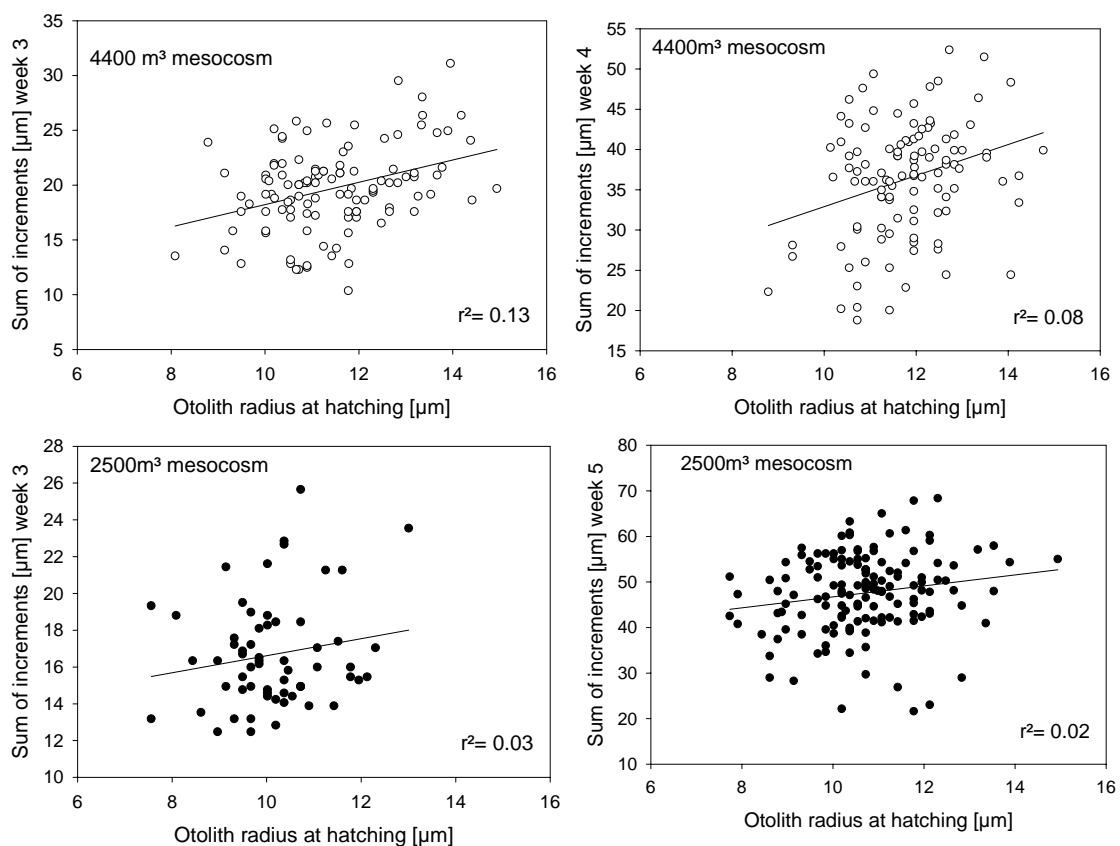


Figure 50. On the upper left and right: relation between otolith radius at hatching and sum of the increments at week 3 and 4 in the large mesocosm (white circles). On the lower left and right: relation between otolith radius at hatching and sum of the increments at week 3 and 5 in the small mesocosm (black circles).

When the sum of the increments of an otolith was plotted against its radius at hatching (Fig. 50) there was a trend for initially large otoliths to remain larger, however none of the relations was statistically significant (t-test, $p > 0.05$). In the 4400m³ mesocosm, certainly due to the environmental conditions, the small otolith radius at hatching had reached comparatively larger sizes than the same group in the 2500m³ mesocosm, and were also in general larger (Fig. 50 upper and low left).

The relation between hatch-check and otolith growth becomes clear when individuals from the same family and sample were plotted separately according to their hatch-check widths (Fig.51). Individuals with a smaller hatch-check had grown slower than those with a larger hatch-check, even within the same family.

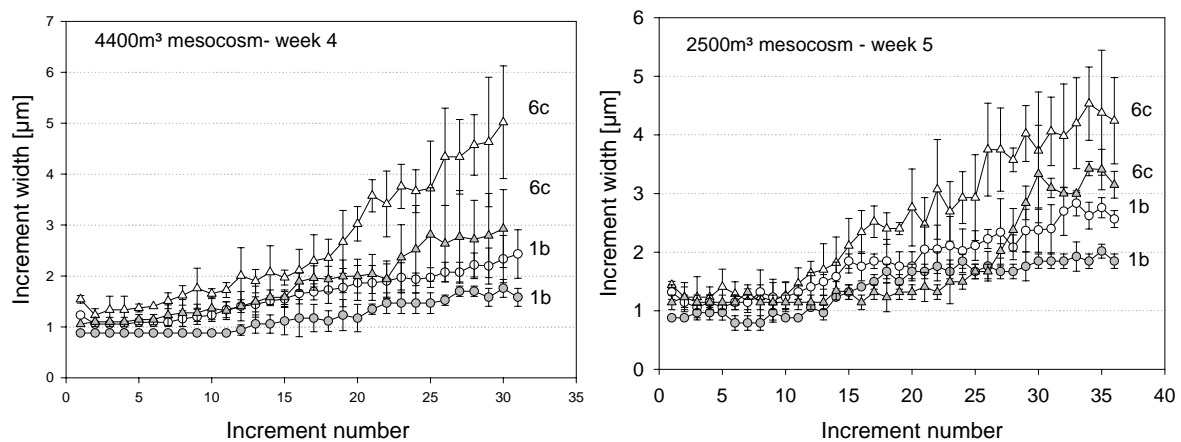


Figure 51. Example of otolith growth (*sagitta*) on dependence of hatch-check width. Gray circles indicate otolith growth (in increment widths) for small eggs' offspring (1b) and small hatch-check (0.87µm). White circles indicate otolith growth for small eggs' offspring (1b) and large hatch-check (1.23-1.41µm). Gray triangles indicate otolith growth (in increment widths) for large eggs' offspring (6c) and small hatch-check (1.05 µm). White triangles indicate otolith growth for large eggs' offspring (6c) and large hatch-check (1.45 µm). On the left: data from 4400m³ mesocosm at week 4. N= 3, 3, 5, 8 for 1b and 6c upwards. On the right: data from 2500m³ mesocosm at week 5. N= 2, 2, 3, 7 upwards.

In the 2500m³ mesocosm, otoliths from large individuals (e.g. families 6c, 8b and 8c) started comparatively larger. But, after four or five days, they decreased growth, maintaining the trend until middle of May, when they caught up growth, ending larger than their counterparts (eg. families 1b and 6b) (Fig. 52, left).

In the 4400m³ mesocosm, otoliths from large individuals started large and maintained this trend until the end of the period. However, the difference among families was not so accentuated as in the 2500m³ mesocosm (Fig. 52, right). Otoliths from small individuals (eg. families 1b and 6b) remained comparatively smaller, from the beginning until the end of the mesocosm period, but have reached larger sizes (Fig.52).

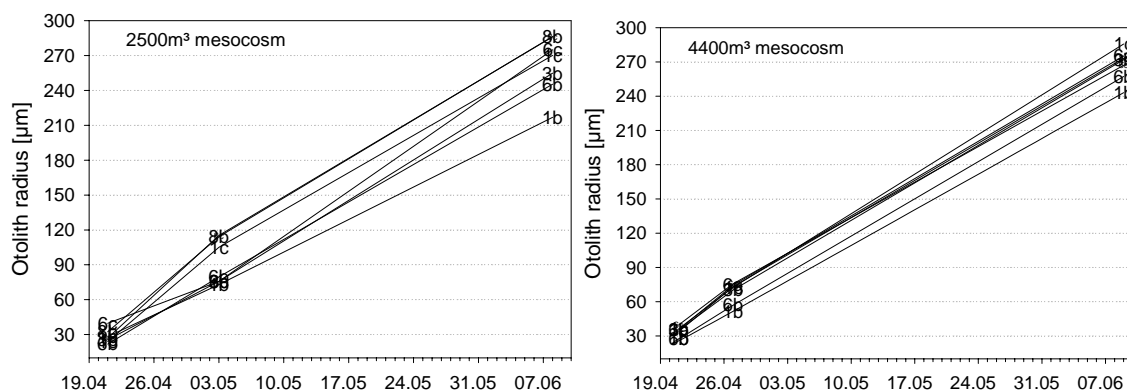


Figure 52. On the left: mean otolith radius of selected families during sampling weeks 3, 5 and 10 in the 2500m³ mesocosm. On the right: mean otolith radius of selected families during sampling weeks 3, 4 and in the 4400m³ mesocosm. Note: to avoid superposition only main families are shown.

In spite of the precarious feeding conditions in the last two weeks within the large mesocosm, the otoliths ended up larger than in the small mesocosm (Fig.53, left). Unfortunately, increment widths from week 10 are not available. Due to an agreement in MACOM they were responsibility of the Danish partner and only otolith radius was estimated. Those otoliths were grinded down and discarded, and therefore, the week 10 material could not be re-read by the author.

It's characteristic for cod otoliths that from the beginning of the juvenile stage onwards, *lapilli* grow slowly, while *sagittae* keep growing at higher rates. Thus, to confirm whether *Lapillus* had grown larger in the 4400m³ mesocosm than in the 2500m³, *sagittae* from the same individuals were weighted. The results validated the lapillar findings and confirmed that otoliths from 4400m³ were larger for all families, with exception of families 6c and 8c (Fig.53, right).

The fact that offspring in the 4400m³ mesocosm had grown smaller in body size at week 10, while otolith radius grew larger has characterized a decoupling between otolith and somatic growth for the large mesocosm individuals (Fig.54 right).

Comparison of fish weight and size at week 10 in both mesocosms (Fig.55 left), indicated that fishes from the 2500m³ mesocosm were much larger than those from 4400m³ mesocosm, and had a larger variability in sizes as already seen. However, those otoliths were smaller and better related to body size than in 4400m³ mesocosm (Fig. 55 right). The same was true for the relation between otolith weight and offspring weight at week 10 (Fig. 56).

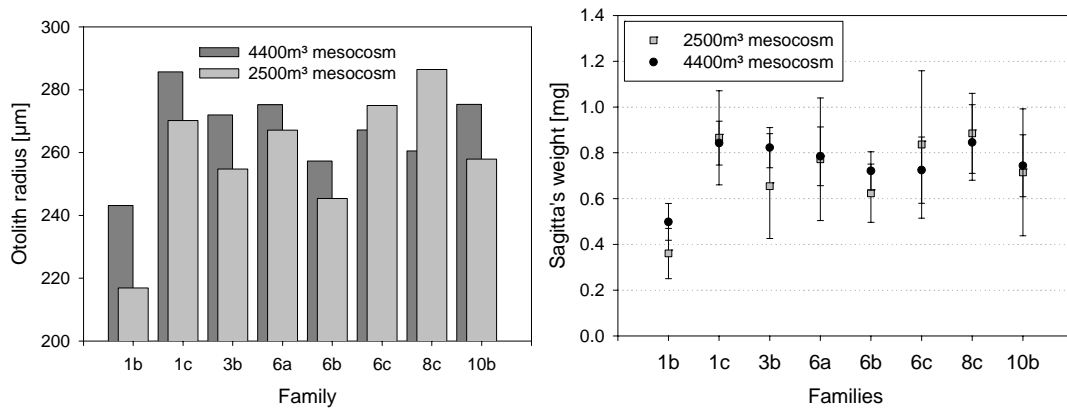


Figure 53. On the left: gray bars indicate the mean otolith radius (*Lapillus*) among families in the 2500 m³ mesocosm, while dark gray bars indicate the mean otolith radius (*Lapillus*) among families in the 4400 m³ mesocosm at week 10. On the right: otolith weight (Sagittae) from the same individuals used for *Lapillus* measurements.

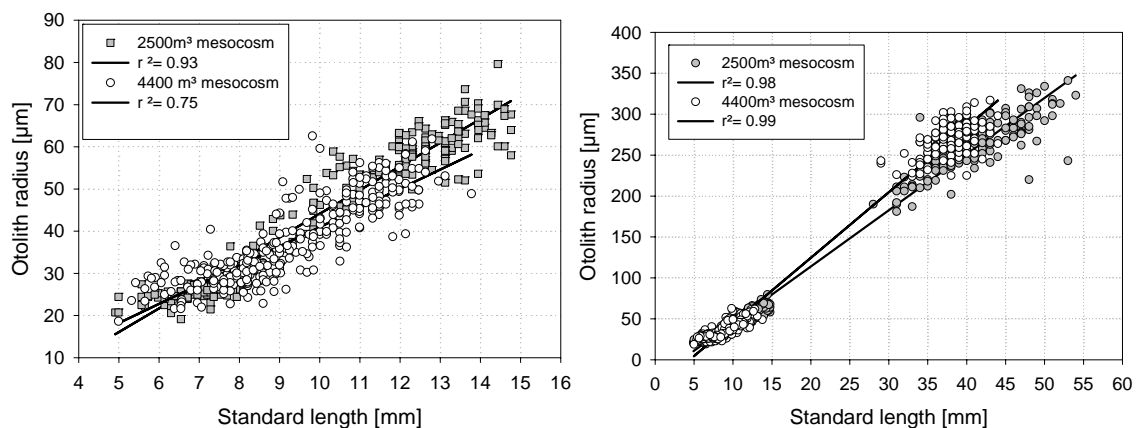


Figure 54. On the left: gray squares indicate the relation between otolith radius and body size for weeks 3 and 5 in the 2500m³ mesocosm. White circles indicate the relation between otolith radius and body size for weeks 3 and 4 in the 4400m³ mesocosm. On the right: the relation between otolith radius and body size for weeks 3, 4, 5 and 10 in the 2500m³ mesocosm (gray circles) and 4400m³ mesocosm (white circles).

When otolith radius was plotted against fish size (SL) for the individual families in the 2500m³ and 4400m³ mesocosm (Fig.57), data were quite bewildering. The best relation was observed for families 1b ($r^2 = 0.83$), 3b ($r^2 = 0.71$) and 6c ($r^2 = 0.64$) in the 2500m³ mesocosm.

The worst relations between otolith and body size were determined for families 3b ($r^2= 0.07$), which was even negative; 6b ($r^2= 0.03$) and 6c, which had no relation. In this last three families there were small individuals, which ended up with especially large otoliths. Mann-Whitney U-test was used for comparison of otolith and size differences between mesocosms, and has shown that the end size reached by families 1b and 3b was not significantly different ($p>0.05$), while otolith sizes were significantly different ($p< 0.05$). For families 1c, 6b, 6c and 8c body size was significantly different ($p>0.05$) between mesocosms, while the otolith size was not.

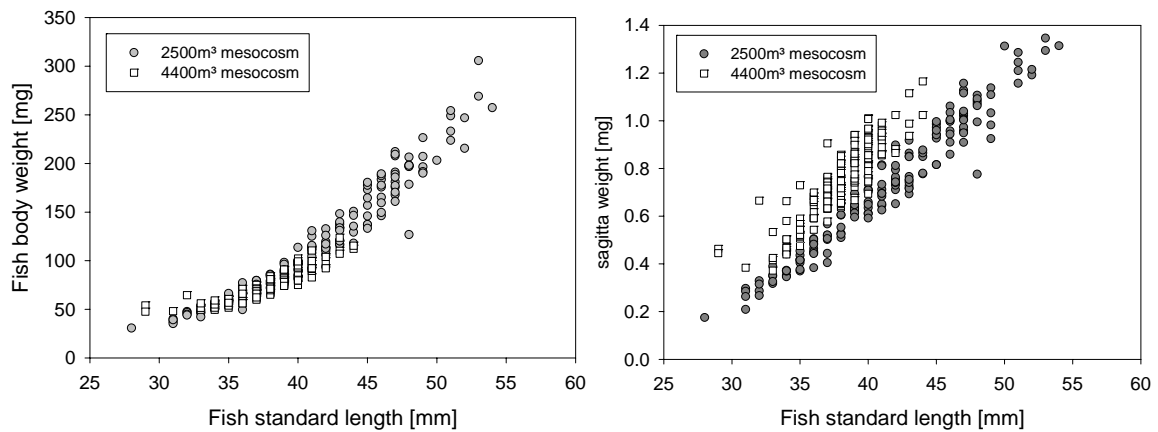


Figure 55. On the left: relation between fish weight and size in the 2500m³ (gray circles) and 4400m³ (white squares) mesocosm. On the right: relation between otolith weight and fish size in the 2500m³ (gray circles) and 4400m³ (white squares).

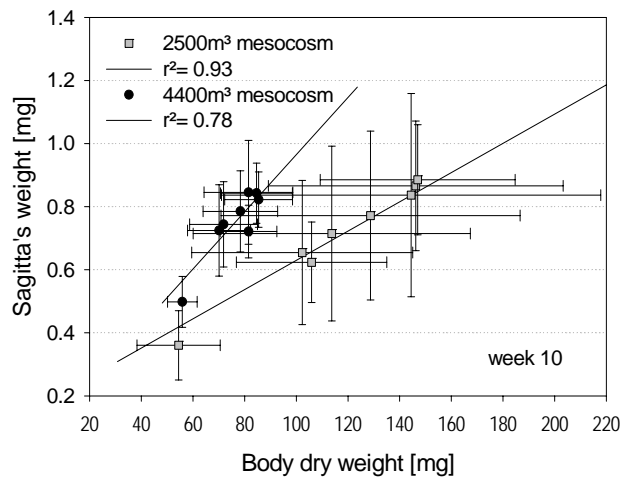


Figure 56. Bi-directional bars give the mean sagitta weight at mean body weight for the different families in the 2500m³ mesocosm (gray squares) and 4400m³ mesocosm (black circles). Error bars give the standard deviation from the mean.

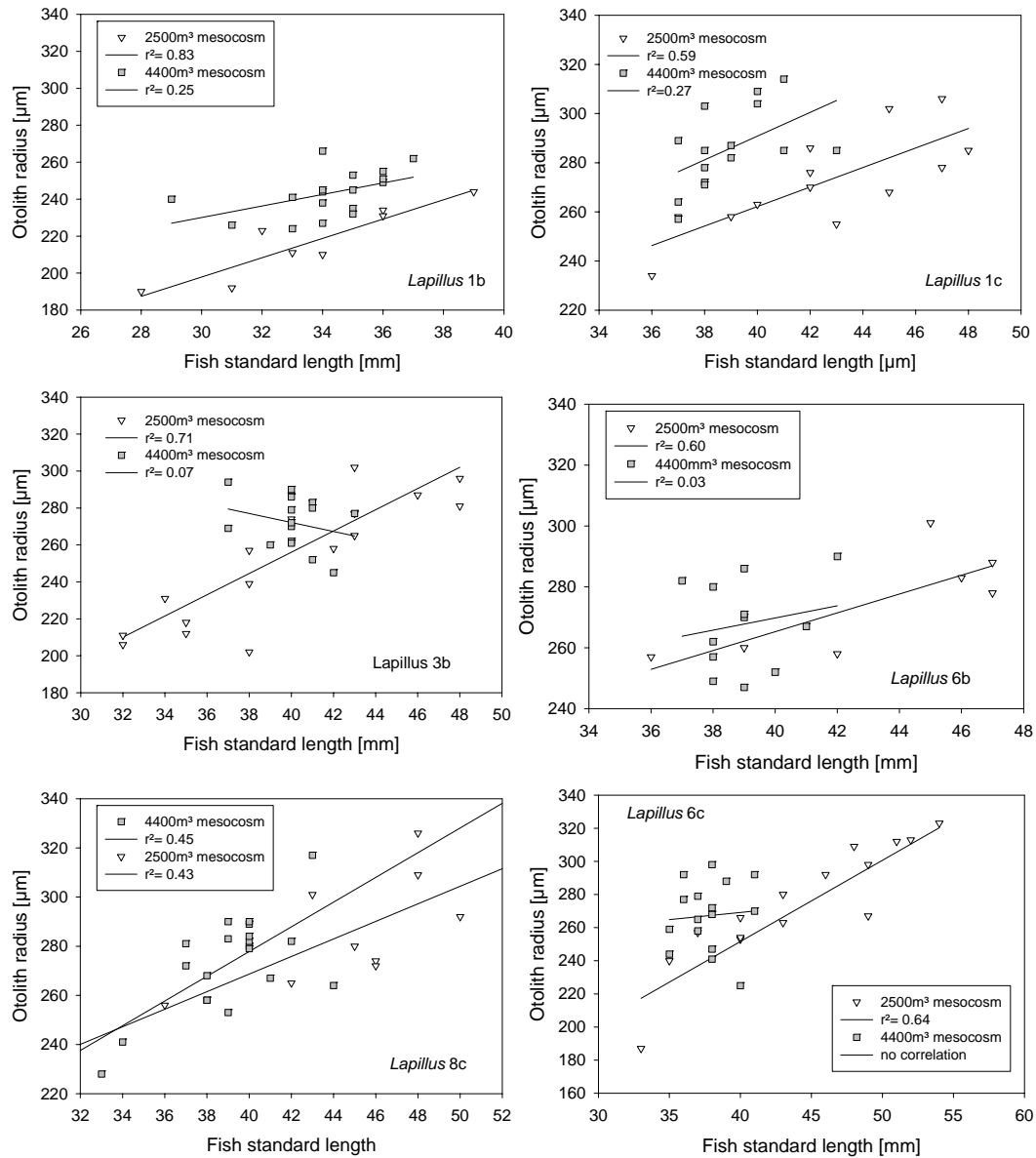


Figure 57. Relation between otolith radius (*Lapillus*) and fish body length. Each one of the gray squares gives the individual data between otolith and fish for the 4400m³, while inverted white triangles give the same relation for the 2500m³ mesocosm. Families are indicated in the graphs. From left upper to last lower figure on the right: 1b, 1c, 3b, 6b, 8c and 6c respectively. Note that family 10b is not shown because of the lower number of individuals.

Concluding, at the end of the mesocosm experiment (week 10), the standard length of individuals recovered in the 4400m³ mesocosm ranged from 34 to 40 mm, while in the 2500m³ it ranged from 32 to 46 mm (Fig.58). As already seen, variation in the 2500m³ was caused by the large difference in mean end sizes among families. Dry weight ranged between 57 and 97 mg in the 4000m³ mesocosm. However, the differences in weight were larger in the 2500m³ mesocosm, ranging from 49 to 178 mg (Fig.58 b).

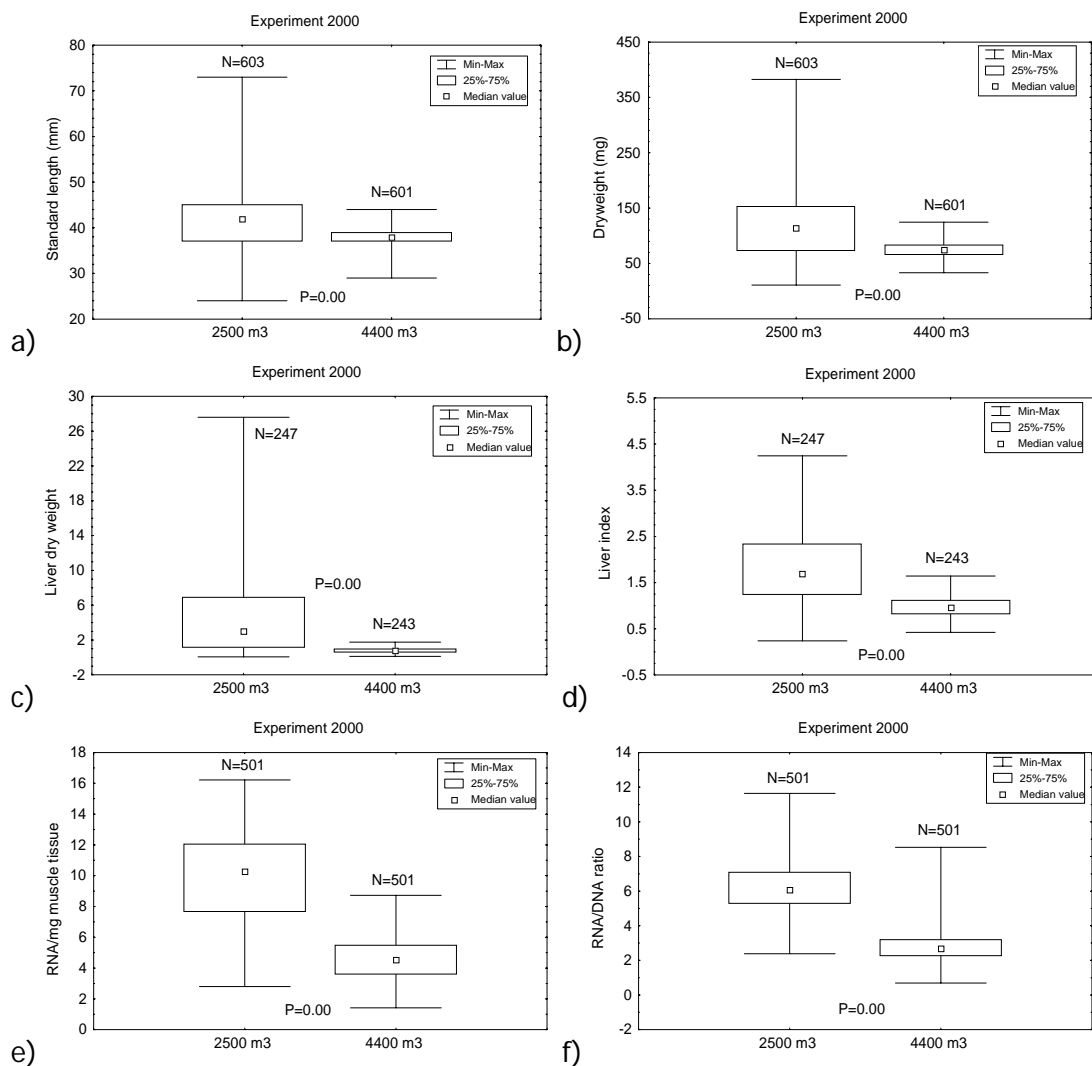


Figure 58. Comparison among individuals from 2500m³ and 4400m³ mesocosm. a) Standard length, b) dry-weight, c) liver weight, d) liver-index, e) RNA concentration in the muscle tissue, and f) RNA/DNA ratio. The box represents the interquartile range, while the bars denote the highest and lowest values and square indicates the median. Data were analysed by Mann-Whitney U-test. Plots from C. Clemmesen.

All measurements (standard length, dry weight, liver weight, liver index, RNA and RNA/DNA ratio) indicated that individuals from the 2500m³ mesocosm were significantly larger, heavier and in better nutritional condition than those from the 4400m³ mesocosm (Fig.58), reflecting the variation in feeding conditions observed between the mesocosms.

