Large Scale Production

of

Baltic Sea Cod

Bornholm 1992-1994

By

Philip Prince

Preface

Due to a decrease in the eastern Baltic cod stock in the beginning of the 1990s the Ministry of Agriculture and Fisheries initiated a project in 1992 with the purpose of developing a breeding method leading to a large-scale production of Baltic cod larvae.

The purpose was to increase the future natural cod population in the Baltic Sea by a restocking with larvae. The project was based on a collaboration between the Ministry of Agriculture and Fisheries, the 'Organization of Danish Fishermen' coordinated by the Danish Institute for Fisheries research.

The project was intended to last for 2 years, but the trial period was extended to 3 years. It was initiated in May 1992 by the establishment of a plant in Østre Flak 4, Neksø, Bornholm. The plant consists of an outdoor semi-intensive production department and an indoor recirculation (?) production department. The design of the production plant has been elaborated in accordance with the current methods of semi-intensive and intensive rearing of marine fish spawn. The theoretical production capacity of the semi-intensive department is about 60,000 larvae, and that of the intensive department about 100,000 larvae, in both cases of the size of 3cm.

Findings from 1992 proving that the collection of cod eggs is difficult led to an agreement with the Fishery Laboratory of Bornholm in 1993 aiming at building up a recirculation plant for breeding fish. Until summer 1993 the running of the plant was performed by the Fishery Laboratory of Bornholm whereupon the running was taken over by the Danish Institute for Fisheries Research. During the years 1993 and 1994 the plant has been delivering cod eggs for the breeding experiments.

The aim of this paper is to give a summary of the findings and results in the years 1992, 1993 and 1994.

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1. Breeding 1992

In this year the intention was to start the feeding experiments in both the semi-intensive and the intensive production department. Due to the late start of the project in the end of May it was impossible to initiate the actual feeding experiments as the temperatures in the plant were too high. In the beginning of June, for instance, when the first hatching experiments with cod eggs were carried out the temperature in the semi-intensive department had become as high as 18° C which was the minimum temperature registered in the project period until 17 August. The maximum temperature in the period was 24° C.

In the trial period the Royal Veterinary and Agricultural High School of Denmark took part in the project supervising the health of the cod eggs and the larvae. It was found that cod eggs from the Baltic Sea have got a low toleration to high temperatures. At 12° C and a salinity of 16 ‰ the hatching per cent was 20. Eggs that were incubated at 16° C and 20° C in 16‰ seawater did not hatch at all. Temperature toleration experiments with Baltic cod larvae exhibited a mortality rate of 100 % within 5 days at 16° C and at a salinity of 16‰. (Buchmann, K. et al, 1992). Experiments with North Sea cod eggs have all shown that at an incubation temperature of 12° C the eggs will be lost before the end of the blasto stage (Thomsen, B.M., 1981).

In the beginning of June the temperature in the intensive feeding experiment plant was 16° C, and the maximum temperature registered in the experiment period was 25° C. For that reason it was also impossible to carry out feeding experiments in this plant.

From the outset of the project it had been the intention that the breeding fish should be stripped in the field to collect eggs for the hatching experiments. It turned out, however, to be impossible to collect eggs in a quantity relevant for production. Baltic cod are batch spawners, and each individual spawns within a period of about 3 months. For that reason it is most difficult to catch females with a large quantity of eggs in a maturation state ready to be stripped. Furthermore,

the difficulties were increased by the fact that the cod catches were rather poor this year (Prince P., 1993)

Due to all this only a few hatching experiments were carried out, in the majority of which the egg quality was very poor. In most cases the mortality rate of the eggs was very high - up to 100 %. The fertilization rate was mostly below 30%.

During the project a considerable quantity of eggs with an erroneous cell division were found which may be one of the causes of the high mortality rate. These egg batches primarily originated from spawners landed by fishermen whereafter the stripping took place at the quay. The large number of deformities may well be due to the fact that the spawners were dead before being landed, the eggs being exposed to a lack of oxygen and/or excessive heat during the transportation that took place in the summer heat.

In all it was difficult to handle the eggs in the summer heat and to avoid temperature raising (Prince, P., 1993).

The hatching of the eggs was carried out in 15-16‰ seawater pumped up from a depth of 70 metres. The hatchery is the only breeding system attached to a refrigerating plant. The system has been described by P. Prince in 1993. Even though the plant was cooled it was impossible to maintain anything like a fairly stable range of temperature. The lowest temperature measured was 4.5° C and the highest one 10.5° C. In certain hatching experiments a temperature fluctuation of X° was measured from the start of the incubation (?) period to the hatching. It is most likely that the temperature fluctuations in the hatchery have had a negative influence the on hatching results.

In the group of newly hatched umbilical sac larvae individuals with epithelium damages were found in a frequency of 18-35%. It is conjectured that these damages were caused by mechanical manipulation of the larvae such as turbulence in the hatching cylinders. In certain cases

lordosis and scoliosis were found in a frequency of up to 20%. In all probability such diseases are caused by the presence of destructive agents in the eggs due to unsatisfactory conditions during the development of the eggs or to bad oxygen conditions in the hatching cylinders (Buchmann, K. et al., 1992). Certain larvae batches exhibited frequencies of 25% or more of fungoid growth or bacteria infections. It was impossible, however, to prove that the infections were lethal.

To preclude that the hatching procedure should be influenced by such potentially negative factors it was decided that the hatching of the eggs should be carried out in the feeding experiment plant in 1993. The tanks of the plant that contain 600 l each take in water in the bottom promoting the feasiblility of changing the water rapidly without furthering turbulence - especially because the eggs float in the surface. In accordance with the programme a quantitative and qualitative monitoring of the zooplankton that was being led into the plant was carried out in the semi-intensive production plant aiming at testing the production potential of the method. The theoretical yielding potential was 20 kg of zooplankton based on net weight when dry (Rasmussen, K., Brun,I., 1992). The actual yielding potential was 5 kg of zooplankton based on net weight when dry (Prince, P., 1993). The difference between the theoretical and the actual yielding potential was mainly due to the fact that the drumfilter was only in operation 25% of the time due to a clogging up with filamentous algae.

After the trial period in 1992 it was found that it would be necessary to procure a large, stable delivery of high-quality fertilized eggs by establishing a stock of breeding fish to accomplish a large scale production of spawn. To go through with the actual feeding experiments it would be necessary to connect a refrigerating plant to the intensive production plant. To go through with the feeding experiments in the semi-intensive plant it would be necessary to have disposal of high-quality, newly hatched larvae as early as in April to avoid temperature problems.

The hatchery which is a copy of a hatchery at the West Coast which has successfully

hatched Nordsø cod eggs did not fulfil the expectations. The fact that a large quantity of the larvae had epithelium damages and also lordosis and scoliosis (Buchmann,K., Larsen, S.L., Dalsgaard, I., 1993) is indicative of an exposure to too much turbulence during the incubation phase, and also of the fact that the environmental conditions in the hatching cylinders had not been satisfactory. Experiments with Nordsø cod eggs have proven that they are most vulnerable to physical influence like turbulence, especially in the beginning of the incubation period, which leads to a high mortality rate (Rollefsen, G. 19??), (Rollefsen, G., 1932). It is not unlikely that Baltic cod eggs are even more vulnerable to physical influence like turbulence. Partly they develop at a high depth in which the physical influence is quite limited, and partly their chorion is thinner than that of the North Sea cod eggs, 3,34 - 4,79 μm and 6-9 μm respectively, (Nissling, A., Westin, L., 1991) due to an adaption to the physical conditions in terms of buoyancy and a low salinity level. North Sea cod eggs develop in the surface water in which they are exposed to turbulence to a much greater extent due to the influence of the wind.

2. Breeding 1993

The actual feeding experiments in the intensive and semi-intensive plants were set on foot in 1993.

The semi-intensive plant.

The feeding experiments in 1992 had been unsuccessful partly because the first eggs were spawned 15 April. Accordingly the first larvae for the experiments were not hatched until around 1 May when the temperature in the semi-intensive plant was already 10-12° C. For that reason it was obviously impossible to produce spawn at the size of 3-5-cm within the proper range of temperature tolerance for the larvae. Cod larvae must be hatched 1 April at the latest as otherwise it will not be possible to produce fingerlings before the temperature in the plant reaches 20° C ,

that is in the middle/end of May (Rasmussen, K., Brun, I. 1993)

As in 1993 the first eggs were spawned 15 April and in 1994 1 May it follows that the semi-intensive breeding technique is unsuitable for the production of Baltic cod larvae due to the fact that it is impossible to adjust the temperature in the semi-intensive plant.

The quantitative and qualitative monitoring of the zooplankton led into the plant was carried on for the rest of the season. It was found that the quantity produced was about 50% of the quantity of 1992. The zooplankton, however, was of a very high quality and possibly suitable as first-feed in the intensive plant (Rasmussen, K., Brun, I., 1993).

2.1 Breeding fish stocks

In the autumn of 1992 the Fishery Laboratory of Bornholm, the 'Danish Fishermans Organization' and the Danish Institute for Fisheries Research collaborated in designing a plant for a stock of breeding fish to produce eggs for the experiments. The financing and construction of the plant was attended to by the Fishery Laboratory of Bornholm along with the daily administration and running until the middle of June whereafter the administration and running was taken over by the Danish Institute for Fisheries Research.

The plant has been constructed as a recirculation plant consisting of:

3 breeding tanks each at the size of $8m^3$

A sedimentation tank

An immersed biofilter unit

An eddy separator

A pump swamp + a pump

A charcoalfilter

An oxygen tower

A pressure tank

The size of the plant is 98m³ in all. It has been erected in a cold-storage room, and the cooling of the water is performed by a regulation of the air temperature. An egg gatherer consisting of a plankton netbag the size of 100 l immersed in a vessel of the same size has been attached too each tank.

In the spawning period the leachate from the three breeding fish tanks is drained off from the surface through egg gatherers collecting the eggs in bags.

The fish were caught in February by nets off Snogebæk at a depth of 10-20 m, landed in tanks with water and transferred to the tanks in the plant which had previously been filled with natural seawater from the Baltic Sea with a salinity of 7-8‰.

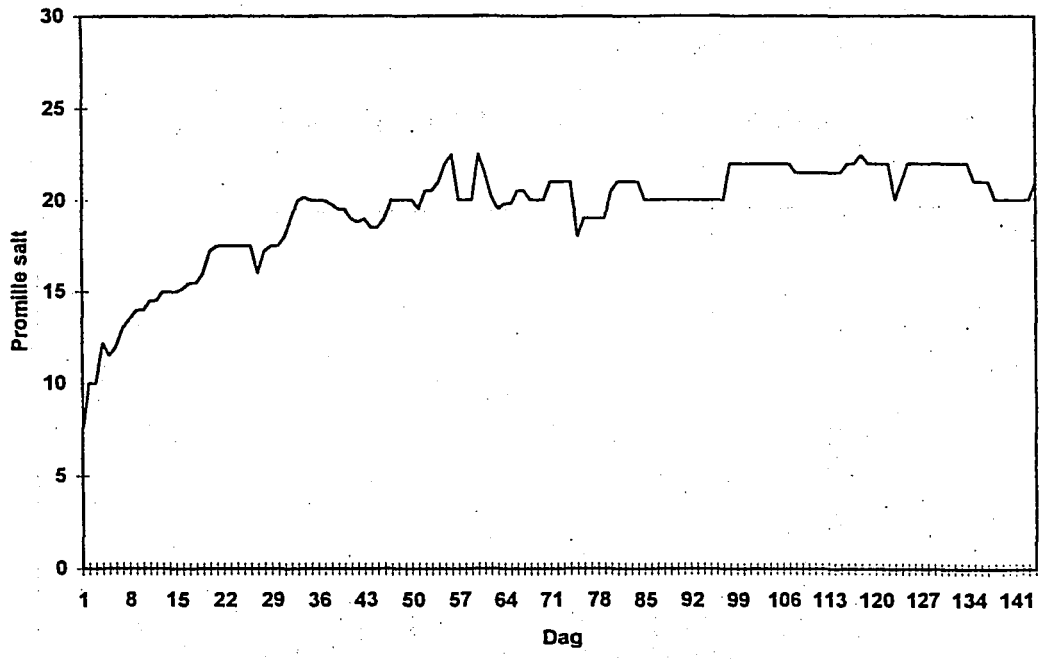
2.2 Physical parameters in the breeding fish plant

Salinity

In the period between 1 March and the middle of April the salinity in the plant was increased from 7-8‰ to 20‰. As fungoid growth was sometimes found in the eggs the salinity was increased to 22‰ in June to see whether this may rectify the problem. However, no difference could be observed. The salinity was measured by a model YSI 33.

The resalting was carried out gradually by adding a supply of syntetic salt (LW - Marinemix) to the eddy separator.

Fig. 1 Salinity, breeding fish plant 1993 in the period 1.03 - 22.07.



The great number of fluctuations in fig 1 were caused by periodic supplies of large quantities of 14‰ Østersø water pumped up from a depth of about 70 m and landed by "Jens Væver". After each supply the readjustment of the salinity would go on for some time.

Ammonia

The ammonia content in the breeding fish plant during the trial period appears in fig 2.

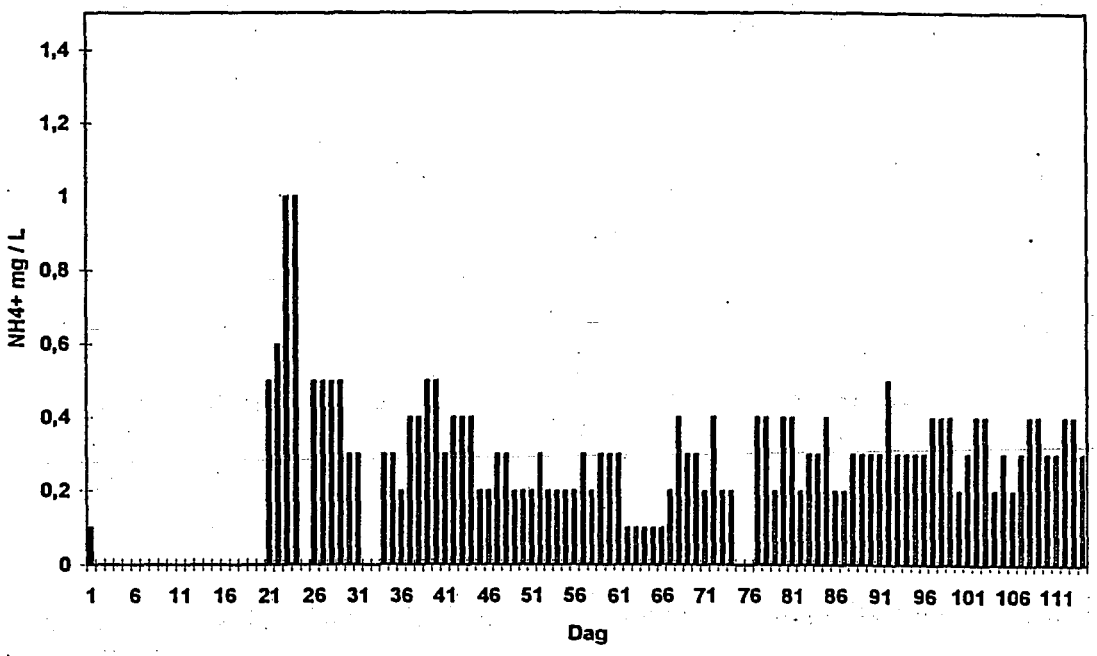
The measurings show the concentration of total ammonia, and were carried out by a Merck Ammonium Test Kit. The measurings were carried out in the water from the eddy separator, that is the leachate from the breeding fish. The test method is not a scientific one, but is often used for aqua culture purposes as it is an excellent indicator. The toxicity of total ammonia depends on the quantity of non-ionized ammonia in the water. The level of concentration depends on the temperature, but even more on the pH value. The higher the temperature and the pH value the higher the concentration of non-ionized ammonia.

FISH - $NH_3 + H_2O = NH_4OH = NH_4 + OH^-$

Non-ionized - toxic ionized - non toxic

dependent on pH value

Fig. 2 Ammonia, breeding fish plant in the period 01.04 - 22.07



At 8° C and a pH value of 7.8 the content of NH_3 is 0.994% (Piper, R.G. et al, 1982) which means that 1 mg/l total ammonia corresponds to a concentration of NH_3 of 0,00994 ppm. It has been found that a NH_3 concentration of 0.0125 ppm leads to growth reductions and furthermore to damages of the gill epithelium, the kidneys and the liver in trout (Piper, R.G. et al, 1982). On the other hand it has been found (Alderson, R., 1979) that growth reduction did not occur in sole when exposed to 16° C, 33‰ salt water and a concentration of 0.045 mg NH_3/l during 42 days. Likewise, turbot exhibited no sign of growth reduction when exposed to the same conditions at a concentration of 0,14 mg NH_3/l in period of 11 days.

It appears in fig. 2 that the concentration of total ammonia was at its highest on 22- 23 April as the concentration level was 1 mg/l corresponding to a content of 0,00994 ppm NH_3

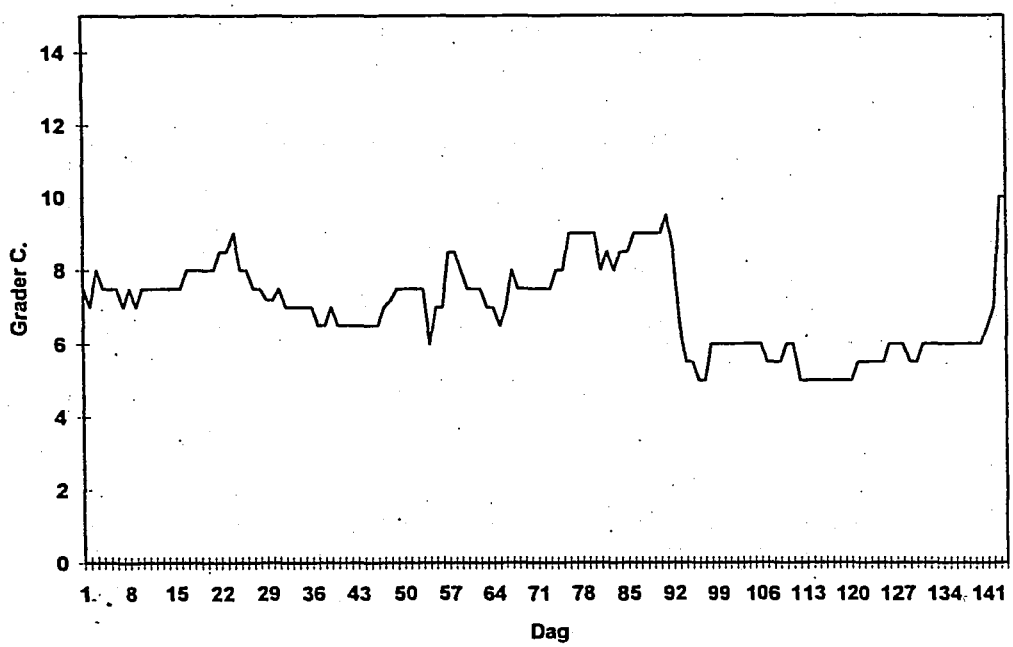
which is close to the level proven to be damaging to trout. In the rest of the period the concentration level was between 0.2 - 0.4 ppm which corresponds to 0.001988 - 0.0040241 ppm NH3.

The upper tolerance level for Baltic cod and eggs is unknown, but for the rest of the season the concentration level was very much below the level damaging to trout.

Temperature

The temperature fluctuations during the spawning season appear in fig. 3. The temperature was measured by a YSI Model 57 oximeter.

Fig. 3 Temperature, breeding fish plant in the period 01.03 - 22.07



The figure shows that the temperature had been fluctuating between 7-9° C until the beginning of June, whereafter it was lowered to a stable level of 5-6° C until the end of the spawning season. The high temperature level in the period March to June was due to the fact that the cold-storing room in which the plant had been placed was not running in this period, but was partly substituted by cooling through an open door from a cold-storing room that was running next to it. Thus the cooling has neither been efficient nor constant. During June the cooling of the very room was

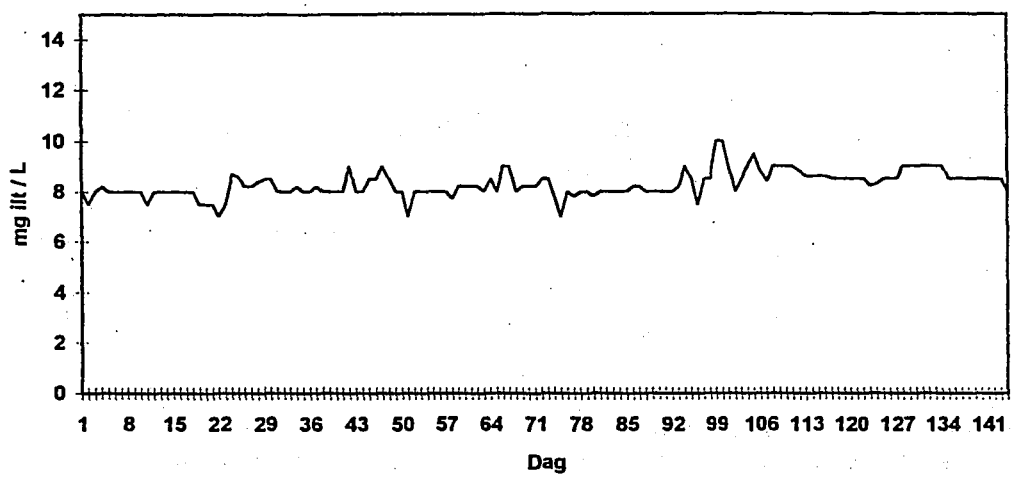
initiated and the temperature lowered to a level of 5-6° C which is identical to that in the spawning banks in The Baltic Sea (Bagge, O.; pers.comm.).

Oxygen

The oxygen conditions in the plant during the trial period appear in fig 4. The oxygen content lingers between 7-10 mg/l with an average content of 8 mg/l, a level which is satisfying. The oxygen level was measured by a YSI Model 57 oximeter.

Under normal circumstances the need for oxygen of trout is 8 mg/l the minimum level being 5ml/l (Christensen, N.O., 1980). Salmonids are among the fish species that have the greatest need for oxygen.

Fig. 4 Oxygen level in the period 01.03 - 22.07, breeding fish group 2

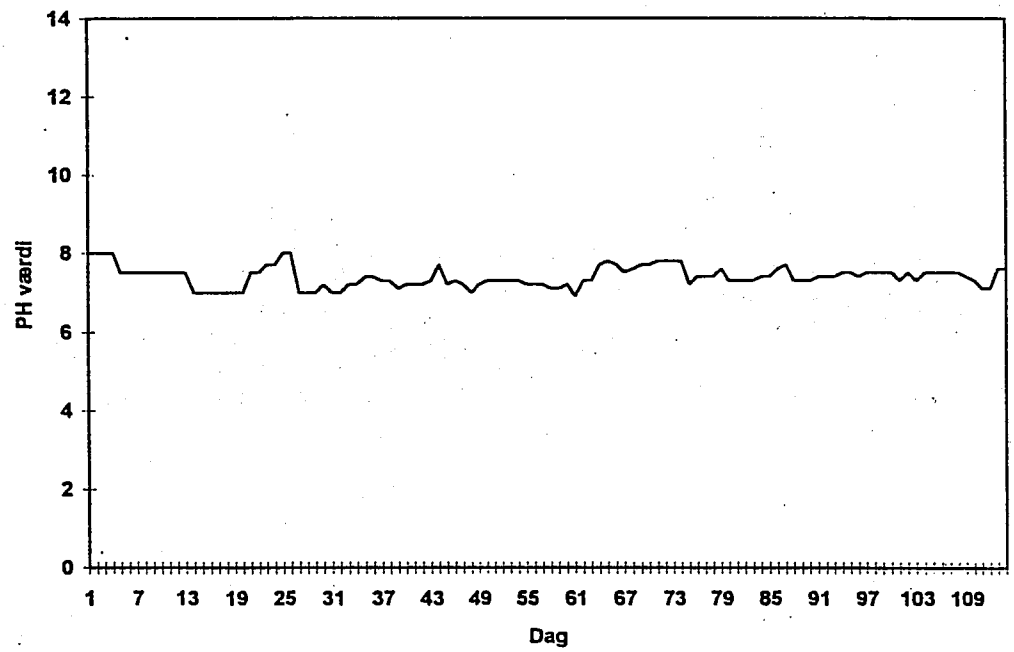


pH values

The pH values during the trial period, fig 5, lingered between 7.8 - 8.0, pH values a little lower than those in the feeding plants and the pH value in the upper water layers of the Baltic Sea that was measured to be 8.2 during the trial period. Seawater of 33‰ has got a pH value of 7.9 (Alderson, R., 1979)

The difference between the pH-values in the breeding fish plant and the feeding plant may be explained by the use of different measuring methods. The pH values in the breeding fish plant were measured with pH-indicator strips and those in the feeding plant with a pH-meter, Knick Portmass 751.

Fig. 5 pH values, breeding fish plant in the period 01.04 - 22.07



2.3 The spawning biology of the breeding fish

Quantity of spawned eggs

The egg gatherers of each of the three groups were emptied once a day, and the daily quantity of each group was measured in ml. The length of the day in the plant was adjusted by a timer according to the natural length of the day of the season. The quantity of eggs produced by the 3 breeding fish groups is shown in fig 6

Fig 6.1 Daily quantity of eggs in the spawning period. Breeding fish group 1

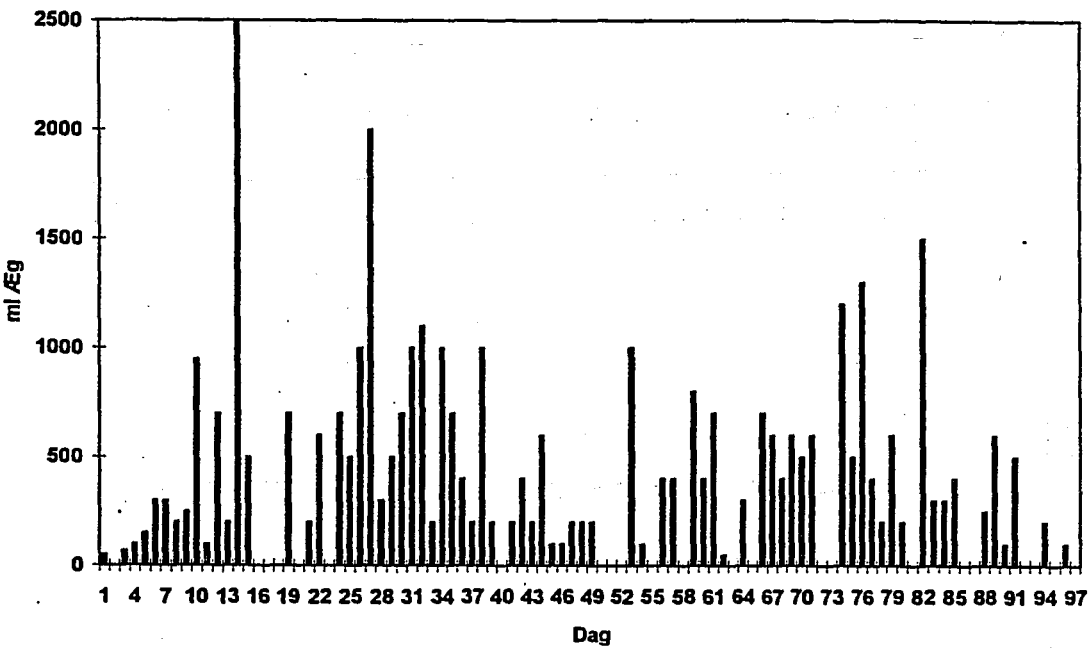


Fig 6.2 Daily quantity of eggs in the spawning period. Breeding fish group 2

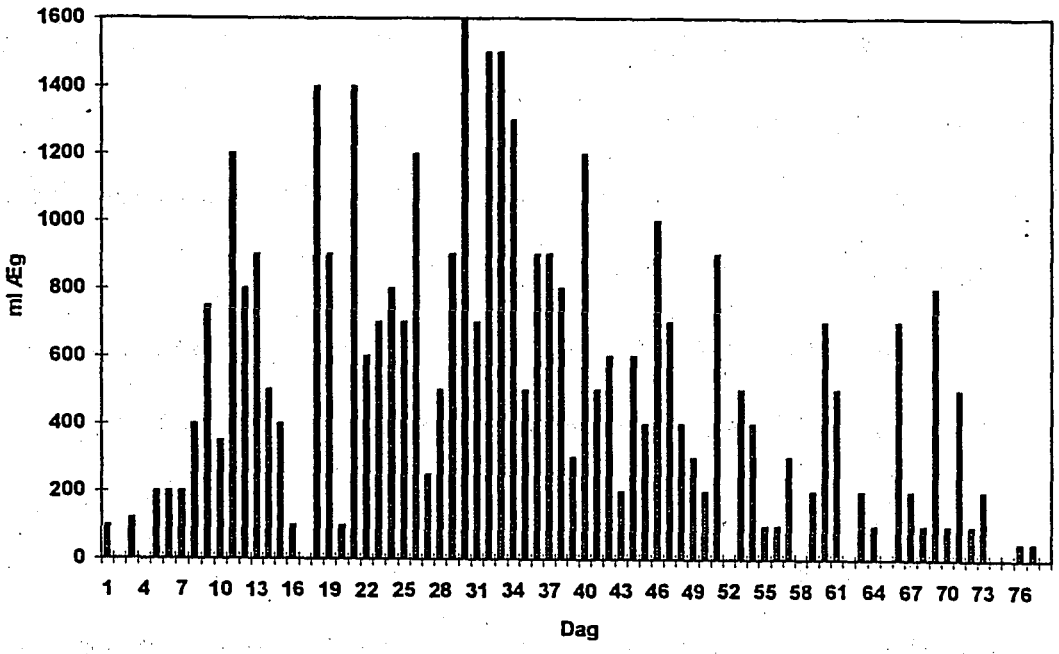
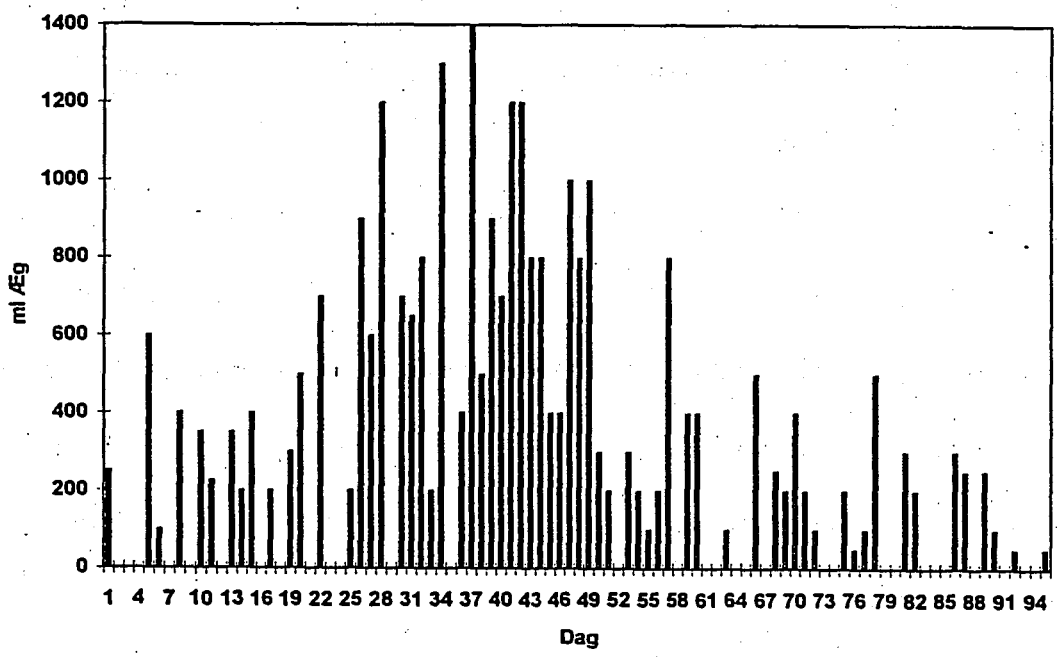


Fig. 6.3 Daily quantity of eggs in the spawning period. Breeding fish group 3



In total 37870 l eggs were spawned by group 1 in a season of 97 days corresponding to 9,008,800 eggs for the whole group or 1,035 l eggs = 248,000 eggs per one kg female cod the

average weight of each individual being 2.180 kg. The group consisted of 11 females and 7 males in total in the end of the spawning period. 1 female died in the period.

Group 2 spawned 38,720 l eggs in total in a season of 76 days corresponding to 9,292,800 eggs or 0.990 l eggs = 237,600 eggs per one kg female cod the average weight of each individual being 2,600 kg. The group consisted of 15 females and 13 males in total at the end of the spawning period. 1 female died in the period.

In a season of 94 days group 3 delivered 30,625 l eggs in total, in all 7,350,000 eggs or 0.640 l = 153,600 eggs per one kg female cod the average weight of each individual being 2.500 kg. The group consisted of 19 females and 5 males in total at the end of the spawning period. I female died during the period.

As regards group 1 the spawning peaked twice, once in the first half of the period and once in the second, at the end of April and of June respectively; the first peak a little bigger than the second. As regards the 2 other groups the spawning only peaked once in the first half of the period, in the middle of May. Furthermore the spawning period of group 2 was about 20 days shorter than that of the other.

It has been claimed (Muller, A., Bagge, O., 1984) that the natural spawning stock in the Bornholm Basin spawns from March to June with a peak in May. Furthermore a reference is given to an other survey (Kandler, 1938) in which the presence of two spawning peaks was observed, one in June and one in September. Finally the possibility that there are two species of cod is being discussed: a western one that is an early spawner, and an eastern one that spawns later in the season. It is impossible to tell whether the groups consist of a mixture of the species, the eastern and the western ones mixed in group 1, and the western one being predominant in group 2 and 3 - especially in group 2 in which the spawning season was shorter.

It has been calculated (Botros van Gurgis, 1962) by ascertaining the weight of the roe before the spawning that a western Baltic cod of 2.700 kg contains 790,000 of eggs per kg while a North Sea cod of the same size contains 728,000 eggs per kg. It has been found that the spawning quantity of a North Sea cod of 2.800 kg spawning in captivity was 964,285 eggs per kg or 370 l/kg female cod. The spawning period lasted for 50 days, and the fish spawned 17 times in all (Kjesbu, O.S., 1989) In both surveys the scientists found that there was a positive correlation between the size of the breeding fish and the quantity of spawned eggs.

A breeding group of North Sea cod consisting of 50 females and 50 males spawn for a period of 75 days while a single female of 6 kg spawns for about 50-60 days (McVey, J.P., 1991). The spawning season in 1993 of the breeding cod at Bornholm was a little longer in group 1 and 3 than it would normally be expected from a group of breeding fish consisting of North Sea cod.

No positive correlation, however, was found between the size of the breeding fish and the quantity of eggs. Group 1 in which the average weight was smallest (2.180 kg) delivered the largest quantity of eggs compared to the body weight.

Fertilization per cent

The daily fertilization per cent of each breeding fish group was measured during a period of 2 months until the end of the spawning season. A daily sample of 5 ml or about 1,200 eggs was taken from the eggs that were collected from the three batches.

A part of the sample, about 200-300 eggs, was taken out and examined in a stereo microscope. Only fertilized eggs with a normal cell division were registered as useful for further investigation. The roe had previously been fixed in a mixture of glacial acetic acid and 8‰ distilled water. The mixture colours the cell surface dark brown which makes it much easier to distinguish fertilized and unfertilized eggs from each other. At the same time it becomes easier to see whether the cell division of the fertilized eggs is normal. The assessment of the eggs was performed in

accordance with the description by Eyjolfur Friogeirsson of the development of cod eggs (Friogeirsson, E., 1978). The eggs that were examined were found to be within the interval between the two-cell division stage and the morula stage.

Fig 7 shows the daily fertilization per cent of the eggs from the three breeding fish groups.

Fig. 7.1 Daily fertilization per cent in the spawning period. Breeding fish group 1.

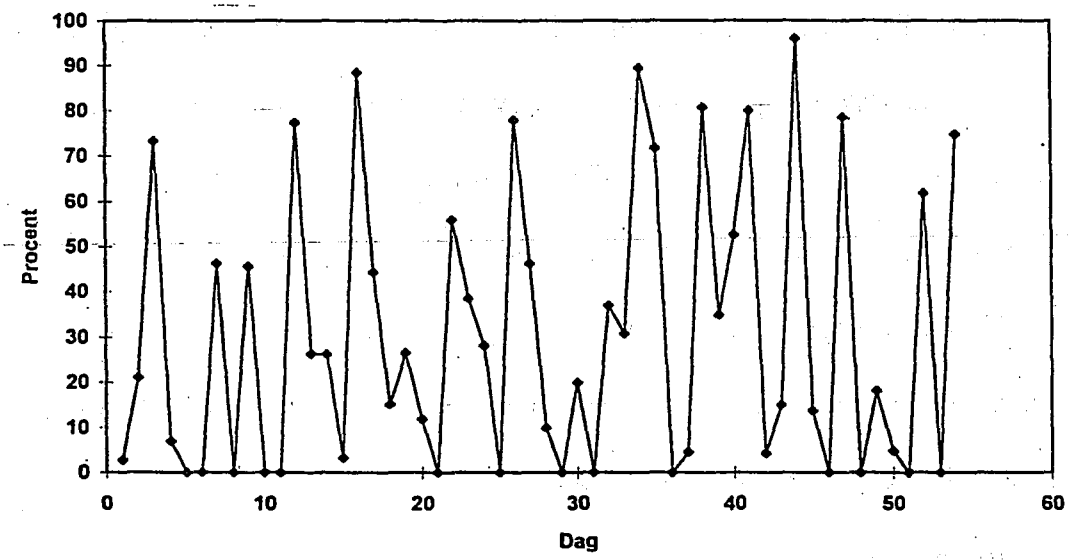


Fig. 7.2 Daily fertilization per cent in the spawning period. Breeding fish group 2.

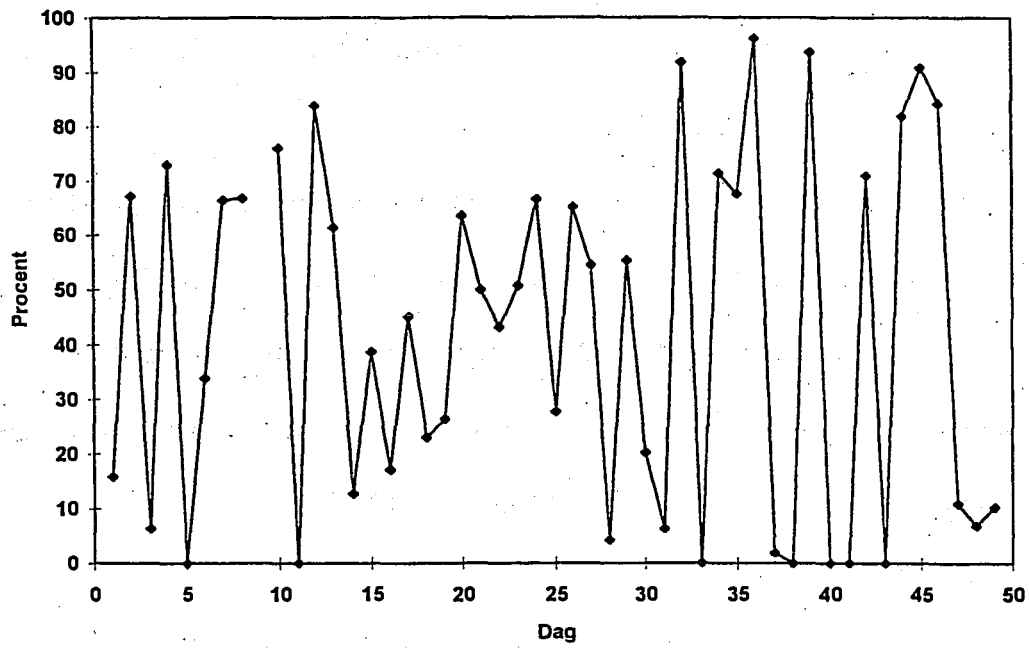
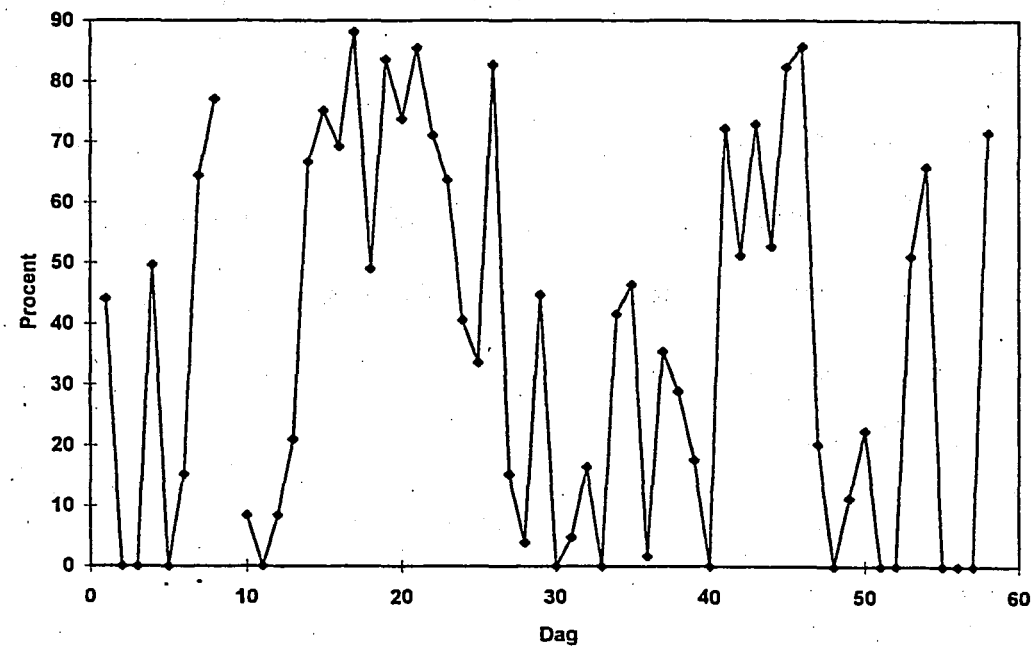


Fig. 7.3 Daily fertilization per cent in the spawning period. Breeding fish group 3.



As seen in the figure the fertilization per cent varies from day to day between about 90% to 0%. The average fertilization per cents are 30% for group 1, 44% for group 2 and 40% for

group 3 respectively.

In this survey the fertilization per cent did not depend on the number of males. In group 3 in which there was the smallest number of males (5) and females (19) the second best fertilization per cent was found (40%).

It is possible that high and fluctuating temperatures lingering between 6-10° C in the first two thirds of the trial period may have influenced the results negatively. Stress may also be the cause of poor fertilization per cents and of unequal spawning rhythms resulting in an excessive keeping back of the eggs in the ovaries, the roe growing over mature leading to a decrease in the fertilization ability (Kjesbu, O.S., 1989), (Holm, J.C., 1991).

2.4 Feeding experiments 1993

Due to the fact that in 1992 the hatching of cod eggs in the hatchery had been rather unsuccessful the feeding experiment plant was used for the hatching of the eggs in 1993 as well as for the actual feeding. The plant consists of the following elements and has got a volume of 18m3 in all:

A sand filter 1.5 m^3

A bio-screen unit; 1 eddy separator 0.6 m^3, 2 immersed screens each at the size of 1,2 m^3

A refrigerating unit + cooling storage unit (pump swamp)

A UV-sterilizer

Oxygen tower

10 conical feeding tanks each the size of 600 l

An eddy separator

The advantage of incubating the eggs in the feeding plant is that a large quantity of eggs may be incubated at the same time - up to 500 ml or 120,000 eggs in each tank. Besides, the turbulence in the tanks is insignificant. Water is added along the walls of the tanks near the bottom

and circles up to the outlet which is placed close to the surface. Hereby the eggs are pushed around in the tanks very carefully without being exposed to turbulence - even at a high waterflow - to secure optimum oxygen conditions. During all the hatching experiments the oxygen saturation level was between 80-95%. The disadvantage of this hatching method is that it is impossible to carry out any kind of efficient collection of dead eggs as the tanks are of a depth of about 1 m. For that reason it is impossible to estimate the precise amount of deceased eggs.

Due to the great volume of the tanks it is also impossible to estimate the amount of newly hatched larvae as they do not disperse homogenously. A homogenous dispersal of the larvae in a tank of 600 l would demand that they were being exposed to powerful turbulence. Cod larvae are very sensitive to changes in the physical conditions (Thorsen, B.M., 1981).

The strategy for the feeding experiments was to offer the cod larvae a wide range of feed to make it as likely as possible that their need for nourishment would be met, as the needs of Baltic cod larvae in terms of food are unknown.

For the purpose of the feeding experiments the algae Rhodomonas sp., Isocrysis galbana, Paulova lutheri and Skeletonema costatum were produced and used as feed from day 1. Several scientists have proven that cod larvae incept phyto plankton immediately after the hatching (Homme, J.M., 1991), (Ellertsen, B. et al, 1976), (Meeren, T. van Der, 1991), (Eilertsen, H.C., 1992), (Tilseth, S. et al, 1987), (Pedersen, T. et al, 1989), (Tilseth, S., 1990).

Polyunsaturates are essential to the survival and growth of marine fish larvae. The two polyunsaturates 20:5 (n-3) and 22: (n-3) are particularly important (Wantable, T. et al 1983), and constitute up to 42.0 - 50.5% of the content of polar lipids in cod larvae (Pedersen, T. et al, 1989).

Marine phyto plankton has a high content of polyunsaturates, and for that reason it is rather suitable for the purpose of nourishment for marine fish larvae either as food in itself or as

an enrichment for other food organisms as for instance rotifers and Artemia as the content of the essential polyunsaturates in these organisms is hereby enlarged (Watanabe, T. et al, 1983), (Lubzens, E., 1987).

North Sea cod larvae have been successfully fed with algae and also rotifers and Artemia enriched by algae. (Huse, J. et al , 1983). By using a similar diet (Howell, B. R., 1984) a survival level of 10% until the metamorphosis was obtained whereafter the mortality rate was insignificant.

It has been proven, however, that in breeding experiments with turbot, plaice and flo- under the use of prey produced in laboratories will often lead to a pigmentation defect in a certain percentage of the fish. By adding a certain quantity of natural zooplankton, however, this problem is can be avoided in the breeding of flounder (Seikai, T., 1985)

Algae as well as rotifers, Acartia tonsa and Artemia were used for the feeding experiments.

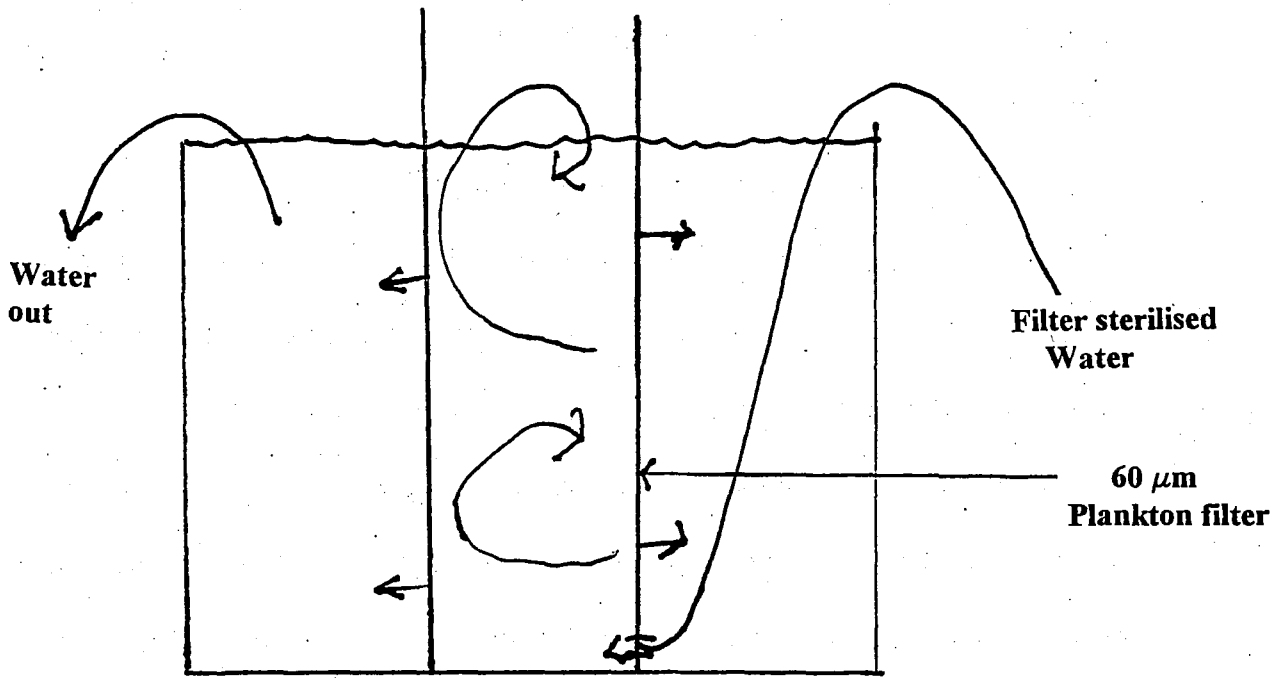
The production of the algae was carried out in an air-conditioned room at 16° C in plastic bags at the size of 30 l. The algae were given light to by a lighting plant with 40 fluorescent tubes. The attenuation water for the cultures was seawater from the breeding experiment plant which had been filter-sterilized and resalted . The water was acclimatized in the air-conditioned room before the attenuation. The enrichment of the algae was performed directly in the algae bags.