In this study, the activity of the glycolytic enzymes lactate dehydrogenase (LDH), and pyruvate kinase (PK) were used as a sensitive proxy for growth and condition. The PK activity has shown a clear response, and was significantly higher for individuals from the 2500m³ mesocosm, reflecting their higher nutritional condition (Fig.59, right). In addition, RNA/DNA ratio and PK enzyme activity determined for the same individual, have shown that both indexes were be positively and significant related to each other ($r= 0.53$, $p=0.0000$). However, concerning the LDH activity, which is a measure of anaerobic metabolism, no significant difference was found between mesocosms (Fig. 59, left). RNA/DNA ratio and LDH were not related.

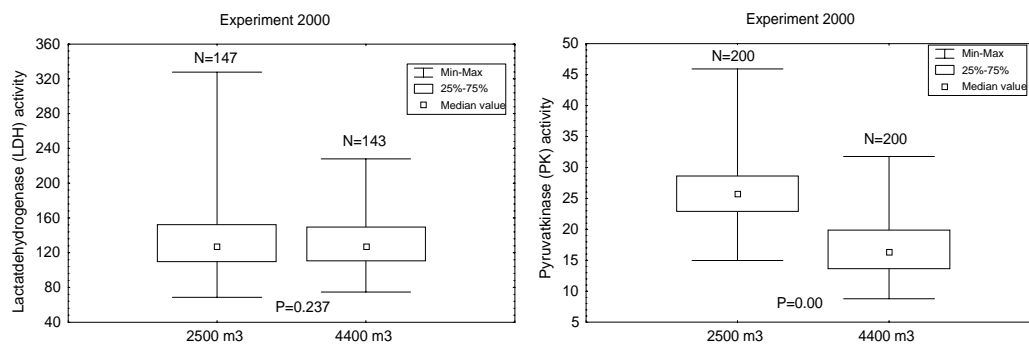


Figure 59. On the left: Comparison of lactate dehydrogenase activity between 2500m³ and 4400m³ at week 10 cod. On the right: Comparison of pyruvate kinase activity between 2500m³ and 4400m³ mesocosm at week 10 cod. The box represents the interquartile range, while the bars denote the highest and lowest values and square indicates the median. Data were analysed by Mann-Whitney U-test. Plots from C. Clemmesen.

Regarding spawning groups, offspring from first spawners have a significantly higher liver dry weight and liver index than those from repeat spawners, in both mesocosms at week 10 (Fig. 60 a,b). Offspring from first spawners have a significantly higher RNA content in their muscles than those from repeat spawners in the 2500m³ mesocosm, but not in the 4400m³ mesocosm (Fig. 60 c). The same was found for the RNA/DNA ratio (Fig. 60d). LDH activity was significantly higher on offspring from repeat than first spawners in both mesocosms, indicating that offspring from first spawners have invested more energy in metabolic processes than those from repeat spawners. Additionally, there was no significant difference in PK activity levels between offspring of the spawning groups (Fig. 60 e,f).

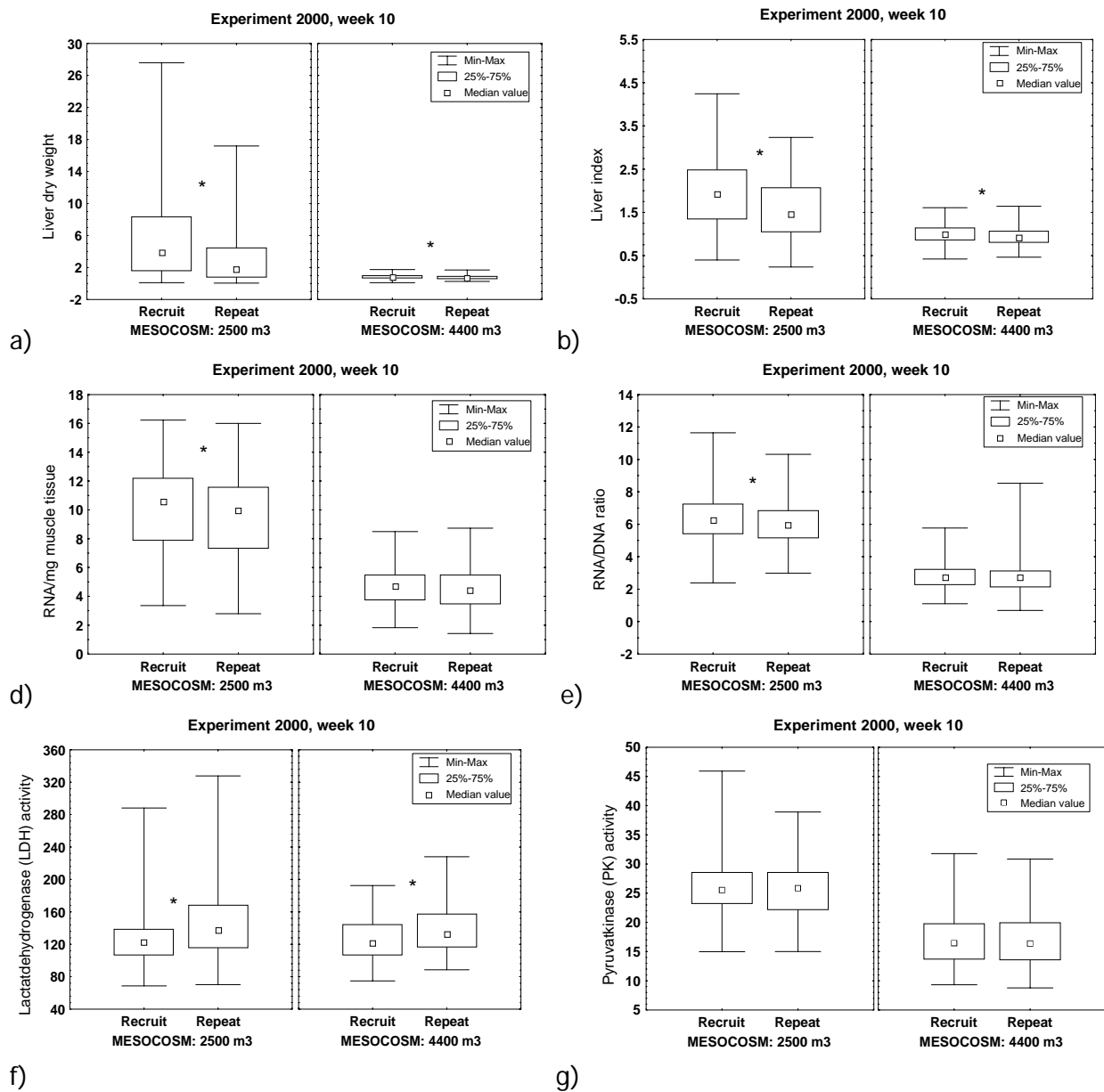


Figure 60. Comparison of liver dry weight, liver index (liver wet weight/total body wet weight), RNA/mg muscle tissue, RNA/DNA ratio, lactate dehydrogenase and pyruvate kinase activity of week 10 cod juveniles reared in the 2500m³ and 4400m³ mesocosms in 2000, subdivided into recruit and repeat spawners. The box represents the inter-quartile range, the bars denote the highest and lowest values, the square indicates the median, * denotes significant differences based on Mann-Whitney U-test. Plots from C. Clemmesen.

RNA/DNA ratios have shown a good match between the offspring's nutritional condition and food availability in both mesocosms (Fig. 61). Otolith radius, reflected the growth at feeding conditions in all sampled weeks, with exception of week 10 in the 4400m³ mesocosm, when a decoupling between otolith and somatic growth took place (Fig.61, left).

In addition, RNA/DNA ratio was poorly related to offspring size at weeks 3 and 4 ($r^2= 0.07$) in the 4400m³ mesocosm (Fig.62, left), and positively related to offspring size ($r^2= 0.33$) at weeks 3 and 5 in the 2500m³ mesocosm. This leads to the conclusion that it was advantageous to be large during the poor initial feeding weeks in the 2500m³ mesocosm since those individuals were better conditioned.

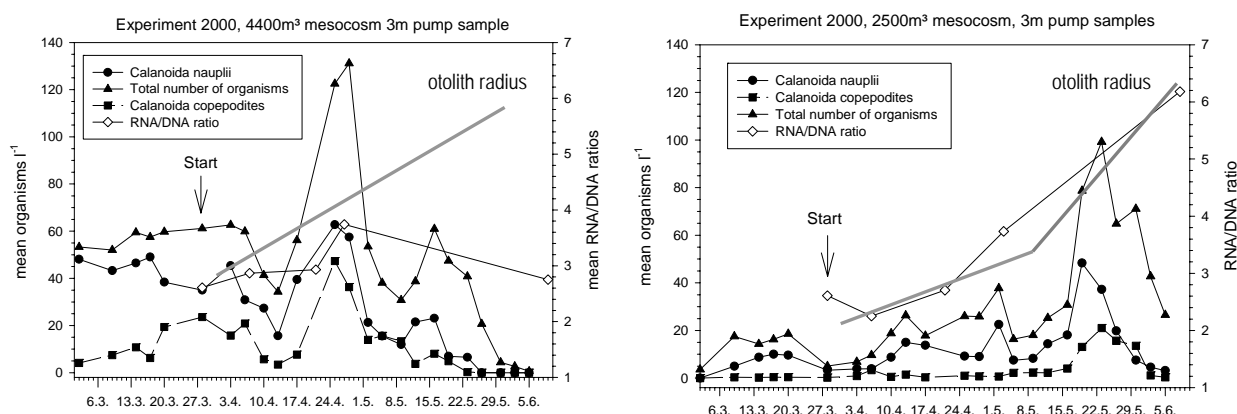


Figure 61. RNA/DNA ratios and Otolith radius in comparison to the zooplankton density found in the 4400m³ mesocosm (left) and 2500m³ mesocosm (right) during the experiment 2000. Note that the axis for otolith radius is not shown.

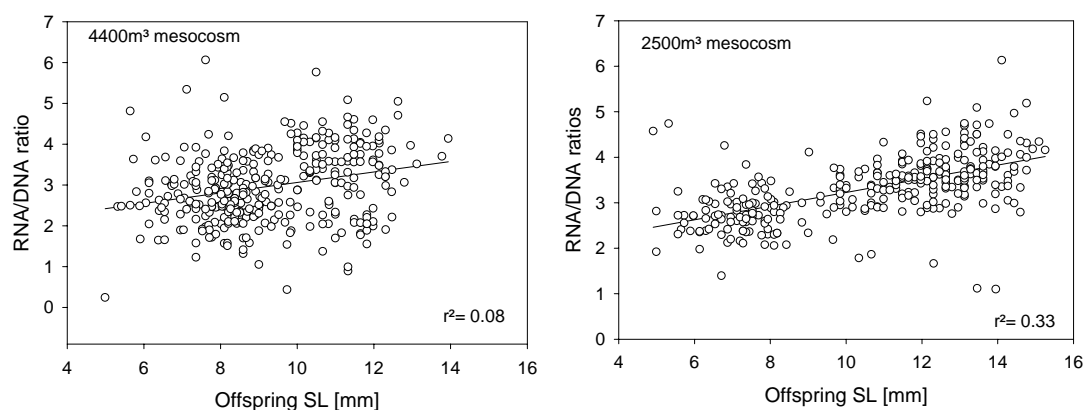


Figure 62. Relation between RNA/DNA ratio and offspring size in weeks 3 and 4 in the 4400m³ mesocosm (left) and 3 and 5 (right) in the 2500m³ mesocosm.

In addition, RNA/DNA ratios were positively related to offspring size at week 10 in the 2500m³ mesocosm ($r^2= 0.19$); and there was no relation between RNA/DNA ratios and offspring size at the same week in the 4400m³ mesocosm.

Otolith radius and RNA/DNA ratios were slightly positively related in all sampling weeks, with exception of week 10 in the 4400m³ mesocosm (week 3 and 4, $r^2=0.03$) The best relation was found for week 3 in the small mesocosm ($r^2=0.08$). However, the relation dropped in the following weeks to $r^2=0.02$. The relation between otolith hatch-check and RNA/DNA was also poor ($r^2=0.04$) and did not improve by using otolith radius at hatching, although there was a slight tendency for individuals with a wider hatch-check to be better conditioned.

To test which factors might have influenced egg size, a multiple regression model was run by J. Gröger (University of Massachusetts Dartmouth, US), fed with following parameters: female weight, female size, female age, female condition before and after spawning, batch volume, batch number, spawning group. The best variables which explained most of the variance on egg size were female age, female size and condition after spawning ($R^2=0.80$).

Another multiple regression model was run to test factors affecting larval size and involved the parameters egg size, temperature (degree days), age, system (mesocosm), and spawning experience.

On week 1, variance of larval size ($R^2=0.18$) and weight ($R^2=0.41$) was best explained by egg size, temperature (degree days), age and system (mesocosm).

On week 3, variance of larval size ($R^2=0.26$) and weight ($R^2=0.51$) was best explained by egg size, age, system, and spawning experience

On week 4 and 5, variance of larval size ($R^2=0.31$) and weight ($R^2=0.42$) was best explained by egg size, temperature (degree days), and system (mesocosm).

Finally, on week 10, variance of juvenile size ($R^2=0.27$) and weight ($R^2=0.31$) was again best explained by the same factors. However, when looking at the R^2 for egg size throughout the weeks, it is possible to see that the influence of egg size on larval sizes has gradually decreased while the influence of environment (system) increased, indicating that maternal effects slowly were getting lost (Fig.63).

To conclude, maternal effects were still affecting growth of 2500m³ mesocosm fish, while in the 4400m³, they have lost strength and the environment started to play a more important role.

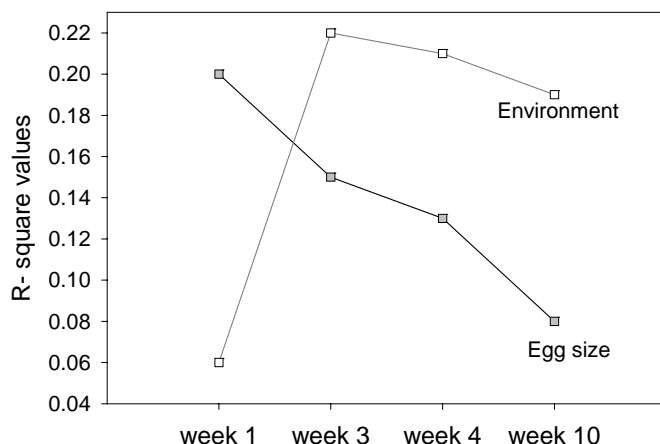


Figure 63. Gray squares indicate the values of R^2 for the parameter egg size during weeks 1, 3, 5 and 10. White squares indicate the R^2 for the mesocosm influence (environment) during weeks 1, 3, 5 and 10 of the mesocosm period.

3.5 Tanks Period

After the termination of the mesocosm period, a high mortality took place in the group from the 4400m³ mesocosm and 50% of the fish died within a week (Fig. 63 right), while in the 2500m³ mesocosm, only 4.5% of the fish died. Fish from the 4400m³ mesocosm were in very bad conditions (mean Fulton Index 0.36). The animals were prostrate for many days, laying in the surface of the tanks and did not respond to artificial feeding. After a week, 50% of fishes from the 4400m³ mesocosm had died (Fig. 64).

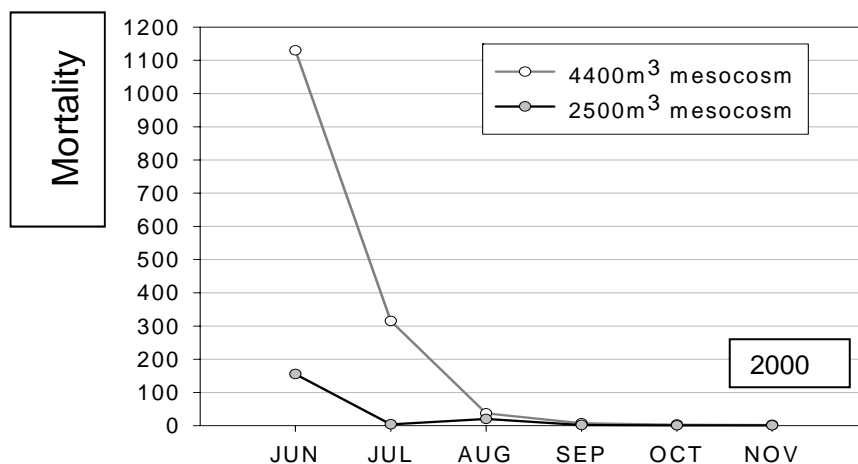


Figure 64. Mortality is giving in total number of individuals per month, after fish were transferred from the mesocosms to indoor tanks. Note: only natural mortality is shown.

The remaining group started to recover. Fish were hand fed for many weeks and after that, on 17.08.2000, they started successfully to feed on 0.6mm pellets (Dana Feed) from automatic feeders positioned in one of the corners of the tank.

During the whole tanks period, i.e., from middle of June 2000 to April 2002, the temperature oscillated between 5 and 13°C (Fig. 65). On July 2000, fishes from 4400m³ had a mean Fulton Index of 0.6 and those from the 2500m³ had a mean Fulton Index (FI) of 0.9. On November 2000, fishes from the 4400m³ mesocosm had a FI of 0.97 and those from the 2500m³ mesocosm of 1.02. By the tagging, on January 2001, fishes from both mesocosms were similar in sizes and condition (Fig. 66).

600 individuals from each mesocosm were randomly selected, tagged, genotyped and placed in larger indoor tanks (Fig. 66). Unfortunately, some hours after the author had tagged the last fish, i.e., at the same night, a big tragedy occurred. The main water source stopped, and the lack of oxygen had killed 450 fishes from the 2500m³ mesocosm, just a small group of 150 individuals from this group have remained. Sizes of the fish that had died and the remaining fish were not significantly different (t-test, $p > 0.05$).

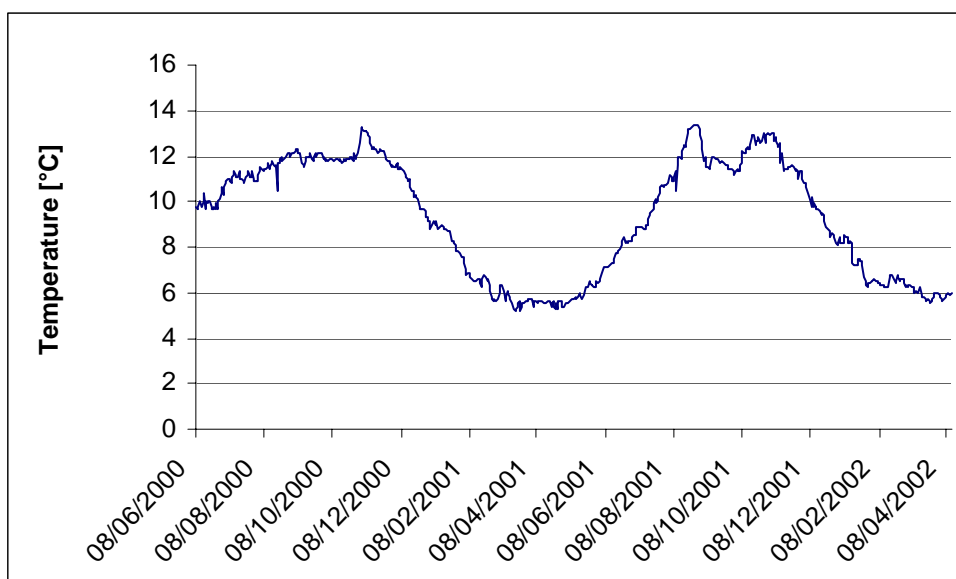


Figure 65. Temperature oscillation during the tank period (June 2000 to April 2002).

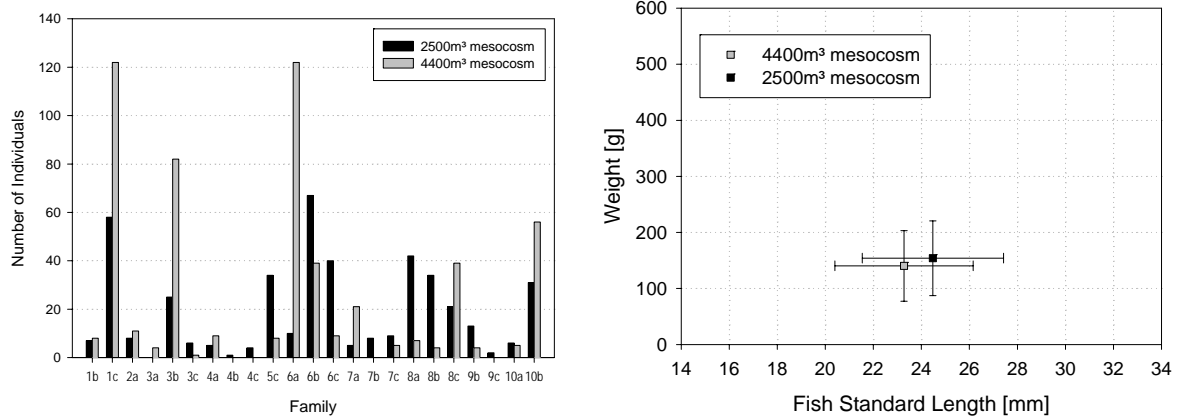


Figure 66. On the left: frequency of individuals that were randomly selected for tagging on January 2001. On the right: weight and length relations of fishes used for tagging. Squares in gray represent the fishes from 4400m³ mesocosm, and Black squares represent the fishes from 2500m³ mesocosm. Error bars give the standard deviation from the mean.

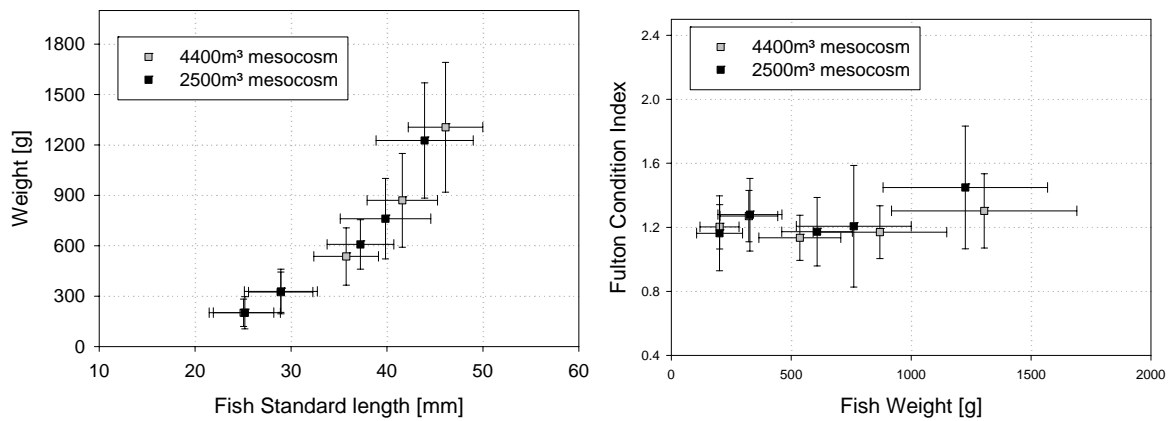


Figure 67. On the left: relation between weight and standard length for fishes from the 4400m³ mesocosm (squares in gray) and 2500m³ mesocosm (black squares) during the whole tanks period. On the right: Fulton Condition calculated for whole tanks period against fish weight. 4400m³ mesocosm (squares in gray), and 2500m³ mesocosm (black squares). Error bars give the standard deviation from the mean.

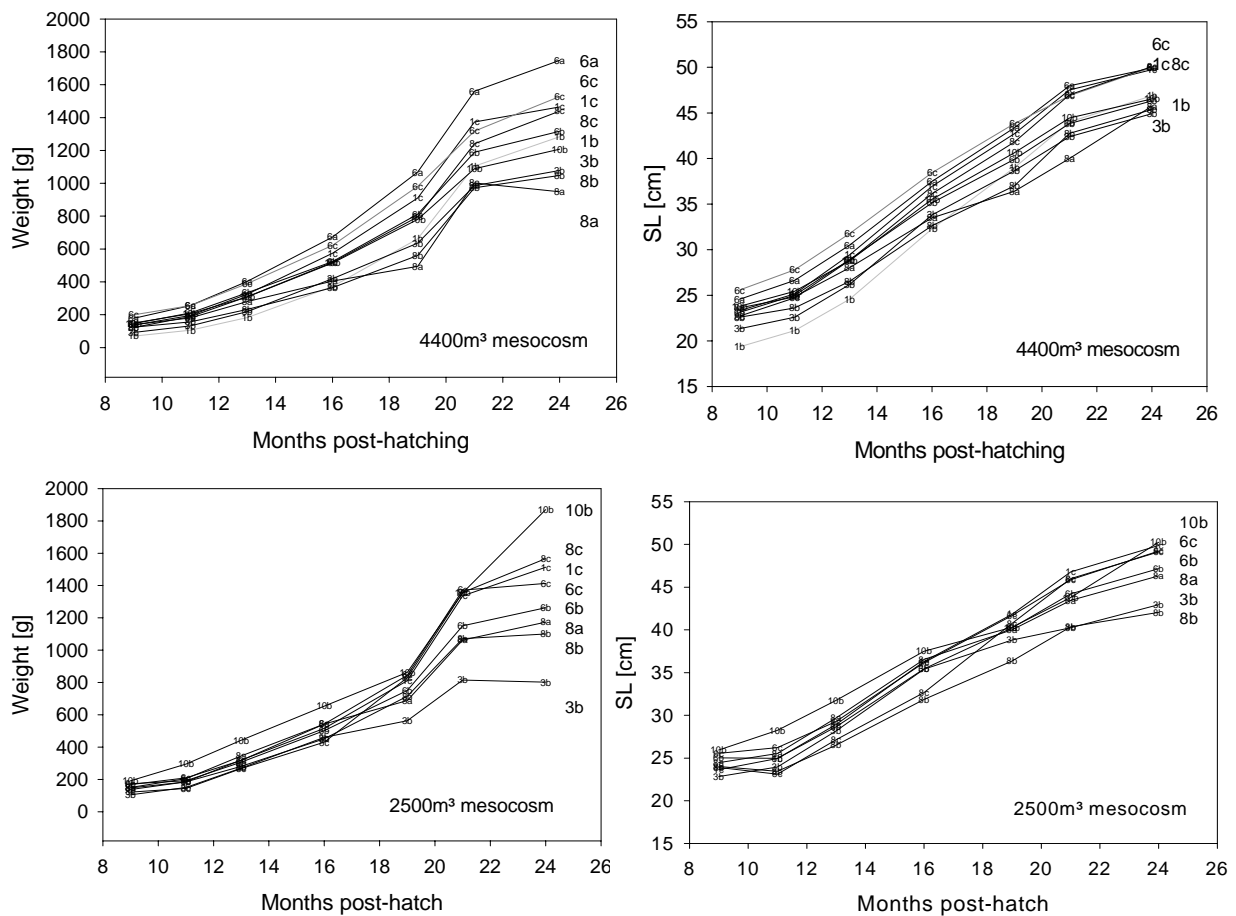


Figure 68. Individual growth trajectories from the mean of individuals belonging to a certain family. On the upper left and right: development of weight and length for offspring from the 4400m³ mesocosm. On the lower left and right: development of weight and length for offspring from the 2500m³ mesocosm.

From the 1200 individuals that started the tanks period, 456 remained. From those 216 were females, while 239 were males. Probably due to the lower density in the tanks, fishes from the 2500m³ mesocosm were slightly better conditioned than those from the 4400m³ mesocosm. However, the difference was not significant (t-test, $p > 0.05$) (Fig. 67). Indeed, both groups ended up with similar size and weight (Fig. 68). By the slaughtering, offspring from the 4400m³ mesocosm had a mean size of 48.6 cm (SD=3.87), and mean weight of 1448g (SD= 419), while offspring from the 2500m³ mesocosm had a mean size of 46.9 cm (SD=5.24), and mean weight of 1404g (SD= 812) in total. When looking at the difference between sexes, males (1479.44 g, SD= 40.1) were slightly, but not significantly, heavier than females (1425.59 g, SD= 48.1) (t-test, $p > 0.5$).

Family 6a was the largest (49.9 cm, SD= 3.12) and heaviest (1747g, SD= 386.99) offspring from 4400m³ mesocosm, while family 8a had the smallest mean weight (948.8g, SD= 437.69), and family 3b had the smallest mean size (44.8 cm, SD= 4.73) (Fig. 69, upper left and right).

From the 2500m³ mesocosm, the largest (50.3 cm, SD= 0.86) and heaviest (1870g, SD= 143.08) offspring was from family 10b. The lightest (801.7g, SD= 345.04) and second smallest (42.9 cm, SD= 5.58) was from family 3b. The reason for the small sizes of offspring 8b (42 cm, SD= 6.03) was the presence of some dwarfs in the family (Fig. 69, lower left and right). Because of the presence of dwarfs, family 8b artificially obtained the highest condition factor among all groups (mean Fulton of 1.50). When this factor was taken in consideration, family 6b and 6a had the highest Fulton condition factor with a mean of 1.42 and 1.41, respectively.

12 different families remained until the end of the experiment, with at least 10 individuals each. When all families containing at least 10 individuals were analysed together, the new generation of females (generation II) daughters from repeat spawners (generation I) were in average heavier, had a higher condition factor (Fulton) and higher potential and relative fecundity than females (generation II) from recruit spawners (generation I) (Table 5.) However, those differences were caused by family 6a, not only the largest and best conditioned of all females, but also, the second most numerous group, with 92 individuals.

No significant difference was found on potential fecundity among individuals from 4400m³ and 2500m³ mesocosm (ANOVA, $p > 0.05$). The average for all groups was 1.20 millions of vitellogenic oocytes. Family 6a had clearly the largest mean value with 1.62 millions. Family 4a had the lowest potential fecundity, with 0.78 millions. Additionally, these two groups had also the largest and smallest relative fecundity, with values of 933 n.g⁻¹ and 674 n.g⁻¹.

Oocyte diameter, which is negatively correlated with the time that spawning starts (A. Thorsen, pers. comm.), has also shown differences among families. Mean size for all fish was 467 μm , while the mean value for 1c, which was the most advanced group, was 528 μm . Families 3b and 6a had the lowest diameters, with 424 and 425 μm , respectively.

The characteristic used to identify whether a female would or would not mature in the year 2002 (two years after hatching) was the presence of vitellogenic oocytes (A. Thorsen, pers. comm.). 84% of the females analysed had the presence of vitellogenic oocytes (Table 6), while the remaining other 16% were immature (pre-vitellogenic). 96% of females from families 1c and 6a presented vitellogenic oocytes, while family 3b had only 62% of vitellogenic oocytes. Thus, the presence or absence of vitellogenic oocytes was significantly influenced by family origin. Females with a large amount of vitellogenic oocytes were relatively heavier and better conditioned (Table 7).

Table 5 . Weight, length, Fulton condition factor, potential fecundity, relative fecundity and oocyte diameter in 2 year old offspring from recruit and repeat mother fish of the year 2000 tank experiment. H_0 tests for significance between values for recruit and repeat spawners.

	Recruit			Repeat			H_0
	Mean	SD	n	Mean	SD	n	P
Weight (g)	1365	369	235	1502	438	221	0.0004
Length (cm)	48.1	4.4	235	48.5	3.8	221	0.255
F. Condition factor	1.21	0.19	235	1.28	0.21	221	0.0001
P. Fecundity (millions)	1.01	0.31	87	1.37	0.48	82	< 0.0001
R. Fecundity (n/g)	707	139	87	839	189	82	< 0.0001
Oocyte diameter (μm)	494	76	87	434	66	82	< 0.0001

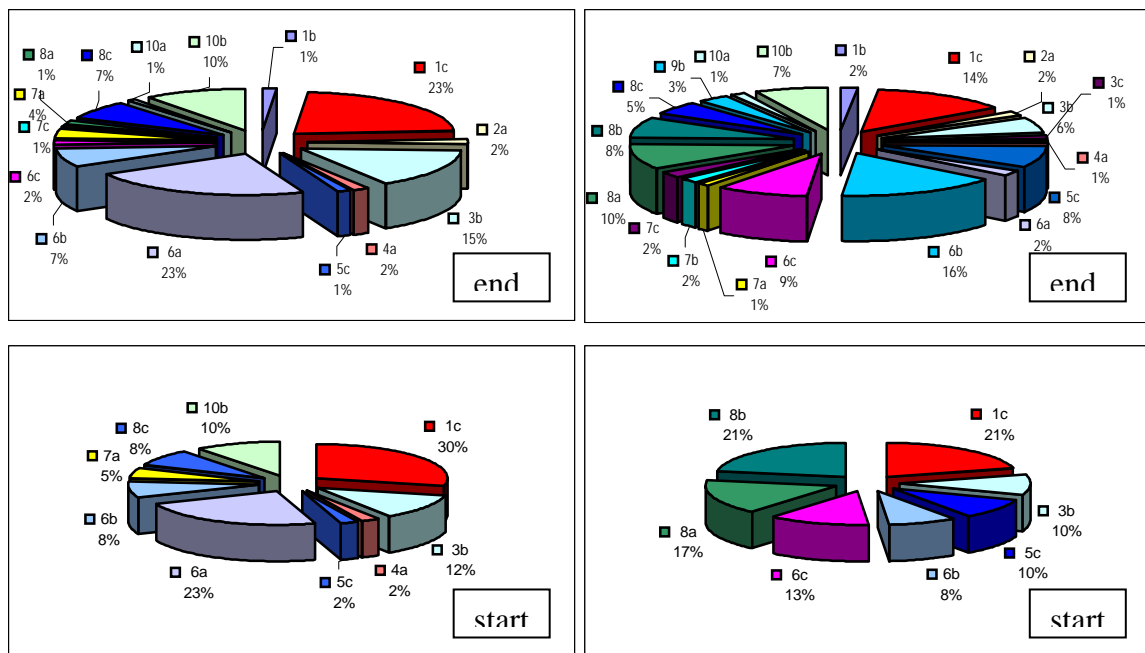


Figure 69. Proportion of families from tagging day (start) and after two years of tanks experiment (end). On the left: fish from 4400m³ mesocosm. On the right: fish from 2500m³ mesocosm.

Table 6. Number (A) and percent fraction (B) of fishes with previtellogenic and vitellogenic oocytes of different families at termination of the year 2000 tank experiment. Only family groups with 10 or more female fishes at termination were included.

A	10b	1c	3b	6a	6b	8c	Totals
Previtellogenic	5	2	9	2	3	3	24
Vitellogenic	11	46	15	39	7	9	127
Totals	16	48	24	41	10	12	151

B	10b	1c	3b	6a	6b	8c	Totals
Previtellogenic	31	4	38	5	30	25	16
Vitellogenic	69	96	63	95	70	75	84
Totals	100	100	100	100	100	100	100

Table 7. Weight, length and Fulton Index of female fish at termination of the year 2000 tank experiment split by ovary stage. PV = Previtellogenic and V = Vitellogenic.

	Mean	Std. Dev.	Count	Min.	Max.
Weight (g), Total	1441	414	216	389	2722
Weight (g), PV	1164	425	42	470	2519
Weight (g), V	1526	373	168	389	2722
Length (cm), Total	48.4	4.1	216	33.0	57.0
Length (cm), PV	46.2	4.9	42	35.0	56.0
Length (cm), V	49.1	3.6	168	33.0	57.0
Fultons CF, Total	1.25	0.20	216	0.78	2.28
Fultons CF, PV	1.15	0.26	42	0.84	2.28
Fultons CF, V	1.27	0.18	168	0.78	1.91

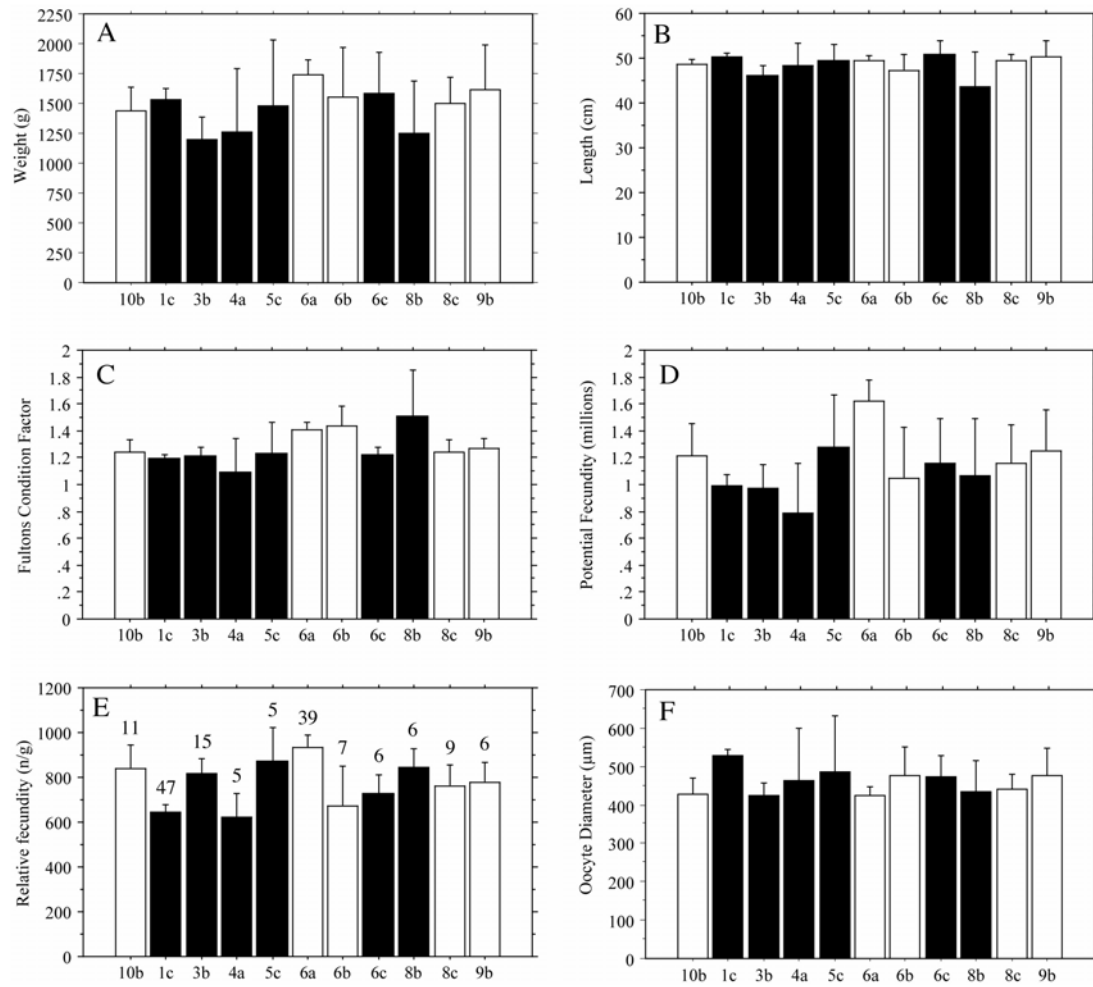


Figure 70. Mean termination values for the mature females in the tank experiment. Black and white bars represent offspring from recruit and repeat spawners, respectively. Histogram "E" indicates the number of individuals available to all plots. Note that only groups with at least five mature females were included. Plots from A. Thorsen, IMR-Norway.

4. Discussion

4.1 Exploitation & changes on the demographic structure

Exploitation results in increased mortality, and as a consequence, stock numbers and biomass decrease, while age structure within the population is shifted towards the dominance of youngers, caused by removing the larger individuals. Commercially exploited stocks that have experienced declines in population abundance have responded similarly, by altering life history traits of growth and maturation (Stearns & Crandall 1984, Rijnsdorp 1989, Heino & Gødo 2002).

Increased growth rates and early maturation with biomass decline were observed, between others, for haddock, *Melanogrammus aeglefinus* (Templeman & Bishop 1979), Atlantic herring, *Clupea harengus* (Sinclair et al. 1980), and Baltic Sea cod (Cardinale & Modin 1999).

The Northeast Arctic cod (NEAC) caught in the 1930s were on average 10-12 years old. In the 1980s this average has dropped to 7-8 years old cod, while size at maturation has declined on average from 89 to 74 cm, corresponding to 42% decrease in weight (Gødo 2000). Norwegian fisheries traditionally take place on spawning grounds. Due to the fishing methods used until the 1930's, fish were selected by time at arrival on the spawning grounds and not by size. Thus, cod with delayed maturation had a reduced mortality risk, could improve size and, after maturation, increase fertility. This historical selection pressure is believed to be responsible for the maturation traditionally observed in this stock (Law & Gray 1989). In the 1940's 50% of the cod reached maturity at 11- 15 years old (T. Jakobsen, IMR, Bergen Norway, pers. comm.). When the modern trawler fishery began, harvesting became selective for larger fish and, fisheries were also extended to the feeding grounds, favoring indirectly selection for earlier maturation.

Although the reasons for this phenomenon are poorly understood, theoretical studies pointed out that the decreases in age- and size-at-maturation have negative consequences on the sustainability of NEAC (Heino 1998). Concerning reproductive strategy, young and small females have less reproductive ability, and therefore, it will cost them more energy to grow large eggs. Oganesyanyan (1993), reported a high proportion of females with spawning omission, indicating that the general assumption that cod spawn annually, after first spawning, is not correct. Besides, according to Kjesbu (1991), it is very difficult to distinguish among immature, recovering or spent cod based on visual inspection, which makes the detection of such anomalies very difficult. Thus, in order to improve predictions, it would be necessary to understand which environmental and physiological processes trigger the onset of maturation in cod fishes. Moreover, the only way to recover cod fisheries is to develop a strategy that helps cod to meet the demographic structure they had held before overexploitation became an issue; and therefore it is necessary to recognize how such mechanisms as early maturation take place.

One hypothesis suggests that earlier maturation is an evolutionary response to the increased mortality, since reduced adult survival will select for earlier maturation and increased reproductive effort (Reznick et al. 1990). Under high mortality risk, the expected number of spawnings become very small for later maturing individuals, and natural selection favors early maturing phenotypes, even if these have lower age-specific fertility. However, it could be that earlier maturation is a phenotypic plasticity response. A decrease in population density results in more resources being available per individual. The consequence of less

competition for resources may alter the life history of species, concerning survival, growth and reproduction (Roff 1984). Therefore, when growth rate increases, fish attain the required size for maturation earlier. In Northeast arctic cod, the decrease in age at maturity was related to the increase of growth stimulated by population decline, suggesting that growth is density dependent in this stock (Jørgensen 1990).

There is no consensus whether organisms mature at a fixed age or at a fixed size. Selection seems to operate on the shape of age/size maturation trajectories. In a study on flatfishes, Roff (1982) suggested that age could be more important than size for early maturing species and vice-versa for late maturing species.

Fish need extra energy to be able to start the maturation processes. This energy is obtained by predation or by burning out energy stored in body tissue (Love 1970). Therefore, the onset of maturation only starts, when fish have accumulated enough energy in the body tissues to be able to fulfill maturation and spawning. Stearns and Crandall (1984) suggested that populations have evolved particular trajectories of age and size at maturity that reflect a genetically fixed component of growth and maturity rate and an adaptive component that respond to environmental factors. Density-dependent processes, such as competition, can act to emphasize phenotypic differences among individuals in the breeding population and determine reproductive output. Moreover, small changes in early development can permanently and dramatically alter developmental trajectories (Bernardo 1996).

Cod from the Barents Sea is traditionally a long migrating, and at high age spawning fish. Migrations back and forth, between the polar front and spawning grounds, may represent a distance of several thousand Kilometers, demanding high energetic resources in addition to those needed for gonadal development.

As swimming performance increases with size and age, repeat spawners are able to perform long migration routes, while younger fish remain more or less stagnated. Sundby et al. (1983) reported that older females arrive on the spawning grounds earlier than their younger counterparts do. Nowadays, many of the first spawners do not reach the appropriate spawning sites, while some of them do not even migrate anymore (Solemdal pers. comm.).

Since time at first maturation is highly variable for cod, it indicates that the onset of maturation is not deterministic, but is a complex process that is also influenced by other factors than age and size (Bernardo 1993). The fact that some individuals at certain age and size class may mature, whereas others do not, illustrates the probabilistic nature of the maturation process. Nevertheless, the probability of maturing is generally expected to increase with age. Additionally, swimming performance of NEAC increases with age, and younger age groups (up to four years old) have restricted migratory potential (Bergstad et al. 1987), which suggests that those processes might be somehow coupled.

4.1.2 Evolutionary consequences of the shift in demographic structure

One of the most disastrous consequences related to decline in population density is the loss of genetic variability. Loss of diversity at the genetic level may occur by extinction; by hybridization that may cause re-arrangement of co-adapted genes and loss of adaptability to local conditions; by fisheries induced selection (Heino & Gødo 2002), or due to decrease in population size resulting in inbreeding (Laikre & Ryman 1996). When populations become severely overfished, disruptions in migrations, low numbers of effective population (N_e), and

consequently inbreeding, become a serious issue. The effect of demographic alterations on genetic diversity will depend on the gene flow between populations, and the smaller the population, the faster the loss of genetic variation.

Physical and life history traits (phenotype) are generated by the genetic makeup of the individual, by the environment in which it lives (food availability, temperature, etc.) and by the interaction between genes and environment. Data from many studies have shown that removing large fish appears to promote spread of slow-growing fish (Smith 1999, Law 2000). Models regarding a decline in age-at-maturation suggest that phenotypic response is consistent with the selectively induced deterioration of genetic diversity (Heino 1998, Law & Gray 1999, Barot et al. 2004). Studying the effect of selective fishing on grayling (*Thymallus thymallus*), Haugen & Vøllestad (2001) observed for a period of only 10 years, a rapid evolution towards early age-at-maturity, reduced length at maturity, faster early growth, slower late growth, and increased size-specific fecundity. However, when selection intensity was relaxed, age and length-at-maturity increased and length-at-age increased, indicating that the genes were still present in the population. In the absence of direct genetic evidence, the dependency of phenotypes in relation to the environment can be measured by "reaction norms". The reaction norm predicts the phenotype that follows from a single genotype as a function of the environmental conditions.

Dieckmann et al. (2002) believe that changes in reaction norms of NEAC have a genetic basis, and although the phenotypic plasticity may also play an important role in this stock, changes in reaction norms explain a larger portion of the age and size-at-maturation than changes in growth. However, this assumption has been criticized by the lack of empirical or field support. In addition, other studies did not find evidence for genetic variation in different phenotypic plasticity traits (Laurila et al. 2002). On the other hand, the NEAC stock has a wide distribution with substantial geographical and environmental heterogeneity. Cod is susceptible to wide temporal and climatic changes, and it is plausible that under such circumstances they have developed evolutionary traits, which allow them to adapt to changes in growth and survival (Gødo 2002). A comparative study by Myers et al. (1997), demonstrated that age at maturity of 20 cod stocks was significantly related to bottom temperature, while Godø & Moksness (1987) observed that differences in growth and maturation of Northeast arctic cod are influenced by environmental conditions rather than genetic variation.

Although the problem concerning genetic losses is still uncertain, one thing is for sure, cod is not only becoming mature at an earlier age but also, the majority of the stock comprises fishes with no previous spawning experience, known as first-time spawners. That was the main concern of this study, since at long term, the best strategy for this stock would be to return to the characteristics of unexploited stock, i.e. maturation at larger sizes and later age. There are many advantages for delaying maturation: Larger and heavier fish will be better conditioned for spawning, have higher fecundity and larger eggs that are more viable (Trippel & Morgan 1994). Harvesting at delayed recruitment enables the stock to maintain a larger SSB with an expanded age structure while supporting a sustainable fishery. If the stock responds to continued exploitation by shifting maturity to an earlier stage, fish will spawn at smaller sizes. They will produce smaller eggs, and consequently small and less viable larvae, so that the contribution to the spawning stock biomass will be less than expected. It is impossible to rebuild the stock, if there are no guarantees of a high quality offspring survival.

The present study (MACOM) has followed offspring's growth trajectories and survival from age zero up to two years old, when individuals became mature, in a attempt to clarify how

much maternal effects accrue to offspring performance, and how far the environment has the determinant role.

4.2 The influence of maternal effects

Maternal effects have received a considerable attention in recent years, particularly under the evolutionary point of view, as maternal effects have been increasingly identified as adaptative factors, rather than simply an annoying source of variation masking the underlying genetic effects (Mosseau & Fox 1998). Maternal effects have been shown to determine offspring life history traits, and they appear to be ubiquitous across a diversity of taxa and often contribute to a considerable portion to the variance of many characters, especially those expressed in early development (Roach & Wulff 1987, Cheverud & Moore 1994, Mousseaux & Fox 1998). They provide the most common mechanism by which environmental variation in one generation affects the phenotype of individuals in subsequent generations. In egg laying animals, maternal effects have large influence on early growth (egg size and early development). In fact, many environmental influences are mediated by maternal effects (Falconer 1965, Kirkpatrick & Lande 1989). An individual's phenotype reflects the size and composition of the egg it developed from, and thus its mother's physiological state (mediated by her phenotype and environment) rather than its own, or its father's phenotype (Flemming & Gross 1990, Reznick 1991, Fox & Mosseau 1996).

Environmentally based maternal effects are ecologically important (Mousseau & Dingle 1991, Rossiter 1996). They are assumed to be a source of time-lagged effects on population dynamics, in which the per capita rate of increase in a population is influenced by the environment experienced by a previous generation (Rossiter 1996). These time lags may result in population cycles, with population destabilization and possible extinction. Maternal effects may accelerate or impede responses to selection, i.e., they result in large time lags in evolutionary response to selection, by delaying in one or more generations. Although studies on maternal effects have shown that the maternal phenotype accounts for more than half of the variance in characters expressed in early life (Wolf 2000), very few studies have examined how long those variations, inherited via maternal effects, persist within populations. The ecological consequences of egg-size variation, for example, remain still poorly understood (Bernardo 1996). Current evidences suggested a direct link between hormones in plasma and eggs produced by the female. Hormones like thyroxine, triiodo-thyronine, estradiol, and cortisol are transferred to the egg during gametogenesis and drive offspring growth prior to embryo self-supply (Hwang et al. 1992, Barry et al. 1995). The levels of cortisol in breeding females will affect the developmental rates of offspring. A higher cortisol level, i.e., over the normal threshold will lead to markedly small larvae at hatching (McCormick 1998, 1999). In contrast, an increase of female androgens will not only produce larvae with larger yolk-sac, but it will also improve the efficiency of yolk utilization by the larvae (McCormick 1999). Female androgens might also influence later growth of offspring, sexual attractiveness, aggression, among others (Clark & Galef 1995, McCormick 1999). Maternal influences through the endocrine system seem to be responsible for much of the variability found in larval morphology at hatching.

4.2.1 Detecting maternal effects in this study

Since repeat and first spawners had similar size and condition in this study, the classical differences described by other studies regarding spawning experience (Palumbi 2004), could not be tested. However, female size was positively related to female age ($r^2 = 0.48$) which was expected, since fish grow indefinitely and size increases with age.

Female size and age were positively related to egg size, which has been confirmed by many studies (Roff 1992, Einum & Fleming 2002). For repeat spawners, egg size was better related to female size ($r^2 = 0.21$), while for first spawners, egg size was better related to female age ($r^2 = 0.24$).

Berkeley et al. (2004), suggested that female age is a more important factor influencing egg size than female size. The possible reason is that elder females might count on increased resources available for gametogenesis, and this will favor the increase in egg sizes. The higher energetic efficiency achieved by older specimens may be a consequence of their improved ability in sequestering resources from the environment (Roff 1992).

In Gadoids, maturation seems to be determined by condition since in years of poor feeding a large amount of repeat females can skip spawning (Oganesyan 1993, Marshall et al. 1998). This decrease in investment per offspring is usually interpreted as a direct result of the maternal nutritional stress rather than an adaptive adjustment of maternal investment per offspring. Maternal nutritional stress decreases the amount of nutritive substances per egg and consequently affects the newly-hatched size and growth.

Despite of this assumptions, the results revealed that female condition (Fulton Index) was negatively related to egg size ($r^2 = 0.16$ before spawning and $r^2 = 0.30$ after spawning season). Such a relation was also found by Kjesbu (1996). However, in the experiment performed by Chambers & Waiwood (1996) female condition (Fulton's Index) was positively related to egg size. Condition factor is known to be an important determinant of reproductive output in iteroparous fish, including cod (Trippel & Harvey 1989, Kjesbu et al. 1991, 1996), but the influence on egg size is less clear. Trippel (1993) observed that fish in low condition have produced the largest *ova* among populations of lake trout. Morita et al. 1999, suggested that the relationships between egg size and maternal size are generally weaker than relationships between egg number and maternal size. This is largely explained by the fact that eggs are produced in proportion to a fish body volume, which is proportional to the cube of its length (Palumbi 2004).

Egg size was not related to maternal weight before spawning, probably due to the long time span between the last measurement and spawning (3 months). However, egg size was positively related to maternal weight of first and repeat spawners after spawning (first spawners $r^2 = 0.37$, and repeat spawners $r^2 = 0.42$). The literature on this aspect is rather conflicting; some authors found a positive relation between egg size and maternal weight (Zastrow et al. 1989), while others did not (Chambers and Waiwood 1996).

Kjesbu et al. (1996) suggested that past investments in Atlantic cod influence future investments, i.e., they found indications that the high fecundity in one season has negatively affected female body weight and egg production in the following season. Based on the observations of Kjesbu et al. (1996), maternal size at age induces increasing of egg sizes stepwise, i.e., skipping one or two spawning seasons. Actually, life history predicts that in variable environment the appropriate measure of fitness is not the arithmetic mean offspring size, but the geometric mean, which is weighted very strongly by years of low productivity (Roff 1992). This is probably the explanation to what has happened in this study. Female

repeat spawners had already spawned in captivity a year before the MACOM experiment has taken place. Thus, they had to recover their body reserves and could not invest the same energy as the first spawners.