The production of rotifers was carried out in 6 conical tanks at the size of 300 l at 25° C in 22‰ filter-sterilized, resalted sea water. The rotifers in production belonged to the S - type which is the smaller one of the two main types, the adult size being 100-200 μm. The adult size of the other type is 130-340 μm. Due to its smallness the smaller type is most suited as a start diet for small marine fish larvae (Fukusho, K., 1989). The enrichment was carried out by an addition of the algae Rhodomonas and Isocrysis in a quantity of 50 l/day/tank plus of a commercial enrichment product, Culture Selco according to the prescriptions of the company. The enrichment

of the rotifers was carried out every sixth hour day and night.

Before starting the feeding with the rotifers they were filtered through a standard 60 umfilter. It soon appeared, however, that the procedure was unsuitable for filtration of large quantities of organisms as thefilter would clog up due to the presence of large quantities of suspended particles of algae in the water from the production tanks. For that reason a cylindrical back pressure filter of 30 l was constructed (fig 8).

Fig. 8 Cylindrical back pressure filter



The advantage of this filter is the presence of a very large filter surface. Besides, the continuous addition of filter-sterilized water creates a rotation of the filtrate which leads to an effective straining off of secondary material. The water in the tank in which thefilter is immersed creates a counterpressure that brings about a careful filtration of the rotifers.

After the filtration the rotifers were tapped into 5 l bottles and transferred to the eddy separators to be acclimatized before the feeding.

The Artemia eggs were hatched in 4 conical tanks of 300 l at 25°C in 35‰ sterile-screened, resalted seawater. Before the hatching of the eggs which lasted for 24 hours under the existing circumstances the eggs were opened, that is the outer part of the two shells was removed. The procedure is carried out in the following way:

1. Hydrogenation of the cysts.
2. Treatment in a hypochlorite solution.
3. Washing off and deactivation of chlorine residues.
4. Hatching.

The technique has been described in detail elsewhere (McVey, J.P.,1983).

The advantage of opening the eggs before the hatching is that the hatching per cent is increased as it becomes easier for the nauplius larvae to break through the shells. At the same time the treatment with hypochlorite disinfects the cysts (Campton, D.E., 1989).

After the hatching the nauplius larvae were filtered through a 125 μm screen along the lines of the principles described in fig 8. The screen, however, was a commercial standard product.

The acclimatization of the newly hatched nauplius larvae was carried out in conical tanks of 1 l immersed in the eddy separator with a fresh air supply. In that way it was possible to store them for up to 24 hours in a concentration of 15,000 nauplius larvae/ml. Previous experiments have proven that nauplius larvae can be stored in that way at 0-4° C in 48 hours with very little energetic loss (McVey, J.P., 1983).

In the later stages of the feeding experiments the newly hatched nauplius larvae were enriched for either 24 or 48 hours depending on the desired size. The enrichment was carried out with Super Celco (commercial product) and the algae Isocrysis and Rhodomonas.

Acartia tonsa eggs were produced in HØI in Charlottenlund, preserved and sent to Bornholm where the eggs were hatched in conical tanks of 1 l with a fresh air supply. The hatching was carried out in 16‰ resalted seawater at 20°C. The nauplius larvae were acclimatized in the same way as the Artemia nauplius larvae and besides fed with Rhodomonas.

In the period between 18 April and 30 June a quantity of 4,045,000 fertilized eggs with a normal cell division had been incubated in the feeding experiment plant at the time of measuring. In this period it was observed that the hatching success was fluctuating to a considerable extent. In certain egg batches the mortality rate was close to 100 %, in other it was quite small as only a very small amount of dead eggs were observed at the bottom. Swedish surveys with Baltic Cod also prove that the quality of the eggs varies (Pickova, J. et al, 1992). Apart from the varying hatching per cents it was characteristic that the worse the hatching results the more fungoid growth could be found in the batches. In certain egg batches in which a high hatching per cent was found no fungoid growth could be observed at all. It seems that fungoid growth arose secondarily when the eggs were of a poor quality. As an effort to prevent fungoid growth a few egg batches were dipped in oxytetracycline without any visible effect.

In the previous year it had been found that fungoid growth may be prevented by a raise of the salinity level (Buchmann, K. et al, 1992). For that reason the eggs were incubated in 20-40 % resalted seawater. The water in the tanks of the plant was 14‰ Baltic seawater pumped up from a depth of about 70 m. The resalting was performed with Instant Ocean (synthetic salt), the same kind of salt that was used for the resalting of the water for the production of prey. The salinity in the plant was like that of the breeding fish plant except for minor variations.

Apparently the choice of salinity did not affect the quantity of fungoid growth, but it has been found that the spermatozoa mobility in Baltic cod is at its best at 20-26‰ (Westin, L. et al, 1991). From that point of view the choice of strategy seems to be correct.

On the basis of a feeding schedule for the feeding of sea bass larvae the distribution of prey was performed along the following lines:

Larval age days	Number of rotifers/ l/day	Number of Artemia l/day
1-3 days	1000	
4-6 days	2000	
7-9 days	3000	
10-11 days	4000	
12-13 days	7000	400 newly hatched
14-17 days	9000	1250-1750 newly hatched.
18-20 days	4000	2000-3000 newly hatched
21-24 days		2500 n.h.+1500 enriched
24-30 days		7000-9000 enriched.

Besides algae of the types Isocrysis, Skeletonema and Paulova were added in a quantity of up to 5 l/day. In the rotifer phase an addition of up to 1000 pieces of Acartia/l/day was carried out too. The addition of algae went on from day 1 and 3 and 14 days ahead. In some experiments less food than prescribed in the schedule was added, in others more. The temperature was kept between 5.5-6.5° C in some of the experiments; in other the temperature was heightened from 5.5° C to 8.5° C during the feeding experiments in a period of 8 days starting at the umbilical sac absorption phase. The experiments were brought to an end after a max of 2 weeks except for the last batches. In all the experiments only a few larvae or none at all were alive after a period of 2 weeks.

In the last experiment session which was initiated 30 June and lasted until 10 August 192 cod fingerlings at a size of 30-40 mm were produced out of 7 batches. The pigmentation of the

cod was normal and there were no visible skeleton deformities. The temperature scheme had been as follows:

30 of June - 7 of July:	6.5° C
8 of July - 17 of July:	7.5° C
19 of July - 3 of August:	8.5° C
4 of August - 10 of August:	10.0° C

In the same period the salinity was lowered from 22‰ to 13‰.

In the period between 27 May and 19 June the total ammonia content lingered between 0.2-0.5 mg/l. From the 19 June to 10 August it lingered between 0.1-0.3 mg/l. During the whole trial period the pH value was 8.3-8.4.

It is impossible to explain why the hatching results varied as much as they did and why the larval survival rate was so low. In the feeding experiment period it was noticeable that the larval mortality arose as early as day 1, and that after 10-12 days only a very small number of larvae were still alive. Normally the first dangerous stage will be expected to set in about the time of the first feeding and the next one at the time of the metamorphosis (Thorisson, K., 1992). In the present experiment the larvae willingly incepted food. After the fourth day the first larvae with food in the stomach could be observed. But as most of them died before 10-12 days they never reached the metamorphosis stage. The larvae that grew older than 12 days frequently exhibited a peculiar pattern of behaviour. The larvae tried to reach the surface while turning on their own axis, this was done in a frenzy of energy. After an activity phase like that the larvae returned to passivity falling down through the water column whereafter the attempt to reach the surface would be repeated. Frequently this pattern would be repeated several times and would always end by a passive remaining at the bottom of the tank. There was no doubt that these larvae were at the point of expiring. Furthermore, the larvae grew very dark with very little or no food at all in the intestinal canal. The reason for this behaviour pattern that was called the death pirouette by the

staff of the plant is unknown.

3. Breeding 1994

In 1994 the intention was to combine the two breeding methods, the semi-intensive and the intensive ones by using the filtration technique of the first one to collect natural zooplankton from the sea and use this for the feeding experiments with the cod larvae in a protected environment in the intensive plant to exclude the possibility of malnutrition as the cause of the larval mortality. Natural zooplankton which is the normal diet of the larvae in their natural growth area should be the most nutritious diet. On the other hand it is not known whether the diversity of the species or the content of essential substances in the zooplankton close to the coast is similar to that of the zooplankton in the growth areas.

A quantitative and qualitative monitoring of the eggs from the two groups of breeding fish was implemented. In group A the average weight was 2.4 kg, and in group B it was 3.3 kg. For that reason it appeared that they were of 2 different years.

To elucidate whether the size of the eggs depends on the time of spawning or on the size of the breeding fish determinations of the dry-matter percentages and diameter measurements were carried out during the spawning period.

To examine whether a correlation between the hatching per cent and the content of certain environmental poisons could be found samples were taken out from every single egg batch in the experiment for further analyses later on. The analyses comprehended 9 PCB-congenes and also DDD, DDE and DDT

3.1 The breeding fish stocks

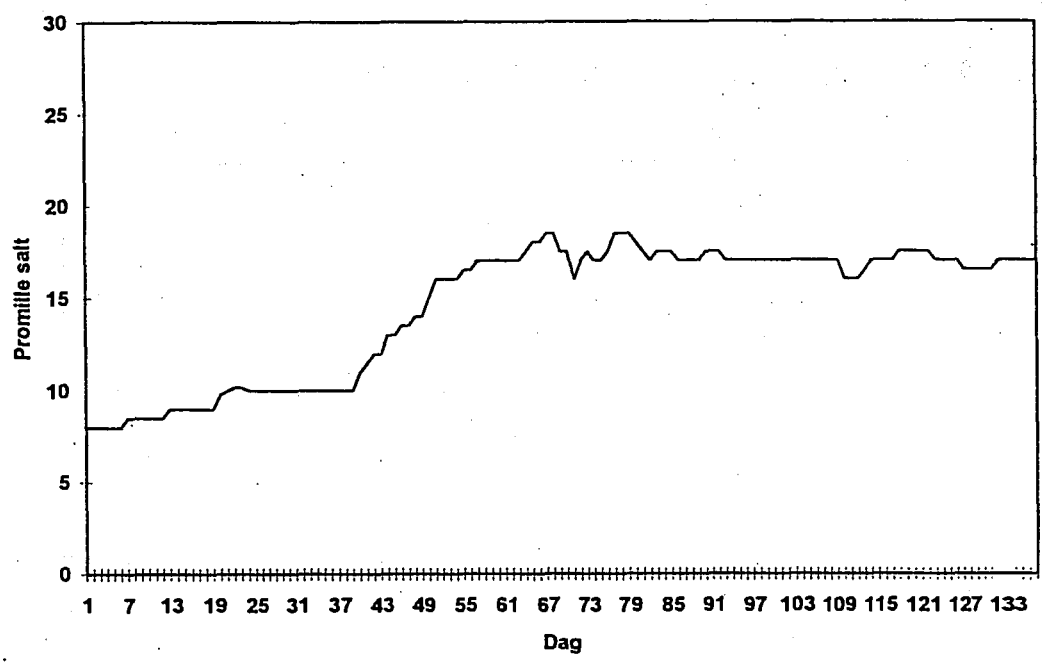
In 1994 the breeding fish plant was improved by an enlargement of the bio-screen unit of 3 m^3. As in 1993 the fish for the experiments were caught off Snogebæk in February. This time the catching was performed with long lines at a depth of 10-20 m. The cod were placed in tanks

with seawater and transferred to the tanks in the plant that were filled with 7-8‰ seawater from the Baltic Sea.

3.2 Physical parameters in the breeding fish plant

Salinity

Fig 9 Salinity, breeding fish plant 01.03 - 15.07 1994



In a period of 2 months the salinity was raised from 7-8‰ to 16-17‰. It had been proven by experiments the previous year that fungoid growth could to be avoided at a salinity of 22‰ and that the number of attacks would be varying. For that reason it was decided that the work should be carried out at a salinity natural to the Baltic cod. The buoyancy of the Baltic cod eggs varies the ideal salinity for Baltic cod being 12.3-16.9‰ depending partly on the qualities of the single breeding fish that spawns the eggs and partly on the number of the batch (Nissling, A., 1991).

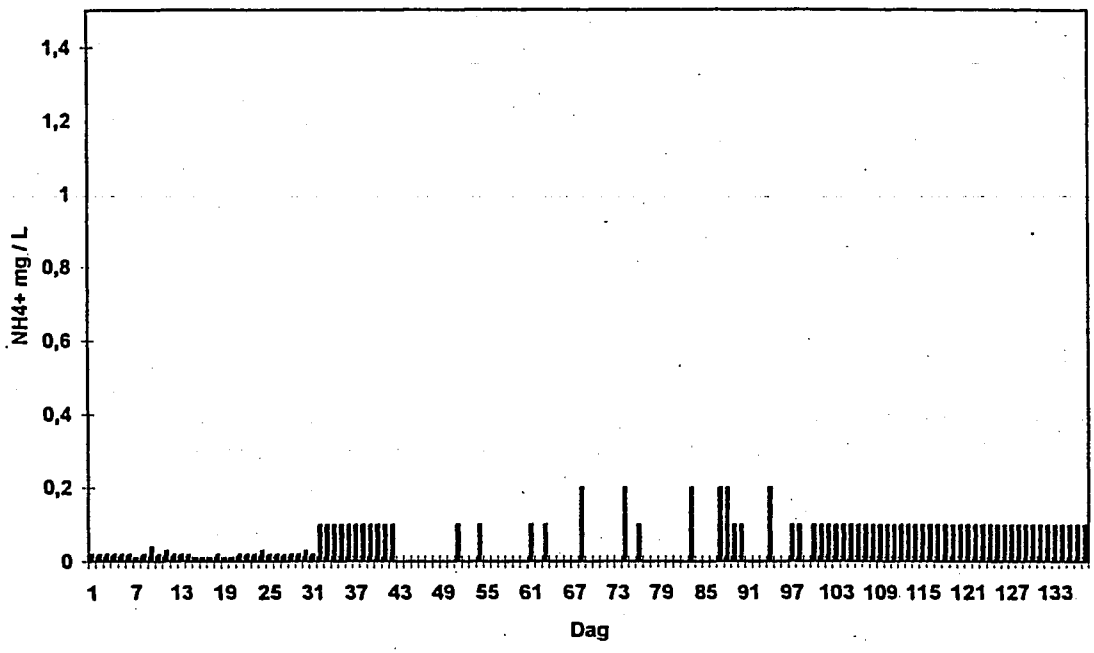
The resalting was done by adding Instant Ocean (synthetic salt).

The salinity level was relatively constant in the trial period. Smaller fluctuations are due to a current replacement of some of the water in the plant. The water used for the substitution was 14‰ Baltic Sea water pumped up from a depth of about 70 m.

Ammonia

The ammonia content in the breeding fish plant is shown in fig. 10, and was measured in the same way as in the previous year.

Fig. 10 Ammonia, breeding fish plant 01.03-15.07 1994



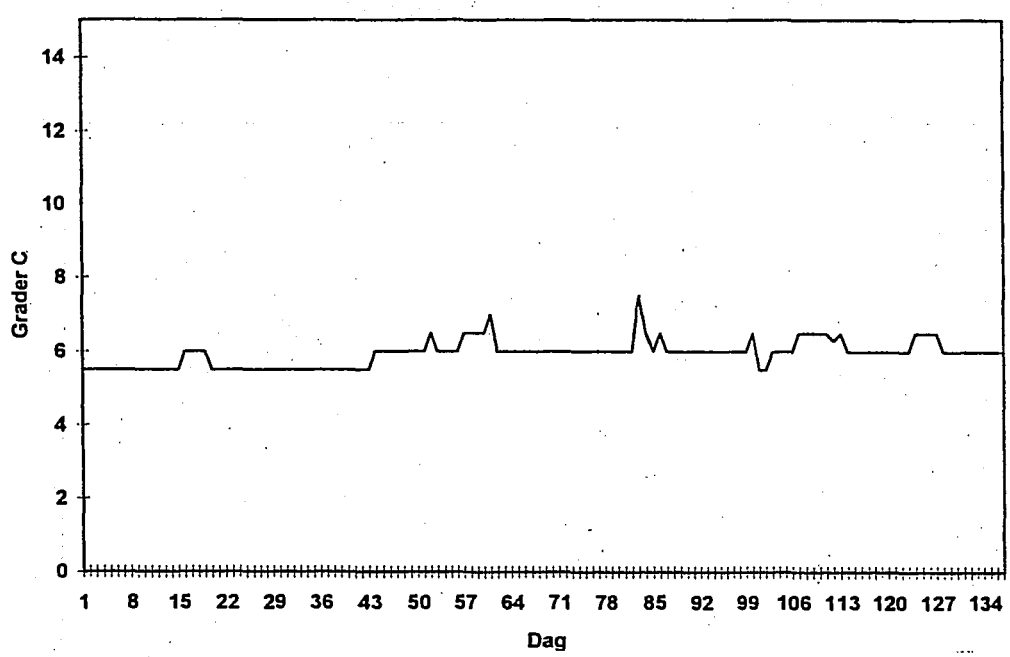
It appears that the concentration level is considerably lower that in the previous year- 0-0.2 mg/l with an average below 0.1 mg/l. This is partly due to an enlargement of the bio-screen capacity and partly to the fact that thefilters had been inoculated with Nitrosomas and Nitrobacter bacteria the main capacity of which being the ability to convert ammonia and nitrite into nitrate.

Temperature

In fig. 11 the temperature fluctuations during the season are marked. The temperature was kept between 5.5-6.5°C with a single period of 7° C in a few days. Thus the temperature was

much more constant and the level much more ideal than in the previous year. This was due to the fact that the cooling system was working in the whole period.

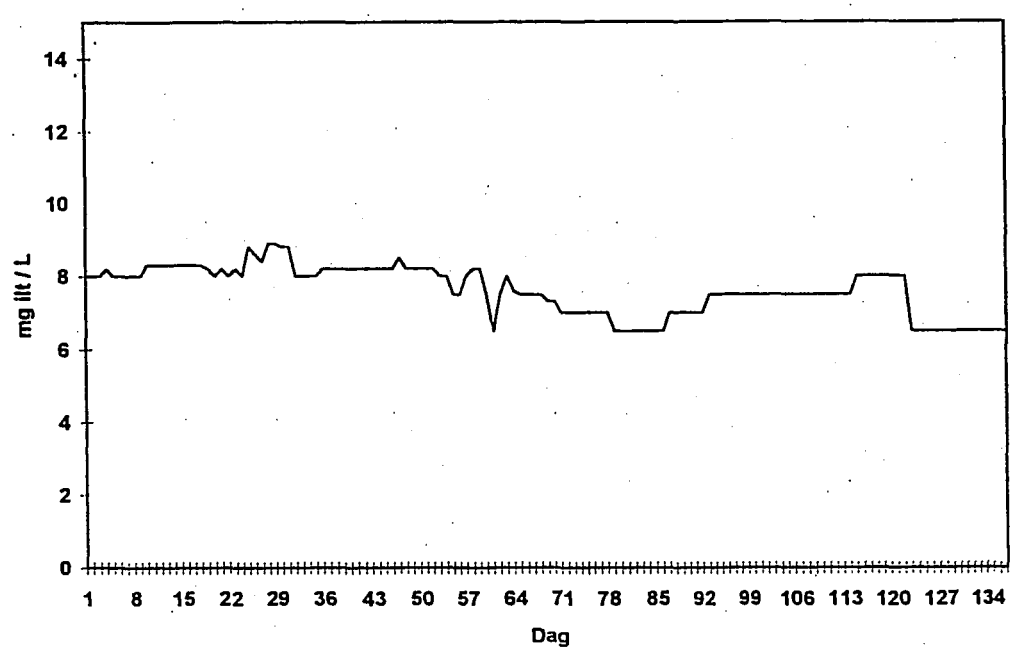
Fig. 11 Temperature, breeding fish plant 01.03 - 15.07, 1994



Oxygen

The oxygen level in the plant in the trial period appears in fig 12 the level being about 7-9 mg/l, an oxygen content that must be considered satisfactory.

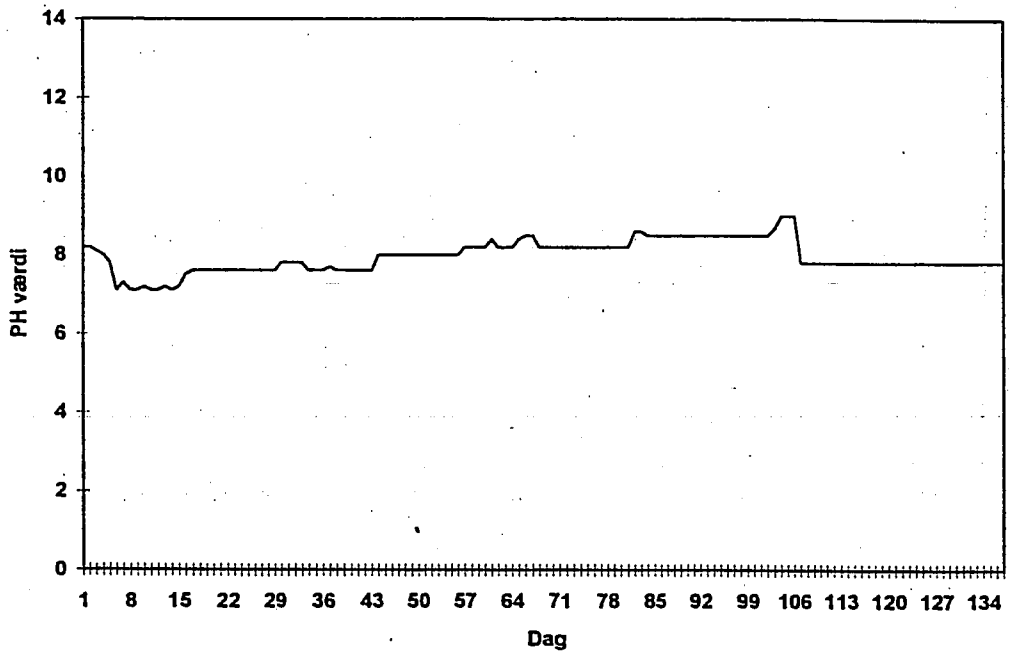
Fig. 12 Oxygen content in the water 01.03-15.07, breeding fish plant, 1994



pH values

The pH value in the trial period which can be seen in fig 13 was about 8 as in the previous year.

Fig. 13 pH values, breeding fish plant 01.03-15.07, 1994



3.3 The spawning biology of the breeding fish

Egg production

The breeding fish were divided into two groups for the experiments. In group A the average weight was 2.4 kg, and in group B it was 3.3 kg. The amount of eggs spawned by the two groups is marked in fig 14.

Fig 14.1 Daily amount of eggs in the spawning period. Breeding fish group A (14 females, 11 males)

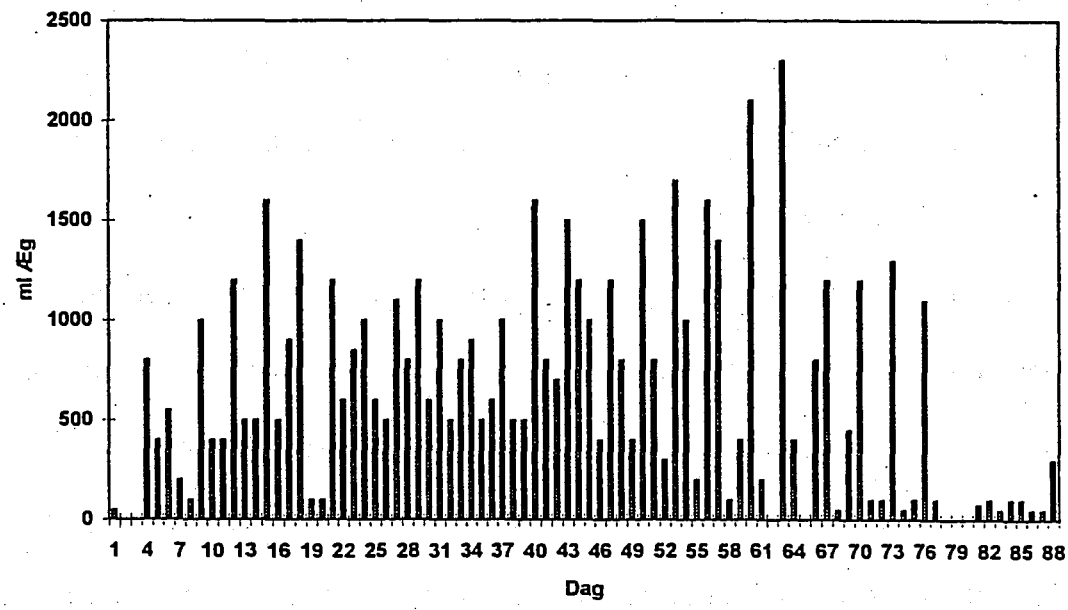
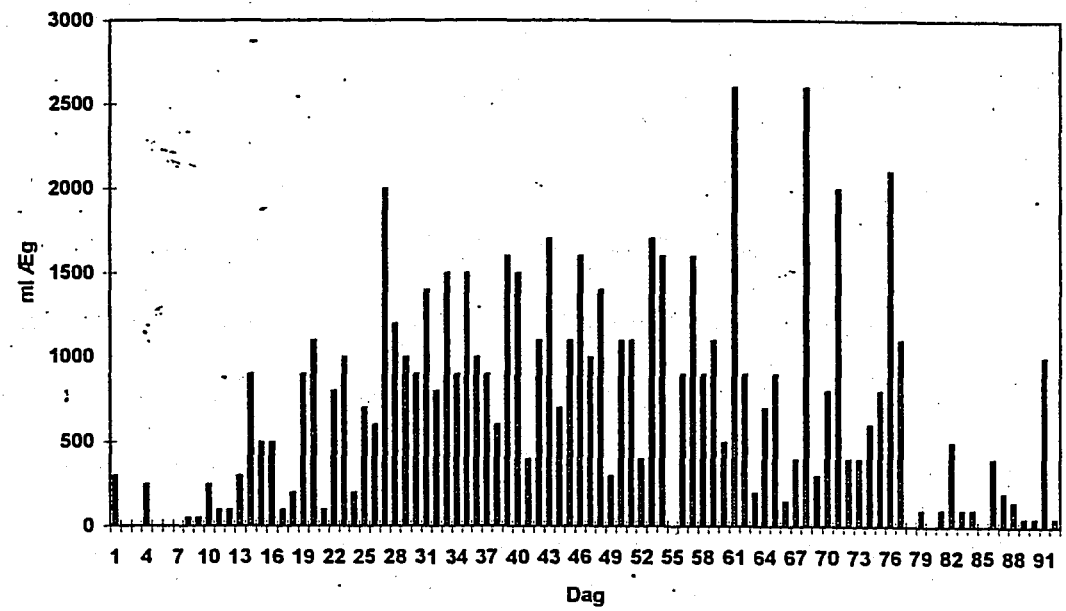


Fig. 14.2 Daily amount of eggs in the spawning period. Breeding fish group B (8 females, 8 males)



56,425 l of eggs in total were spawned by group A in a period of about 90 days which corresponds to 13,542,000 eggs or 1.881 l eggs = 451,400 eggs pr kg female. The group consisted of 14 females and 11 males at the end of the spawning season. No females died in the period.