Egg sizes were larger in the initial batches, but became smaller towards the end of the spawning season. This phenomenon has been confirmed by several other studies (Kjesbu et al. 1991, Solemdal 1997, Marteinsdottir & Steinarsson 1998, Trippel 1998, Ouillet et al. 2001). This difference in egg sizes suggests that maternal nutrition affects maternal investment per offspring in addition to total reproductive output. Seasonal declines in egg sizes may have a physiological basis. In cod, mobilization of protein and lipids from muscles and liver to the ovaries occur between successive egg batches. Females that spawn a larger number of batches, over a broader period, tend to increase their chances of producing a progeny that would match the relatively brief periods of abundant food, whereas the short spawning duration of first-time spawners reduces the chances that the offspring will match up good conditions for survival (Chambers & Waiwood 1996).

The oocytes swell substantially through uptake of water shortly before they are released from the body cavity (Kjesbu et al. 1991). Females often spawn more than their own body volume in eggs summed over all batches (Trippel 1998, Fordham & Trippel 1999). Batch spawning may therefore place especially physiological demands on individuals, which could account for the successive declines observed in egg diameter between initial and terminal batches.

Since cod do not feed during the spawning season, maternal nutrition achieved before will affect both, number of eggs and egg size. However, intraspecific variation in the size of the ripe gonads is much larger than intraspecific variation in the size of the eggs, which indicates that the number of offspring varies much more than the maternal investment per offspring, and that maternal investment per offspring is maintained over a wide range of maternal nutrition (Lessios 1987). Hence, variation in maternal nutrition has larger effects on fecundity than on quality of individual offspring (Bertram & Strahman 1998). Nevertheless, poor feeding conditions of the mother decreases the investment per offspring in many ectotherm species (Bayne et al. 1978, Thompson 1982, Guisande & Harris 1995).

4.2.1.1 Egg quality parameters

Determination of what makes a good egg remains a difficult task. Egg quality has been measured by mortality, fertilization rates, eyeing, hatching and first feeding (Bromage et al. 1992); morphological features of larvae (Kjorsvik 1994); the shape of the egg, its transparency and distribution of oil globes (Kjorsvik 1990); and by their ability to float or to sink in sea water (McEnvoy 1984; Kjorsvik et al. 1990). However, according to Brooks et al. (1997), the quality of an egg is determined by the intrinsic properties of the egg itself, by its genes and by maternal mRNA transcripts and nutrients contained within the yolk, which are provided by the mother. Components that do affect egg quality include the endocrine status of the female during the growth of the oocyte in the ovary, the diet of the brood females, the complement of nutrients deposited into the oocyte and the physiochemical conditions of the water in which the eggs are incubated. Effects of maternal nutrition on eggs and offspring are common for animals with lack of parental care and include egg-size, growth rate, performance and survival (Hislop et al. 1978, Bertram & Strahman 1998, Lambert et al. 2002). Moreover, an egg is the final product of the oocyte growth and development (Tyler & Sumpter 1996). Once ovulated, only water and some diluted chemicals are able to pass into an egg (Holliday & Jones 1965). Hence, all contents of an egg that will determine its quality must be incorporated while the oocyte is within the ovary.

Egg size was positively related to egg dry-weight and energy content ($r^2= 0.92$ and $r^2= 0.87$, respectively) indicating that larger eggs also have a higher content of nutritional substances. There was no relation between lipid content and egg size. However, egg size and amount of free amino acids (FAA) were highly positively related ($r^2= 0.82$). The yolk consists of 80% protein and 20% fat (Plack et al. 1971), and FAA constitute up to 50% of the total amino-acid pool (Thorstein et al. 1993). Thus, due to their higher concentration, proteins have probably produced a clearer signal in the analysis.

Concerning lipids, phospholipids are all contained in membrane structures, while triglycerids (TAG) are used as storage. Although not significantly related, there was a positive trend between egg size and TAG. Cholesterol is the base of all steroid hormones synthesis in fishes as well as in mammals (Teshima 1989). However, the peroxidation of unsaturated fatty acids and of cholesterol results in toxic products, which are harmful to the organisms (Peterson & Lignell 1996, Pickova 1998). In addition, highly polyunsaturated fatty acids are the most susceptible to oxidative reactions (Cosgrove et al. 1987) and therefore the molecules, which usually initiate the free radical chain reactions. Because of this, they are present in lower and variable concentrations (Rønestad et al. 1998) and therefore, did not show a visible signal in this study.

4.2.1.2 The offspring

Yolk-sac size, larvae SL and egg size were positively related ($r^2= 0.32$ and $r^2= 0.26$, respectively) indicating that large eggs have produced larger larvae and higher yolk content. Since the biochemical composition of FAA comprise the major substrate of aerobic metabolism during the earliest developmental phases (Finn 1994), it becomes clear why in terms of quality, bigger eggs might be better. The advantages of offspring originating from large eggs have been demonstrated over a wide range of taxa. Offspring from large eggs have shown improved survival and growth in many studies (Roff 1992, Mosseau & Fox 1998, Trippel 1998).

Offspring size was highly positively related to maternal size during all sampled weeks in the 2500m³. In the initial weeks when environmental conditions were adverse, larvae from larger mothers had a clear advantage over the small one. They were better conditioned, they had grown faster, they have shown a wide and quick response of compensatory growth and they ended up at larger sizes than larvae from smaller mothers. The same was observed in the 4400m³ mesocosm. However, the signal was lost in the last two weeks, when environment started to play a larger role. Independently of environment, offspring from larger mothers were most successful in both environments.

4.2.1.3 Effect on otoliths

As already observed by other studies (Geffen 1995, Otterlei et al. 2002) there was no significant relation between otolith radius and body size in newly-hatched larvae. The lack of relation among otolith radii and larval sizes could be explained due to the high relative size variation in early stages, since the relation improved when otolith radii reached sizes larger than 40 μm .

Nevertheless, in general speaking, large mothers had larger larvae, and otoliths from larger larvae tended to have wider hatch-checks. Otoliths with wider hatch-checks have shown larger growth potential. Larvae with larger hatch-check seem to become larger individuals later. Besides, otoliths from large eggs (families 6c, 8b) have also shown larger resistance

against decoupling and produced wider increment, especially in periods of recovering (compensatory growth. (Please, not that both phenomena will be discussed in more detail further down).

The reason for the variation in hatch-check widths can be only speculated, since very little is known about this subject. However, recent studies indicated that increment width is positively related to fish metabolism rather than to somatic growth. (Armstrong et al. 2004). Therefore, a wider hatch-check might be related to a higher metabolism. The physiological reasons for that variation are certainly related to maternal effects. Although very little is known about “genetic make-up” (Davidson 1986, Hales et al. 1994, Nagahama 1994), studies looking at the ontogeny of the endocrine system have so far indicated that fish larvae are physiologically immature, with little or no capacity to produce certain enzymes, growth factors and hormones, until the end of the yolk resorption (Tanaka et al. 1995), and therefore fish larvae are dependent on mother resources.

Indeed, maternal hormones may fulfill the regulatory needs of fish larvae until they develop their own endocrine glands (Lam 1994), and thyroid hormones of maternal origin are deposited in the egg yolk (Tagawa & Hirano 1991), and may have significant effects on fish embryo development (Lam 1994). In a unique work, Shiao & Hwang (2004) discovered that thyroid hormones are necessary for otolith growth, and that exogenous triiodothyronine (T3) has increased otolith increment width by 20%. Thus, it might be that hatch-check width is closely related to the levels of T3 within the egg at hatching, which in other words, would mean that newly-hatched with larger hatch-checks also have a higher metabolism, regardless egg size. This would also explain part of the variability found between egg size and hatch-check. However, this is only a new theory from the author and will need further confirmations (Buehler in prep.). Moreover, in spite of absence of predators in the system and feeding abundance, yolk-sac larvae mortality was also high in the 4400m³ indicating that some other intrinsic properties might be related.

4.2.1.4 Biochemical methods

Biochemical methods such as RNA/DNA or glycolytic enzymes in this study have apparently failed as a parameter to detect maternal effects, being rather related to environmental effects as feeding conditions.

4.2.1.5 Tanks data

In spite of all manipulations, as size grading, and feeding *ad libitum*, etc., maternal effects could still be followed until two years after the experiment. There was some inversion of trajectories, i.e., offspring from small eggs (1b) turned out being larger than some offspring from large eggs (eg. 8b), what might have been caused by fact that small fishes, as from family 1b were kept separated and in much lower densities than the large ones. Even though, the most abundant, and fecund females in generation II, were the daughters from older, or large mothers, like families (6a, 6c, 8c, 1c) reinforcing the idea that under a less compromised experimental set-up, maternal effects could have shown a stronger signal than found in this study.

4.3 The connection between maternal effects and environment

The typical question that raises when discussing about maternal effects is: If large eggs have so many advantages, why evolution has not selected only this trait?

Larger eggs tend to have a longer incubation period, making them longer susceptible to predation, and have higher energetic costs to the mother, reducing fecundity and subsequently, parental fitness. The “bigger is worse during incubation” theory (Krogh 1959), assumed a negative effect of egg size on survival rates under lower oxygen condition. This has been accepted for many years without an empirical test, until Einum et al. (2002) proved that larger eggs have actually a larger tolerance to low oxygen levels. Moreover, Vallin & Nissling (2000) observed that in the brackish, low oxygen conditions prevailing in the Baltic Sea, large eggs had a higher survival compared to small eggs. However, they attributed that to a higher buoyancy of large eggs, allowing them to stay in shallower depths, which are oxygen richer.

Additionally, one of the most commonly cited reasons for egg size to not increase continually is that there is a negative relationship between the number and size of eggs. Thus, when feeding conditions are good, larger eggs could be a disadvantage, since the offspring would be in less number. This could be seen in the initial weeks of the 4400m³ mesocosm. On the other side, in poor environments as the initial weeks in the 2500m³ mesocosm, the large individuals were in visible advantage. Ware (1975) argued that if there were no survival differences associated with the egg size, then herring should produce as many small as small. However, this tendency is constrained by the fact that larger eggs, as shown in this study, produce larger larvae, which tend to have higher survival changes, because mortality in the sea is inversely related to body size (Peterson & Wroblenski 1984).

It has been often suggested that maternal effects often provide a mechanism for adaptative trans-generational phenotypic plasticity, in which the environment experienced by the mother is translated into phenotypic variation in the offspring and this relationship can be modeled as a reaction norm. (Mosseau & Fox 1998). In a field situation, mother's experience on the environment can lead to variation in her growth, i.e. body size, condition and physiological state. This may be indirectly transmitted to the offspring via cytoplasmic factors as yolk amount, hormones, and mRNAs in the egg that may directly (via programming) or indirectly via offspring sensitivity to maternally transmitted factors. For example, Tanasichuck & Ware (1987) noted that the water temperature regulates the fecundity and because the mature ovary weight is constant, fecundity therefore determines the egg size. When the temperature is higher than the normal at the decisive period (e.g. 60-90 days before spawning to *Clupea pallas*) the fecundity is higher (higher gonadotropin levels) and the eggs will be smaller. At colder water temperature, the opposite occurs, fecundity is lower and the eggs are larger. In this study, females were kept in captivity under higher temperatures than normally would be found in the Barents during the onset of the maturation. Unfortunately, fecundity was not determined in this project.

4.4 Environmental effects

This study was performed in two large mesocosms that initially were supposed to work as replicates, but that actually had offered two different environments to the on-growing

offspring. Undoubtedly that has influenced offspring growth and survival, and therefore, the influence of environmental conditions on the offspring could also be taken in consideration.

During the initial weeks, the 2500m³ mesocosm was characterized by low zooplankton density, which improved from middle of May 2000, onwards until June 8, when the mesocosm experiment was terminated. In the 4400m³ mesocosm, initial weeks were characterized by a high zooplankton density, which was maintained high until approximately the last two weeks, when feeding conditions dropped to very low levels. Additionally, it is worth remembering that survival might have been influenced by the lack of predators in both mesocosms, and by the fact that bottom topography within the mesocosms was very irregular, endowing individuals with many hiding places. Although, temperature increased towards the end of the experiment, the 2500m³ mesocosm was almost about 2.5° C lower than the 4400m³ mesocosm.

Due to the initial poor feeding conditions, a size selection towards larger larvae occurred in the 2500m³ mesocosm. This was observed through a decrease in diversity, when many of the released families were gone; through a higher mortality, and by the increasing of the hatch-check-widths between the newly-hatched stage and week 3.

In the 4400m³ mesocosm, larvae grew faster and went through the yolk-sac period within one week, while in the 2500m³ mesocosm, the yolk-sac period lasted longer. However, as already mentioned, the temperature was also lower in the 2500m³ mesocosm.

In the 4400m³ mesocosm, size selection was minimized; and a larger variability in condition (RNA/DNA ratios) was observed. This variability was also reflected by the otolith microstructure analysis, in which a large amount of slow growing individuals could be observed; and by a larger amount of offspring from small eggs in this mesocosm.

Individuals from large eggs have grown at higher growth rates until approximately the last two weeks, when zooplankton collapsed and temperature was extremely high. This forced the large individuals to reduce growth. On the other hand, small individuals seemed not to be so affected by the adverse conditions. Consequently, large and small individuals ended-up with similar sizes. In the last weeks it was actually a disadvantage to be large, since those individuals had suffered more under the bad feeding conditions than their smaller counterparts. However, this last aspect should be seen with caution in this special case, because the temperature in the 4400m³ was about four times higher than these individuals would have experienced in their natural environment; and this might have affected fish metabolism in an extreme way.

Due to seasonal oscillations in zooplankton supply, fishes are regularly subjected to periods of starvation during which they have to rely on body reserves. In order to survive starvation the organisms have to spare body resources by reducing total energy turnover (Wieser et al. 1992, Yang & Somero 1993).

The reduction of total energy flux leads to a redistribution of the available energy between energy-consuming processes. The energy is mainly channeled into maintenance functions while other processes such as growth are down regulated, or even arrested. Behavioral changes may also contribute to the reduction of the overall energy requirement, like for example reducing swimming activity during starvation (van Dijk et al. 2002). A general reduction of all energy consuming processes can be achieved by a decrease of body temperature (behavioral hypothermia). Fish temperature is very close to environmental temperature. When exposed to thermal gradients fish are able to regulate body temperature by seeking warmer or colder temperatures. The maximum power principle suggests that fish

should select thermal habitats where surplus power is maximized, i.e., maximum energy is available for growth processes (Crowder & Magnuson 1983). The optimum temperature for growth (T_{opt}) of fish is greatly dependent on food availability. If food is limited T_{opt} decreases, because low temperatures are related to low metabolic rates, and therefore less energy is expended on maintenance processes (Jobling 1994). However, even the coldest water layer was about 12°C, which means about three times higher than this stock is adapted to.

When feeding conditions improve in the 2500m³ mesocosm, fishes recovered quickly their growth in a physiological process known as "compensatory growth" and ended up larger than their counterparts from the 4400m³ that had initially extreme favourable feeding conditions.

Compensatory growth is a widespread phenomenon in nature, in which growth is accelerated to catch-up original growth trajectory after food deprivation has been restored. Studies have shown that expression of this growth regulation are depended on several factors, which include the nature, severity and the duration of "under nutrition" (Hayward et. al 1997, Ali et al. 2003). The most common mechanism whereby compensatory growth takes place is through hyperphagia, which is a rate of food consumption significantly higher than shown by fish continuously fed on *ad libitum* ration (Wotton 1998).

The advantage in the 2500m³ mesocosm was that food deprivation happened during a period of relative lower temperature: 4°C to 8°C, what helped fish organism to decrease their metabolic demands. After that, temperature coincided with improving of feeding conditions, which helped fish to recover growth quickly. This was also confirmed by the otolith microstructure analysis from week 5 in the 2500m³ mesocom, which showed that individuals from large eggs had an extremely slow growth up to days 20- 25 after hatching. Right after, growth was rapidly recovered, by week 4 otoliths radius from the same families were significantly larger in mesocosm 2500m³ than otoliths in mesocosm 4400m³. Due to the switch in feeding conditions fishes from the 2500m³ had ended-up significantly larger than their counterparts in the 4400m³ mesocosm. However, the opposite happened to the otoliths. The linear relation between otolith radius and body size decreased in the 4400m³ mesocosm, and many families had shown a negative relation between otolith and body size. Since otolith growth is mainly affected by temperature, calcium deposition has taken place even during the lack of feed at the final period in the 4400m³ mesocosm.

This problem has been already described by many other studies (Mosegaard et al. 1988, Barber & Jenkins 2001, van der Meeren & Moksness 2003, Munday et al. 2004) and seems to occur when water temperature is outside of the normal gradient expected for an specific species, liked occurred during this study. This phenomenon takes place when otolith and somatic growth did not the same way to the environmental conditions, and it is called "decoupling". In addition, for some still unexplained reason, slow growing fish tend to have larger otoliths for their body sizes, while fast growing fish tend to have smaller otoliths in relation to their body sizes (Campana 1990).

During all mesocosm period, survival was only slightly positively related to larval size. The lack of a more clear signal was probably caused by the fact that the mesocosms were predator free environments. Additionally, due to the mesocosms topography there was enough place for larvae to hide, which probably has prevented cannibalism. Based, on the color of stomachs of the offspring sampled it is possible to observe if cannibalism has taken place or not, since larvae that had fed on other larvae have a gray colored stomach (A. Folkvord, IMR, Norway, pers. comm.). The stomach of very few individuals had shown this characteristic. Besides, individuals from week 10 in the 4400m³ were in very poor condition,

and the letargy caused by extreme temperatures, probably did not corroborate for cannibalism either.

4.5 Analysis on the approach followed by this study

4.5.1 Differences between repeat and first spawners

Although in nature, repeat spawners are known to be larger and better conditioned than first time spawners, such conditions could not be held in MACOM. After one year in captivity, the already small differences between repeat and recruit spawners were completely lost. It would have been more effective to built a set-up reflecting the differences found in nature. However, nowadays, more than 85% of the Northeastern arctic cod stock is constituted by first time spawners (Salthaug, IMR pers. comm.). Due to the actual demographic structure of the stock, we did not manage to catch the typical repeat spawners we expected.

The repeat spawners selected for this study were probably second time spawners. As reproduction imply in body energetic costs, those fish had to recover the energy reserves allocated in reproduction and therefore, they could not reach the same growth (SGR) as the first spawners. Since first spawners had higher growth rates, both groups ended up with similar size.

Since cod is a determinate batch spawner, the amount of female eggs recorded will indicate the amount of eggs produced in the season. Unfortunately, in this study, spawning performance was not monitored until the end of the period and therefore, total egg production could not be estimated. However, Trippel (1998) found that first time spawners released significant fewer egg batches with lower egg viability than repeat spawners. The total number of eggs produced by first spawners was significantly lower than that of repeat spawners. The spawning frequency was less uniform in first spawners, and the spawning duration was significantly higher for repeat spawners.

4.5.2 Mating Selection

In nature, cod presents a complex mating behavior, in which females select the best match. However, in this study, males were randomly placed to the females within the different compartments. No courtship behavior could be observed, and some of the couples have shown hostile conducts. The consequences caused by lack of selection associated to this breeding set-up cannot be predicted (Hutchings et al. 1998). However, it is widely held that females show mate preferences in species where the males provide only sperm to the females. In cod, females seem especially attracted to males with larger back fins (Nordeide & Folstad 2000). Besides, females can differentiate sounds from different males, using them as means to assess the desirability of potential mates (Myrberg et al. 1986). The strength of drumming-muscles, i.e., the muscles responsible for male sounds, is positively related to male size and to fertilization potential since large males produce larger amounts of ejaculate (Fox et al. 1995, Nordeide & Folstad 2000). Fertilization failures occur for cod in the wild, and the high presence of first spawners may contribute to this phenomenon (Westin & Nissling 1991, Trippel 1998). Many studies suggested that females indeed do prefer to mate with older males (Zuk 1988, Manning 1989, Simmons & Zuk 1992, Simmons 1995), and this may happen because viability selection leads older males to higher genotypic quality than younger males (Trivers 1972, Manning 1985, Kirkpatrick 1987, Anderson 1994, Beck &

Powell 2000). Rudolfson et al. (2004) found that mating a female with the right male has increased survival in 74%. Bekkevold et al. (2002), noticed that larger males have a higher contribution on egg fertility. They also suggested that the highly skewed distribution of paternity success observed in cod may be a factor contributing to the low effective population size/census population size ratios observed in this species.

The differential allocation hypothesis predicts that females can increase their reproductive success by investing in reproduction depending on the attractiveness of their current mate (Burley 1986). Because sire effects on offspring phenotype may result from allocation of more resources in the offspring of attractive mates, it has been suggested that they may be confounded by genetic effects (Cunningham & Russel 2000). However, rather than confounding genetic sire effects, differences in the allocation of resources to the offspring may in fact promote sexual selection via an amplification of the genetic sire effects (Sheldon 2000). The differential allocation hypotheses have been supported by many studies (Burley 1986, 1988, Petri & Williams 1993, Wedell 1996, Cunningham & Russel 2000, Kolm 2001).

Although the breeding set-up chosen for this experiment did not allow testing this aspect, we noticed that offspring from the largest male did particularly well during the mesocosm period (family 6c, 6a, 8c). Thus, quality of the embryo might have been also influenced by paternal genes after fertilization. Since maternal choice affects offspring phenotype, one could argue that such maternal preferences for "good genes" could also be considered a maternal effect, because it is the maternal behavior that leads to variation among the offspring.

4.5.3 Egg parameters

During monitoring of the spawning season it became clear that the initial plan of selecting batch numbers five to eight is a quite impossible task to be achieved. First, because repeat spawners started spawning earlier than first spawners, which has been confirmed by other experiments (Marteinsdottir et al. 1993, Chambers & Waiwood 1996, Trippel 1998). Second, the large stress induced females to irregular spawning-intervals in the compartments, in which the most affected were the repeat females. Based on the concept of "female happiness" proposed by Solemdal (1995), it is possible to affirm that many of the females were quite unhappy, and some have died during the spawning period. Kjesbu (1988) observed that regular and irregular spawners have a different behavior. Regular spawners typically swam around the compartment for some time and then rested, while irregular spawners have chosen a compartment corner and stayed. The same behavior was observed in this study.

Comparison among eggs from the different females was biased by the fact that Atlantic cod shows considerable variation among batches within a spawning season (Kjesbu et al. 1996). Egg sizes in a batch are ultimately determined by the energy available in the interval between batches (Kjesbu 1991). Regarding alternatives to overcome this problem, a hormonal induction of spawning could be tried. However, although this has been achieved in other fish species (Lin 1982, Zohar 2003, Garcia-Alonso & Vizziano 2004), very little is known about hormonal control of cod maturation. Cryopreservation is not an option either, since eggs cannot be successfully used after freezing (Bromage & Roberts 1995). To be able to get larvae hatching at the same occasion, early-incubated batches were cooled down 1-2°C inducing different ontogenic stages at hatch. Moreover, the multiple regression model has shown that different hatching dates offered some advantage to older larvae concerning feeding in week 1 ($R^2 = 0.10$), probably due to a larger mouth opening. However, this effect had soon disappeared (week 3, $R^2 = 0.000$).

Although egg size differences have been demonstrated for cod in numerous studies (Chambers & Waiwood 1996), in this study, only 10 eggs were measured for each family. Even though, the standard deviation was quite significant, especially for family 6C. A larger amount of egg measurements would certainly have improved the relations.

Additionally, cod eggs are so small that analysis could not be performed on single eggs, but on samples of at least 10ml eggs, and therefore individual differences were lost.

Parental genes strongly influence fecundity and egg quality, but almost nothing is known about gene expression and on mRNA translation in fish oocytes and embryos, which allows room for future investigations. The experimental set-up performed by MACOM did not allow a direct relation between the nutritional state of eggs and their respective spawners. Moreover, since vitellogenin is synthesized in the liver, maternal liver might give a proxy for maternal reserves. However, neither liver weight nor liver index had any relation to the egg parameters analyzed. Since the organic tissues can accumulate water during starvation, a more sensitive method would be to determine total lipid content in muscle and liver cells, which give accurate measures of energy reserves in fish (Adams 1999, Schulman & Love 1999, Lloret & Planes 2003). Kjesbu et al. 1991, noticed that females withdrew protein from the muscle and fat from the liver during the spawning period. Unfortunately, none of these measures were taken. Nevertheless, egg nutritional content was positively related to egg size, which has proven to be true for many ectotherm species (Clarke 1993, Bridges 1993, Guisande & Harris 1995).

4.6 Evolutionary consequences of maternal effects

The process of adaptation basically involves Darwin's principle of evolution by natural selection in which variation in heritable traits is shaped by natural selection, and individuals that leave the most surviving progeny in the next generation have the highest fitness. Since maternal effects, as egg size for example, confer a strong advantage to the offspring and egg size can be genetically transmitted to offspring, the reproductive trait of egg size should respond to the force of natural selection. However, egg size could affect the reproductive phenotype of the offspring as a non-genetic or "environmental effect".

Certainly, the possibility of maternal traits having both genetic and non-genetic effects on reproductive phenotype of offspring complicates the adaptational analysis of maternal effects. Heritable genetic variation that forms the basis of maternal effects remains confounded by heritable non-genetic variation, unless elaborate mating designs are used to estimate the magnitude of purely additive genetic component, as for example half-sibling designs (Falconer 1981). Such problems are even complicated in natural populations where matings cannot be controlled. Consequently, the influence of maternal effects on natural populations can be over or under-estimated, depending on which degree those factors confound themselves (Sinervo & Denardo 1996).

4.7 Conclusions

Cod was a major trade importance in the middle ages, and have supported large fisheries ever since. However, it also provided one of the most spectacular examples of what can go wrong with fisheries. However, the uncertainties on steadiness of cod stocks began much earlier before fishery pressure has become an issue. Based on Norwegian historical data sets on Northeast arctic cod (NEAC) landings, in 1852, an official commission concluded that the stocks were decreasing. Obviously, the oscillations in yield observed at that time were environmentally driven, and not caused by fisheries. Even though, ideas of helping to rebuild fish stocks through artificial propagation started to take place. Nowadays, scientists agree that stock enhancement is not a solution, since the genetic characteristics of cultivated fish released in the wild can have profound consequences on the natural stocks. Besides, the higher costs involved, do not make the project feasible (Hilborn 1998, Blaxter 2000, Svåsand et al. 2000). Although cod aquaculture has highly improved in the last years, the economic viability remains a difficult task. Cod winning demands high animal protein consumption, and as a result, the final product is not competitive enough for the market prices. Therefore, at least for a while, the only viable commercial source remains the ocean.

With most of the valuable stocks becoming overfished, the major challenge now is to keep fish production on the rise to meet the increasing protein demand of a growing global population. The search for predictive relationships between Spawning Stock Biomass (SSB) and recruitment has been a key issue in fishery sciences (Myers et al. 1996). After many years of study, scientists are finally starting to understand some of the many parameters involved in this complex puzzle. Moreover, the belief that simple mathematic models alone may predict reliable responses, is given place to the general idea that answers are found in the fields, and modeling should be improved with real observations, instead of unrealistic assumptions, which in most cases have conducted to misleads.