By group B 65,751 l eggs were spawned in a period of about 90 days which corresponds to 15,780,000 eggs or 2.481 l eggs = 595,440 eggs pr kg female. The group consisted of 8 females and 8 males at the end of the spawning season. One female died 6 July, that is the amount of eggs pr kg female is actually a little smaller than calculated.

In both groups the spawning peaked slightly in the first part of the spawning period. It has been observed (Kjesbu, O.S., 1989) that the spawning peaked in the last half of the spawning season for a single North Sea cod.

In 1994 the correlation between the size of the breeding fish and the amount of eggs that was spawned was positive as previously found to be the case in both North Sea- and Baltic cod. The amount of spawned eggs pr kg female was much higher in 1994 than in 1993.

The amount of spawned eggs must not be seen as an exact estimation of what can be yielded by a Baltic cod at the size of 2.4 kg and 3.3 kg respectively, but as an average as the size of the breeding fish was not homogenous. For instance, the weight of the smallest fish in group A was 1.292 kg and that of the biggest one 3.354 kg, and in group B the weight of the smallest fish was 1.804 kg and that of the biggest one 4.583 kg.

The fertilization per cent

The fertilization per cent was measured every day from the start of the spawning period 1 May to 15 July. The method in use was the same as in the previous year. It appears in fig 15 that despite the fact that the per cent varies somewhat the difference from day to day is much smaller than in the previous year. In group A the biggest variations can be found at the end of the season, and in group B in the beginning and the end of the season. The average fertilization per cent for

the whole season was 79 % for group A and 72 % for group B, an average much higher than in the previous year.

The improvement of the results from 1993 to 1994 is probably due to the handling of the fish at the catching. In the first year they were caught by nets, and in the second they were hooked by the staff of the project who were most careful with the treatment of the fish. For instance the landing of the fish was carried out very slowly to avoid an exposure of the fish to sudden variations of pressure. Another improvement was that the physical parameters in the plant were much more stable and favourable in 1994. The temperature conditions in particular had not been optimum in 1993 as they had been too high, whereas in 1994 they were close to the optimum, 5.5-6.5°C.

It is worth noticing that in 1993 the spawning season was instituted 15 April while in 1994 it was instituted 1 May, 15 days later. The raise in temperature may well be the cause of an acceleration of the time of the season the fish speeding up the spawning possibly leading to a deterioration of the egg quality.

Fig. 15.1 Daily fertilization level in the spawning period. Breeding fish group A

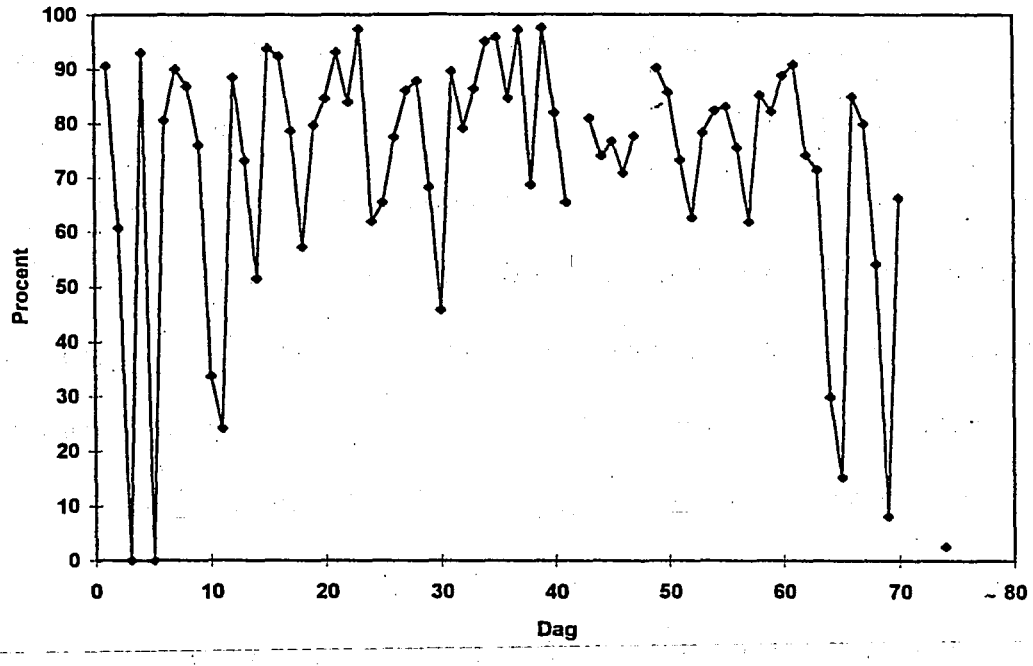
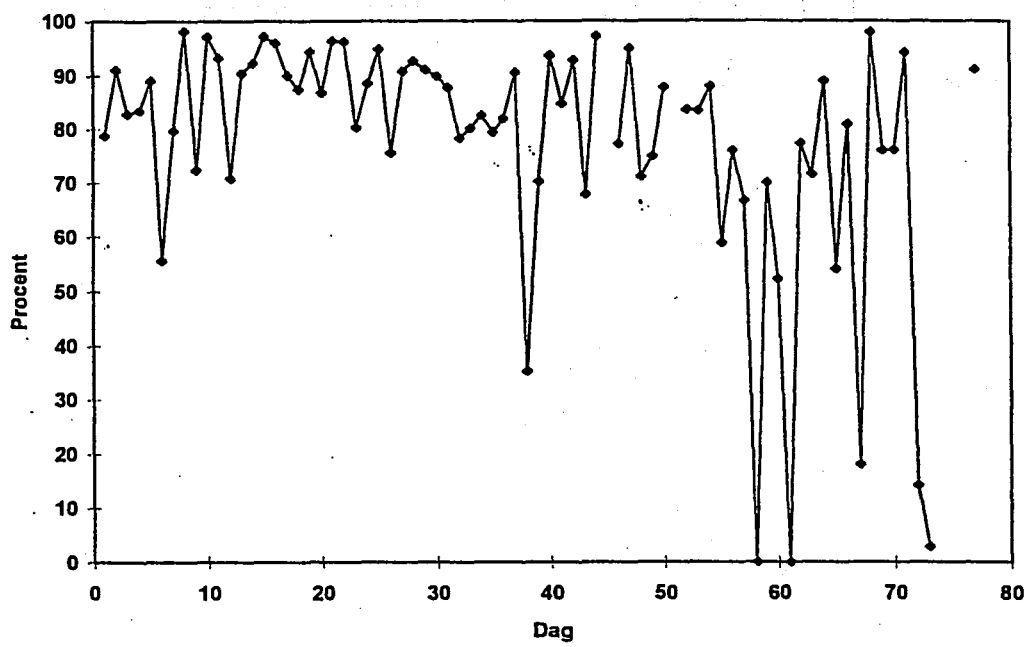


Fig. 15.1 Daily fertilization level in the spawning period. Breeding fish group B



Egg diameter and determination of dry-matter content in relation to time of year and size of breeding fish

The egg diameter was measured by a measuring ocular with 25 units = 1 mm.

The determination of the dry-matter content was performed in a ME thermostatically controlled oven at 57.5° C

± 0.1° C for 24 hours.

In fig 16 the relation between egg diameter and time of spawning can be seen. Each point is based on an average of 20 measurings.

Fig. 16.1 Egg diameter/time. Breeding fish group A

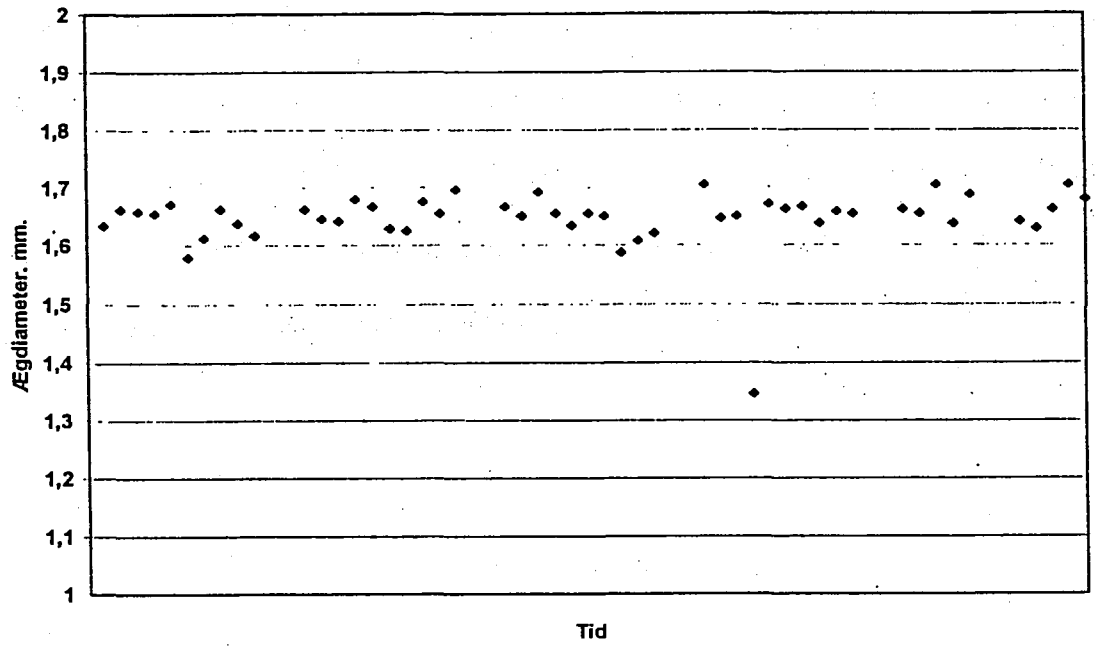
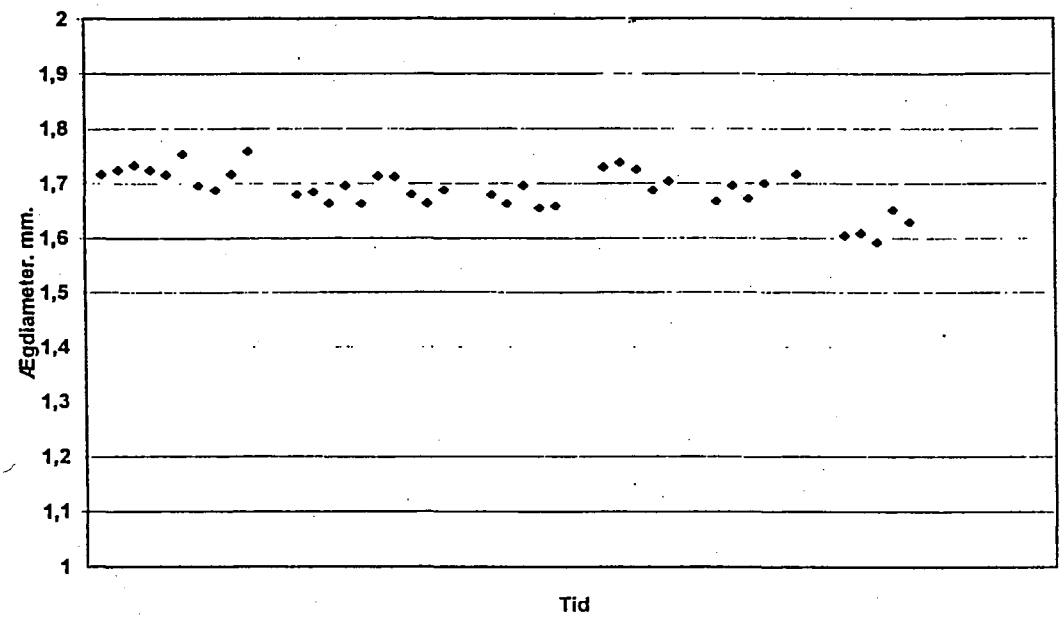


Fig. 16.2 Egg diameter/time. Breeding fish group B



The figure indicates that the egg diameter remains constant during the spawning season, and that the correlation between egg diameter and size of breeding fish is positive. In group A it the average diameter is 1.65 mm while in group B it is 1.72 mm. The presence of some variation between the points in the figure may well be explained by the fact that the two breeding fish groups were not homogenous in terms of size of the females.

It has been found (Kjesbu, O.S., 1989) that in North Sea cod the egg diameter varies according to the time of the season with a peak in the first third of the season with a diameter of 1.38 mm decreasing to a size of 1.26 mm in the end of it. The eggs were spawned by a female of 4.2 kg. Beside variations according to time of season it was found that the egg diameter was much smaller than that of the Baltic cod eggs despite the size of the fish. A size variation of 1.16 - 1.60 mm has been found in North Sea cod eggs collected in the field (Hislaj, J.R.G. et al, 1987).

Data on egg dry-matter content for the eggs are presented in fig 17. The tendency that weight compared to time of season is lineary reappears. Furthermore, the fig indicates a positive correlation between egg diameter and dry-matter content.

Fig. 17.1 Egg dry-matter content/time. Breeding fish group A

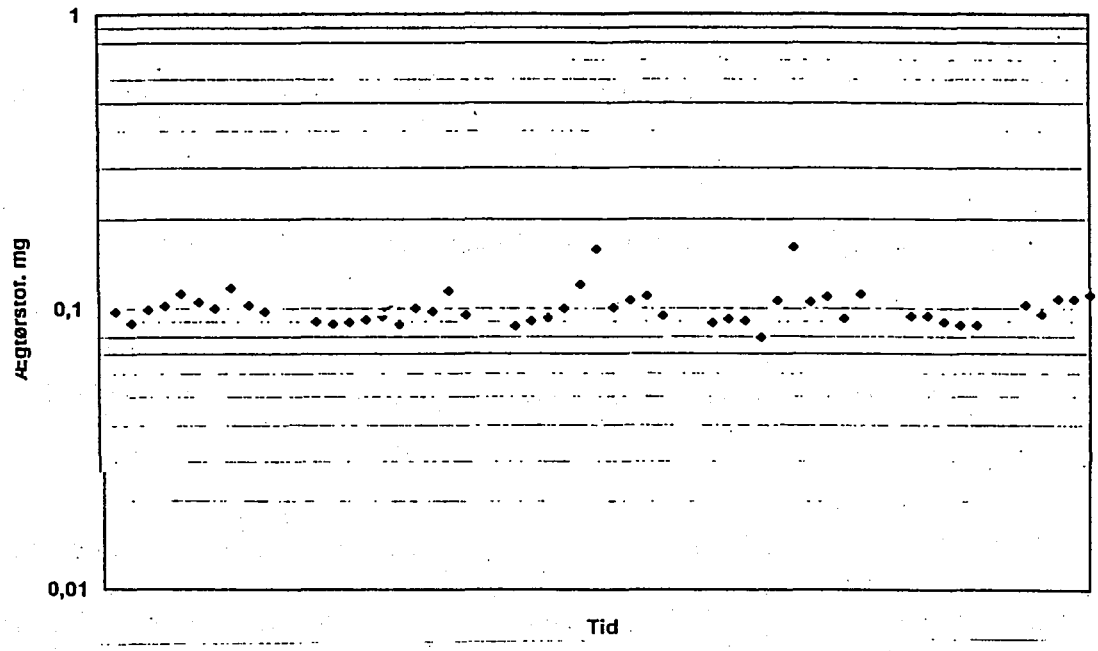
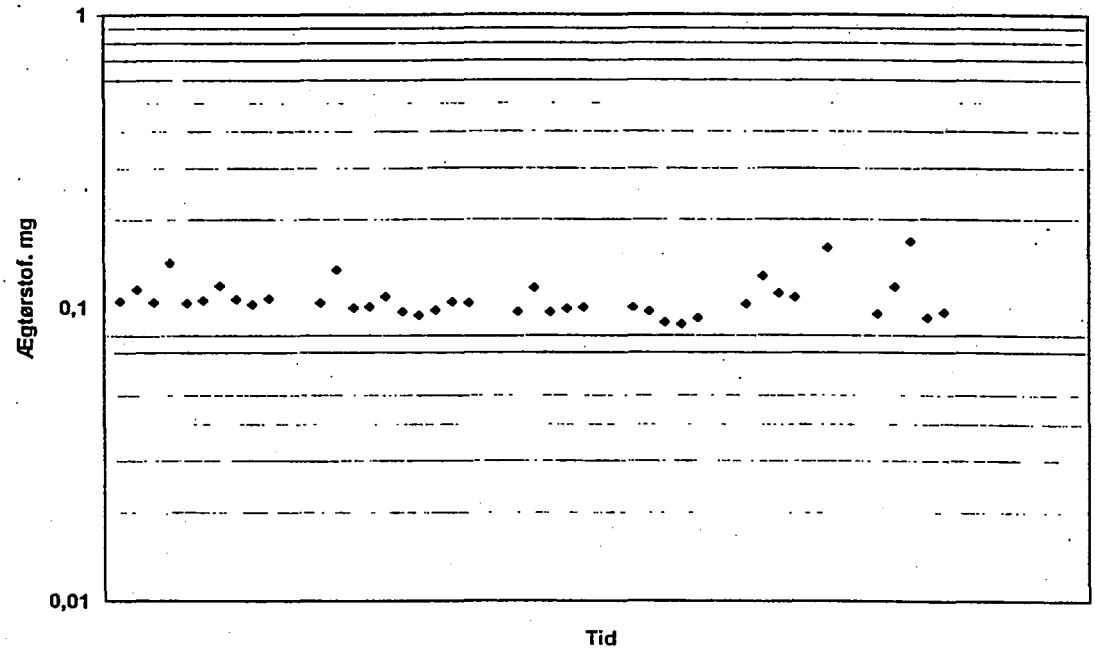


Fig. 17.2 Egg dry-matter content/time. Breeding fish group B



The dry-matter content in group A is about 0.100 mg, and in group B about 0.120 mg. North Sea cod eggs at the size of 1.4 mm have got a dry-matter content of 0.110 mg (Knutsen, G.M. et al 1985) and are thus heavier than Baltic cod eggs compared to size of diameter. The explanation of this is the adaption of the Baltic cod to the particular hydrographic conditions in the Baltic Sea such as for instance low salinity. A big diameter and a low density enhances the buoyancy.

Determination of the length of newly hatched larvae in relation to time of season and size of breeding fish.

The determination of the length of the newly hatched larvae was performed by an ocular with 25 units = 1 mm.

In fig 18 the relation between the length of the larvae and the time of season is depicted. Each point is based on an average of 50 measurings. The trend in the figure is not entirely linear. For instance there is a tendency towards a fall in the end of the season. This may be due to an error in the collection of samples. The hatching of the eggs is not synchronic but lasts for up to 2 days. Furthermore the trend is that the larvae from group B are longer except for the last measuring.

Fig. 18. 1 Lenth of larvae/time Breeding fish group A

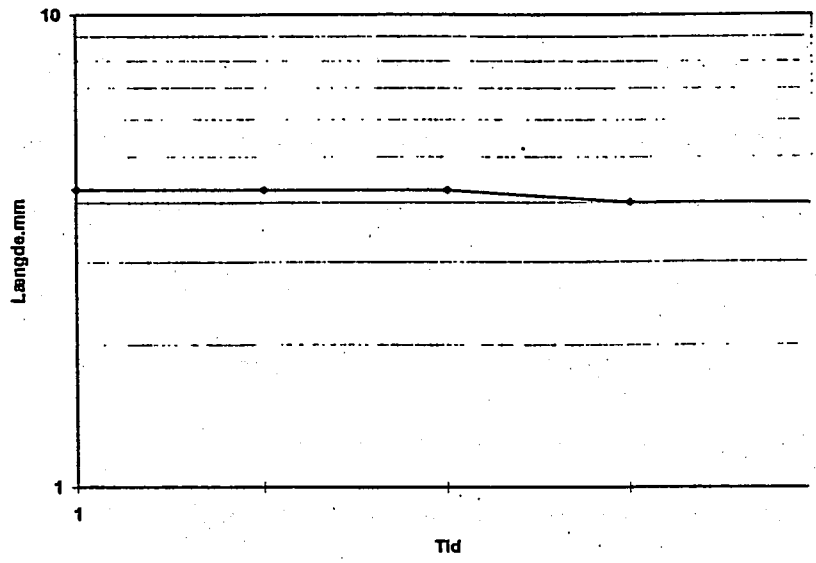
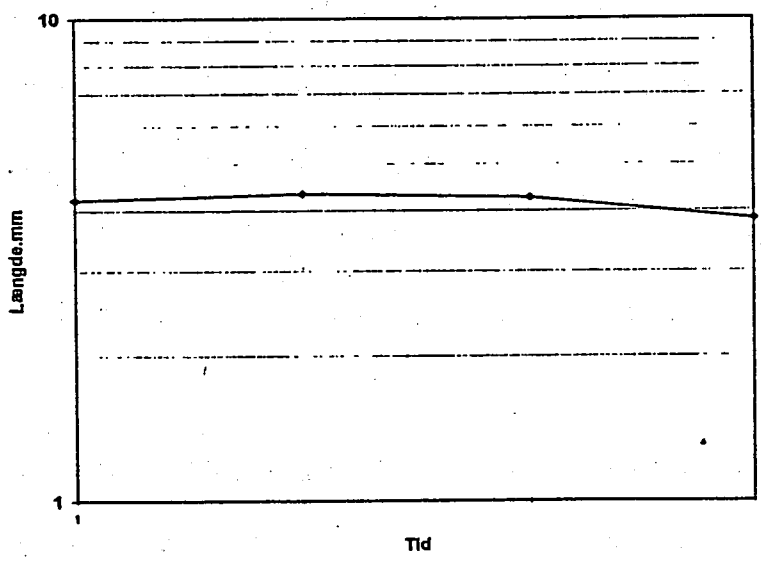


Fig. 18. 2 Lenth of larvae/time Breeding fish group B



3.4 Hatching experiments

As in 1993 the hatching results had been fluctuating a monitoring was effected in 1994 of the mortality during the incubation phase.

The hatchery from 1992 was changed. 10 cylindrical tanks were constructed, each of them with a diameter of 30 cm and a length of 50 cm with a bottom of 500 μm plankton nets for

the collection of dead eggs. The cylinders were attached to the refrigerating plant directly and were equipped with separate, immersed water supply units. The water in the hatchery was filtered through a charcoal filter, exposed to a uv-treatment, cooled and sterilized through a series offilters the size of 5 μm, 2 μm and 0,2 μm respectively and oxidized in an oxygen tower before being led back into the incubators. All the water in the hatchery, 3.5 m^3 was sterilized once an hour. The water consumption for the hatching experiments was considerably lower than the filtration capacity, and for that reason the water that was finally led into the incubators had been filter sterilized and oxidized several times before the use. The conditions should thus be optimum.

5 hatching experiments were performed with eggs from breeding fish group A and 4 with eggs from group B.