This study clearly had shown that maternal effects are an issue, they have an effect on cod recruitment and this should be taken in consideration in the future fisheries management. Because the bottom line of this analysis is or we do something about the actual demographic structure of the cod stocks; and improve spawning stock biomass, or for cod's sake we have to stick to the salad!

5. Summary

The aim of this study was to compare the viability of the Atlantic cod (*Gadus morhua*) offspring of recruit (first-time) female spawners with that of the offspring of repeat (elder) female spawners. This was achieved by rearing offspring from first-time and repeat spawning females in large marine enclosures (mesocosms: 2500 and 4400m³), which had their Parental origin assigned through genetic fingerprinting. Survival, growth, and condition among families were compared. After termination of the mesocosm period, individual growth was followed until maturation, when fecundity (egg production) was measured and compared.

In contrast to what is found in natural populations, in this study, recruit spawners became better conditioned than repeat spawners during captivity, masking the expected spawning experience effect. Offspring from recruit spawners were significantly larger and heavier than the offspring of repeat spawners. Even though, a significant relation between female size and egg size was found. Larger eggs had a higher energetic content. Larvae from larger eggs were also larger at hatch and had a larger yolk sac and larger otoliths. They were also more resistant to starvation.

The differences in temperature and feed densities found in the two mesocosms had strongly influenced mean offspring size, weight and nutritional condition (RNA/DNA ratio, RNA/mg muscle tissue, and pyruvate kinase activity). There was no relation between RNA/DNA ratios and maternal effects, indicating that condition was rather a function of environmental influences. The amount of lactate dehydrogenase activity measured did not show any significant differences between mesocosms. However, there were significant family differences within mesocosms. Although environmental conditions had played an important role, the variability in offspring sizes could still be addressed to maternal origin until the end of the mesocosm experiment. Differences in egg size and egg energy content were reflected in differences on growth during the whole mesocosm period, with exception of week 10 in the 4400m³ mesocosm, when environmental influences on offspring increased, and maternal effects were gradually weakened.

Offspring size and survival were only slightly positively correlated. A possible explanation to this could be that small individuals might have been privileged by the absence of predators in the system (mesocosms).

To conclude, maternal effects played an important role in the initial larval stages of this study and could be followed until week 10 in the 2500m³ mesocosm. However, in the 4400m³ mesocosm, they tended slowly to disappear, being masked by the extreme environmental conditions experienced by the juveniles at the end of experiment. Nevertheless, those extreme temperatures experienced by the juveniles in this experiment would never occur in nature, and maybe maternal effects would last for a larger period. Indeed, in spite of manipulation (eg. vaccination, feed ad libitum and size grading) a slight signature of maternal effects was still visible two years after the start of the experiment. This might indicate that in the field, an initial advantage given by the mother might have a decisive influence on late survival and therefore, this aspect should be taken in consideration on the future fishery management of Gadoids.

6. Zusammenfassung

Die Zielsetzungen dieser Studie waren ein Vergleich der Lebensfähigkeit der Nachkommen von "recruit spawners" (unerfahrenen, erstmaligen Laichern) mit "repeat spawners" (bereits erfahrenen Laichern) des Atlantischen Kabeljaus (*Gadus morhua*). Die Nachkommen verschiedener Laichpaare, welche diesen durch Genetischen Fingerabdruck zugeordnet werden konnten, wurden in grossen Becken (Mesokosmen mit 2500 bzw. 4400 m³ Fassungsvermögen) aufgezogen. Überlebensrate, Wachstum, und Kondition des Nachkommen wurde zwischen den Familien verglichen. Nach Beendigung der Mesokosmen-Experimente wurde das Wachstum der Tiere bis zu deren Laichreife verfolgt und ihre Fruchtbarkeit (Eiproduktion) ermittelt und verglichen.

Im Gegensatz zu den Ergebnissen früherer Studien an natürlichen Populationen zeigten die Nachkommen der unerfahrenen Laicher eine bessere Kondition als die der erfahrenen Laicher. Die Nachkommen der erstmaligen Laicher waren größer und schwerer als die von erfahrenen Laichern. Allerdings gab es einen signifikanten Zusammenhang zwischen der Grösse der Weibchen und der Eigrösse. Größere Eier hatten einen höheren Energiegehalt. Larven von größeren Eiern waren ausserdem größer beim Schlupf und hatten größere Dottersäcke und Otolithen, und waren zudem hungerresistenter.

Die schwankenden Temperaturen und Nahrungsdichten, die in den beiden Mesokosmen angetroffen wurden, hatten einen starken Einfluss auf die durchschnittlichen Werte für Grösse, Gewicht und Ernährungszustand (RNA/mg Muskelgewebe, RNA-DNA Verhältnis und Pyruvatkinase Aktivität), die in den Larven und Juvenilen gemessen wurden. Die RNA/DNA Verhältnisse zeigten keine signifikanten Unterschiede zwischen Nachkommen von erfahrenen und unerfahrenen Laichern. Dies kann als Hinweis dafür gesehen werden, dass der Ernährungszustand des Nachwuchses zum Zeitpunkt der Probennahme eher von den Umweltbedingungen als von der mütterlichen Abstammung beeinflusst wurde. Die ebenfalls ermittelten Laktatdehydrogenase Aktivitäten zeigten keine signifikanten Unterschiede zwischen den Mesokosmen, obgleich es Unterschiede zwischen den Familien innerhalb der Mesokosmen gab.

Obwohl die vorherrschenden Umweltbedingungen eine wichtige Rolle gespielt haben, konnte die Variabilität in der Grösse des Nachwuchses bis zur Beendigung des Mesokosmen-Experimentes der mütterlichen Abstammung zugeordnet werden. Unterschiede in Grösse und Energiegehalt der Eier spiegelten sich in unterschiedlichem Wachstum des Nachwuchses während der gesamten Mesokosmenphase wider. Eine Ausnahme stellt in diesem Zusammenhang die 10. Woche im 4400m³ Mesokosmos dar, da zu diesem Zeitpunkt der Einfluss der Umweltbedingungen zugenommen hat (Hungernot), während die mütterlichen Effekte geschwächt wurden. Nachwuchsgrösse und Überlebensrate waren nur schwach positiv korreliert. Eine mögliche Erklärung hierfür könnte sein, dass die kleinen Individuen durch das nicht vorhanden sein von Prädatoren in den Mesokosmen eventuell einen Vorteil genossen haben.

Zusammenfassend betrachtet ließ sich zeigen, dass der mütterliche Effekt zu Anfang des Experimentes deutlich nachzuweisen war und im 2500m³ Mesokosmos bis Woche 10 verfolgt werden konnte. Im 4400m³ Mesokosmos hingegen zeigte der Einfluss der mütterlichen Effekte eine langsam abnehmende Tendenz, da diese Effekte vermutlich durch die extremen Umweltbedingungen zum Ende der Versuchsphase maskiert wurden (extreme Temperaturen und Hungernot). Allerdings würden derart hohe Temperaturen wie sie am Ende der Mesokosmenphase auftraten unter natürlichen Bedingungen normalerweise nie auftreten, und vielleicht hätten die mütterlichen Effekte unter anderen Temperaturbedingungen länger angehalten.

Tatsächlich konnte trotz zahlreicher Manipulationen der Versuchstiere (z.B. Impfung, Sortierung nach Grössenklassen, Fütterung *ad libitum*, u.s.w.) noch zwei Jahre nach Beginn des Versuchs ein schwacher mütterlicher Einfluss festgestellt werden. Dies könnte als Hinweis dafür gesehen werden, dass in der Natur ein von der Mutter vorgegebener Anfangsvorteil entscheidenden Einfluss auf das spätere Überleben haben könnte.. Daher sollte der Einfluss der mütterlichen Abstammung bei zukünftigen Managementstrategien für Gadiden in Erwägung gezogen werden.

7. References

- Adams, S. M. 1999. In: Lipids in freshwater ecosystems. M. T. Arts, and B. C. Wainman, Editors. New York: Springer- Verlag. pp 132-160.
- Ahnelt, H., and Keckeis, H. 1994. Breeding tubercles and spawning behavior in *Chondrostoma nasus* (Teleostei: Cyprinidae): a correlation? Ichthyol. Explor. Freshwaters, 5: 321–330.
- Ajiad, A. Jakobsen, T. and Odsen, N. 1999. Sexual maturation in Northeast arctic cod. J. Northw. Atl. Fish Sci. 25: 1-15.
- Ali, M., Nicieza, A. Wootton, R. J. 2003. Compensatory growth in fishes: A response to growth depression. Fish and Fisheries 4: 147- 190.
- Anderson, J. T. 1988. A review of size dependent survival during pre-recruitment stages of fishes in relation to recruitment. J. Northw. Atl. Sci. 8:55-66.
- Anderson M. 1994. Sexual Selection. Princeton, NJ: Princeton University Press.
- Armstrong J.D., Fallon-Cousins P.S., Wright P.J. 2004. The relationship between specific dynamic action and otolith growth in pike. Journal of Fish Biology. Vol. 64, no. 3, pp. 739-749(11).
- Arnold, S. J. 1994. Multivariate inheritance and evolution: a review of concepts. In: C.R.B. Boake (Ed.) Quantitative genetic studies on behavioural evolution. The University of Chicago Press, Chicago, U.S.A. pp. 17-48.
- Arnold G.P., Walker, M.G., Emerson, L.S. 1994. Movements of cod (*Gadus morhua* L.) in relation to the tidal streams in the southern North Sea. ICES J. Mar.Science 51 (2): 207-232.
- Asano, M. & Mugiya, Y. 1993. Biochemical and calcium binding properties of water soluble proteins isolated from otoliths of tilapia *Oreochromis niloticus*. Com. Biochem. Physiol. 104B: 201-205.
- Astrup, J. & Möhl, B. 1998. Discrimination between low and high repetition rates of ultrasonic pulses by cod. J. Fish Biol. 52: 205-208.
- Avault, J.W. 2001. Selecting new species for aquaculture. Aquaculture 5-6: 55-57.
- Baba, K., Shimizu, M., Mugiya, Y., Yamada, J. 1991. Otolith matrix proteins of walleye Pollock: biochemical properties and immuno-histochemical localization in the saccular tissue. In: Suga, S. Nakahara, H. ed. Mechanisms and phylogeny of mineralization in biological systems. Tokyo Springer Verlag. 57-61.
- Bagenal, T.B. 1969. The relationship between food supply and fecundity in brown trout *Salmo trutta* L. J. Fish. Biol. 1: 167-182.
- Bailey, KM. Houde, E.D. 1989, Predation on eggs and larvae of marine fishes and the recruitment problem. Adv. Mar. Biol. 25, 1-83.
- Barber, M.C. & Jenkins, G. P. 2001. Differential effects of food and temperature lead to decoupling of short-term otolith and somatic growth rates in juvenile King George whiting. J. Fish Biol. 58: 1320-1330.
- Barnung, T. & Grahl-Nielsen, O. 1987. The fatty acid profile in cod (*Gadus morhua* L.) eggs and larvae. Developmental variations and responses to oil pollution. Sarsia, **72**, 415-417.

- Barot S, Heino M, O'Brien L, Dieckmann U. 2004. Estimating reaction norms for age and size at maturation when age at first reproduction is unknown. *Evolutionary Ecology Research* 6 (5): 659-678.
- Barry, James P., Chuck H. Baxter, Rafe D. Sagarin and Sarah E. Gilman. 1995. Climate-related, long-term faunal changes in a California rocky intertidal community. *Science* 267:672-675
- Bayne, B. L., D. L. Holland, M. N. Moore, D. M. Lowe, and J. Widdows. 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. *Journal of the Marine Biological Association of the United Kingdom* 58: 825-841.
- Beck CW, Powell LA. 2000. Evolution of female choice based on male age: Are older males better mates? *Evolutionary Ecology Research* 2: 107–118.
- Bekkevold, D., Hansen, M.M., Loeschcke, V. 2002. Male reproductive competition in spawning aggregations of cod (*Gadus morhua* L.) *Molecular Ecology* 11, 91-102.
- Belchier, M. et al. 2004. Recruitment studies: Manual on precision and accuracy tools. ICES Techniques in Marine Environmental Sciences. Cooperative Research Report N°.33, 35 pp.
- Benoit, H.P., Pepin, P 1999. Interaction of rearing temperatures and maternal influence on egg development rates and larval size at hatch in yellowtail flounder (*Pleuronectes ferrugineus*). *Can. J. Fish. Aquat. Sci.* 56(5): 785-794
- Bergmeyer, H.U. & Bernt, E. 1970. Lactat-dehydrogenase, UV-test mit Pyruvat und NADH. In: Bergmeyer, H U (ed) *Methoden der enzymatischen Analyse* 533-538. Verlag-Chemie, Weinheim, Germany.
- Bergstad, O.A., T. Jørgensen, O. Dragesund 1987. Life history and ecology of the gadoid resources of the Barents Sea. – *Fisheries Research* 5:119-161.
- Bernardo, J. 1993. Determinants of maturation in animals. *Trends in Ecology & Evolution (TREE)* 8:166-173.
- Bernardo, J. 1996. Maternal effects in animal ecology. *Am. Zool.* 36(2): 83-105.
- Bertram, D.F., Chambers, R.C., Leggett, W.C. 1993. Negative correlations between larval and juvenile growth rates in winter flounder: implications of compensatory growth for variation in size-at-age. *Mar. Ecol. Prog. Ser.* 96: 209-215
- Bertram, D.F., Miller, T.J., Leggett, W.C. 1997. Individual variation in growth and development during the early life stages of winter flounder *Pleuronectes americanus*. *Fish. Bull.* 95(1): 1-10
- Bigelow, H.B., Schroeder, W.C. 1953. *Fishes of the Gulf of Maine*. Fish. Bull. Fish Wild. Serv. U.S. 53: 1-577
- Bertram, D.F. & Strahmann, R.R. 1998. Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. *Ecology* 79 (1) 315-327.
- Beverton, R.J.H & Holt, S. J. 1957. On the dynamics of Exploited fish populations. *Fisheries Investigation Series II*, vol. 29, 553 pp.
- Blaxter, J.H.S., Hempel, G. 1963. The influence of egg size on herring larvae (*Clupea harengus* L.) *J. Cons. Perm. Int. Explor. Mer.* 28: 211-240.

- Blaxter, J.H.S., Hempel, G. 1966. Utilization of yolk by herring larvae. *J. mar. biol. Ass. U.K.* 46: 219-234
- Blaxter, J.H.S. 1986. Development of Sense Organs and Behaviour of Teleost Larvae with Special Reference to Feeding and Predator Avoidance. *Trans. Am. Fish. Soc.* 115: 98-114
- Blaxter, J.H.S. 1992. The effect of temperature on larval fishes. *Netherlands Journal of Zoology.* 42: 336-357.
- Blaxter, M. 2000. Genes and genomes of *Necator americanus* and related hookworms. *Int. J. Parasitol.* 30: 347-355.
- Bligh, E. G. & Dyer, W. J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37 : 911-17
- Blaxter, M. 2000. Genes and genomes of *Necator americanus* and related hookworms. *Int. J. Parasitol.* 30: 347-355.
- Bøgstad, B. & Mehl, S. 1997. Interaction between Atlantic cod (*Gadus morhua*) and its prey species in the Barents Sea. In: Forage Fishes in Marine Ecosystems. Alaska Sea Grant Report 97-01. University of Alaska Fairbanks, Fairbanks- AK.
- Boersma, M. 1995. The allocation of resources to reproduction in *Daphnia galeata*. Against the odds? *Ecology* 76: 1251- 1261.
- Bradford, M.J., Peterman, R.M. 1987. Maternal size effects may explain positive correlations between age at maturity of parent and offspring sockeye salmon (*Oncorhynchus nerka*). *Can. Spec. Publ. Fish. Aquat. Sci.* 96: 90-100
- Brawn, V. M. 1961a. Aggressive behaviour in cod (*Gadus callarias* L.) *Behaviour* 18: 107-147.
- Brawn, V. M. 1961b. Reproductive behaviour of cod. (*Gadus callarias* L.) *Behaviour* 18: 176-197..
- Bridges, T. S. 1993. Reproductive investment in four developmental morphs of *Streblospio* (Polychaeta: Spionidae). *Biological Bulletin* 184:144-152.
- Bromage, N.R., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Duston, J. and Barker, G. 1992. Broodstock management, fecundity, egg quality and timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 100, 141-166.
- Bromage, N., Randall, C., Duston, J., Thrush, M. & Jones, J. 1993. Environmental control of reproduction in salmonids. In: Muir, J.F. & Roberts, R.J. (eds.) *Recent advances in aquaculture* vol. 4. Oxford-UK Blackwell scientific publications pp. 55-65.
- Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Dye, J., Smith, P., Gillespie, M. 1994. Egg quality determinants in finfish: the role of overripening with special reference to the timing of stripping in the Atlantic Halibut *Hippoglossus hippoglossus*. *J. World. Aquac. Soc.* 25(1): 13-21
- Bromage, N. and Roberts, R. (eds.) 1995. *Broodstock Management and Egg and Larval Quality*. Blackwell.
- Bromage, N. et al., (1994). Egg quality in finfish: the role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. *J. World Aquacult. Soc.* 25: 13-21.
- Brooker, A.L., Cook, D., Bentzen, P, Wright, J.M and Doyle, R.W. 1994. The organization of

microsatellites differs between mammals and cold water teleost fishes. Canadian Journal of Fisheries and Aquatic Sciences 51: 1959-1966.

Brooks, S., Tyler, C.R., and Sumpter, P. 1997. Egg quality in fish: what makes a good egg? Rev. Fish Biol. Fish. 7: 387-416.

Bucher, T. & Pfeleiderer, G. 1955. Methods in Enzymology, Academic Press, New York 435-440.

Burley, N. 1986. Sexual selection for aesthetic traits in species with biparental care. Am. Nat. 127: 415-445.

Burley, N. 1988. The differential allocation hypothesis: an experimental test. Am. Nat. 132: 611-628.

Butler, J. L. 1992. Collection and preservation of material for otolith analysis, p. 13-17. In Stevenson, D.K. and Campana, S. [ed.]. Otolith microstructure analysis. Can. Spec. Publ. Fish Aquat. 117.

Campana, S. E. 1984. Microstructural growth patterns in the otoliths of larval and juvenile starry flounder, *Platichthys stellatus*. Canadian Journal of Zoology 62, 1507-1512.

Campana, S.E. 1990. How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences 47, 2219-2227.

Campana, S.E. 1996. Year-class strength and growth rate in young Atlantic cod (*Gadus morhua*). Marine Ecology Progress Series 135, 21-26.

Campana, S.E. 1999. Chemistry and composition of fish otoliths; pathways, mechanisms and applications. Mar. Ecol. Prog. Ser. 188: 263-297.

Campana, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59, 197-242.

Campana, S.E. & Neilson, J.D. 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some environmental variables in their production. Canadian Journal of Fisheries and Aquatic Sciences 39(7), 937-942.

Campana, S.E. & Neilson, J.D. 1985. Microstructure of fish otoliths. Canadian Journal of Fisheries and Aquatic Sciences 42, 1014-1032.

Campana, S.E., Gagne, J.A. & Munro, J. 1987. Otolith microstructure data of larval herring (*Clupea harengus*): image or reality? Canadian Journal of Fisheries and Aquatic Sciences 44, 1922-1929.

Campana, S. E., Frank, K.T., Hurley, P.C.F., Koeller, P.A., Page, F.H. & Smith, P.C. 1989. Survival and abundance of young Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) as indicators of yearclass strength. Canadian Journal of Fisheries and Aquatic Sciences 46(Suppl. 1), 171-182.

Cardinale, M. & Modin, J. 1999. Changes in size at maturity of Baltic cod (*Gadus morhua*) during a period of large variations in stock size and environmental conditions. Fish. Res., 41: 285-295.

Chambers, R.C., Leggett, W.C. 1989. Parental influence on early life history traits of winter flounder

(*Pseudopleuronectes americanus*). Rapp. P.-V. Reun. Ciem. 191: 477

Chambers, R.C., Leggett, W.C. 1992. Possible causes and consequences of variation in age and size at metamorphosis in flatfishes (Pleuronectiformes): an analysis at the individual, population and species levels. Netherlands Journal of Sea Research 29(1-3): 7-24

Chambers, R.C., Leggett, W.C. 1996. Maternal influences on variation in egg sizes in temperate marine fishes. Am. Zool. 36(2): 180-196.

Chambers, R.C. & Trippel, E. 1997. In Early life history and recruitment in fish populations. Fish and Fisheries Series 21. Chapman and Hall, London, U.K. pp. 477

Chambers, C. & Waiwood, K.G. 1996. Maternal seasonal differences in egg sizes and spawning characteristics of captive Atlantic cod, *Gadus morhua*. Can. J. Fish. Aquat. Sci. 53: 1986-2003.

Cheverud, J.M., Wagner, G.P., and Dow, M.K. 1989. Methods for the comparative analysis of variation patterns. Syst. Zool. 38: 201-213.

Cheverud, J. M., and A. J. Moore. 1994. Quantitative genetics and the role of the environment provided by relatives in behavioral evolution. Pp. 67-100 in: C. R. B. Boake, ed. Quantitative genetic studies of behavioral evolution. University of Chicago Press, Chicago.

Christiansen, J.S., Fevolden, S.E., Karamushko, O.V., and Karamushko, L.I. 1998. Maternal output in polar fish reproduction. In Fishes of Antarctica: a biological review. Edited by G. di Prisco, E. Pisano, and A. Clarke. Springer-Verlag Italia, Milano. pp. 41-52.

Clark, M. & Galef, B.G. 1995. A Gerbil Dam's Fetal Intrauterine Position Affects the Sex Ratios of Litters She Gestates. Physiology & Behaviour 57(2): 297-299.

Cohen, D.M., Inada, T., Iwamoto, T., Sciallaba, N. 1990. FAO fisheries synopsis. Rome [FAO FISH. SYNOP.], no. 125 vol10, 442 pp.

Congdon, J.D. and Gibbons, J.W. 1987. Morphological constraints on egg size: a challenge to optimal egg size theory? Proc. Natl. Acad. Sci. USA, 84: 4145-4147.

Corlet, J. 1958. Distribution of larval cod in Western Barents Sea. Spec. Publs int. Comm NW. Atlant. Fish (1) 281-288.

Cosgrove J. P., Church D. F., Pryor W. A. 1987. The kinetics of the autoxidation of polyunsaturated fatty acids. Lipids; 22:299-304.

Crowder, L. B., and Magnuson, I. J. 1983. Cost-benefit analysis of temperature and food resource use: a synthesis with examples from the fishes. In: Aspey, W. P. and Lustick, S. I. (eds) behavioral energetics: the cost of survival in vertebrates. Ohio Univ. Press, Columbus, pp.- 189-221.

Cunningham, E.J.A. & Russell, A.F. 2000. Egg investment is influenced by male attractiveness in the mallard. Nature 404 (6773): 74-77.

Cushing, D.H. 1969. The regularity of spawning season in some fishes. Journal do Conseil International pour l' Exploration de la Mer, 33: 81-82.

Cushing, H.D. 1975. Plankton production and year-class strength in fish populations : un update of match-mismatch hypothesis. Advances in Marine Biology, 246: 249-293.

- Davidson, J.E. 1986. Insight and intellectual giftedness. In R.J. Sternberg & J.E. Davidson (Eds.), *Conceptions of giftedness*. New York: Cambridge University Press.
- Dannevig, G. 1963. Artificial propagation of cod. *Fisk.Dir. Skr. Havunders.*, 13(6): 73-79.
- de March, B.G.E. 1993. Genetic aspects of *Arctic charr* culture. SPECIAL SESSIONS ON ARCTIC CHARR CULTURE AND INTENSIVE SALMONID SYSTEMS *Bull. Aquacult. Assoc. Can.* 93(1): 25-30
- Danzmann, R.G. 1997. PROBMAX: A computer program for assigning unknown parentage in pedigree analysis from known genotypic pools of parents and progeny. *Journal of Hereditary* 88: 333.
- Dieckmann, U., Heino, M. and Gødo, O.R., 2002. A quantitative genetics model for the evolution of maturation reaction norms in age- and size-structured populations. *ICES CM* 2002/Y:08.
- Dobson, S.H., Holmes, R.M. 1984. Compensatory growth in the rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* 25(6): 649-656
- Engen, F. & I. Folstad 1999. Cod courtship song: a song at the expense of dance?- *Canadian Journal of Zoology* 77:542-550.
- Einum, S. and Fleming, I.A. 2000. Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature*, 405: 565–567.
- Einum, S. and Fleming, I.A. 2002. Does within-population variation in fish egg size reflect maternal influences on optimal values? *Am. Nat.*, 160: 756–765.
- Einum, S., Hendry, A.P. and Fleming, I.A. 2002. Egg size evolution in aquatic environments: does oxygen availability constrain size? *Proc. R. Soc. Lond. B*, 269: 2325–2330.
- Falconer, D.S. 1965. Maternal effects and selection response. In: S.J. Geerts (ed.) *Genetics Today*, Vol.3, Proceedings of the Eleventh International Congress of Genetics. Pergamon Press Oxford. Pp. 763-774.
- Falconer, D.S. 1981. *Introduction to Quantative Genetics*. Logman, London, 340 pp.
- Farrel, J. & Campana, S.E. 1996. Regulation of calcium and strontium deposition on the otoliths of juvenile tilapia, *Oreochromis niloticus*. *Comp. Biochem. Physiol.* 115 A: 103-109.
- Fay, R.R. & Popper, A. N. 1974. Acoustic simulation of the ear of goldfish (*Carassius aurata*) *J. Expl. Biol.* 61 : 243-260.
- Fay, R.R. 1988. *Hearing in vertebrates : A phycophysics databook*. Hill-Fay Associates, Winnetka, 621pp.
- Ferguson, M.M., Liskauskas, A.P., Danzmann, R.G. 1995. Genetic and environmental correlates of variation in body weight of brook trout (*Salvelinus fontinalis*). *Can. J. Fish. Aquat. Sci.* 52: 307-314
Fisheries Series 21. Edited by R.C. Chambers and E.A. Trippel. Chapman and Hall, London, U.K. pp. 31–62.
- Finn, R. N. 1994. *Physiological energetics of developing fish embryos and larvae*. Dr. Scient. Thesis. University of Bergen.