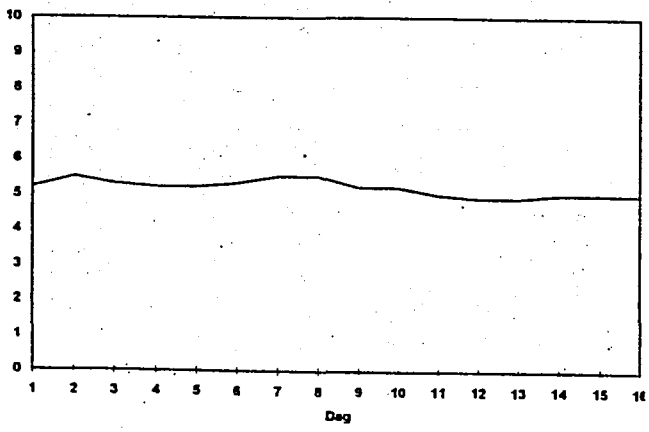
The seawater for the experiments was 7-8‰ Baltic seawater water filtered through a 60 um filter and resalted to 17‰ by an addition of synthetic salt, Instant ocean. After each trial session the hatchery was emptied for water and disinfected with fresh water with an addition of NOAH increasing the pH value to 14. In experiment A1 the hatching per cent is an average of 5 analogous experiments, in A2 an average of 4, in A3 an average of 4, in A4 an average of 2, in A5 an average of 2, in B1 an average of 4, in B2 an average of 2, in B3 an average of 2 and in B4 an average of 2.

Fig 19 exhibits the hatching per cents in every single experiment and the temperature level during the experiment. The term "actual hatching per cent" refers to the fact that the per cent is calculated on the basis of the fact that the fertilization per cent was in no case as high as 100%. All dead eggs were collected every second day, and the number estimated by calculating the amount of eggs in 10 ml for each 50 ml of eggs.

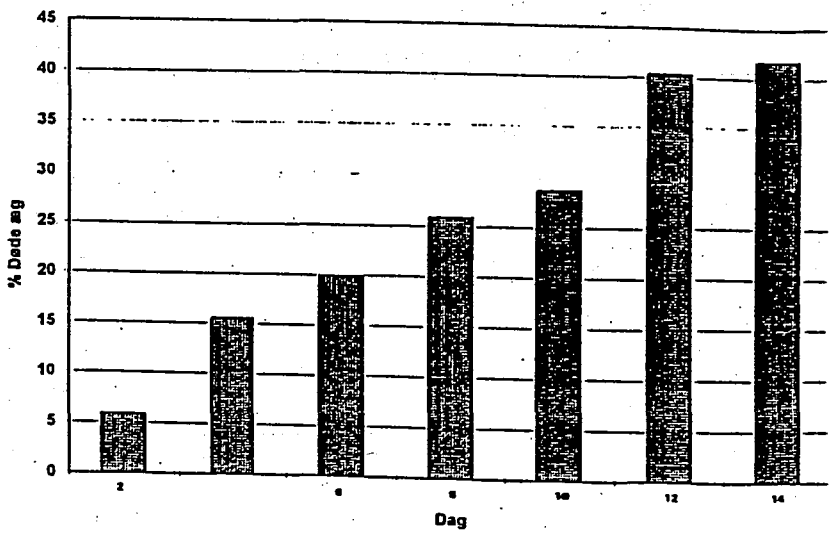
In experiment A2 and A3 the mortality rate is highest in the first half of the incubation phase. In the other experiments in series A the mortality rate is stable during the whole phase.

Fig. 19

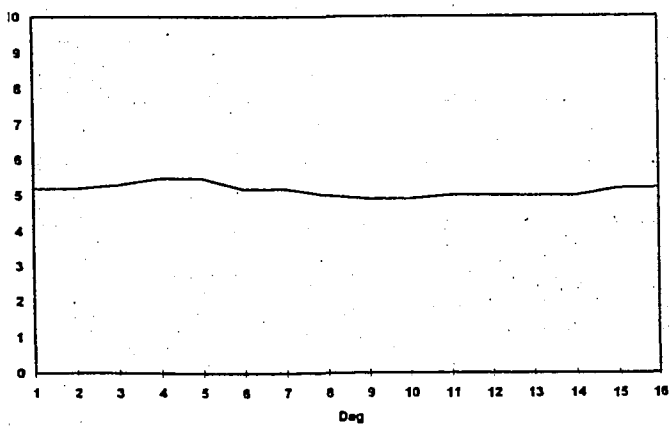
Temperature, hatchery, experiment A1



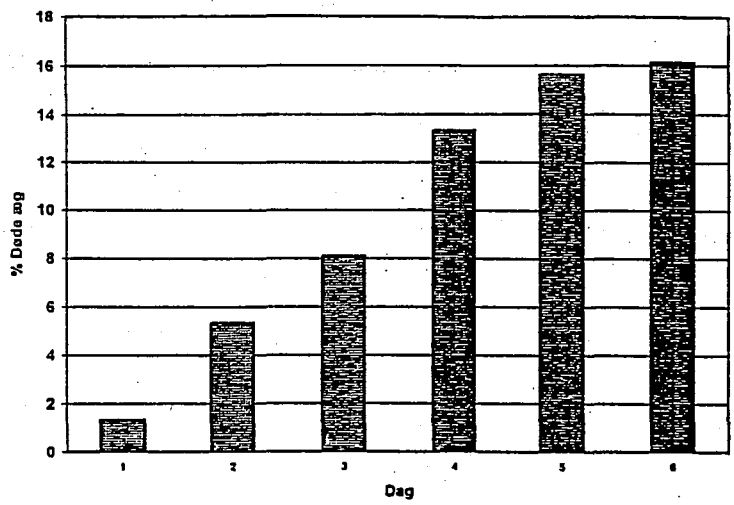
Cumulative egg mortality rate. Experiment A1. Actual hatching per cent 61%



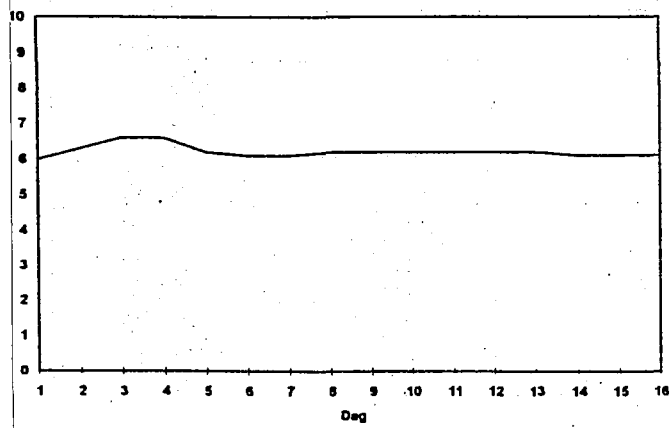
Temperature, hatchery, experiment A2



Cumulative egg mortality rate. Experiment A2. Actual hatching percent 8.i%



Temperature, hatchery, experiment A3



Cumulative egg mortality rate. Experiment A3. Actual hatching per cent 24%

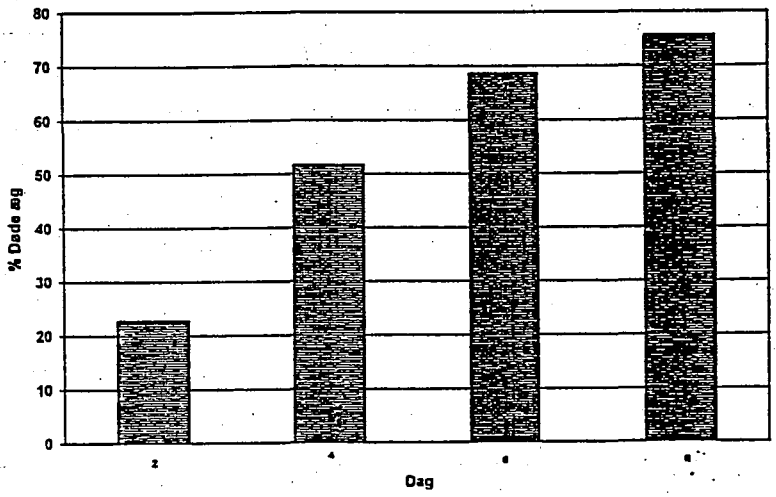
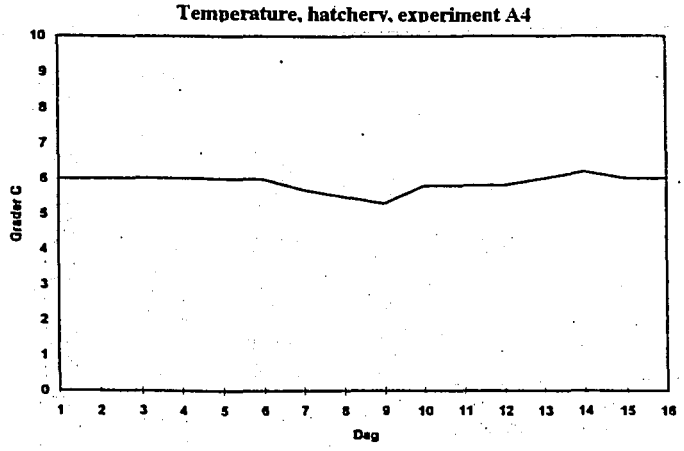
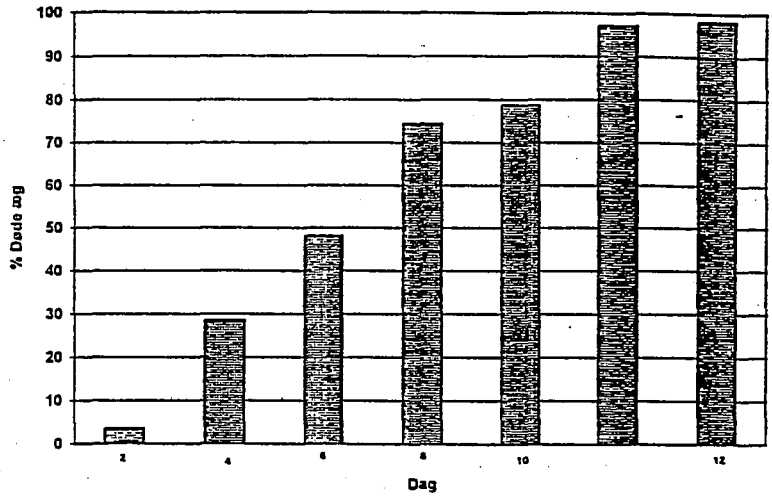


Fig. 19 continued

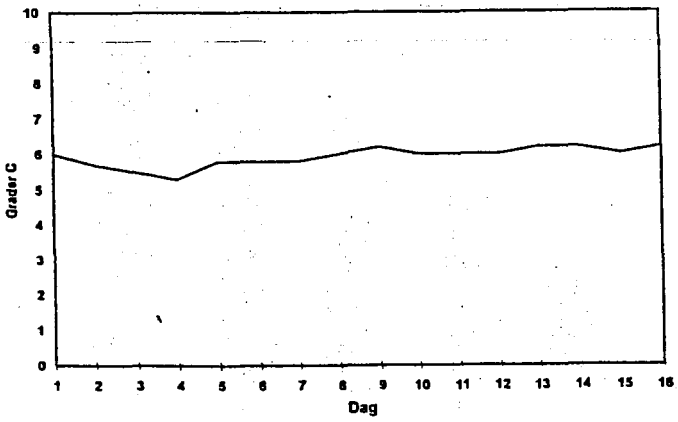
Temperature, hatchery, experiment A4



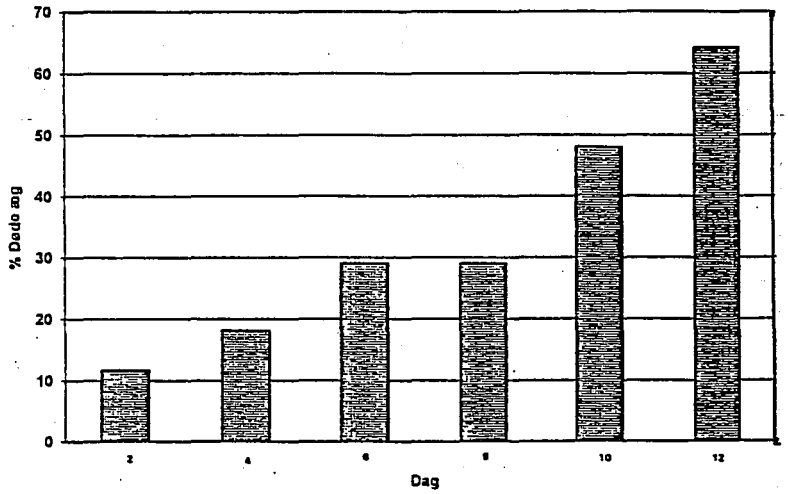
Cumulative egg mortality rate. Experiment A4. Actual hatching per cent 3%



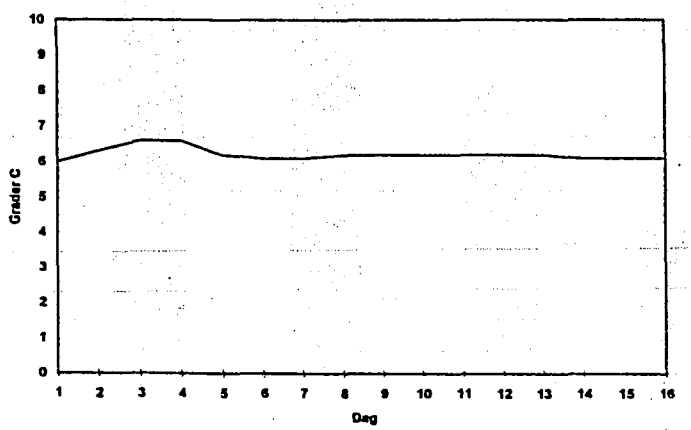
Temperature, hatchery, experiment A5



Cumulative egg mortality rate. Experiment A5. Actual hatching per cent 51%



Temperature, hatchery, experiment B1



Cumulative egg mortality rate. Experiment B1. actual hatching per cent 18%

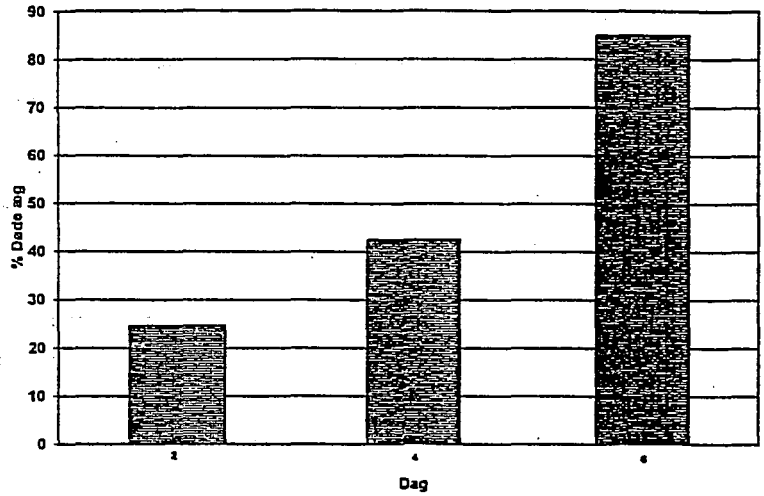
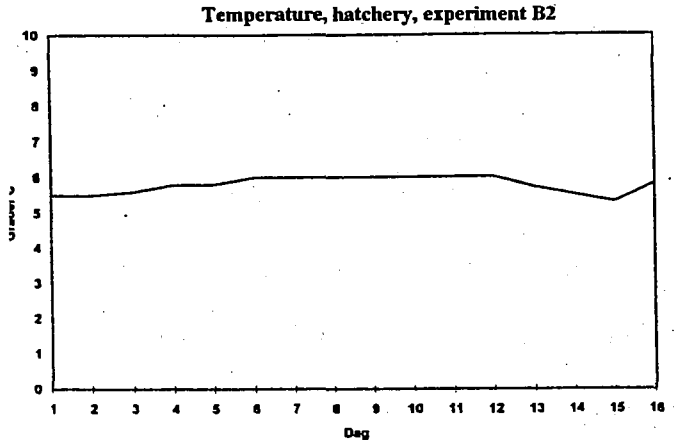
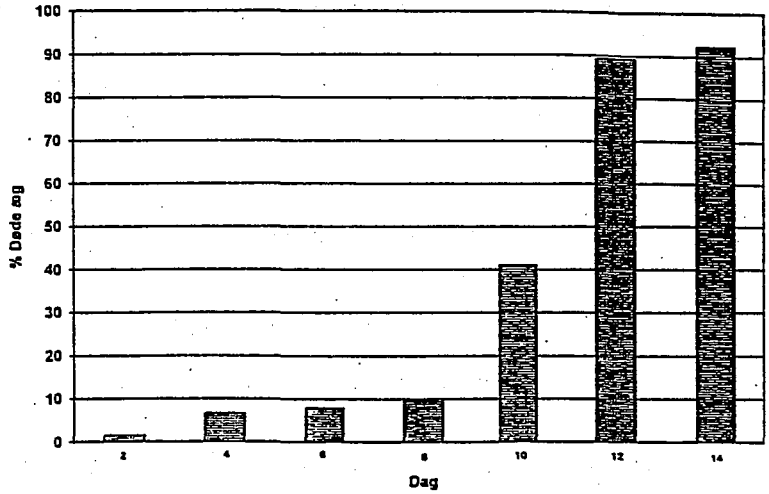


Fig. 19 Continued

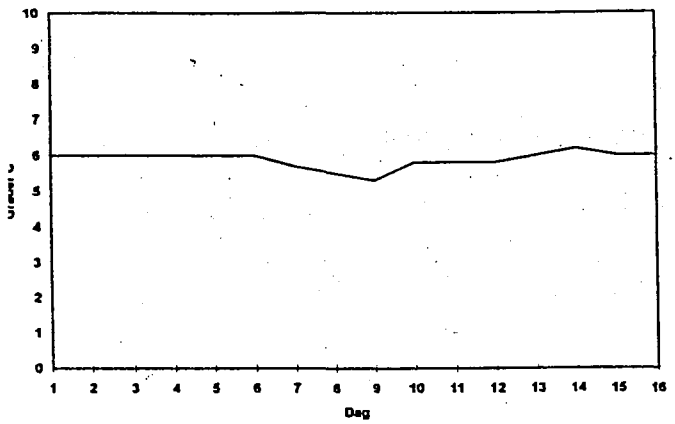
Temperature, hatchery, experiment B2



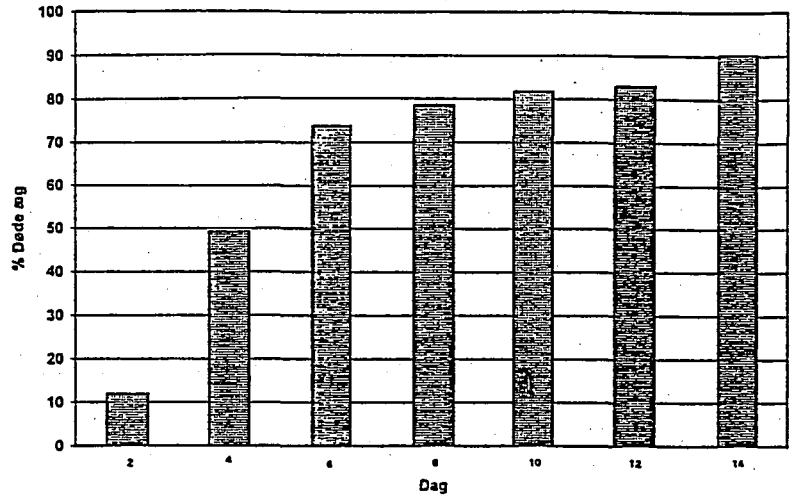
Cumulative egg mortality rate. Experiment B2. Actual hatching per cent 10%



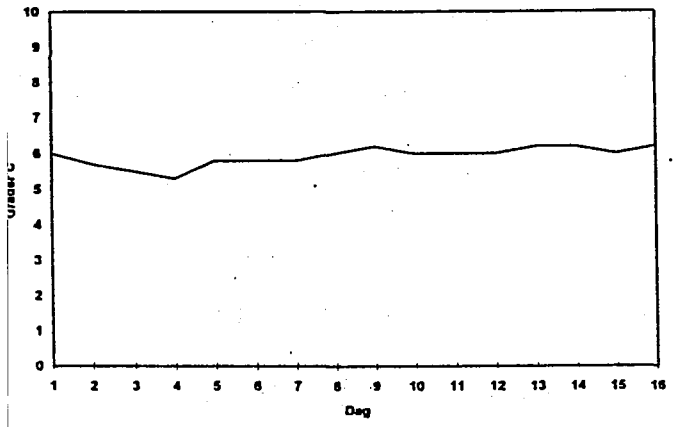
Temperature, hatchery, experiment B3



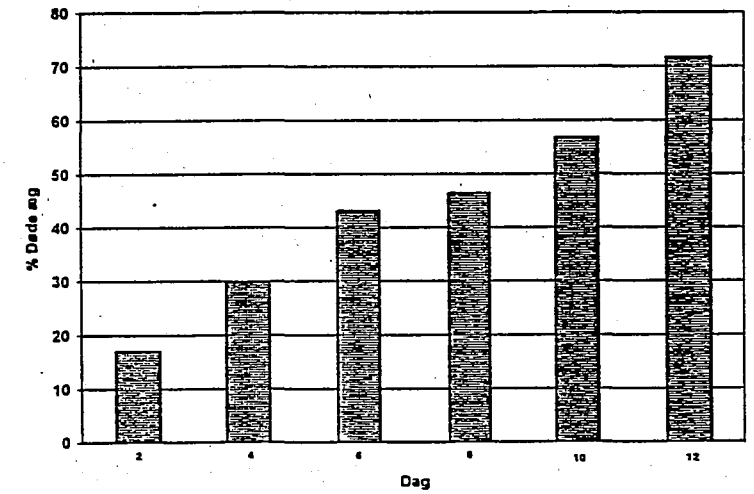
Cumulative egg mortality rate. Experiment B3. Actual hatching per cent 15%



Temperature, hatchery, experiment B4



Cumulative egg mortality rate. Experiment B4. Actual hatching per cent 39%



In the experiments in series B the mortality rate in experiment B1 and B3 peaks in the first half of the period, in experiment series B2 it peaks in the last half. In experiment B4 the mortality rate is constant.

In the case of both series the hatching experiments are characterized by varying hatching per cents. In series B the hatching per cents are generally lower than in series A.

In all 239.000 larvae were produced. In all experiments the temperature level was 5-6,5C the maximum fluctuation being 0,5C in the incubation phase.

In Swedish hatching experiments with Baltic cod no hatching per cents higher than 60% were achieved (Pickova, J. et al, 1992). In hatching experiments with turbot from the western Baltic Sea the highest hatching per cent was 54%.

3.5 Environmental poison content

To examine whether the eggs may be contaminated by environmental poisons affecting the hatching results a sample was collected from each egg batch in which the content of DDD, DDE, DDT and the PCB-congenes CB28, CB52, CB101, CB105, CB118, CB138, CB153, CB170 and CB180 was measured.

The results from these measurings are based on single measurings and thus subject to some uncertainty. The egg quantity was not sufficient for double measurings. The environmental poisons mentioned above are attached to lipoids, and for that reason it is the lipoids of the eggs that have been analyzed. As in the case of other kinds of pelagic marine fish eggs the content of lipoids in cod eggs is very little (Craik, J.G.A. et al, 1987), (Lønning, S. et al, 1988). For that reason quite large quantities of eggs are required to permit an extraction of sufficient quantities of lipoids for double measurings.

209 kinds of PCB-congenes are known all of them breakdown products from PCB. The 9 kinds of congenes included in these analyses come to 70-80% of the total quantity of PCB-

congenes, and are thus used as markers.

In table 1 the concentration of DDD, DDE and DDT in the egg batches appear.

Table 1

Egg batch nr.	p,p DDD	p,p DDE	p,p DDT	µg/kg roe
A1	0.062	1.323	0.282	
A2	0.048	1.023	0.282	
A3	0.057	0.937	0.406	
A4	0.070	2.017	0.336	
A5	0.057	0.942	0.148	
B1	0.045	1.526	0.159	
B2	0.125	3.899	0.741	
B3	0.027	1.625	0.341	
B4	0.082	2.762	0.520	

There is no positive correlation between the hatching per cents and the content of neither DDD, DDE or DDT, not even if the numbers are added up.

A content of DDT of 2.95 mg/kg of roe leads to a high egg mortality rate in trout and later on to a high mortality rate in the umbilical sac spawn. A level of 2.67 mg/kg of roe or less does not affect the hatching per cent or the mortality rate in umbilical sac spawn (Burdick, G.E. et al, 1964).

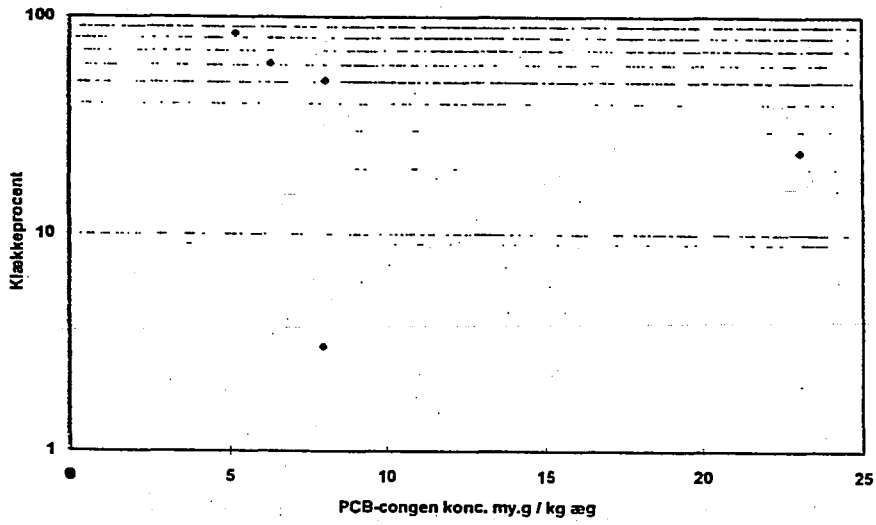
DDT-levels of 1.09 - 2.76 mg/kg of roe from the Coho salmon in Lake Michigan led to a mortality rate of 15-73% in the larvae in 8 weeks while levels of 0.55 - 0.66 mg/kg of roe led to a mortality rate of 1-5% in the same period (Johnson, H.E., 1969) DDE-levels of 18 ng/g fat or more affect the hatching per cent in herring from the western Baltic Sea (Hansen, P.D. et al, 1985). The levels found to be dangerous in these surveys are much higher than those found in the

egg batches of the present experiments.

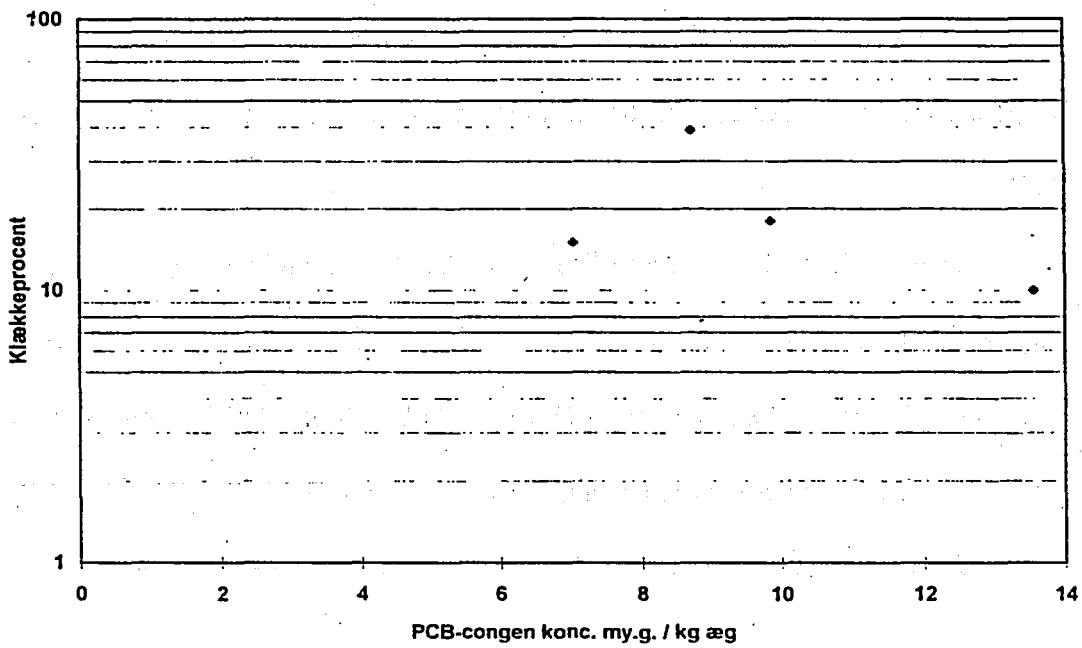
The hatching per cent as a function of the PCB-congen content in the two experiment series A and B can be seen in fig 20. The level of the 9 congenes is marked as an addition.

It appears in the fig that there is no correlation between the hatching per cent and the content of PCB-congenes.

Fig 20.1 Hatching per cent in relation to PCB-congen content in the eggs. Breeding fish group A.



Fif. 20.2 Hatching per cent in relation to PCB-congen content in the eggs. Breeding fish group B.



Thus the correlation coefficients of the experiment series A is: $R^2 = 0.480$ and of the experiment series B: $R^2 = 0.421$. PCB levels of 120 ng/g fat has a negative effect on the hatching per cent in western Baltic herring (Hansen, P.D. et al, 1985). The levels observed in the hatching experiments are considerably lower than the level of 120 ng/g fat mentioned to be critical in herring. Surveys of Coho salmon spawn have shown that PCB levels of 3.9 - 5.2 mg/kg have led to disease as well as death. Several surveys have proven that chlorinated hydrogen carbonate compounds may affect the quality of fish eggs and larvae negatively (Bengtsson, B.E., 1978), (Karås, P. et al, 1991), (Sandstrøm, O. et al, 1988), (Westernhagen, H.V. et al, 1988).

Larvae experiments

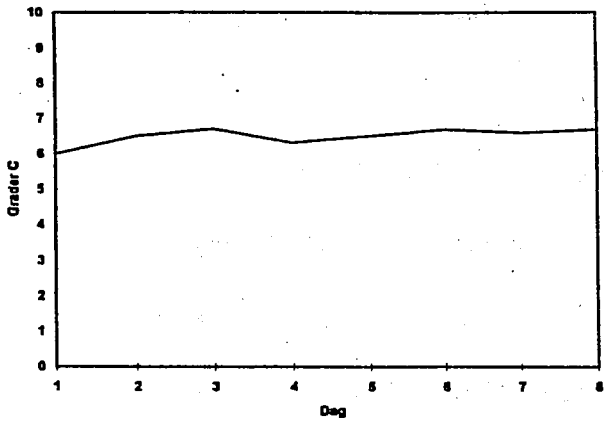
In 1994 2 feeding experiment sessions were carried out, one with the larvae from the hatching experiment A1 with 85,400 larvae and one with the larvae from hatching experiment A2 with 115,300 larvae. The experiments were carried out in 17‰ resalted seawater at 5.5-6.5‰ C. The larvae were fed along the same lines as in the previous year, but instead of food produced in the laboratory natural zooplankton from natural seawater was used.

The zooplankton at the size of 60-150 μm was given as feed along with the algae Tetraselmis, Rhodomonas and Isocrysis. In experiment A1 the larvae survived for 11 days, in experiment B for 12 days. During the dry-matter measurings it was examined whether the larvae in experiment A1 had any food content in their stomachs. On day 4 40 out of 50 had been eating, on day 6 24 out of 50 had been eating. As in the previous year the larvae died within the 2 first weeks although this time they were fed with natural zooplankton. This may indicate that the problem is not a nutritional one. It is worth noticing, however, that the larvae did willingly incept food whereafter most of them stopped to eat within a period of 2 days.

3.7 Starvation experiments with the larvae

To test the survival potential of the larvae a series of starvation experiments were initiated. Larvae from the egg batches A3, A4, A5, B1, B2, B3, and B4 were used for the experiments. 20 larvae from each group were placed in cylinders with a diameter of 22 cm, a length of 30 cm and a bottom of 500 μm plankton nets. The cylinders were immersed in a tank from the production plant and each cylinder was attached to a separate water supply unit. The number of surviving larvae was counted once a day. Fig 21 depicts the larval survival rate of each day and the temperature level during the experiments.

Fig. 21



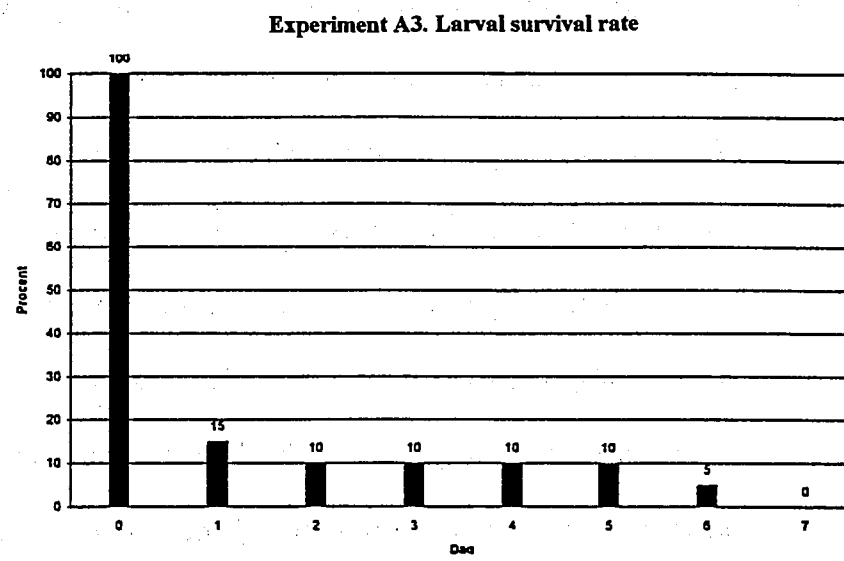
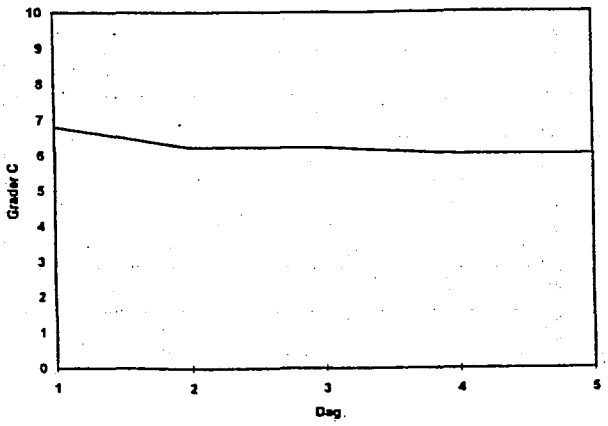
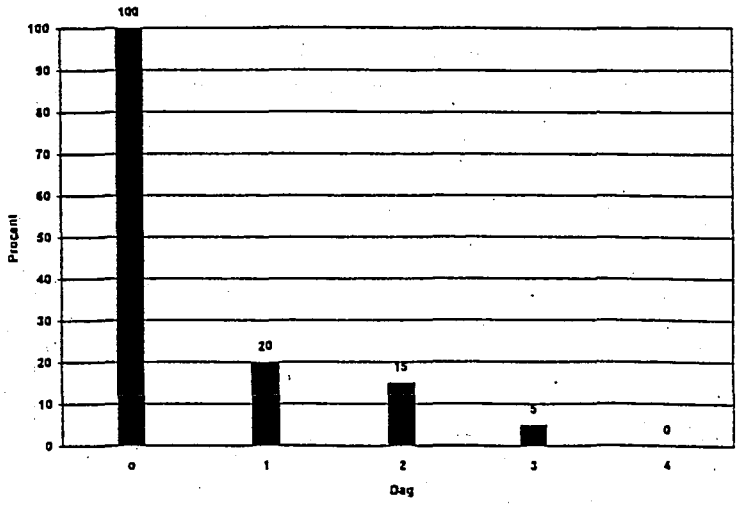


Fig. 21 continued

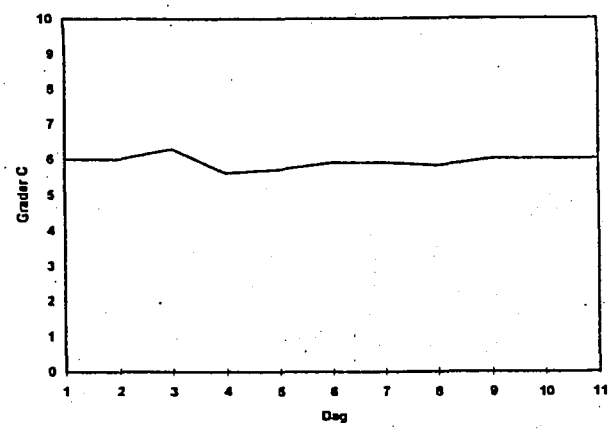
Temperature, experiment A4



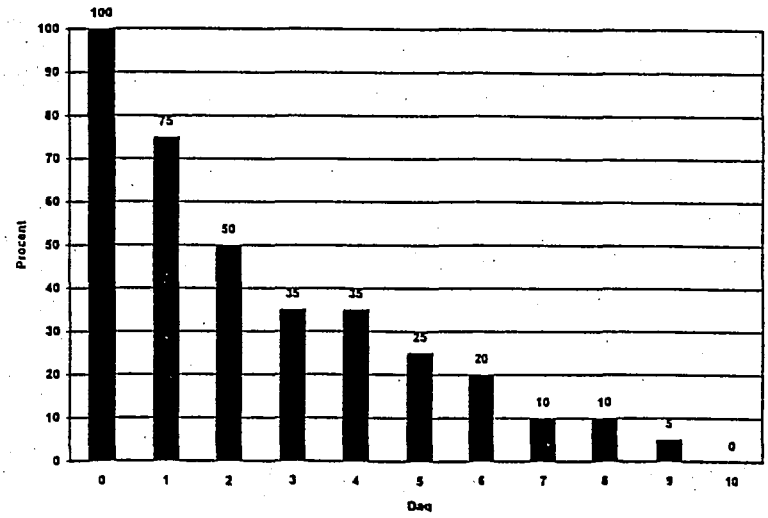
Experiment A4. Larval survival rate



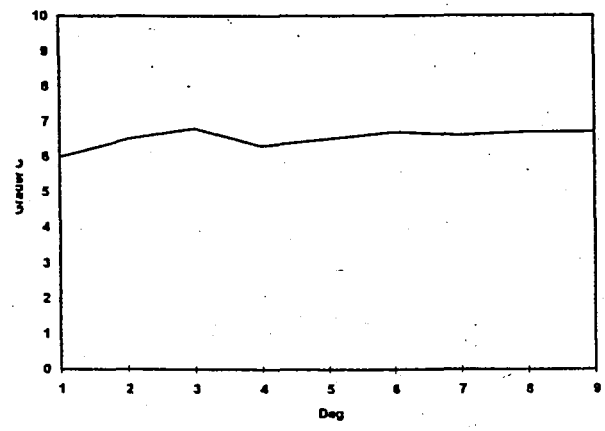
Temperature, experiment A5



Experiment A5. Larval survival rate



Temperature, experiment B1



Experiment B1. Larval survival rate

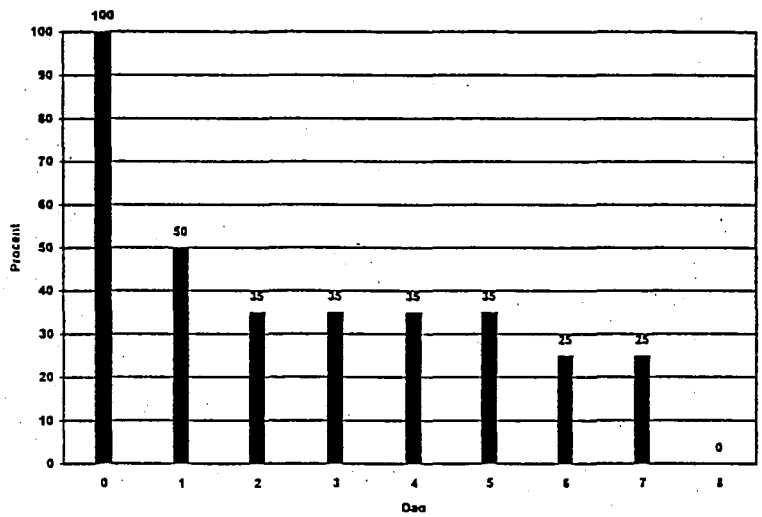
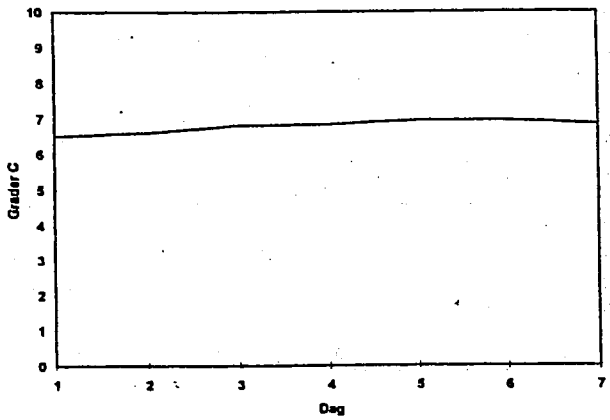
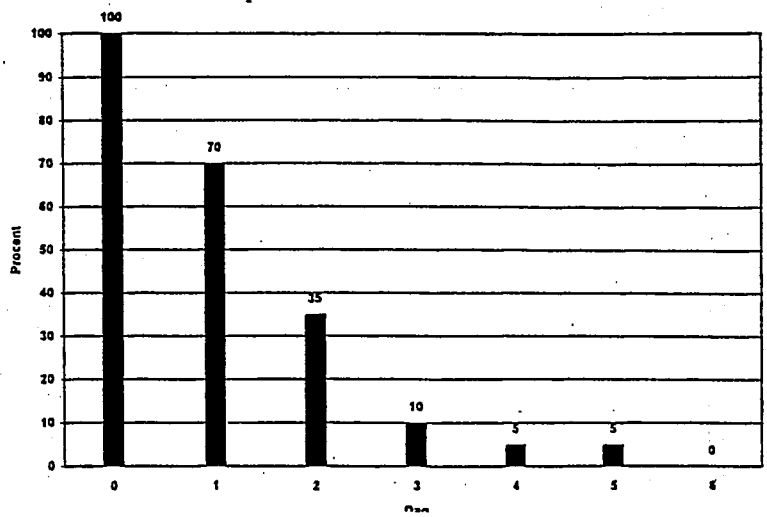


Fig. 21 continued

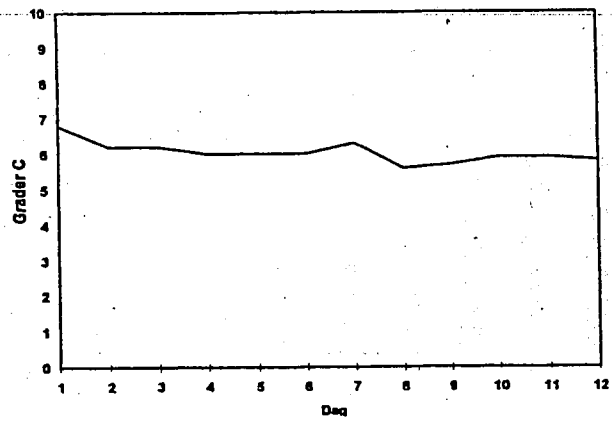
Temperature, experiment B2



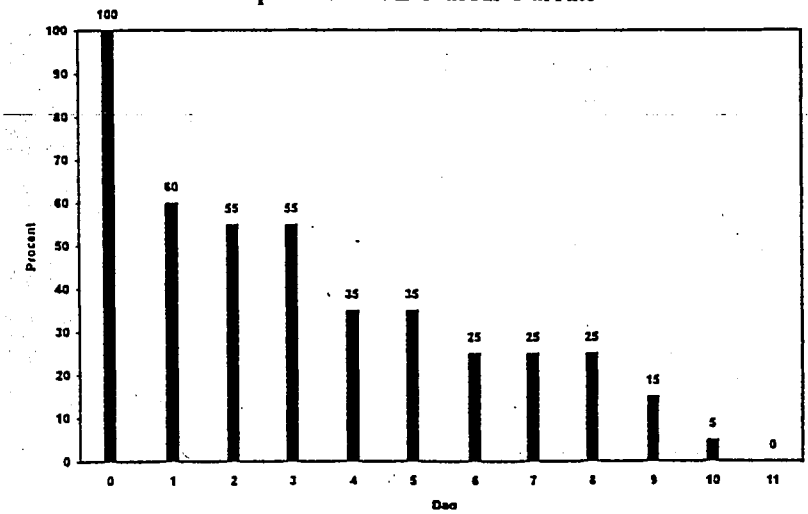
Experiment B2. Larval survival rate



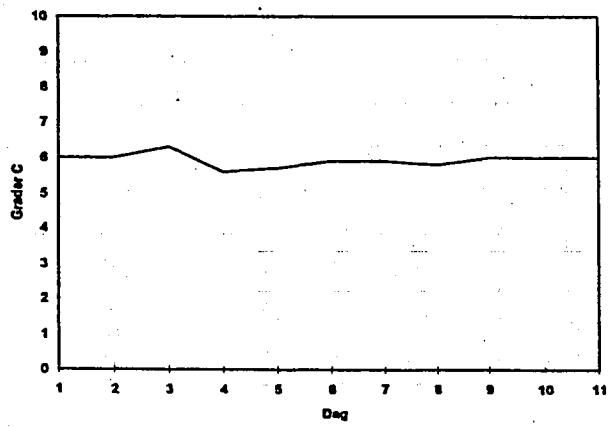
Temperature, experiment B3



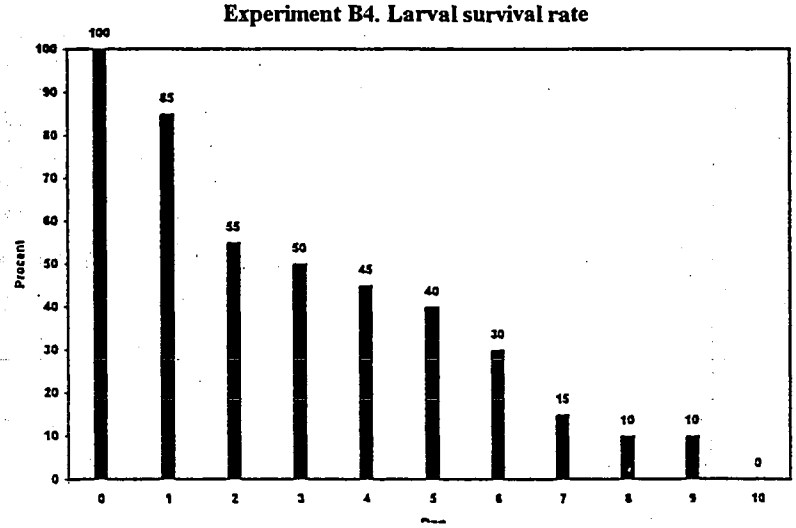
Experiment B3. Larval survival rate



Temperature, experiment B4



Experiment B4. Larval survival rate



The most characteristic feature is the high mortality rate from the outset of day 1. At the umbilical sac phase on day 6 only a few larvae were still alive in most of the experiments. In experiment A3 5%, in A4 0%, in A5 20%, in B1 25%, in B2 0%, in B3 25% and in B4 30%. Experiments with North Sea cod larvae have shown that they can live for up to 16 days at 6,9C without being fed (Yin, M.C. et al., 1987).

The larvae in the experiments shown in fig 21 lived for a much shorter time, the longest survival period being 10 days.

In fig 22 the larval survival rate at the end of the umbilical sac phase, day 6, is shown as a function of the hatching per cent. As depicted in the figure there is a connection between the hatching per cent and the larval survival rate. This may indicate the presence of some unknown factor which influences the hatching per cent and later on the larval survival rate.

Fig. 22.1 Larval survival rate day 6 as a function of the hatching per cent.

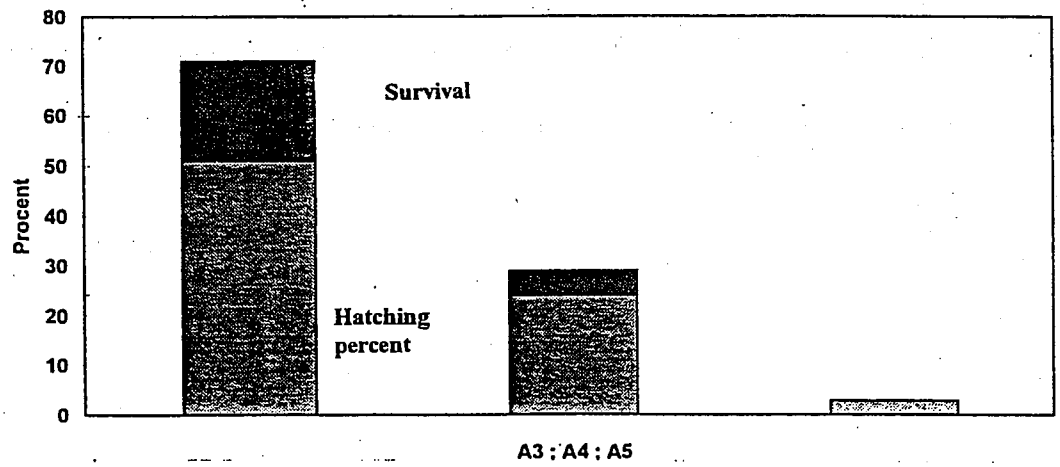
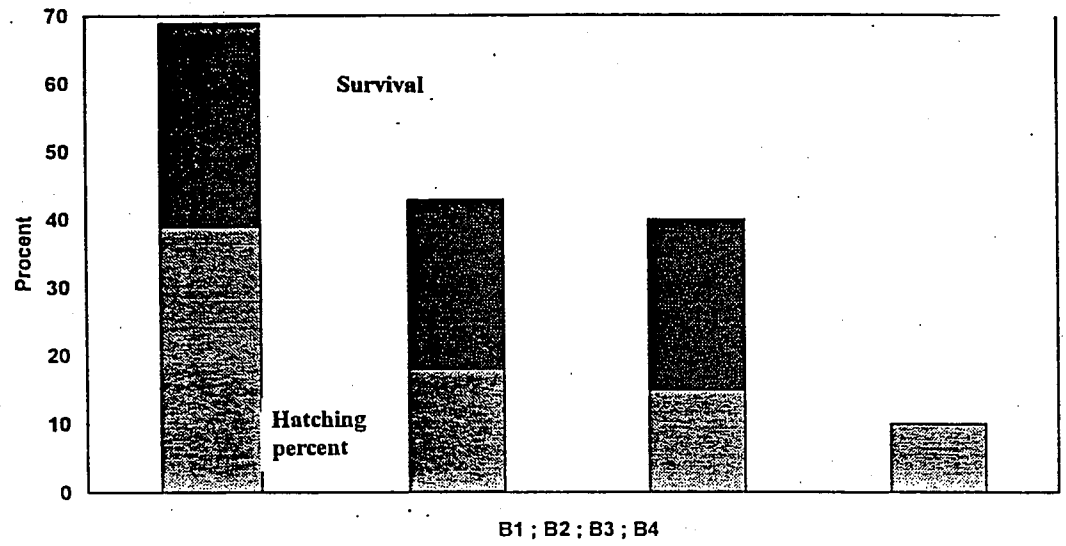


Fig. 22.2 Larval survival rate day 6 as a function of the hatching per cent.