- Fleming, I.A. and Gross, M.R. 1990. Latitudinal clines: a trade-off between egg number and size in Pacific salmon. *Ecology*, 71: 1–11.
- Fleming, I.A., Jonsson, B., Gross, M.R. and Lamberg, A. 1996. An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). *J. Appl. Ecol.*, 33: 893–905.
- Folkvord A. 1991. Growth, survival and cannibalism of cod juveniles (*Gadus morhua* L.): effects of feed type, starvation and fish size. *Aquaculture* 97:41-59.
- Folkvord A, Otterå H. 1993. Effects of initial size distribution, day length and feeding frequency on growth, survival and cannibalism in juvenile Atlantic cod (*Gadus morhua* L.). *Aquaculture* 114:243-260.
- Folkvord A, Ystanes L, Johannessen A, et al. 1996. RNA:DNA ratios and growth of herring (*Clupea harengus*) larvae reared in mesocosms. *Marine Biology* 126 (4): 591-602.
- Fordham, S. E. & Trippel, E. A. 1999. Feeding behaviour of cod (*Gadus morhua*) in relation to spawning. *J. Appl. Ichthyology* 15: 1-9.
- Fox, C. W., Waddel, K.J. Mosseau, T.A. 1995. Parental host plant affects offspring life histories in seed beetle. *Ecology* 76: 402-411.
- Fox, C.W., M.S. Thakar and T.A. Mousseau. 1997. Egg size plasticity in a seed beetle: an adaptive maternal effect. *American Naturalist* 149:149-163.
- Fyhn, H.J., Serigstad, B., Mangor-Jensen, A. 1987. Free amino-acids in developing eggs and yolk-sac larvae of cod, *Gadus morhua* L. *Sarsia* 72: 363-365.
- Fraser, A.J., Gamble, J.C., Sargent, J.R. 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing egg and unfed larvae of cod (*Gadus morhua*). *Marine biology* 99: 307-313.
- Gall, G.A.E. 1974. Influence of egg sizes and age of the female in hatchability and growth of rainbow trout. *California Fish and Game*, 60: 26-35.
- Gallahar NK, Kingsford MJ. 1996. Factors influencing Sr/Ca ratios in otoliths of *Girella elevata*: An experimental investigation *J. Fish Biol.* 48 (2): 174-186 FEB 1996
- García-Alonso, J. & Vizziano, D. 2004. Induction of Oocyte Maturation in the White Croaker *Micropogonias furnieri* (PISCES: SCIAENIDAE) by Human Chorionic Gonadotropin. *Brazilian Journal of Biology*, Vol. 46(1): 73-80.
- Gisbert, E., Williot, P., Castelló-Orvay, F. 2000. Influence of egg size on growth and survival of early stages of Siberian sturgeon (*Acipenser baeri*) under small scale hatchery conditions. *Aquaculture* 183: 83-94.
- Gosh, R.I. 1985. Metabolism of fish sexual products and embryos. *Naukova Dumka*, Kiev. (In Russian.)
- Gjøsæter J., Enersen K. & Enersen S. E. 1996. Ressurser av torsk og andre fisk i fjorder på den norske Skagerrakkysten. *Fisken og Havet* 23, 1-28.
- Godø, O.R. & Moksness, E. 1987. Growth and maturation of Norwegian coastal cod and Northeast arctic cod under different conditions. *Fisheries Research* 5: 235-242.
- Godø, O.R. & Haug, T. 1999. Growth rate and sexual maturity in cod (*Gadus morhua*) and

- Atlantic halibut (*Hippoglossus hippoglossus*). J. Northw. Atl. Sci. 25: 117-125.
- Godø, O.R. 2000. Fluctuation in Stock Properties of Arcto-Norwegian Cod Related to Long-term Environmental Changes. IIASA Interim Report IR-00-23. 20 pp.
- Godø, O. R., Heino, M., Sjøiland, H., Alvarez, J., Dahl, M., de Lange, J., Gullaksen, O., Tangen, Ø. & Torkelsen, T. 2002. Blue whiting survey during spring 2002. Working Document to The Northern Pelagic and Blue Whiting Fisheries Working Group, Vigo, Spain, 29 April-8 May 2002. 27 pp.
- Guisande, C. & Harris, R. 1995. Effect of total organic content of eggs on hatching success and naupliar survival in the copepod *Calanus helgolandicus*. Limnology and Oceanography 40: 476-482.
- Gulland, J.A., 1965. Survival of the youngest stages of fish and its relation to year class strength. Spec. Pub. Int. Comm. Northw. Atlant. Fish 6: 365-371.
- Halaka, K., and Lusk, S. 1995. Mortality in eggs of nase, *Chondrostoma nasus*, during incubation. Folia Zool. 44 (Suppl. 1): 51-56.
- Hales, R. E., Yudofsky, S. C., & Talbott, J. A. (Eds.). (1994). The American Psychiatric Press Textbook of Psychiatry (2nd ed.). Washington, DC: American Psychiatric Press, Inc.
- Hamlin, H.J., von Herbing, I.H., Kling, L.J. 2000. Histological and morphological evaluations of the digestive tract and associated organs of haddock throughout post-hatching ontogeny. J. Fish. Biol. 57: 716-732
- Haugen, T.O. & Vøllestad, L.A. 2001. A century of life-history evolution in grayling. Genetica 112- 113, 475-491 .
- Hayward, R.S., Noltie, D.B. and Wang, N. 1997. Use of compensatory growth to double hybrid sunfish growth rates. Transactions of the American Fisheries Society 126: 316-322.
- Hawkins, A.D. & K.J. Rasmussen 1978. The calls of Gadoid fish. – Journal of the Marine Biology Association of U.K. 58:891-911.
- Hawkins, A. D. 1986. Radio-tracking observations on Atlantic salmon ascending the Aberdeenshire Dee. Scottish Fisheries Research Report, No 36.
- Hawkins, A.D., Amorim, M.C.P. 2000. Spawning sounds of the male haddock, *Melanogrammus aeglefinus*. Exp. Biol. Fish. 59: 29-41
- Hay, D.E. 1982 Fixation shrinkage of herring larvae: effects of salinity, formalin concentration and other factors. Can. J. Fish. Aquat. Sci. 39:1138-1143
- Heath, D.D. and Blouw, D.M. 1998. Are maternal effects in fishes adaptive or merely physiological side effects? In Maternal Effects as Adaptations (T. A. Mousseau and C.W. Fox, eds), pp. 178-201. Oxford: Oxford University Press.
- Heath, D.D., J.W. Heath, C.A. Bryden, R. Johnson and C.W. Fox. 2003. Rapid evolution of egg size in captive salmon. Science 299:1738-1740.
- Heino, M. 1998: Evolution of age schedules of reproduction and environmental feedback. Doctoral thesis, University of Helsinki. 23 pp. ISBN 952-90-9544-9.
- Heino, M., Dieckmann, U. and Godø, O.R. 2002. Measuring probabilistic reaction norms for age and size at maturation, Evolution 56: 669-678.

- Hempel, G. 1965. On the importance of larval survival for the population dynamics of marine fish. In: CalCOfi Reports. Vol. 10: 13-23.
- Hempel, G., & Blaxter, J.H.S. 1967. Egg weight in Atlantic herring (*Clupea harengus* L.). J.Cons.Perm.Int. Explor.Mer, 31(2):170–95
- Hendry, A.P., Day, T. and Cooper, A.B. 2001. Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. Am. Nat., 157: 387–407.
- Heyer, C.J., Miller, T.J., Binkowski, F.P., Caldarone, E.M., Rice, J.A. 2001. Maternal effects as a recruitment mechanism in Lake Michigan yellow perch (*Perca flavescens*). Can. J. Fish. Aquat. Sci. 58(7): 1477-1487
- Hjort, J. 1914. Fluctuation in the great fisheries of northern Europe reviewed in the light of biological research. Rapp. P.-v.. Reun. Cons. Perm. Int. Explor. Mer. 20: 1-228
- Hilborn, R. 1998. The economic performance of marine stock enhancement projects. Bulletin of Marine Science 62: 661-674
- Hislop, J.R.G., Robb, A.P. & Gauld, J.A. 1978. Observations on effects of feeding level on growth and reproduction in haddock, *Melanogrammus aeglefinus* (L.) in captivity. J. Fish Biol. 13, 85-98.
- 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 British waters. J. Fish. Biol. 32(6): 923-930
- Höglund, J. & Atalo, R.V. 1995. Leks. Princeton University Press. Princeton.
- Høie, H. Folkvord, A., Johannessen, A. 1999. Maternal, paternal and temperature effects on otolith size of young herring (*Clupea harengus* L.). J. Exp. Mar. Biol. Ecol. 234: 167-184
- Holliday, F.G.T. & Jones, M.P. 1965. Osmotic regulation in the embryo of herring (*Clupea harengus*). J. Mar. Biol. Ass. UK 45: 305-311.
- Holtby, L.B. and Healey, M.C. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci., 43: 1946–1959.
- Hostache, G., Pascal, M., Tessier, C. 1995. Influence of incubation temperature on male-female relations in the atipa *Hoplosternum littorale*.
- Houde, E.D. 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. 2: 17-29
- Houde, E.D. (1989). Subtle and episodic in the early life of fishes. Journal of Fish Biology 35(A), 25-38
- Hunter, J.R. 1980. The feeding behaviour and ecology of marine fish larvae. P. 287-330. In: Bardach, J.E., Magnuson, J.J., May, R.C., Reinhart, J.M. (eds.): Fish behaviour and its use in the capture and culture of fishes. ICLARM conference proceedings 5
- Hutchings, J.A., and M.E.B. Jones. 1998. Life history variation and growth rate thresholds for maturity in Atlantic salmon, *Salmo salar*. Can. J. Fish. Aquat. Sci. 55 (Suppl. 1): 22-47.
- Hüssy, K., St. John, M.A., Böttcher U., 1997. Habitat utilisation by juvenile O-group demersal cod: Evidence for density dependent processes influencing recruitment? Mar. Ecol. Prog. Ser., 155:199-208

- Hüssy, K., Mosegaard, H., Hinrichsen, H-H., Böttcher, U. 2003. Factors determining variations in otolith microincrement width of demersal juvenile Baltic cod *Gadus morhua*. Mar. Ecol. Prog. Ser. 258: 243-251
- Hwang, P.P., Wu, S.M. Lin, J.H. and Wu, L.S. 1992. Cortisol content of eggs and larvae of teleosts. General Comparative Endocrinology 86: 189- 196.
- Ivlev, V.S. 1961. Experimental ecology of the feeding of fishes. Translated from the Russian by Douglas Scott. Yale University Press, New Haven, Conn.
- Jakobsen, T. 1999. Fisheries management in the Barents Sea. ICES CM 1999/Q:01.
- Jakobsen, T. & Ajiad, A. 1999. Management implications of sexual differences in maturation and spawning mortality of Northeast arctic cod. J. Northw. Atl. Fish. Sci. 25: 125-131.
- Janssen, G. M., G. De Jong, E. N. G. Josse, and W. Scharloo. 1988. A negative maternal effect in springtails. Evolution 42:828-834.
- Jeffrey S. W., Humphrey G. F., 1975, New spectrophotometric equation for determining chlorophyll a, b, c1 and c2, Biochem. Physiol. Pflanz., 167, 194-204.
- Jennings S, Pinnegar JK, Polunin NVC, et al. 2001. Weak cross-species relationships between body size and trophic level belie powerful size-based trophic structuring in fish communities. JOURNAL OF ANIMAL ECOLOGY 70 (6): 934-944.
- Jobling, M. 1988. A review on the physiological and nutritional energetics of cod *Gadus morhua* L., with particular reference to growth under farmed conditions. Aquaculture 70: 1-19.
- Jobling, M & Baardvik, B.M. 1994. The influence of environmental manipulations on inter and intra- individual variation in food acquisition and growth performance of Arctic charr *Salvelinus alpinus*. J. Fish Biol. 44: 1069-1087.
- Jobling, M & Koskela, J. 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and subsequent period of compensatory growth. J. Fish boil. 49: 658-667.
- Johnston, T.A. 1997. Within-population variability in egg characteristics of walleye (*Stizostedion vitreum*) and white sucker (*Catostomus commersoni*). Can. J. Fish. Aquat. Sci. 54: 1006–1014.
- Jonsson, N., Jonsson, B. and Flemming, I.A. 1996. Does early growth cause a phenotypically plastic response in egg production of Atlantic salmon? Funct. Ecol. 10: 89-96.
- Jonsson, N. and Jonsson, B. 1999. Trade-off between egg mass and egg number in brown trout. J. Fish Biol., 55: 767–783.
- Jones, H. 1968. Fish Migration. The Camelot Press Ltd. London. 325 pp.
- Jones, C. 1986. Determining age of larval fish with the otolith increment technique. Fish. Bull. U.S. 84:91-104.
- Jørgensen, T. 1990. Long-term changes in age at sexual maturity of Northeast arctic cod (*Gadus morhua*) Journal du Conseil International pour l'Exploration de la Mer 46: 235-248.
- Kalliio-Nyberg, I., Koljonen, M., Saloniemi, I. 2000. Effect of maternal and paternal line on spatial and temporal marine distribution in Atlantic salmon. Anim. Behav. 60(3): 377-384

- Kalish, J.M. 1989. Otolith microchemistry: validation of the effects of physiology, age and environment on otolith composition. *J. Expl. Mar. Biol. Ecol.* 132: 151-178.
- Kamler, E. 1992. Early life history of fish. An energetics approach. Chapman and Hall, London, U.K.
- Kamler, E., Keckeis, H., and Bauer-Nemeschkal, E. 1998. Temperature induced changes of survival, development and yolk partitioning in *Chondrostoma nasus*. *J. Fish Biol.* 53: 658–682.
- Kato, T., and Kamler, E. 1983. Criteria for evaluation of fish egg quality, as exemplified for *Salmo gairdneri* (Rich). *Bull. Natl. Res. Inst. Aquacult.* 4: 61–78.
- Keckeis, H., Winkler, G., Flore, L., Reckendorfer, W., and Schiemer, F. 1998. Spatial and seasonal characteristics of 0+ fish nursery habitats of nase, *Chondrostoma nasus* in the River Danube, Austria. *Folia Zool.* 46(Suppl. 1): 133–150.
- Kerrigan, B.A. 1997. Variability in larval development of the tropical reef fish *Pomacentrus amboinensis* (Pomacentridae): The parental legacy. *Mar. Biol.* 127(3): 395-402
- King, 1995, *Fisheries Biology, Assessment and Management*. Fishing News Books. 341 pp.
- Kinghorn, B. P. 1983. A review of quantitative genetics in fish breeding. *Aquaculture* 31: 283-304.
- Kirchhofer, A. 1996. Fish conservation in Switzerland — three case-studies. In *Conservation of endangered freshwater fish in Europe*. Edited by A. Kirchhofer and D. Hefti. Birkhaeuser-Verlag, Basel. pp. 135–145.
- Kirkpatrick, M. 1987. Sexual selection by female choice in polygynous animals. *Annual Review of Ecology and Systematics* 18: 43-70.
- Kirkpatrick, M. and Lande, R. 1989. The evolution of maternal characters. *Evolution* 43(3): 485-503.
- Kolm, N. 2001. Females produce larger eggs for large males in a paternal mouth-brooding fish. *Proc. R. Soc. Lond. B*, 268: 2229–2234.
- Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* 350: 33 38.
- Kirkpatrick, M. & Barton, N.H. 1997. The strength of indirect selection on female mating preferences. *Proc. Natl. Acad. Sci. USA* 94: 1282–1286.
- Kjesbu, O. S. 1988. Aspects of the reproduction in cod (*Gadus morhua* L.) : oogenesis, fecundity, spawning in captivity and stage of spawning. Dr. Sc. Thesis, University of Bergen, Norway.
- Kjesbu, O.S. 1989. The spawning activity of cod, *Gadus morhua* L. *J. Fish. Biol.* 34: 195-206.
- Kjesbu, O.S. 1991. A simple method for determining the maturity stages of northeast Arctic cod (*Gadus morhua* L.) by in vitro examination of oocytes. *Sarsia*, vol. 75, no. 4, pp. 335-338.
- Kjesbu, OS; Klungsoyr, J; Kryvi, H; Witthames, PR; Greer Walker, M. 1991. Fecundity, atresia, and egg size of captive Atlantic cod (*Gadus morhua*) in relation to proximate body composition. *CAN. J. FISH. AQUAT. SCI.* vol. 48, no. 12, pp. 2333-2343.
- Kjesbu, OS; Kryvi, H; Sundby, S; Solemdal, P. 1992. Buoyancy variations in eggs of Atlantic cod (*Gadus morhua* L.) in relation to chorion thickness and egg size: Theory and

observations. J. FISH BIOL. vol. 41, no. 4, pp. 581-599.

Kjesbu, O.S. & Holm, J.C. 1994. Oocyte recruitment in first time spawning Atlantic cod (*Gadus morhua*) in relation to feeding regime. Can. J. Fish. Aquat. Sci. 51: 1893-1898.

Kjesbu, O.S., Solemdal, P. Bratland, P. Fonn, M. 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 53: 610-620.

Knutsen, G.M. & Tilseth, S. 1985. Growth, development and feeding success of Atlantic cod larvae *Gadus morhua* related to egg size. Trans. Am. Fish. Soc. 114: 507-511.

Kjørsvik, E. 1994. Egg quality in wild and broodstock cod (*Gadus morhua* L.) Journal of the World Aquaculture Society, 25: 22-29.

Kjørsvik, E., Mangor-Jensen, A., Holmefjord, I. 1990. Egg quality in fishes. Adv. Mar. Biol. 26: 71-113.

Kolm, N. 2001. Females produce larger eggs for large males in paternal mouth-brooding fish. Proc. R. Soc. Lond. B., 268 : 2229-2234.

Kopiejewska, W. 1986. The nase, *Chondrostoma nasus* (Linnaeus 1758). In Ryby sodkowodne Polski. Edited by M. Bryliaska. Pakstwowe Wydawnictwo Naukowe, Warszawa. pp. 233-237.

Koski, K.V. 1981. The survival and quality of two stocks of chum salmon (*Oncorhynchus keta*) from egg deposition to emergence. Rapports et Procés-Verbaux des Réunions Conseil International pour l'Exploration de la Mer, 178: 330-333.

Koski, W.R., Davis, R.A., Miller, G.W., and Withrow, D.E. 1993. Reproduction. In: Burns, J.J., Montague, J., and Cowles, C.J. The bowhead whale. The Society for Marine Mammalogy, Special Publication No. 2. Lawrence, Kansas: Allen Press Inc. 239-274.

Krogh, A. 1959. The Comparative Physiology of Respiratory Mechanisms. Philadelphia, PA: University of Pennsylvania Press.

Kurlanski, M. 1999. Cod: A Biography of the Fish that Changed the World, Penguin Books. 294 pp.

Laikre, L. and Ryman, N. 1996. Effects on intraspecific biodiversity from harvesting and enhancing natural populations. Ambio 25:504-509.

Lam, T.J. 1994. Hormones egg/larval quality in fish . J. World Aquat. Soc. 25: 2-10.

Lambert, Y. & Dutil, JD. 2000. Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to the spawning level of energy reserves. Can. Journ. of Fish. and Aquat. Sci.

Laurila A, Karttunen S, Merila J. 2002. Adaptive phenotypic plasticity and genetics of larval life histories in two *Rana temporaria* populations. Evolution 56 (3): 617-627

Lasker, R. 1981. Marine fish larvae morphology, ecology and relation to fisheries. University of Washington Press. Seattle.

Laurence, G.C., Rogers, C.A. 1976. Effects of temperature and salinity on comparative embryo development of Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*). J. Cons. Int. Explor. Mer. 36(3): 220-228

Laurila, A., Pakkasmaa, S., Crochet, P., & Merilä, J. 2002. Predator-induced plasticity in early

- life history and morphology in two anuran amphibians. *Oecologia*, 132: 524-530.
- Law, R., Grey, D.R. 1989. Evolution of yields from populations with age-specific cropping. *Evolutionary Ecology* 3: 343–359.
- Lee, A.J. 1952. The influence of hydrography on the Bear Island cod fishery. *Rapp.P.-v.Reun.Cons. perm. int .Explor, Mer* : 131: 74-102.
- Leggett, W.C., Deblois, E. 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? *Netherlands Journal of Sea Research* 32(2): 119-134
- Leising, A.W., Franks, P.J.S. 1999. Larval Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) growth on Georges Bank: a model with temperature, prey size and turbulence forcing. *Can. J. Fish. Aquat. Sci.* 56: 25-36
- Lelek, A. 1987. The freshwater fishes of Europe. Vol. 9. Aula Verlag, Wiesbaden.
- Lessios, H.A. 1987. Temporal and spatial variation in egg size of 13 Panamanian echinoids. *Journal of Experimental Marine Biology and Ecology* 114: 217-239.
- Limburg, K.E. 1995. Otolith strontium traces environmental history of sub-yearling American shad (*Alosa sapidissima*). *Mar. Ecol. Prog. Ser.* 119: 25-35
- Lloret J. and Planes S. 2003. Condition, feeding and reproductive success of white seabream (*Diplodus sargus*) as indicators of habitat quality and the effect of protection in the northwestern Mediterranean. *Marine Ecology Progress Series* 248: 197-208.
- Loken, S., Pedersen, T. 1996. Effect of parent type and temperature on vertebrae number in juvenile cod, *Gadus morhua*, in northern Norway. *Sarsia* 80(4): 293-298
- Lorenzen C. J., 1967. Determination of chlorophyll and phaeopigments: spectrophotometric equations, *Limnol. Oceanogr.*, 12.
- Lough, R.G., Potter, D.C. 1993. Vertical migration patterns and diel migrations of larval and juvenile haddock *Melanogrammus aeglefinus* and Atlantic cod *Gadus morhua* on Georges Bank. *Fish. Bull.* 91(2): 281-298.
- Love, R.M. 1980. The chemical biology of fishes. Vol.2: Advances; 1968-1977. Academic Press, London, 943 p.
- Lusk, S., and Hala.ka, K. 1995. Anglers' catches as an indicator of population size of the nase, *Chondrostoma nasus*. *Folia Zool.* 44: 45–46.
- Mann, R.H.K., and Mills, C.A. 1979. Demographic aspects of fish fecundity. *Symp. Zool. Soc. Lond.* 44: 161–177.
- Magnusson, J.V. MS. 1977. Notes on the eggs and larvae of Greenland halibut at Iceland. ICES C.M. Doc. N. F47
- Manning JT. 1985. Choosy females and correlates of male age. *Journal of Theoretical Biology* 116: 349–354.
- Manning JT. 1989. Age-advertisement and the evolution of the peacock train. *Journal of Evolutionary Biology* 2: 379–384.

- Marsch, E. 1986. Effects of egg size on offspring fitness and maternal fecundity in the orange throat darter, *Etheostama spectabile* (Pisces: Percidae). *Copeia*, 1: 18-30
- Marshall, T., Kjesbu, O.S., Yaragina, N.A., Solemdal, P., Ulltang, Ø. 1998. Is spawner biomass a sensitive measure of the reproductive and recruitment potential of the Northeast Arctic Cod? *Can. J. Fish. Aquat. Sci.* 55: 1766-1783.
- Marteinsdottir, G., Jonasdottir, S. and Thorsteinsson, V. 1993. Seasonal variation in egg size and its influence on egg and larval qualities of cod at Iceland. ICES Symposium on cod and climate change 1993/48. Charlottenlund, Denmark.
- Marteinsdottir, G., Steinarsson, A. 1998. Maternal influences on the size and viability of Iceland cod *Gadus morhua* eggs and larvae. *J. Fish. Bio.* 52(6): 1241-1258
- Marteinsdottir, G., Thorarinsson, K. 1998. Improving the stock recruitment relationship in Icelandic cod (*Gadus morhua*) by including age diversity of spawners. *Can. J. Fish. Aquat. Sci.* 55 (6): 1372-1377.
- Maslov, N.A. 1960. Soviet investigations on the biology of cod and other demersal fish in the Barents Sea. In: Soviet fishery investigations in the North European Seas, 185-231. VNIRO/PINRO, Moscow, 468 pp.
- Matteucci, S.D. y Colma, A. 1982. Metodología para el estudio de la vegetación. Monografía 22, OEA, Washington 168 pp. (In Spanish).
- Mayer, L. M., Schick, L. L., Self, R. F., Jumars, L. P. A. Findlay, R. H. Chen, Z, Sampson, S. 1997. Digestive environments of benthic macroinvertebrate guts: Enzymes, surfactants, and dissolved organic matter, *Journal of Marine Research* 55:785-812
- McCormick, M.I. 1998. Behaviour induced maternal stress in a fish influences progeny quality by a hormonal mechanism. *Ecology* 79(6): 1873-1883
- McCormick, M.I. 1999. Experimental test of the effect of maternal hormones on larval fish of a coral reef fish. *Oecologia* 118: 412-422.
- McEnvoy, B. L. A. 1984. Ovulatory rhythms and over-ripening of eggs cultivated turbot, *Scophthalmus maximus* L. *J. Fish Biol.* 24, 437-448.
- McGinley, M.A. 1989. The influence of a positive correlation between clutch size and offspring fitness on the optimal offspring size. *Evol. Ecol.*, 3: 150-156.
- Mertz, G., and R. A. Myers. 1994. Match/mismatch predictions of spawning duration versus recruitment variability. *Fish. Oceanogr.* 3: 236-245.
- Mertz, G. & Myers, R.A. 1995. Estimating the predictability of recruitment. *Fish. Bull. US.* 93: 657-665.
- Miller, T.J., Crowder, L.B., Rice, J.A., and Marschall, E.A. 1988. Larval size and recruitment mechanisms in fishes: toward a conceptual framework. *Can. J. Fish. Aquat. Sci.* 45: 1657-1670.
- Miller, R.B. 1957. Have the genetic patterns of fishes been altered by introductions or by selective fishing? *Journal of the Fisheries Research Board of Canada* 14: 797-806.
- Miller, T.J., Herra, T., Leggett, W.C. 1995. An individual-based analysis of the variability of eggs and their newly hatched larvae of Atlantic cod (*Gadus morhua*) on the Scotian Shelf. *Can. J. Fish. Aquat. Sci.* 52: 1083-1093.

- Miller, K.M., Le, K.D. and Beacham, T.D. 2000. Development of tri- and tetranucleotide repeat microsatellite loci in Atlantic cod (*Gadus morhua*). *Molecular Ecology* 9: 237-244.
- M'intosh, W. C. & Price, E. 1980. On the development and life histories of teleostean food and other fishes. *Trans. R. Soc. Edinb.* 35: 665-994.
- Moksness, E. 1982. Food uptake, growth and survival of capelin larvae (*Mallotus villosus* Müller) in outdoor constructed basin. *Fisk. Dir. Skr. HavUnders.* 17: 267-285.
- Monteleone, D.M., Houde, E.D. 1990. Influence of maternal size on survival and growth of striped bass *Morone saxatilis* (Walbaum) eggs and larvae. *J. Exp. Mar. Biol. Ecol.* 140 (1-2): 1-11
- Morgan, M.J. & Trippel, E.A. 1996. Skewed sex ratios in spawning shoals of Atlantic cod (*Gadus morhua*). *ICES ICES J. Mar. Sci.* Vol. 53, no. 5, pp. 820-826. 1996.
- Morgan, M.J., Wilson, C.E. and Crim, L.W. 1999. The effect of stress on reproduction in Atlantic cod. *J. Fish Biol* 54(3): 477-488.
- Morita, K., Yamamoto, S., Takashima, Y., Matsuishi, T. Kanno, Y and Nishimura, K. 1999. Effect of maternal growth history on egg number and size in wild white-spotted char (*Salvelinus leucomaenis*). *Can. J. Fish. Aquat. Sci.* 56: 1585-1589.
- Mortensen, A., Damsgaard, B. 1993. Compensatory growth and weight segregation following light and temperature manipulation of juvenile Atlantic salmon (*Salmo salra* L.) and Arctic charr (*Salvelinus alpinus* L.). *Aquaculture* 114(3-4): 261-272
- Mosegaard, H., Svedäng, H. and Taberman, K. 1988. Uncoupling of somatic and otolith growth rates in Arctic char (*Salvelinus alpinus*) as an effect of difference in temperature response. *Can. J. Fish. Aquat. Sci.* 45: 1514-1524.
- Mosegaard, H. 1990. What is reflected by otolith size at emergence? - a reevaluation of the results in West and Larkin (1987). *Canadian Journal of Fisheries and Aquatic Sciences* 47:225-228.
- Mosegaard, H. and Morales-Nin, B. (2000) Section 1: Otolith physiology and morphology. *Fisheries Research* 46, 3-4
- Mosseau, T.A. & Dingle, H. 1991. Maternal effects in insect life histories. *Annu. Rev. Entomol.* 36: 511-534.
- Mosseau, T.A. & Fox, C.W. 1998. *Maternal effects as adaptations*. Oxford University Press. NY.
- Mugiya, Y. 1968. Calcification in fish and shellfish-VIII histochemical similarities between the otolith and the macula region of the sacculus in juvenile rainbow trout, with special reference to the otolith formation of fish. *Fish. Bull. Japn. Soc. Sci. Fish.* 34 : 1096-1106.
- Munday, P.L., Hodges, A., Choat, J.H. and Gust, N. (2004) Sex-specific growth effects in protogynous hermaphrodites. *Canadian Journal of Fisheries and Aquatic Science*, 61: 323-327.
- Murayama, E., Okuno, A., Ohira, T. Takagi, Y. And Nagasava, H. 2000. Molecular cloning and expression of an otolith matrix protein cDNA from the rainbow trout, *Onchorhynchus mykiss*. *Comp. Biochem. Physiol.* 126: 511- 520.

- Muus, B.J., Nielsen, J.G. 1999. Die Meeresfische Europas. Franckh-Kosmos Verlag, Stuttgart.
- Myers, R.A., Hutchings, J.A. and Barrowman N.J. 1996. Hypothesis for decline of the cod in the North Atlantic. Mar. Ecol. Prog. Ser. 138; 293-308.
- Myers, R.A. & Worm, B. 2003. Rapid worldwide depletion of predatory fish communities. Nature 423: 280 –283.
- Myers, R.A., Hutchings, J.A., Barrowman, N.J. 1997. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecological Applications 7: 91-106.
- Myrberg, AA., Mohler, M., Catala, JD. 1986. Sound production by males of a coral fish (*Pomatocentrus partitus*): its significance to females. Anim. Behav. 34: 913-923.
- Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. Int. Dev. Biol. 38:217-229.
- Nagler, J.J., Parsons, J.E., Cloud, J.G. 2000. Single pair mating indicates maternal effects on embryo survival in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 184(1-2): 177-183
- Neilson, J.D., Perry, R.I., Valerio, P. Wiawood, K.G. 1986. Condition of Atlantic cod *Gadus morhua* after the transition to exogenous feeding: Morphometrics, buoyancy and predator avoidance. Mar. Ecol. Prog. Ser. 32(2-3): 229-235
- Nissling, A. & Vallin, L. 1996. The ability of Baltic eggs to maintain neutral buoyancy and the opportunity for survival in fluctuating conditions in the Baltic Sea. J. Fish. Biol. 48, 217-227.
- Nordeide, J.T. 1998. Coastal cod and north-east Arctic cod – do they mingle at the spawning grounds in Lofoten?- Sarsia 83:373-379.
- Nordeide, J.T. & I. Folstad 2000. Is cod lekking or a promiscuous group spawner?- Fish and Fisheries 1:90-93.
- Nordeide, J.T. & E. Kjellsby 1999. Sound from spawning cod at their spawning grounds.- ICES Journal of Marine Science 56:326-332.
- Oganesyan, M.Y. 1993. Periodicity of the Barents Sea cod reproduction . ICES C.M. G:64 Demersal Fish Committee.
- Otterlei E, Folkvord A, Nyhammer G. 2002. Temperature dependent otolith growth of larval and early juvenile Atlantic cod (*Gadus morhua*). ICES Journal of Marine Science 59: 401-410.
- Otterå H, Folkvord A. 1993. Allometric growth in juvenile cod (*Gadus morhua* L.) and possible effects on cannibalism. Journal of Fish Biology 43:643-645
- Ouilett, P., Lambert, Y., Berubé, I. 2001. Cod egg characteristics and viability in relation to low temperature and maternal nutritional condition. ICES J. Mar. Sci. 58 : 672-682.
- Overholtz, W.J. 1987. Factors relating to the reproductive biology of Georges Bank haddock *Melanogrammus aeglefinus* in 1977-83. J. Northw. Atl. Fish. Sci. 7: 145-154
- Pakkasmaa, S., Peuhkuri, N., Laurila, A., Hirvonen, H., Ranta, E. 2001. Female and male contribution to egg size in salmonids. Evol. Ecol. 15(2): 143-153
- Palumbi, S. R. 2004. Why mothers matter. Nature 430, 621-622.

- Panagiotaki, P., Geffen, A.J. 1992. Parental effects on size variation in fish larvae. *J. Fish Biol.* 41(Suppl. B): 37-42
- Panella, G. 1971. Fish otoliths; daily growth layers and periodical patterns. *Science* 173: 1124-1127.
- Parker, G.A. and Begon, M. 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. *Am. Nat.*, 128: 573–592.
- Partridge, L. and M. Farquhar. 1981. Sexual activity reduces lifespan of male fruitflies. *Nature* 294:580-582.
- Partridge, L. & Harvey, P.H. 1985. Costs of reproduction. *Nature* 316: 20.
- Pedersen, T. 1984. Variation of peak spawning of Arcto- Norwegian cod (*Gadus morhua* L.) during the time period 1929-1982 based on indices estimated from fishery statistics. *Floedevigen Rapp.*, 1: 301-316.
- Pedersen, T., Elliasen JE, Eilertsen HC, Tande KS, Olsen RE. 1989. Feeding, growth, lipid composition, and survival of larval cod (*Gadus morhua* L.) in relation to environmental conditions in a enclosure at 70° N in northern Norway. *Papp. P.-v. Reun. Cons. Int. Explor. Mer* 191 : 409-420.
- Pelletier, D., Guderley, H., and Dutil , J.D. 1993. Effect of growth rate, temperature and season on glycolytic enzyme activities in white muscle of cod *Gadus morhua*. *J.Expl. Zool.* 265: 477-487.
- Pelletier, D., Dutil, J.D., Blier, P.U. and Guderley, H., 1994. Relation between growth rate and metabolic organization of white muscle, liver and digestive tract in cod *Gadus morhua*. *J. Comp. Physiol. B. Biochem. Syst. Envir. Physiol.* 164: 179-190.
- Pepin, P., Myers, R.A. 1991. Significance of egg and larval size to recruitment variability of temperate marine fish. *Can. J. Fish. Aquat. Sci.* 48: 1802-1828
- Petterson, A & Lignell, Å. 1996. Decreased astaxanthin levels in the Baltic Salmon and the M74 syndrome. In: Report from the second workshop on reproduction disturbances in fish. (Bengston, BE, Hill, C. and Nellbring, S. eds.) Swedish Environmental Protection Agency, Report 4534: 28-29.
- Petrie, M. & Williams, A. 1993. Peahens lay more eggs for peacocks with larger trains. *Proc. R. Soc. Lond. B* 251: 127–131.
- Pickova J., Dutta P.C., Larsson P.-O. & Kiessling A. 1997. Early embryonic cleavage pattern, hatching success, and egg-lipid fatty composition: comparison between two cod (*Gadus morhua*) stocks. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 2410–2416.
- Pickova J., Kiessling A., Pettersson A. & Dutta P.C. 1998. Comparison of fatty acid composition and astaxanthin content in healthy and by M74 affected salmon eggs from three Swedish river stocks. *Comparative Biochemistry and Physiology B* 120, 265–271.
- Plack, P.A. Pritchard, D.J. and Fraser, N.W. 1971. Egg proteins in cod serum. Natural occurrence and induction by injections of oestradiol 3-benzoate. *Biochem. J.* 121: 847-856.
- Popper, A. & Plath, C. 1993. Inner ear and lateral line of bony fishes. In: *Physiology of Fish.* DH. Evans, ed. CRC Press Boca Raton, Florida, pp.99-136.

- Popper, A. N. and Lu, Z. 2000. Structure-function relationships in fish otolith organs. *Fisheries Res.* 46:16-25.
- Porter MJR, Stefansson SO, Nyhammer G, Karlsen O, Norberg B, Bromage NR 2000. Environmental influences on melatonin secretion in Atlantic cod (*Gadus morhua* L.) and their relevance to commercial culture. *Fish Physiology and Biochemistry* 23 (3): 191-200.
- Quinn, T.P., Hendry, A.P. and Wetzel, L.A. 1995. The influence of life history trade-offs and the size of incubation gravels on egg size variation in sockeye salmon (*Oncorhynchus nerka*). *Oikos*, 74: 425–438.
- Raciborski, K. 1987. Energy and protein transformation in sea trout (*Salmo trutta* L.) larvae during transition from yolk to external food. *Pol. Arch. Hydrobiol.* 34: 437–502.
- Rakitin, A., Ferguson, M.M., Trippel, E.A. 1999. Sperm competition and fertilization success in Atlantic cod (*Gadus morhua*): effect of sire size and condition factor on gamete quality. *Can. J. Fish. Aquat. Sci.* 56: 2315-2323
- Rana, K.J. 1985. Influence of egg size on the growth, onset of feeding , point-of-no-return and survival of unfed *Oreochromis mossambicus* fry. *Aquaculture* 46: 119-131
- Ré, P., Gonçalves, E. 1993. Growth of *Sprattus sprattus* larvae in the German Bight (North Sea) as inferred by otolith microstructure. *Mar. Ecol. Prog. Ser.* 96: 139-145.
- Reznick, D.A. and J.A. Endler. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata*). *Evolution* 36:160-177.
- Reznick, D.A., H. Bryga, and J.A. Endler. 1990. Experimentally induced life-history evolution in a natural population. *Nature* 346:357-359.
- Reznick, D. N. 1991. Maternal effects in fish life histories. Pages 780-793 in E. C. Dudley, editor. *The unity of evolutionary biology. Volume II. Dioscordies, Portland, Oregon, USA.*
- Reznick, D; Callahan, H; Llauredo, R. 1996. Maternal effects on offspring quality in poeciliid fishes. *American Zoologist*, vol. 36, no. 2, pp. 147-156
- Rice, J. A., T. J. Miller, L. B. Crowder, E. A. Marschall, A. S. Trebitz, and D. L. DeAngelis. 1993. Growth rate variation and larval survival: inferences from an individual-based, size-dependent predation model. *Canadian Journal of Fisheries and Aquatic Sciences* 50:133-142.
- Rice, J., Shelton, P.A., Rivard, D., Ghislain, A.C. and Frechet, A. 2003. Recovering Canadian Atlantic Stocks: The shape of things to come? *Int. Counc. Expl. Sea* CM 2003/U:06.
- Rideout, R.M., Trippel, E.A. and Litvak, M.K. 2004. Paternal effects on haddock (*Melanogrammus aeglefinus*) early life history traits. *Journal of Fish Biology* 64:695-701.
- Ricker, W. E. 1954. Stock and recruitment. *Journal of the Fisheries Research Board of Canada* 11: 559-623.
- Riis-Vestergaard, J. 2002. Energy density of marine pelagic fish eggs. *J.Fish Biol.* 60: 1511-1528.
- Rijnsdorp, A.D. 1989. Maturation of male and female North Sea plaice (*Pleuronectes platessa* L.). *ICES J. Cons.*, 46 : 35-51.
- Riveiro, I., Guisande, C., Lloves, M., Maneiro, I., Cabanas, J.M. 2000. Importance of parental effects on larval survival in *Sardina pilchardus*. *Mar. Ecol. Prog. Ser.* 205: 249-258

- Roach, D.A. & Wulff, R.D. 1987. Maternal effects in plants. *Annual Review of Ecology and Systematics* 18:209–235.
- Rudolfson, G., Figenschou, L., Folstad, I., Nordeide J. T. and Sørensen E. 2005. Potential fitness benefits from mate selection in the Atlantic cod (*Gadus morhua*). *Journal of Evolutionary Biology* 18:1, 172-179.
- Roff, D.A. 1982. Reproductive strategies in flatfish: a first synthesis. *Can. J. Fish. And Aquat. Scien.* 39: 1686-1698.
- Roff, D.A. 1984. The evolution of life history parameters in teleosts. *Canadian Journal of Fisheries and Aquatic Science* 41: 989–1000.
- Roff, D.A. 1992. *The Evolution of Life Histories: Theory and Analysis*. New York: Chapman & Hall.
- Sakai, S. and Harada, Y. 2001. Why do large mothers produce large offspring? Theory and a test. *Am. Nat.*, 157: 348–359.
- Rollefsen, G. 1930. The otoliths of cod. *Fiskeridir. Skr. Havunders*, 4 (3): 1-18.
- Rollefsen, G. 1934. The cod otolith as a guide of race, sexual development and mortality. *Rapp. Pv-Reunion Cons. Int. Expl. Mer.* 88: 1-6.
- Rønestad, I., Koven, W.M., Tandler, A., Harel, M., Fyhn, H.J. 1998. Utilisation of yolk fuels in developing eggs and larvae of European Sea Bass (*Dicentrarchus labrax*). *Aquaculture* 162:157-170.
- Rønestad, I., Thorsen, A. and Finn, R.N. 1999. Fish larval nutrition : a review of recent advances in the roles of aminoacids. *Aquaculture* 177: 201-216.
- Rossiter, M.C. 1996. Incidence and Consequences of inherited environmental effects. *Annu. Rev. Ecol. Syst.* 27: 451-476.
- Rossiter MC. 1998. The role of environmental variation in parental effects expression. In: Mousseau TA, Fox CW, eds. *Maternal Effects as Adaptations*. Oxford, UK: Oxford University Press, 112–134.
- Sargent, R.C., Taylor, P.D. and Gross, M.R. 1987. Parental care and the evolution of egg size in fishes. *Am. Nat.*, 129: 32–46.
- Sarvala, J., and Helminen, H. 1995. Significance of egg size variation in the year-class fluctuations of vendace (*Coregonus albula*). *Arch. Hydrobiol. Spec. Issues Adv. Limnol.* 46: 187–194.
- Schulman, G. E. & Love, R. M. 1999. *The biochemical Ecology of Marine Fish*. *Advances in Marine Biology*. Vol.36. Academic Press, New York, N.Y.
- Secor, D.H., Dean, J.M., and Laban, E.H. 1991. *Otolith Removal and Preparation for Microstructural Examination: A users Manual*.
- Sheldon, BC. 2000. Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* 15: 397-402.
- Semlitsch, R.D., Schmiedehausen, S. 1994. Parental contributions to variation in hatchling

size and its relationship to growth and metamorphosis in tadpoles of *Rana lessonae* and *Rana esculenta*. *Copeia* 2: 406-412

Seoka, M., Takii, K., Takaoka, O., Furuta S., Nakamura, M., and Kumai, H. 1997. Chemical and enzyme comparison between red sea bream eggs with high and low hatchability. *Suisanzoshoku*,

Seymour, R.S. and Bradford, D.F. 1995. Respiration of amphibian eggs. *Physiol. Zool.*, 68: 1–25.

Shepperd, J.G. 1982. A versatile new stock-recruitment relationship for fisheries, and the construction of sustainable yield curves. *Journal du Conseil, Conseil International pour la Exploration de la Mer* 40: 65-75.

Shiao, J.C. & Hwang, P-P. 2004. Thyreoid hormones are necessary for teleostean otolith growth. *Marine Ecology Progress Series* 278: 271-278.

Shields, R.J., Brwon, N.P., Smith, S.G. 1997. Blastomere morphology as a predictive measure of fish egg viability. *Aquaculture* 155: 1-12

Simmons LW. 1995. Correlates of male quality in the field cricket. *Gryllus campestris* L.: Age, size, and symmetry determine pairing success in field populations. *Behavioural Ecology* 6: 376–381.

Simmons LW, Zuk M. 1992. Variability in call structure and pairing success of male field crickets, *Gryllus bimaculatus*: The effects of age, size and parasite load. *Animal Behaviour* 44: 1145–1152.

Sinclair, A. , Sinclair, M. and Iles, T.D. 1980. An analysis of some biological characteristics of the 4X juvenile herring fishery. *CAFSAC Res. Doc.*, No. 20, 28 p.

Sinclair, M. 1988. *Marine populations: an essay on population regulation and speciation*. University of Washington Press, Seattle. 252 p.

Sinervo, B. and DeNardo, D.F. 1996. Cost of reproduction in the wild: path analysis of natural selection and experimental tests of causation. *Evolution* 50(3):1299-1313.

Shirota, A. 1970. Studies on the mouth size of fish larvae. *Bull. Jap. Soc. Sci. Fish.*36 : 353-367

Smith, C.C. and Fretwell, S.D. 1974. The optimal balance between size and number of offspring. *Am. Nat.*, 108: 499–506.

Smith, P. 1999. Haddock advance – Canadians move nearer in joint project. *Fish Farm. Int.* 26(3): 5

Solemdal, P. Kjesbu, O.S., Fonn, M. 1995. Egg mortality in recruit- and repeat spawning cod-an experimental study. *Int. Coun. Expl. Sea* CM 1995/G: 35.

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

Springate, J.R.C. & Bromage, N.R. 1985. Effects of egg size on early growth and survival in rainbow trout (*Salmo gairdneri Richardson*) *Aquaculture* 47, 163-172.

Stearns, S.C. & Crandall, R. E. 1984. Plasticity for age and size of sexual maturity: a life-

history response to unavoidable stress. In: G. Potts & R. J. Wootton (eds.) Fish reproduction. Academic Press: London. Pp. 13-33.

Steen, R.P. and Quinn, T.P. 1999. Egg burial depth by sockeye salmon (*Oncorhynchus nerka*): implications for survival of embryos and natural selection on female body size. *Can. J. Zool.*, 77: 836–841.

Stenseth, N.C., Mysterud, A., Ottersen, G., Hurrell, J., Lima, M. 2002. Ecological effects of climate fluctuations. *Science*, Vol 297, Issue 5585, 1292-1296

Sundby, S. 1983. A one-dimensional model for the vertical distribution of pelagic fish eggs in the mixed layer. *Deep-Sea Res.*, 30, 645–661

Sundby, S. and Bratland, P. 1987. "Spatial Distribution and Production of Eggs from Northeast-Arctic Cod at the Coast of Northern Norway 1983-1985". *Fisken Hav*. 1: 1-58.

Sundby, S., H. Bjørke, A. V. Soldal, and S. Olsen. 1989. Mortality rates during the early life stages and year class strength of the North east Arctic cod (*Gadus morhua* L.). *Rapport et Proces-verbaux des Reunions du Conseil International pour l'Exploration de la Mer* 191:351-358.

Su, G.S, Liljedahl, L.E, and Gall, G.A.E. 1997. Genetic and environmental variation of female reproductive traits in rainbow trout (*onchorhynchus mykiss*). *Aquaculture* 154: 115-124.

Svåsand, T., Jørstad, K.E., Otterå, H. and Kjesbu, O.S. 1996. Differences in growth performance between Arcto-Norwegian and Norwegian coastal cod reared under identical situations. *J. Fish Biol.* 49: 108-119.

Svåsand, T., Kristiansen, T., Pedersen, T., Salvanes, A.G.V., Engelsen, R, Nævdal, G. and Nødtvedt, M. 2000. The enhancement of cod stocks. *Fish and Fisheries* 1: 173-205.

Tagawa M, Hirano T. Changes in tissue and blood concentrations of thyroid hormones in developing chum salmon. *Gen. Comp. Endocrinol.* 1989; 76: 437–443.

Tagawa, M. and Hirano, T. 1991. Effects of thyroid hormone deficiency in eggs on early development of the medaka, *Oryzias latipes*. *J. Exp. Zool.* 257, 360-366.

Taggart, J. B., Hynes, R. A., Prodöhl, P. A. and Ferguson, A. 1992. A simplified protocol for routine total DNA isolation from salmonid fishes. *J. Fish Biol.*, 40, 963-965.

Takagi, Y. & Takahashi, A. 1999. Characterization of otolith soluble matrix producing cells in the saccular epithelium of rainbow trout (*Onchorhynchus mykiss*) inner ear. *The Anatomical Record* 254: 322-329.

Takagi, Y. 2002. Otolith formation and endolymph chemistry: a strong correlation between the aragonite saturation state and pH in the endolymph of the trout otolith organ. *Mar. Ecol. Prog. Ser.* 231: 237-245 2002

Takizaw, K., Fujita, Y., Ogushi, Y., Matsuno, S. 1994. Relative change in body length and weight in several fish larvae due to formalin fixation and preservation. *Fish. Sci.* 60(4): 355-359

Tanaka, M., Tanangonan, M., Togata, E.G., de Jesus, H., Nishida, R. and Hirano, T. 1995. Develop of the pituitari, thyreoid and internal glands and applications of endocrinology to the improved rearing of marine fish larvae. *Aquaculture* 135: 111-126.

- Tave, D. 1993. Genetics for fish hatchery managers. 2nd ed., AVI Book, Van Nostrand Reinhold, New York
- Theilacker, G.H. 1978. Effect of starvation on the histological and morphological characteristics of jack mackerel, *Trachurus symmetricus*, larvae. Fish. Bull. 76(2): 403-414
- Theilacker, G.H. 1981. Effect of feeding history and egg size on the morphology of jack mackerel, *Trachurus symmetricus*, larvae. Rapp. P.-v.. Reun. Cons. Perm. Int. Explor. Mer 178 : 432-440
- Theshima, S. 1989. Sterol of marine invertebrates. In Kayama T. (ed.). Sogo-Shitshitsu-Kagaku, Tokio. p. 142-145
- Teshima, S. 1997. Phospholipids and sterols. In: D'Abramo, LR, Connlin, DE. Akiyama, DM. (eds). Advances in World Aquaculture. Vol.6: Crustacean nutrition 1997: 85-197.
- Thompson, R.J. 1982. The relationship between food ration and reproductive effort in green sea urchin, *Strongylocentrotus droebachiensis* Oecologia 56: 50-57.
- Templeman, W. & Bishop, CA. 1979. Age, growth, year class strength and mortality of haddock, *Melanogrammus aeglefinus*, on St. Pierre Bank in 1948-1975 and their relation to the haddock fishery in this area. ICNAF Research Bulletin 14: 85-99.
- Thorpe, J.E., Miles, M.S. and Keay, D.S. 1984. Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture 43: 289- 305.
- Thorpe, J.E. 1994. Performance thresholds and life history flexibility in salmonids. Conserv. Biol. 8: 877-879.
- Thorsen, A., Fyhn, HJ, Wallace, RA. 1993. Free aminoacids as osmotic effectors for oocyte hydration in marine fishes. In: Walter, BT., Fyhn, HJ (eds.) Physiological and biological aspects of fish development. P- 94-98.. University of Bergen, Norway.
- Thorsen, A. & KJESBU, O.S. , 2001. A rapid method for estimation of follicle size and potential fecundity in Atlantic cod using a computer-aided particle analysis system. J. Sea Res. 46: 295-308.
- Trippel, E.A. 1993. Relations of fecundity, maturation and body size of lake trout, and implications for management in northwestern Ontario lakes. N. Am. J. Fish Manag. 13: 64-72.
- Trippel, E. A. 1998. Egg size and viability and seasonal offspring production of young Atlantic cod. Trans. Am. Fish. Soc. 127: 339-359.
- Trippel, E.A. 1999. Estimation of stock reproductive potential : history and challenges for Canadian Atlantic gadoid stock assessment. J. Northw. Atl. Fish. Sci. 25: 61-81.
- Trippel, E.A. 2003. Estimation of male reproductive success of marine fishes. J. Northw. Atl. Fish. Sci. 33: 81-113.
- Trippel, E.A., Castell, J.D., Neil, S.R.E., Blair, T.J. 2000. Assessment of egg quality of haddock (*Melanogrammus aeglefinus*) in paired matings. In: Reproductive biology of fish, pp. 405-407, 6. Int. Symp. on the Reproductive Physiology of Fish, Bergen (Norway), 4-9 Jul 1999
- Trippel, E.A., Kjesbu, O.S., and Solemdal, P. 1997. Effect of adult age and size structure on reproductive output of marine fishes. In Early life history and recruitment in fish populations. Chambers, RC and Trippel, EA. (eds.) Chapman & Hall, London. Pp. 31-62.

- Trippel, E. A. & Morgan, M.J. 1994. Age specific paternal influences on reproductive success of Atlantic cod (*Gadus morhua* L.) from the Grand Banks, Newfoundland. ICES Mar. Sci. Symp. 198 : 414-422.
- Trippel, E.A. & Harvey, H.H. 1989. Missing opportunities to produce: an energy dependent or fecundity gaining strategy in white sucker (*Catostomus commersoni*) C. J. Zool. 67:2180-2188.
- Trivers RL. 1972. Parental investment and sexual selection. In: Campbell B, editor. Sexual Selection and the Descent of Man—1871–1971, 136–179. Chicago II: Aldine Press.
- Truett, G.E., Heeger, P., Mynatt, R.L., Truett, A. A., Walker, J.A. & Warman, M.L. 2000. Preparation of PCR- quality mouse genomic DNA with hot sodium hydroxide and Tris (HotSHOT) BioTechniques 29: 52-54.
- Tyler, C.R. and Sumpter, J.P. (1996). Oocyte growth and development in teleosts. Reviews in Fish Biology and Fisheries. 6:287-318.
- Ulvund, K.A. & Grahl-Nielsen, O. 1988. Fatty acid composition in eggs of Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 45, 898-901.
- Vallin, L., Nissling, A. 1999. Potential factors influencing reproductive success of Baltic cod *Gadus morhua*. Ambio 28: 92-99
- Vallin, L., Nissling, A. 2000. Maternal effects on egg size and egg buoyancy of Baltic cod, *Gadus morhua*. Implications for stock structure effects on recruitment. Fish. Res. 49(1): 21-37
- van den Berghe, E.P. and Gross, M.R. 1989. Natural selection resulting from female breeding competition in a Pacific salmon (coho: *Oncorhynchus kisutch*). Evolution, 43: 125–140.
- van Dijk, P.L.M., Staaks, G., Hardewig, I. 2002. The effect of fasting and refeeding on temperature preference, activity and growth of roach, *Rutilus rutilus*. Oecologia 130, 496-530.
- van Oppen M.J.H., Rico, C., Deutsch, J.C., Turner, G.F. & Hewitt, G.M. 1997. Isolation and characterisation of microsatellite loci in the cichlid fish *Pseudotropheus zebra*. Molecular Ecology 6. 387-388
- van der Meeren, T; Naess, T. 1993. How does cod (*Gadus morhua*) cope with variability in feeding conditions during early larval stages?. MAR. BIOL. vol. 116, no. 4, pp. 637-647
- van der Meeren, T. & Næs, K.E. 1996. Development of rearing techniques using large enclosed ecosystems in the mass production of marine fish fry. Rev. Fish. Sci. 5: 367-390.
- van der Meeren, T. & Moksness, E. 2003, Growth and mortality patterns evaluated from otolith microstructure in Atlantic cod *Gadus morhua* larvae reared on different feeding regimes in mesocosms. Marine Ecology Progress Series 264: 95-107.
- Vetter, R.A.H & Buchholz, F. 1997. Catalytic properties of two pyruvate kinase isoforms in Nordic Krill, *Megacyctiphanes norvegica*, with respect to seasonal temperature adaptation. Comp. Biochem. Physiol. A. 116: 1-10.
- Vinje, F., Heino, M., Dieckmann, U., Gødo, O.R., Mork, J. 2003. Spatial structure in length at age of cod in the Barents sea. J. Fish. Biol. 62: 549-564.