Conclusion

It has been found in the experiment period of 3 years that it is possible to make Baltic cod spawn in captivity, that it is possible to produce large quantities of eggs, but that, to a great extent, the hatching per cents are subject to variations. It is possible to produce larvae in a quantity relevant for production. The larvae incept food already from day 4, but the mortality rate is rather high very soon after the hatching. The mortality rate is almost 100% after 2 weeks. In both 1993 and 1994 the Royal Veterinary and Agricultural High School of Denmark performed disease monitoring of eggs and larvae without being able to relate the mortality rate to pathogenic infections.

It is possible to produce spawn without visible defects in a small number. Apparently the larvae do not die from malnutrition. Besides there is a correlation between the hatching per cent and the larval survival rate.

The problems connected to the extent of the egg and larval mortality must be elucidated by further investigation before a large-scale production of spawn can be accomplished.

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TORSKEYNGELPROJEKT BORNHOLM

RESULTATER 1992 - 1994

Philip S. Prince

Danmarks Fiskeri- og Havundersøgelser
Hirtshals

januar 1995

FORORD

På baggrund af den i begyndelsen af 1990'erne lave bestand af torsk i den østlige Østersø iværksatte Fiskeriministeriet i 1992 et pilotprojekt med det formål at udvikle en opdrætsmetode til storskala produktion af Østersø torskeyngel.

Hensigten var igennem udsætning af yngel på længere sigt at ophjælpe den naturlige bestand af torsk i Østersøen.

Projektet blev udarbejdet som et samarbejde mellem Fiskeriministeriet, Dansk Fiskeriforening og Danmarks Havfiskeriforening med Danmarks Fiskeri- og Havundersøgelser som koordinator.

Projektet, som fra starten var berammet til 2 år, siden udvidet til 3 år, fik sin opstart i maj måned 1992 med etablering af et pilotanlæg på Østre Flak 4, Neksø, Bornholm. Anlægget består af en udendørs semiintensiv produktionsafdeling samt en indendørs recirkuleret produktionsafdeling. Anlægget er konstrueret som et produktionsanlæg efter gængse principper vedrørende semiintensiv og intensiv opdræt af marin fiskeyngel. Anlæggets teoretiske produktionskapacitet er for semiintensiv afdelingens vedkommende ca. 60.000 stk. yngel 3-5 cm lange og for intensiv afdelingen ca. 100.000 stk. yngel 3-5 cm.

På baggrund af negative erfaringer i 1992 med indsamling af torskeæg i felten blev der i 1993 indgået et samarbejde med Bornholms Fiskerilaboratorium om opbygning af et recirkuleret anlæg til hold af moderfisk. Driften af anlægget blev indtil sommeren 1993 varetaget af Bornholms Fiskerilaboratorium, hvorefter Danmarks Fiskeri- og Havundersøgelser overtog driften. Anlægget har igennem sæsonerne 1993 og 1994 leveret æg til opdrætsforsøgene.

I det følgende vil der blive redegjort for de indhøstede erfaringer og resultater igennem sæsonerne 1992, 1993 og 1994.

Philip S. Prince

Danmarks Fiskeri- og Havundersøgelser
Hirtshals

1. Opdræt 1992

Det var hensigten at foretage startfodringsforsøg i både den semiintensive og intensive produktionsafdeling. På grund af projektets sene start i slutningen af maj måned umuliggjorde høje temperaturer i anlægget en egentlig startfodring. Således var temperaturen i semiintensivanlægget i begyndelsen af juni, hvor de første klækkeforsøg med torskeæg blev foretaget, steget til 18°C, hvilket var den laveste temperatur, der blev registreret i projektperioden frem til den 17. august. Højeste registrerede temperatur i perioden var 24°C.

Forsøg udført af den Kgl. Veterinær- og Landbohøjskole, som igennem forsøgsperioden var tilkoblet projektet for at overvåge torskeæggenes og larvernes sundhedstilstand, har vist, at Østersø torskeæg har en lav tolerance over forhøje temperaturer. Ved 12°C og en salinitet på 16%o registreredes en klækkeprocent på 20. Æg inkuberet ved 16°C og 20°C i 16%o havvand klækkede overhovedet ikke. Temperaturtoleranceforsøg med Østersø torskelaver viste ved 16°C og 16%o salinitet 100% dødelighed efter 5 døgn. (Buchmann, K. et al., 1992). Forsøg med Nordsøtorskeæg har vist, at ved en inkubationstemperatur på 12°C dør alle alle æggene inden blastulastadiet er overstået (Thomsen, B.M., 1981).

Temperaturen i det intensive startfodringsanlæg var i begyndelsen af juni 16°C og højeste registrerede temperatur i forsøgsperioden var 25°C. Det var derfor heller ikke muligt at gennemføre startfodringsforsøg i dette anlæg.

Fra projektets start var der satset på at afstryge moderfisk i felten for at skaffe æg til klækkeforsøgene. Det viste sig dog umuligt at indsamle æg i produktionsrelevant skala. Østersø torsk er batch gydere, og de enkelte torsk gyder over en periode på ca. 3 mdr. Man skal derfor være meget heldig for at fange hunner med en større portion helt afstrygningsmodne æg. Situationen blev ydermere forværret af, at torskefangsterne var meget lave det år (Prince, P., 1993).

Der blev derfor kun gennemført ganske få klækkeforsøg, oftest med æg af dårlig kvalitet, hvor der i de fleste tilfælde blev observeret meget store ægdødeligheder - op til 100%. Befrugtningsraten var som regel lavere end 30%.

Under projektet blev der observeret en del æg med fejlagtig celledeling medførende en høj mortalitetsrate. Disse ægbatches stammede primært fra moderfisk ilandbragt af fiskerne, hvorefter afstrygningen foregik på kajen. Graden af misdannelser kan meget vel skyldes, at moderfiskene var døde, inden de nåede land, og æggene derfor udsat for iltmangel og/eller temperaturstigning under transporten, som foregik i sommervarmen.

I det hele taget var det et problem at håndtere æggene i sommervarmen og undgå temperaturstigninger (Prince, P., 1993).

Klækning af æggene foregik i 15-16‰ havvand oppumpet fra 70 meters dybde. Klækkeriet var det eneste opdrætssystem med påmonteret køleanlæg. Systemet er beskrevet i (Prince, P., 1993). Trods køling var det ikke muligt at holde bare et nogenlunde stabilt temperaturniveau. Laveste registrerede temperatur var 4,5°C og højeste 10,5°C. I visse klækkeforsøg blev der registreret en temperaturvariation på 4,0°C fra inkubationsperiodens start og frem til klækning. Det kan ikke udelukkes, at den varierende temperatur i klækkeriet har påvirket klækkeresultaterne i negativ retning.

Blandt nyklækkede blommesækslarver blev der observeret individer med epithelskader på frekvenser mellem 18-35%. Disse skader menes fremkommet ved en mekanisk påvirkning af laverne forårsaget af turbulente forhold i klækkecylindrene. Der blev observeret lordose og scoliose med en frekvens på i visse tilfælde op til 20%. Det antages, at sådanne lidelser skyldes tilstedeværelsen af fosterskadelige stoffer i ægget på grund af utilfredsstillende forhold under ægudviklingen eller dårlige iltforhold i klækkecylindrene (Buchmann, K. et al., 1992). Visse larvebatches udviste frekvenser på 25% eller mere af infektioner med svamp eller bakterier. Det kunne dog ikke påvises, at infektionerne var lethale.

For at udelukke disse potentielle negative påvirkninger af klækkeresultaterne blev det besluttet at gennemføre klækningen af æggene i startfodringsanlægget i 1993 sæsonen. Tankene i anlægget, som er på 600 l har vandindtag i bunden, hvilket muliggør at have en høj vandudskiftningsrate uden at skabe turbulente forhold - ikke mindst fordi æggene flyder i overfladen.

I det semiintensive produktionsanlæg blev der som planlagt gennemført en kvantitativ og kvalitativ monitering af det til anlægget indpumpede zooplankton med det formål at teste metodens produktions potentiale. Det beregnede afhøstningspotentiale var 20 kg zooplankton baseret på tørvægt (Rasmussen, K.; Brun, I., 1992). Den reelle afhøstning var 5 kg zooplankton baseret på tørvægt (Prince, P., 1993). Forskellen mellem den teoretiske og den reele, afhøstede mængde zooplankton skyldes primært, at tromlefiltret kun var i drift i 25% af tiden på grund af tilkoblingsproblemer med trådalger.

Konklusionen på resultaterne efter forsøgsperioden i 1992 blev, at hvis en større produktion af yngel skulle kunne gennemføres, ville det være nødvendigt at sikre en jævn og større tilgang af befrugtede æg af en god kvalitet ved etablering af et moderfiskehold. For at gennemføre de egentlige startfodringsforsøg ville det være nødvendigt at etablere køling på det intensive produktionsanlæg. For at gennemføre startfodringsforsøg i semiintensivanlægget ville det være nødvendigt at have nyklækkede larver af god kvalitet til rådighed allerede i april måned for at undgå temperaturproblemer.

Klækkeriet, som er en kopi af et klækkeri brugt til succesfuld klækning af Nordsø torskeæg på Vestkysten, viste sig ikke at leve op til forventningerne. Det store antal af larver med epithelskader samt lordosis og scoliosis (Buchmann, K.; Larsen, S.L.; Dalsgaard, I., 1993) tyder på, at forholdene dels har været for turbulente under inkubationsfasen samt, at miljøet i klækkecylindrene har været utilstrækkeligt. Forsøg med Nordsø torskeæg har vist, at de specielt i starten af inkubationsfasen er meget sårbare overfor fysiske påvirkninger som turbulens medførende en høj mortalitetsrate (Rollefsen, G., 19),(Rollefsen, G., 1932). Det er højst sandsynligt, at Østersø torskeæg er endnu mere påvirkelige af fysisk påvirkning som turbulens. Dels udvikler de sig på store dybder, 70-80 m, hvor de fysiske forhold er meget stationære, dels har æggene på grund af deres tilpasning med hensyn til flydeevne og lavere salinitet en tyndere chorion end Nordsø torskens æg, henholdsvis 3,34-4,79 um og 6-9 um (Nissling, A.; Westin, L., 1991). Nordsø torskeæg udvikles i de overfladenære lag, hvor de i langt højere grad er udsat for turbulente forhold på grund af vindens påvirkning.

2. Opdræt 1993

De egentlige startfodringsforsøg i intensiv og semiintensivanlæggene startede i 1993.

Semiintensivanlægget

Startfodringsforsøgene i semiintensivanlægget mislykkedes dels på grund af, at de første æg blev gydt den 15. april, hvilket medførte, at de første larver til forsøgene først var klækkede omkring 1. maj, hvor temperaturen i semiintensivanlægget allerede var nået op på 10-12°C. Det var derfor klart, at det ikke ville være muligt at producere yngel på 3-5 cm inden for larvernes temperaturtoleranceområde. Torskelarverne skal være klækket senest den 1. april, da det ellers ikke vil være muligt at producere fingerlings, inden temperaturen i anlægget når ca. 20°C, dvs. midten/slutningen af maj. (Rasmussen, K.; Brun, I., 1993).

Da de første producerede æg i 1993 kom den 15. april og de første producerede æg i 1994 kom den 1. maj må det konkluderes, at den semiintensive opdrætsteknik ikke er brugbar til produktion af Østersø torskeyngel på grund af manglende mulighed for at manipulere med temperaturen.

Resten af sæsonen fortsatte den kvantitative og kvalitative monitering af det indpumpede zooplankton. Resultaterne viser, at den afhøstede mængde var ca. 50% af mængden i 1992, men at zooplanktonet var af høj kvalitet og muligvis velegnet som startfoder i intensivanlægget (Rasmussen, K.; Brun, I., 1993).

2.1 Moderfiskehold

I samarbejde med Bornholms Fiskerilaboratorium, Dansk Fiskeriforening og Danmarks Fiskeri- og Havundersøgelser blev der i efteråret 1992 designet et anlæg til hold af en moderfiskebestand med det formål at producere æg til forsøgene. Financiering og opførelse af anlægget blev varetaget af Bornholms Fiskerilaboratorium, ligesom den daglige ledelse og drift frem til medio juni, hvorefter ledelsen og driften blev overtaget af Danmarks Fiskeri- og Havundersøgelser.

Anlægget er opbygget som et recirkuleret anlæg bestående af:

3 opdrætskar á 8 m3
Sedimentationstank
Neddykket biofilterenhed, 1,5 m3
Hvirvelseperator
Pumpesump + pumpe
Kulfilter
Beluftningstårn
Højtank

Anlægget er ialt på 98 m3 og er opført i et kølerum og nedkøling af vandet sker ved at kontrollere lufttemperaturen.Til hvert bassin var der monteret en ægopsamler bestående af en planktonnetpose på 100 liter nedsænket i en beholder af samme dimension.

I gydeperioden blev afløbsvandet fra de 3 moderfiskekar ledt igennem hver sin ægopsamler som overfladeafledning, hvorved æggene blev opsamlet i poserne.

Fiskene blev indfanget i februar måned med garn ud for Snogebæk på 10-20 meters dybde; ilandbragt i tanke med vand og overført til bassinerne i anlægget, som forinden var fyldt med naturligt havvand fra Østersøen med en saltholdighed på 7-8%o.

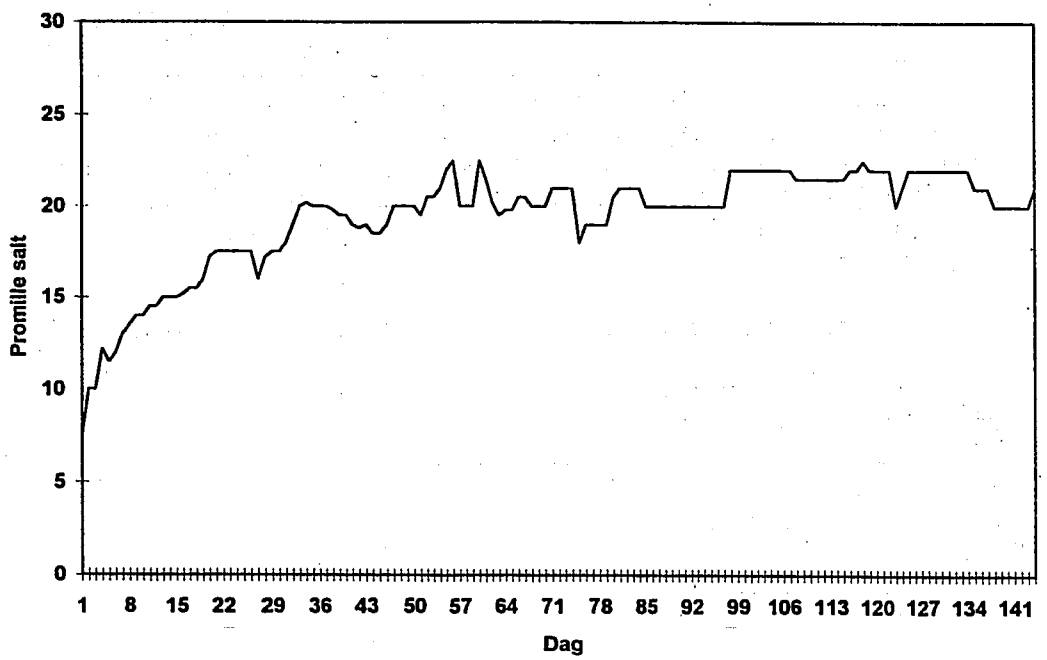
2.2 Fysiske parametre i moderfiskeanlægget

Salinitet

Saliniteten i anlægget, fig. 1, blev i perioden fra 1. marts til midten af april hævet fra 7-8%o til ca. 20%o. Da der blandt de gydte æg til tider blev observeret svamp, blev saliniteten i juni måned hævet til ca. 22%o for at se, om dette kunne afhjælpe problemet. Der kunne dog ikke observeres nogen forskel. Saliniteten blev målt med en model YSI 33.

Opsaltning foregik ved gradvist at tilsætte syntetisk salt (LW - Marinemix) til hvirvelseperatoren.

Fig. 1.



De store udsving på fig. 1 skyldes, at der periodevis blev tilsat større mængder 14%o Østersøvand oppumpet fra ca. 70 meters dybde og ilandbragt af "Jens Væver". Herefter gik der en periode med at få justeret saliniteten.

Ammoniak

Ammoniakindholdet i moderfiskeanlægget igennem forsøgsperioden fremgår af fig. 2.

Målingerne viser koncentrationen af totalammonium og er foretaget med et Merck Ammonium Test Kit. Målingerne blev foretaget på vandet fra hvirvelseperatoren, dvs. afløbsvandet fra moderfiskene. Testmetoden er ikke en videnskabelig testmetode, men bruges ofte i akvakultur øjemed som en god indikationsmetode. Giftigheden af totalammonium er afhængig af, hvor stor en andel ikke-ioniseret ammonium, der forefindes i vandet. Koncentrationen er bestemt af temperaturen og primært pH. Jo højere temperatur og pH desto større koncentration af ikke-ioniseret ammonium:

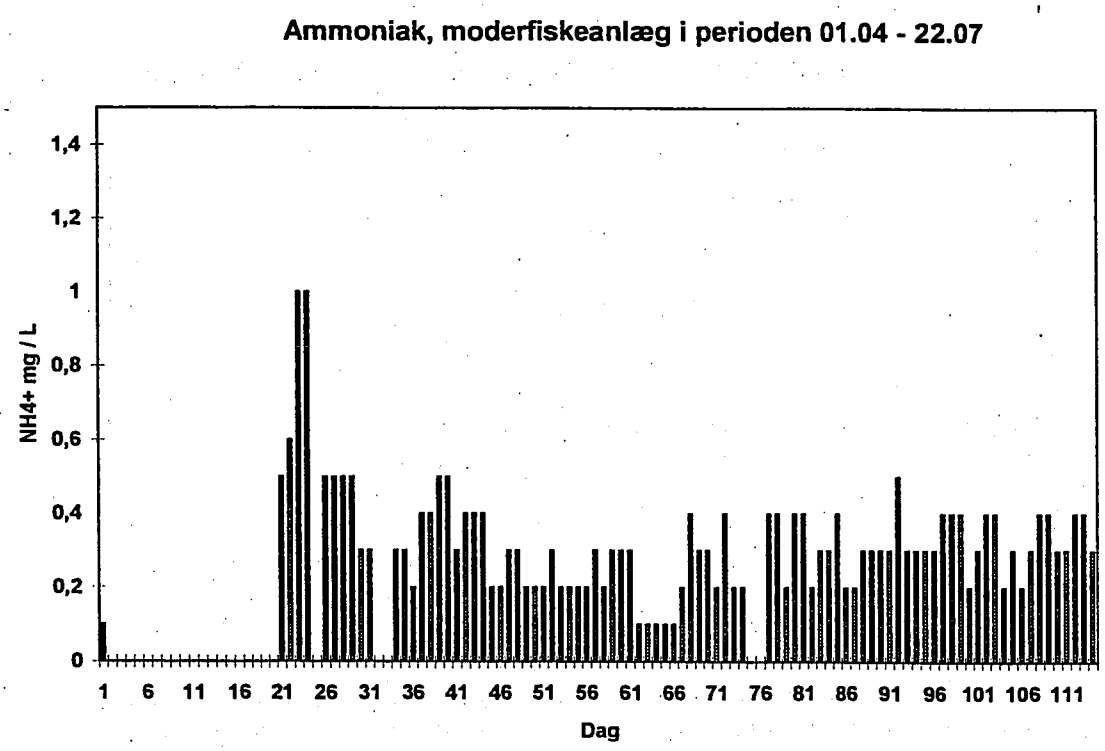
Fisk - $NH_3 + H_2O = NH_4OH = NH_4^+ + OH^-$

IKKE-ioniseret - giftig

ioniseret - ikke giftig

ph afhængig

Fig. 2.



Ved 8°C og en pH på 7,8 er indholdet af NH3 0,994% (Piper, R.G. et al., 1982), dvs. at 1 mg/l totalammonium svarer til en koncentration af NH3 på 0,00994 ppm. Ved en NH3 koncentration på 0,0125 ppm sker der en vækstreduktion hos ørreder samt skader på gælleepithel, nyrer og lever (Piper, R.G. et al., 1982). Derimod fandt (Alderson, R., 1979), at søtunge ved 16°C, pH 7,9, 33‰ saltvand og en koncentration på 0,045 mg NH3/l over en periode på 42 dage ikke viste tegn på nedsat vækst. Pighvarre udviste ikke nedsat vækst under samme forhold ved en koncentration på 0,14 mg NH3/l over en periode på 11 dage.

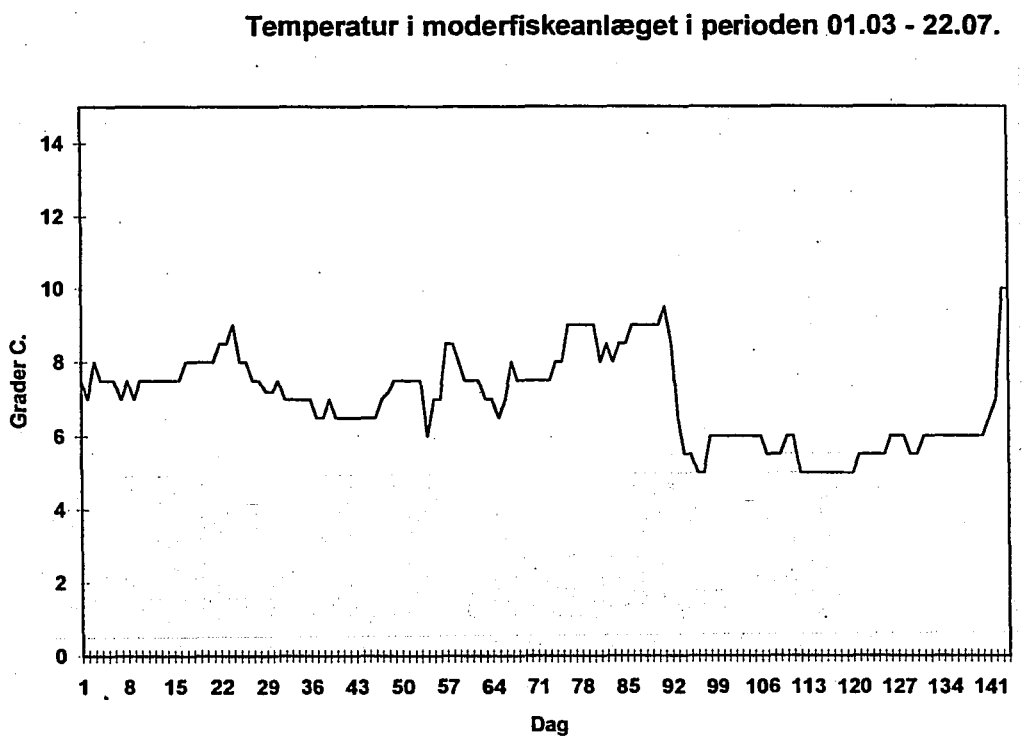
Af fig. 2 fremgår det, at koncentrationen af totalammonium toppede 22-23. april med en koncentration på 1 mg/l svarende til et indhold på 0,00994 ppm NH3, hvilket er tæt på kritisk niveau for ørred. Resten af perioden lå koncentrationen på mellem 0,2-0,4 ppm svarende til 0,001988-0,0040241 ppm NH3.

Tolerancegrænserne for Østersø torsk og æg er ukendte, men koncentrationen resten af sæsonen lå væsentligt under den for ørreder beskrevne kritiske grænse.

Temperatur

Temperaturforløbet igennem gydesæsonen er vist i fig. 3 , temperaturen blev målt med et YSI Model 57 oxymeter.