- Voellestad, L.A., Lillehammer, T. 2000. Individual variation in early life-history traits in brown trout. *Ecol. Freshwat. Fish* 9(4): 242-247
- Yang, T.-H. & Somero, G. N. 1993. Effects of feeding and food deprivation on oxygen consumption, muscle protein concentration and activities of energy metabolism enzymes in muscle and brain of shallow-living (*Scorpaena guttata*) and deep-living (*Sebastolobus alascanus*) Scorpenaenid fishes. *Journal of Experimental Biology* 181,213–232.
- Ware, D.M. 1975. Relation between egg size, growth, and natural mortality of larval fish. *Journal of the Fisheries Research Board of Canada* 32: 2503-2512.
- Wedekind, C., Müller, R., Spichers, H. 2001. Potential genetic benefits of mate selection in whitefish. *J. Evol. Biol.* 14: 980-986
- Wedell, N. 1996. Mate quality affects reproductive effort in a paternally investing species. *The American Naturalist* 148: 1075-1088
- West, W.Q.B 1970. The spawning biology and fecundity of cod in Scottish waters. Ph.D. University of Aberdeen, Scotland.
- Westin, L. & Nissling, A. 1991. Effects of salinity on spermatozoa mobility, percentage of fertilized eggs and egg development of Baltic cod (*Gadus morhua*), and implications for cod stock fluctuations in the Baltic. *Mar. Biol.* 108: 5-9.
- Wieser, W., Forstner, H., Medgyesy, N. and Hinterleitner, S. 1988. To switch or not to switch: partitioning of energy between growth and activity in larval cyprinids (Cyprinidae: Teleostei). *Funct. Ecol.* 2, 499–507.
- Wieser, W., Krumschnabel, G., Ojwang-Okwor, J.P. 1992. The energetics of starvation and growth after refeeding in juveniles of three cyprinid species. *Envir. Biol. Fish* 33: 63-71.
- Winkler, G., Keckeis, H., Reckendorfer, W., and Schiemer, F. 1998. Temporal and spatial dynamics of 0+ *Chondrostoma nasus*, at the inshore zone of a large river. *Folia Zool.* 46(Suppl.1): 151–168.
- Wootton, R.J. 1979. Energy costs of egg production and environmental determinants of fecundity in teleost fishes. *Symp. Zool. Soc. Lond.* 44: 133–159.
- Wolf, J.B. 2000. Genetic Interactions from Maternal Effects. *Evolution* 54 (6), pp. 1882-1898.
- Wootton, R. J. 1998. *Ecology of Teleost Fishes* 2nd ed. Kluwer, Dordrecht. 392pp.
- Wootton, R.J. 1994. Life histories as sampling devices: optimum egg size in pelagic fishes. *J. Fish. Biol.* 45: 1067-1077
- Zastrow, C.E., Houde, E.D., and Sanders, E.H. 1989. Quality of striped bass (*Morone saxatilis*) eggs in relation to river source and female weight. *Rapports et Procès-Verbaux des Réunions, Conseil International pour l'Exploration de la Mer* 191 :34-42.
- Zohar, Y; Zmora, O; Findiesen, A; Lipman, E; Stubblefield, J; Hines, AH, Davis, JLD. 2003. Hatchery mass production of blue crab (*Callinectes sapidus*) juveniles. *Journal of Shellfish Research*. Vol.22, n.1, p.363.
- Zou, Z., Cui, Y., Gui, J. Yang, Y. 2001. Growth and feed utilization of gibel carp, *Carassius auratus gibelio*: Paternal effects in a gynogenetic fish. *J. Appl. Ichtyol.* 17(2): 54-58

Zuk M. 1988. Parasite load, body size and age of wild-caught male field crickets (*Orthoptera: Gryllidae*). Effects on sexual selection. *Evolution* 42: 969–976.

Table of Figures:

Figure 1.	<i>Gadus morhua</i> L	1
Figure 2.	Typical pictures from old times: Boys posing with huge codfishes (left, copyright Ryan Shannon DFO, Canada and right, picture from the Greenpeace archive.)	2
Figure 3.	Distribution of the economical zones relevant to the NEAC fishery management in the Barents Sea (from Jakobsen 1999)	4
Figure 4.	Distribution areas and major migrations of NEAC. On the left, main migrations to the spawning groups. Right, return of the spent fish from the spawning to the feeding areas (after Jones, 1968).	5
Figure 5.	The courtship behaviour of cod. Sequence shows the subsequent moments when the female enters the male territory, the male flaunting display to ventral mount and spawning. (From Brawn 1961)	6
Figure 6.	Inner ear of a teleost fish with respective otoliths within the labyrinth systems (after Secor et al. 1991)	11
Figure 7.	External tanks used for spawning during MACOM at the facilities of Parisvatnet. On the right, detailed view of a compartment with a spawning couple inside (photos by V. Burhler)	13
Figure 8.	a) Jon Kåre (IMR Bergen) collecting eggs from the compartments. b) the incubations at the facilities of Parisvatnet (photos by V. Buehler)	14
Figure 9.	Air photograph of Flødevigen Marine Station in southern Norway showing the mesocosms location (photo from Flødevigen archive)	15
Figure 10.	Small juvenile cod being tagged with soft α -tags. On the left: position where the injector's needle is placed. On the right: how it looks like after tagging (photo Ø. Paulsen)	20
Figure 11.	a) Yolk-sac measurements done on newly-hatch larvae (larva from family 7c, photo by V. Buehler) and b) formula applied to calculate yolk sac volume	22
Figure 12.	Specific Growth Rate (SGR) of females calculated for the period January 1999 to January 2000	28
Figure 13a.	Monitoring of the spawning season 2000. The families are indicated on the right upper corner of the graphs. Y axis represents the mean egg size in mm, while X axis indicates the spawning date. White squares represent the spawning sequence of first spawners. The squares in gray indicate the spawning sequence of repeat spawners. Bars give the standard error. The circle indicated with batch was incubated for each family	30
Figure 13b.	Monitoring of the spawning season 2000. The families are indicated on the right upper corner of the graphs. Y axis represents the mean egg size in mm, while X axis indicates the spawning date. White squares represent the spawning sequence of first spawners. The squares in gray indicate the spawning sequence of repeat spawners. Bars give the standard Error. The circle indicated with batch was incubated for each family	31
Figure 14.	Squares (white= first spawners and gray= repeat spawners) indicate the mean egg size produced by the mothers during the spawning season 2000 (all batches were included). Triangles indicate the mean egg size from the batches incubated for the mesocosm experiment (with= first spawners, invert gray= repeat spawners). Error bars give the standard derivation from the mean	32
Figure 15.	Relation between mean egg sizes produced by the females during the spawning season 2000 and the females Specific Growth Rate (SGR). On the left, first spawners (white squares). On the right, repeat spawners (squares in gray).	32
Figure 16.	On the upper left: Relation between female size and age. On the	

- upper right: Relation between egg size and female age. On the lower left: Relation between egg size and female age. White squares represent first spawners and gray circles represent repeat spawners. On the lower right: Relation between egg size and female condition for both first and repeat spawners. Triangles in gray indicate Fulton Index before spawning, while inverted white triangles indicate the Fulton Index for females after the spawning season, weighted without gonads. 33
- Figure 17. On the upper left: Relation between egg size and egg wet-weight ($r=0.99$). On the upper right: Relation between egg size and egg dry-weight. On the lower left: Relation between egg size and energy content. On the lower right: Relation between amount of FAA and egg size. Equations combine egg measurements from first and repeat spawners 34
- Figure 18. On the left: Relation between egg size and larvae size at hatching. On the right: relation between egg size and yolk-sac volume. Main families are shown in the regression. Bi-directional bars give the standard derivation from the mean for y and x values, respectively 35
- Figure 19. On the upper left: relation between larval size and dry-weight. Linear regression. On the upper right: relation between newly-hatched yolk-sac volume and body size. Linear regression. On the lower left: relation between RNA/DNA ratio and newly-hatched dry-weight. Linear regression. On the lower right: relation between RNA/DNA ratio and newly-hatched size 36
- Figure 20. Lapillus of a five weeks old cod larva. Observe the hatch – a bright circle (ring) around the core. The core is the central area within the hatch-check (diagonal white line). The otolith radius is the distance from the center to the outer border (white horizontal line), while the small vertical parallel lines indicate the daily increment widths 37
- Figure 21. On the left: relation between otolith radius at hatching and egg size. On the right: relation between otolith radius at hatching and newly-hatched standard length. Bi-directional bars give the standard derivation from the mean for y and x values, respectively. Main families are indicated 37
- Figure 22. On the left: relation between hatch-check and egg size. On the right: relation between hatch-check and larval size. Bi-directional bars give the standard derivation from the mean for the y and x values, respectively. Main families are shown 38
- Figure 23. On the upper left: relation between otolith core size and Newly-hatched standard length. On the upper right: relation between RNA/DNA ratio and otolith core. On the lower left: relation between otolith hatch-check width and core size. On the lower right: relation between otolith hatch-check and yolk-sac volume. Note that only main families are shown 38
- Figure 24. On the left: relation between otolith radius and newly-hatches standard length. Bi-directional bars give the standard derivation from the mean for y and x values, respectively. On the right: linear regressions plotted for individual families 39
- Figure 25. On the left: White circles and black triangles indicate the weekly salinity variation within the 2500m³ and 4400 m³ mesocosms, respectively. On the right: same symbols indicate the oxygen content in the mesocosms 40
- Figure 26. On the left: White circles and black triangles indicate the daily average temperature in the 2500m³ and 4400m³ mesocosms, respectively. On the right: Black lines, white circles and black triangles indicate the amount of chlorophyll-a in the 2500m³ and 4400m³ mesocosms, respectively. Grey lines, black circles and white

- triangles indicate the amount of phaeo-pigments for the same samples, in the 2500m³ and 4400m³ mesocosms 40
- Figure 27. Phytoplankton composition in the mesocosms (based on the 100-cells method). On the left: data from the 2500m³ and on the right, data from the 4400m³ mesocosms. Legend: black line and black circles = *Thalassiosira* sp., dotted line and white circles = *Skeletonema costatum*, black line and inverted black triangles = *Chaetoceros similis*, semi-dashed back line and white inverted triangles = *Eucampia zodiacus*, black line and black squares = flagellates 41
- Figure 28. Zooplankton density from samples pumped at 3m depth. On the left: data from the 2500m³ and on the right, data from the 4400m³ mesocosms. Legend: black line and gray triangles = total organisms found in the samples per liter, black line and black circles = calanoida nauplii per liter, black line and white squares = calanoida copepodites per liter 42
- Figure 29. Reduction of estimated abundance of larvae over time. White diamonds indicate the amount of larvae estimated for the 4400m³, while gray diamonds indicate de amount of larvae for the 2500m³ mesocom. Note that releasing and termination numbers are known. 43
- Figure 30. On the left: relative frequencies of recruit and repeat spawner´s offspring in the 4400m³. On the right: relative frequencies of recruit and repeat spawner´s offspring in the 2500m³ mesocosm before (a) and after (b) removal of recruit family 1c. Figure adapted from L. Hauser, Univ. Hull 44
- Figure 31. Percentage of larvae recovered on the first sampling week in the 2500m³ mesocosm (left) and 4400m³ mesocosm (right) 44
- Figure 32. Histograms indicate the relative frequency of the families sampled during the week 1, 3, 5 and 10 in the 2500m³ mesocosm 45
- Figure 33. Histograms indicate the relative frequency of the families sampled during the week 1, 3, 4 and 10 in the 4400m³ mesocosm. 45
- Figure 34. Shannon´s evenness calculated for family frequencies in the 2500m³ and 4400m³ mesocosm during sampling weeks. Figure adapted from L. Hauser 46
- Figure 35. Relation between family survival and egg size at the sampling weeks 1, 3, 5 and 10 in the 2500m³ mesocosm experiment 2000. Note that there was no correlation on weeks 3 and 5 47
- Figure 36. Relation between mean family survival in (%) and egg size at the sampling weeks 1, 3, 4 and 10 (no correlation) in the 4400m³ mesocosm experiment 2000 48
- Figure 37. On the left: Triangles in gray represent the relation between egg size and mean survival of families in (%) during sampling weeks 1, 3, 5 and 10 in the 2500m³ mesocosm. On the right: Black square represent the same relation for weeks 1, 3, 4 and 10 in the 4400m³ mesocosm 48
- Figure 38. Relation between offspring SL and egg size in the different sampling weeks. Each symbol gives the mean between a certain family offspring size and the respectively egg size mean. On the left: triangles in gray show results from the 2500m³ mesocosm, while black squares indicate results from the 4400m³ mesocosm 49
- Figure 39. Relation between maternal size and mean offspring SL in the different sampling weeks. On the left: triangles in gray indicate results for the 2500m³ mesocosm. On the right: black squares indicate results for the 4400m³ mesocosm 50
- Figure 40. Relation between family survival (%) and mean offspring size during sampling weeks 1, 3, 5 and 10 in the 2500m³ mesocosm 51
- Figure 41. Relation between family survival (%) and mean offspring size during

	sampling weeks in the 4400m ³ mesocosm	52
Figure 42.	Relationship between egg size and offspring's Specific Growth Rate (SGR) in the 4400m ³ mesocosm	52
Figure 43.	Relationship between egg size and offspring's Specific Growth Rate (SGR) in the 2500m ³ mesocosm	53
Figure 44.	Comparison of standard length, dry weight and RNA/DNA ratio of cod larvae and juveniles from recruit spawners and repeat spawners reared in the mesocosm. Data points indicate mean values. For 2500m ³ mesocosm, gray circles and gray triangles represent the offspring from recruit and repeat spawners, respectively. For 4400m ³ mesocosm, black squares and black inverted triangles represent the offspring from recruit and repeat spawners, respectively. Note that first spawners are called "recruit"	54
Figure 45a.	Comparison of standard length among families. On the upper left: recruit spawner's offspring from the 2500m ³ mesocosm. On the upper right: repeat spawner's offspring from the 2500m ³ mesocosm. On the lower left: recruit spawner's offspring from the 4400m ³ mesocosm. On the lower right: repeat spawners from the 4400m ³ mesocosm	57
Figure 45b.	Comparison of dry weight among families. On the upper left: recruit spawner's offspring from the 2500m ³ mesocosm. On the upper right: repeat spawner's offspring from the 2500m ³ mesocosm. On the lower left: recruit spawner's offspring from the 4400m ³ mesocosm. On the lower right: repeat spawners from the 4400m ³ mesocosm	58
Figure 45c.	Comparison of RNA/DNA ratio among families. On the upper left: recruit spawner's offspring from the 2500m ³ mesocosm. On the upper right: repeat spawner's offspring from the 2500m ³ mesocosm. On the lower left: recruit spawner's offspring from the 4400m ³ mesocosm. On the lower right: repeat spawners from the 4400m ³ mesocosm	59
Figure 46.	On the left: Mean Specific Growth Rates for the different families offspring in the 2500m ³ mesocosm. On the right: Mean Specific Growth Rates for the different families offspring in the 4400m ³ mesocosm. Note: to avoid superposition only some families are shown	59
Figure 47a.	Mean increment width at age from otoliths obtained from the same individuals samples at weeks 3, 4 and 5 in the mesocosms. On the left: reading obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m ³ mesocosm, respectively. White triangles and inverted black triangles indicate widths of week 3 and 5 of the 2500m ³ mesocosm, respectively. White triangles and inverted black triangles indicate increment widths of week 3 and 4 of the 4400m ³ mesocosm, respectively. Error bars give the standard derivation (SD) from the mean. Note that sagittal increments are wider than lapillar increments	60
Figure 47b.	Mean increment width at age from otoliths obtained from the same individuals samples at weeks 3, 4 and 5 in the mesocosms. On the left: reading obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m ³ mesocosm, respectively. White triangles and inverted black triangles indicate widths of week 3 and 5 of the 2500m ³ mesocosm, respectively. White triangles and inverted black triangles indicate increment widths of week 3 and 4 of the 4400m ³ mesocosm,	

- respectively. Error bars give the standard derivation (SD) from the mean. Note that sagittal increments are wider than lapillar increments. Some families are missing in some sampling weeks
- Figure 47c. Mean increment width at age from otoliths obtained from the same individuals samples at weeks 3, 4 and 5 in the mesocosms. On the left: reading obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m³ mesocosm, respectively. White triangles and inverted black triangles indicate widths of week 3 and 4 of the 4400m³ mesocosm, respectively. Error bars give the standard derivation (SD) from the mean. Note that sagittal increments are wider than lapillar increments. Some families are missing in some sampling weeks
- Figure 47d. Mean increment width at age from otoliths obtained from the same individuals samples at weeks 3, 4 and 5 in the mesocosms. On the left: reading obtained from the Lapillar otolith. On the right: readings obtained from the Sagittal otolith. Black squares and white circles indicate increment widths of week 3 and 5 of the 2500m³ mesocosm, respectively. White triangles and inverted black triangles indicate widths of week 3 and 4 of the 4400m³ mesocosm, respectively. Error bars give the standard derivation (SD) from the mean. Note that sagittal increment are wider than lapillar increments. Some families are missing in some sampling weeks
- Figure 48. On the left: hatch-check widths observed in otoliths from newly-hatched larvae. On the right: Hatch-check widths observed in otoliths of larvae samples at week 3. Gray squares represent the family means for the 2500m³ mesocosm, and black circles represent the means for the 4400m³ mesocosm. Error bars give the standard derivation from the mean
- Figure 49. Comparison of mean increment width (in interval of five increment) among families. On the upper left and right: offspring sampled in the 4400m³ mesocosm, during weeks 3 and 4 respectively. On the lower left and right: offspring sampled in the 2500m³ mesocosm, during weeks 3 and 5 respectively
- Figure 50. On the upper left and right: relation between otolith radius at hatching and sum of increments at week 3 and 4 in the large mesocosm. On the lower left and right: relation between otolith radius at hatching and sum of increments at week 3 and 5 in the small mesocosm
- Figure 51. Example of otolith growth (*sagitta*) on depece of hatch-check width. Gray circles indicate otolith growth (in increment widths) for small eggs offspring (1b) and small hatch-check (0.87 μm). While circles indicate otolith growth for small eggs offspring (1b) and large hatch-check (1.23-1.41 μm). Gray triangles indicate otolith growth (in increment widths) for large eggs offspring (6c) and small hatch-check (1.05 μm). White triangles indicate otolith growth for large eggs offspring (6c) and large hatch-check (1.45 μm). On the left: data from 4400m³ mesocosm at week 4. N=3, 3, 5, 8 for 1b and 6c upwards. On the right: data from 2500m³ mesocosm at week 5. N=2, 2, 3, 7 upwards
- Figure 52. On the left: mean otolith radius of selected families during sampling weeks 3, 5 and 10 in the 2500m³ mesocosm. On the right: mean otolith radius of selected families during sampling weeks 3, 4 and in the 4400m³ mesocosm. Note: to avoid superposition only main families are shown
- Figure 53. On the left: gray bars indicate the mean otolith radius (*Lapillus*) among families in the 2500m³ mesocosm, while dark bars indicate

- the mean otolith radius (*Lapillus*) among families in the 4400m³ mesocosm at week 10. On the right: otolith weight (Sagittae) from the same individuals used for *Lapillus* measurements 68
- Figure 54. On the left: gray squares indicate the relation between otolith radius and body size for weeks 3 and 5 in the 2500m³ mesocosm. White circles indicate the relation between otolith radius and body size for weeks 3 and 4 in the 4400m³ mesocosm. On the right: the relation between otolith radius and body size for weeks 3, 4, 5 and 10 in the 2500m³ mesocosm (gray circles) and 4400m³ mesocosm (white circles) 68
- Figure 55. On the left: relation between fish weight and size in the 2500m³ (gray circles) and 4400m³ (white squares) mesocosm. On the right: relation between otolith weight and fish size in the 2500m³ (gray circles) and 4400m³ (white squares). 69
- Figure 56. Bi-directional bars give the mean *sagitta* weight at mean body weight for the different families in the 2500m³ mesocosm (gray squares) and 4400m³ (black circles). Error bars give the standard derivation from the mean 69
- Figure 57. Relation between otolith radius (*Lapillus*) and fish body length. Each one of the gray squares give the individual data between otolith and fish for the 4400m³, while inverted white triangles give the same relation for the 2500m³ mesocosm. Families are indicated in the graphs. From left upper to last lower figure on the right: 1b, 1c, 3b, 6b, 8c and 6c respectively. Note that family 10b are not show because of the lower number of individuals 70
- Figure 58. Comparison among individuals from 2500m³ and 4400m³ mesocosm. a) Standard length, b) dry-weight, c) liver weight, d) liver-index, e) RNA concentration in the muscle tissue, and f) RNA/DNA ratio. The box represents the interquartile range, while the bars denote the highest and lowest values and squares indicates the median. Data were analyzed by Mann- Whitney U-Test. Plots from C. Clemmesen 71
- Figure 59. On the left: Comparison of lactate dehydrogenase activity between 2500m³ and 4400m³ at week 10 cod. On the right: Comparison of pyruvate kinase activity between 2500m³ and 4400m³ mesocosm at week 10 cod. The box represents the interquartile range, while the bars denote the highest and lowest values and square indicates the median. Data were analyzed by Mann- Whitney U-test. Plots from C. Clemmesen 72
- Figure 60. Comparison of liver dry weight, liver index (liver wet weight/total body wet weight), RNA/mg muscle tissue. RNA/DNA ratio, lactase dehydrogenase and pyruvat kinase activity of week 10 cod juveniles reared in the 2500m³ and 4400m³ mesocosms in 2000, subdivided into recruit and repeat spawners. The box represents the inter-quartile range, the bars denote the highest and lowest values, the square indicates the median, *denotes significant differences based on Mann-Whitney U-test. Plots from C. Clemmesen 73
- Figure 61. RNA/DNA ratios and Otolith radius in comparison to the zooplankton density found in the 4400m³ mesocosm (left) and 2500m³ mesocosm (right) during the experiment 2000. Note that the axis for otolith radius is not shown. 74
- Figure 62. Relation between RNA/DNA ratio and offspring size in at weeks 3 and 4 in the 4400m³ mesocosm (left) and 3 and 5 (right) in the 2500m³ mesocosm 74
- Figure 63. Values of R-square for egg size (gray symbols) and for environment (white symbols), during the mesocosm period 76
- Figure 64. Mortality in the months after fish were transferred from the

	mesocosms to indoor tanks. Note: only natural mortality is shown.	76
Figure 65.	Temperature oscillation during the tank period (June 200 to April 2002)	77
Figure 66.	On the left: frequency of individuals that were randomly selected for tagging on January 2001. On the right: weight and relations of fishes used for tagging. Squares in gray represent the fishes from 4400m ³ mesocosm, and black squares represent the fishes from 2500m ³ mesocosm	78
Figure 67.	Relation between weight and length during the whole tank period. Squares in gray represent data from 4400m ³ mesocoms while black squares represent data for 2500m ³ mesocosm. Error bars give the standard deviation from the mean	78
Figure 68.	Individual growth trajectories from the mean of individuals belonging to a certain family. On the upper left and right: development of weight and length for offspring from 4400m ³ mesocosm. On the lower left and right: development of weight and length for offspring from 2500m ³ mesocosm	79
Figure 69.	Proportion of families from tagging day (start) and after two years of tanks experiment (end). On the left: fish from large mesocosm. On the right: fish from small mesocosm	81
Figure 70.	Mean termination values for the mature female in the tank experiment. Black and white bars represent offspring from recruit and repeat spawners, respectively. Histogram "E" indicates the number of individuals available to all plots. Note that only groups with at least five mature female were included. Plots from A. Thorsen, IMR-Norway	83

List of Tables:

Table 1.	Number of larvae from the different families released in the mesocosms: Family, Group (concerning spawning experiment), volume of eggs incubated, incubation date, hatching date, releasing date, fertilization rate, percent of larvae mal-formation, amount of larvae released in the 2500m ³ mesocosm, amount of larvae released in the 4400m ³ mesocosm and total larvae released	17
Table 2.	Characteristics of the broodstock utilized in the experiment 2000. The families were named after the compartments in which male and female pairs were placed during the spawning season. Note: First spawners were called "First spawners"	27
Table 3a.	Comparison of mean values of standard length (SL) between recruit (first spawners) and repeat spawners offspring. Significance was tested by Student's t-test and significant differences are highlighted in bold	54
Table 3b.	Comparison of mean values of dry weight (DW) between recruit (first spawners) and repeat spawners offspring. Significance was tested by Student's t-test and significant differences are highlighted in bold	55
Table 3c.	Comparison of mean values of RNA/DNA ratio between recruit (first spawners) and repeat spawners offspring. Significance was tested by Student's t-test and significant differences are highlighted in bold	55
Table 4.	Size of the samples (N) used for all plots concerning biochemistry and otolith data. For otoliths symbols represent (<i>lapillus</i>) and (<i>sagitta</i>) respectively.	56
Table 5.	Weight, length, Fulton condition factor, potential fecundity, realized fecundity and oocyte diameter in 2 year old offspring from recruit and repeat mother fish of the year 2000 tank experiment	81
Table 6.	Number (A) and percent fraction (B) of fishes with previtellogenic and	

vitellogenic oocytes of different families at termination of the year 2000 tank experiment. Only family groups with 10 or more female fishes at termination were include 82

Table 7. Weight, length and Fulton Index of female fish at termination of the year 2000 tank experiment split by ovary stage. PV = Previtellogenic and V = Vitellogenic 82