Fig. 3.



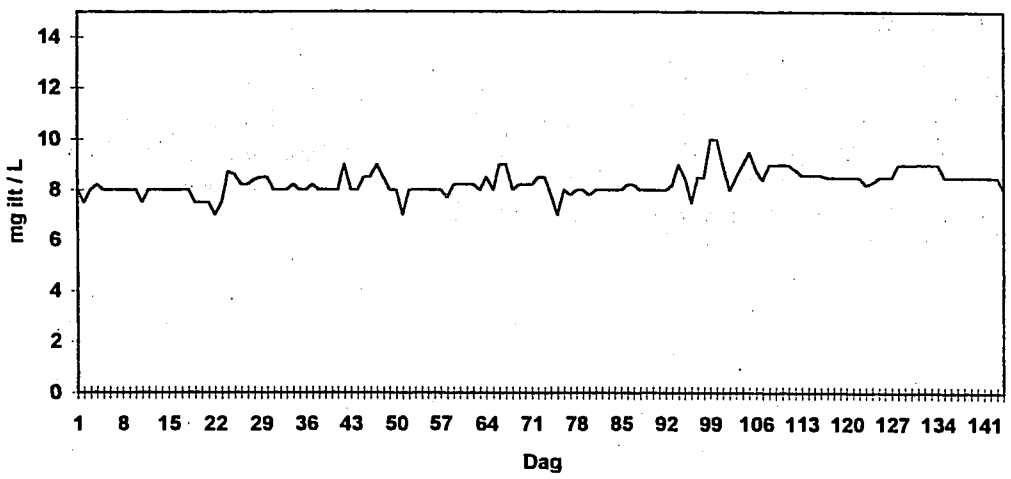
Det fremgår af figuren, at temperaturen har svinget mellem 7-9°C frem til begyndelsen af juni måned, hvorefter den blev sænket og holdt sig stabil mellem 5-6°C indtil gydesæsonens afslutning. Grunden til den højere temperatur i månederne marts til juni skyldes, at kølerummet, som anlægget var placeret i, ikke var i drift i denne periode, men blev nedkølet sekundært ved, at en dør stod åben ind til et kølerum i drift ved siden af. Kølingen har dermed ikke været effektiv og konstant. I løbet af juni blev der startet op for kølingen af selve rummet, og temperaturen sænket til 5-6°C, hvilket er det naturlige temperaturforhold på gydepladserne i Østersøen (Bagge, O., pers. comm.).

Ilt

Iltforholdene i anlægget igennem forsøgsperioden fremgår af fig. 4. og ligger mellem 7-10 mg/l med et gennemsnit på ca. 8 mg/l og må derfor siges at være tilfredsstillende, ilten blev målt med et YSI Model 57 oxymeter.

Ørredens normalbehov for ilt er 8 mg/l med en minimumsgrænse på 5 mg/l (Christensen, N.O., 1980). Laksefisk er blandt de fiskearter som har det højeste behov for ilt.

Fig. 4. **Iltindhold i vandet i perioden 01.03 - 22.07, moderfisk gruppe 2.**



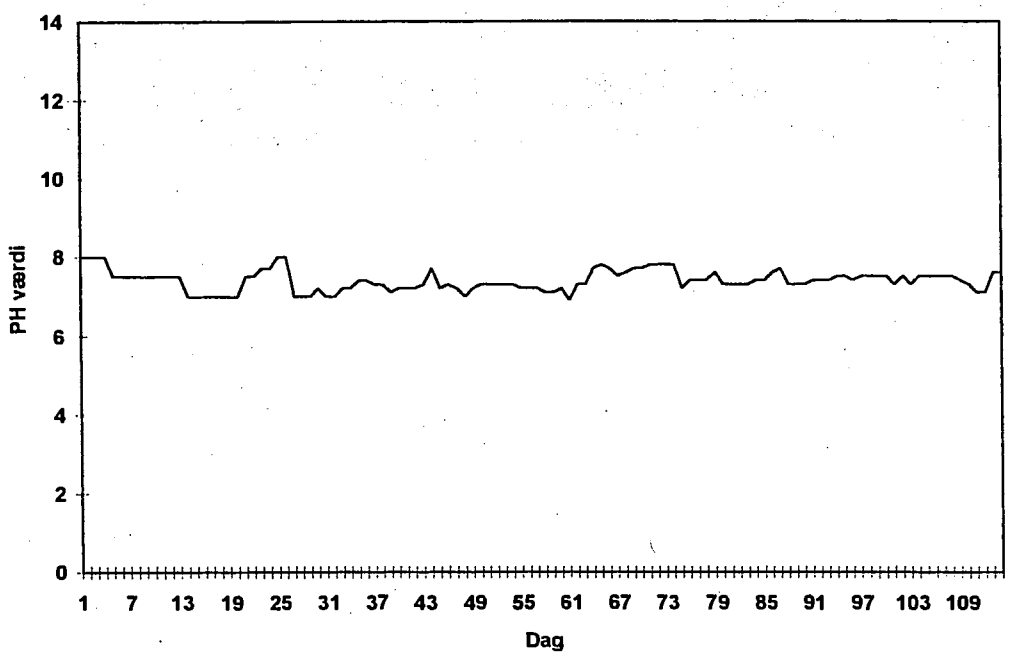
pH

pH-forholdene gennem forsøgsperioden, fig. 5, har ligget mellem 7,8-8,0, hvilket er lidt lavere end pH i startfodringsanlægget og pH i de øvre vandlag i Østersøen, som i forsøgsperioden blev målt til 8,2. Havvand på 33%o har en pH på 7,9 (Alderson, R., 1979).

Forskellen mellem pH-værdierne i moderfiskeanlægget og startfodringsanlægget kan skyldes forskelle forårsaget af målemetoderne. pH i moderfiskeanlægget blev målt med pH-indikator strips og i startfodringsanlægget med et pH-meter, Knick Portmass 751.

Fig. 5.

PH forhold, moderfiskeanlæg i perioden 01.04 - 22.07

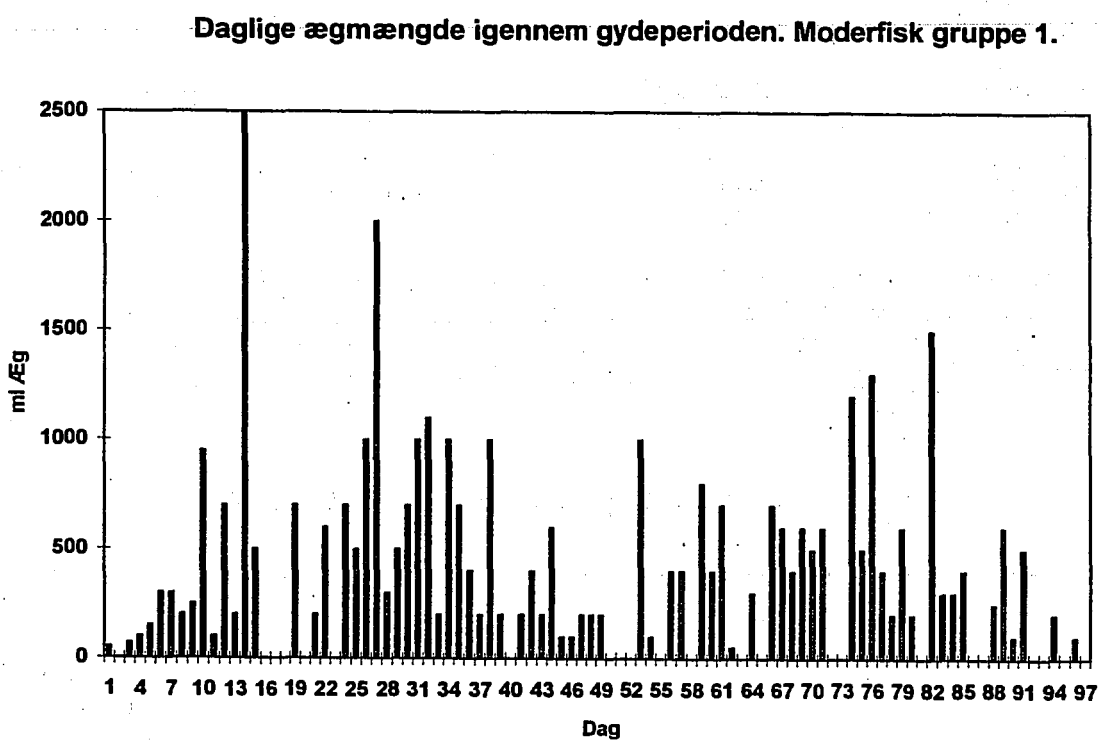


2.3 Moderfiskenes gydebiologi

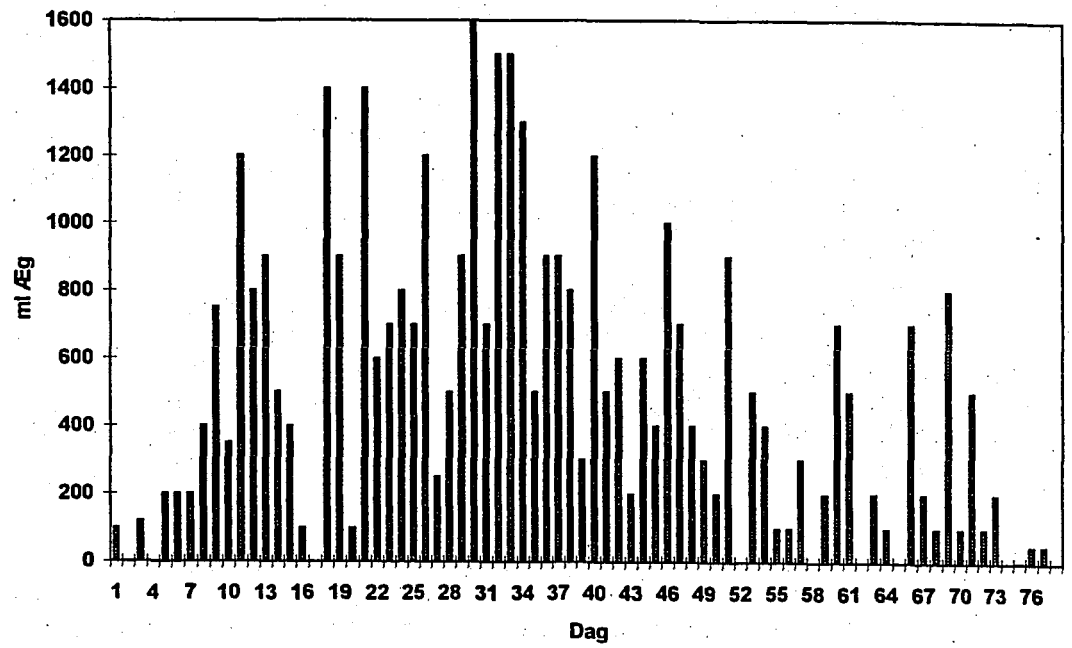
Produceret ægmængde

Ægopsamlerne for hver af de 3 grupper blev tømt en gang dagligt og den daglige, producerede mængde æg for hver gruppe blev opmålt i ml. Dagslængden i anlægget blev reguleret af en timer og svarede til den for årstiden naturlige dagslængde. Mængderne af æg, som blev produceret af de 3 moderfiskegrupper, er vist i fig. 6.

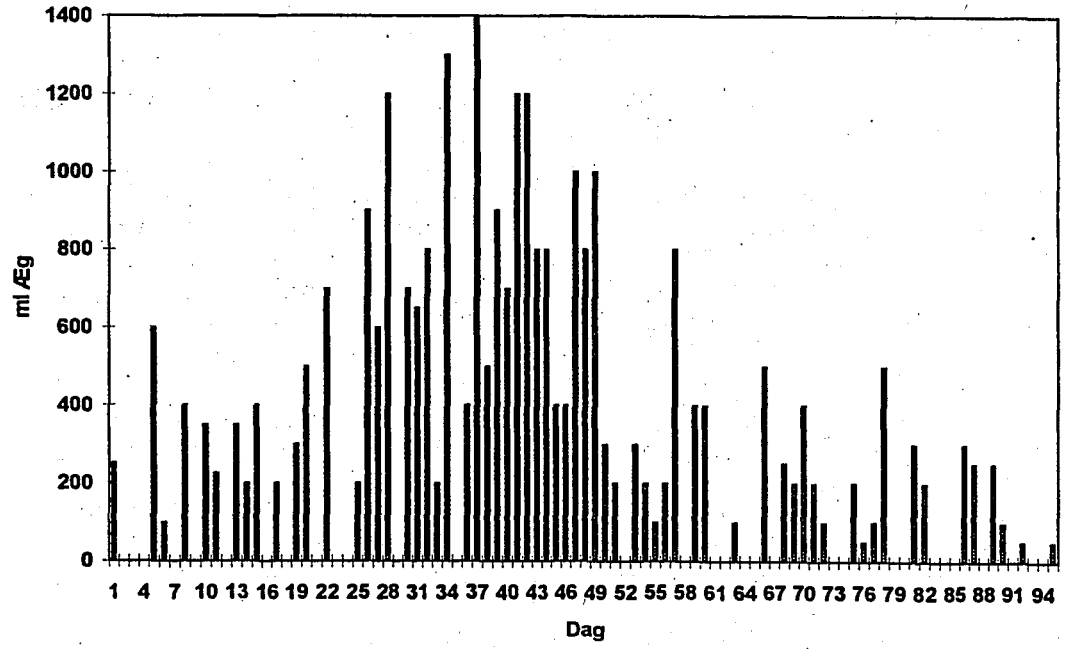
Fig. 6.



Daglige ægmængde igennem gydeperioden. Moderfisk gruppe 2.



Daglige ægmængde igennem gydeperioden. Moderfisk gruppe 3.



Gruppe 1 gød ialt 37.870 l æg gennem sæsonen, som varede 97 dage, hvilket svarer til ialt 9.008.800 æg for hele gruppen eller 1.035 l æg = 248.400 æg pr. kg hun med en gennemsnitsvægt på 2,180 kg. Gruppen bestod af ialt 11 hunner og 7 hanner ved gydeperiodens afslutning. 1 hun døde i løbet af perioden.

Gruppe 2 gød ialt 38.720 l æg gennem sæsonen, som varede 76 dage, ialt 9.292.800 æg eller 0,990 l æg = 237.600 æg pr. kg hun med en gennemsnitsvægt på 2,600 kg. Gruppen bestod af ialt 15 hunner og 13 hanner ved gydeperiodens afslutning. I løbet af gydeperioden døde 1 hun.

Gruppe 3 producerede ialt 30.625 l æg igennem perioden, som varede 94 dage, ialt 7.350.000 æg eller 0,640 l = 153.600 æg pr. kg hun med en gennemsnitsvægt på 2,500 kg. Gruppen bestod af ialt 19 hunner og 5 hanner ved gydeperiodens afslutning. I løbet af gydeperioden døde 1 hun.

For gruppe 1 var de 2 toppunkter for gydningen et i første halvdel og et i anden halvdel, henholdsvis slutningen af april og slutningen af juni; det første lidt større end det andet. For de 2 andre gruppers vedkommende havde de kun et toppunkt, som lå i første halvdel af perioden, medio maj. Gruppe 2 havde endvidere en gydeperiode som var ca. 20 dage kortere end de andres.

(Müller, A.; Bagge, O., 1984) beskriver, at den naturlige gydebestand i Bornholms Dybet gyder fra marts til og med juni med toppunkt i maj måned. Forfatterne refererer til (Kändler, 1938), som beskriver to toppunkter, et i juni og et i september måned. Muligheden for tilstedeværelsen af to torskeracer diskuteres; en vestlig, som gyder tidligt og en østlig, som gyder senere på året. Om der er tale om en opblanding af to forskellige racer, en østlig og en vestlig i gruppe 1 og en primært vestlig i gruppe 2 og 3, her specielt gruppe 2 med den korteste gydesæson, vides ikke.

(Botros van Gurgis, 1962) beregnede ved at veje ugydt rogn fra vestlige Østersø torsk, at en hun på 2,700 kg indeholdt 790.000 æg pr. kg kropsvægt, mens en Nordsø torsk af samme størrelse indeholdt 728.000 æg/kg kropsvægt. Ægmængden fra en Nordsø torsk på 2,800 kg, som gød i fangenskab blev beregnet til 964.285 æg/kg eller 370 l/kg kropsvægt; gydeperioden forløb over 50 dage; fisken gød ialt 17 portioner (Kjesbu, O.S., 1989). I begge undersøgelser fandt forfatterne desuden en positiv korrelation mellem moderfiskestørrelse og ægmængde.

En gydebestand af Nordsøtorsk bestående af 50 hunner og 50 hanner vil gyde over en periode på 75 dage, mens en enkelt hun på 6 kg vil gyde over 50-60 dage (McVey, J.P., 1991). Gydesæsonens længde for moderfiskene på Bornholm var i 1993 lidt længere for gruppe 1 og 3 end, hvad man normalt vil forvente af en gruppe moderfisk bestående af Nordsø torsk.

Derimod var der ingen positiv korrelation mellem moderfiskenes størrelse og den producerede ægmængde. Gruppe 1 med den mindste gennemsnitsvægt (2,180 kg) producerede den største mængde æg i forhold til kropsvægten.

Befrugtningsprocent

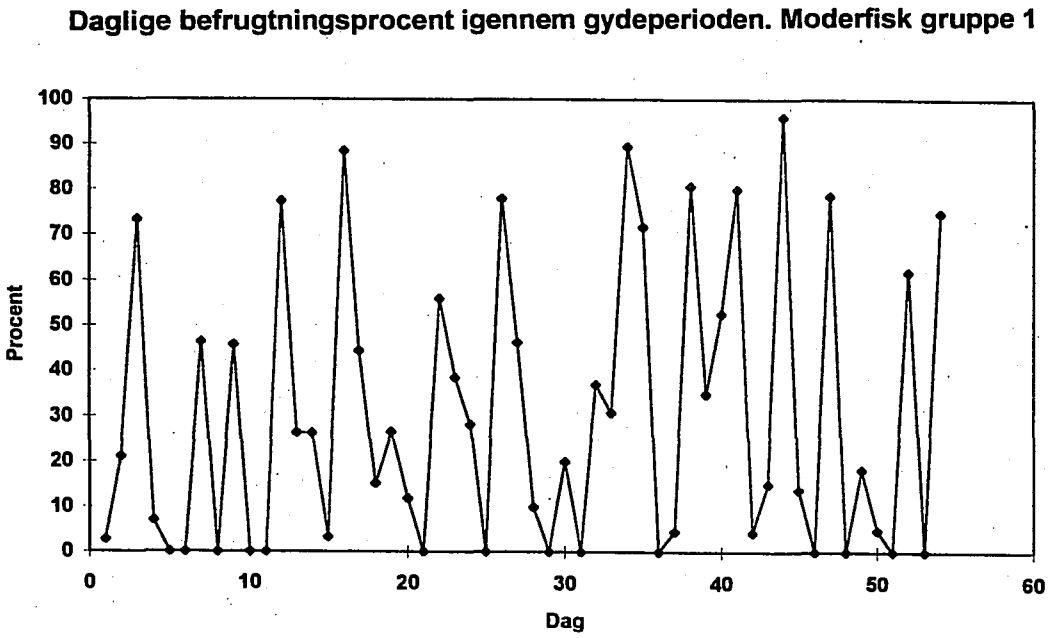
Den daglige befrugtningsprocent for hver af

moderfiskegrupperne blev bestemt gennem en periode på 2 måneder indtil gydesæsonens afslutning. Af de indsamlede æg blev der udtaget 5 ml eller ca. 1.200 æg fra hver af de 3 batches.

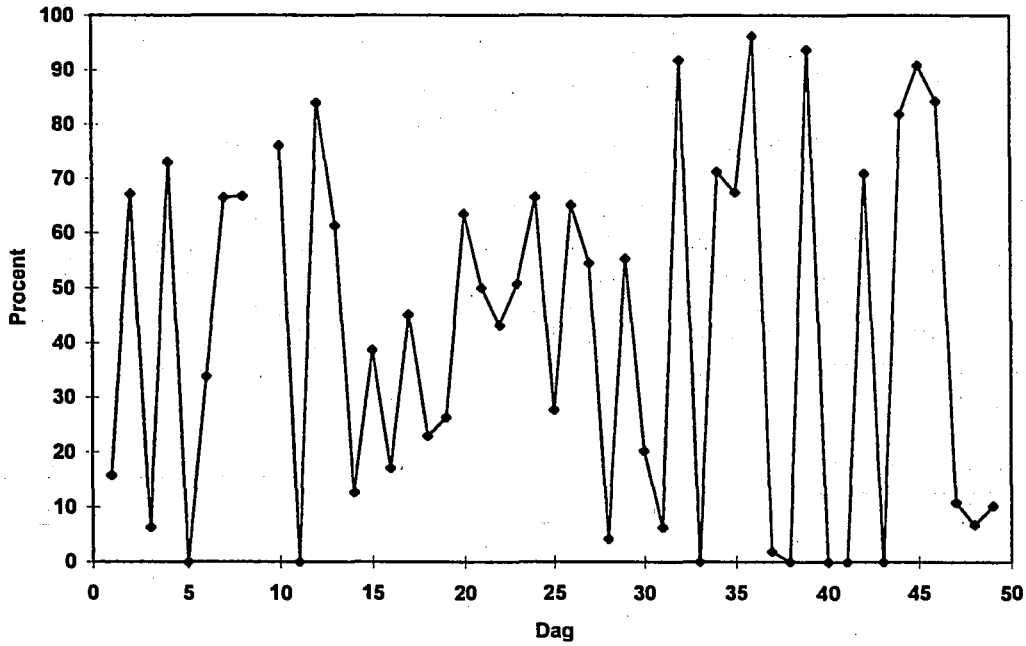
En delprøve på 200-300 æg blev herefter udtaget og undersøgt under stereomikroskop. Kun befrugtede æg med korrekt celledeling blev registreret som gode æg. Æggene blev forinden fikseret i en blanding af iseddikesyre og 8%o destilleret vand. Blandingen bevirker, at celleoverfladen bliver mørkebrun, hvilket gør det væsentligt nemmere at skelne befrugtede og ubefrugtede æg fra hinanden. Det er samtidig nemmere at se, om de befrugtede æg har korrekt celledeling. Vurderingen blev foretaget efter Eyjolfur Friogeirssons beskrivelse af torskeæggets udvikling (Friogeirsson, E., 1978). Æggene, som blev undersøgt, var fra tocellestadiet frem til morulastadiet.

Fig. 7 viser den daglige befrugtningsprocent for æggene fra de 3 moderfiskegrupper.

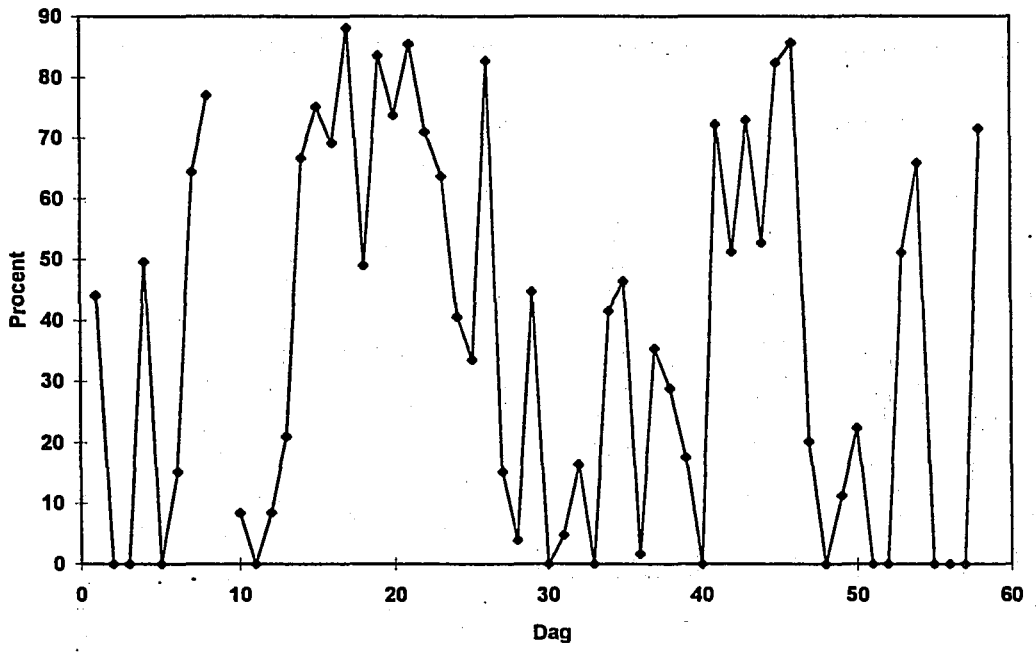
Fig. 7.



Daglige befrugtningsprocent igennem gydeperioden. Moderfisk gruppe 2



Daglige befrugtningsprocent igennem gydeperioden. Moderfisk gruppe 3



Som det ses af figuren varierer befrugtningsprocenten fra dag til dag og svinger lige fra ca. 90% til 0%. Den gennemsnitlige

befrugtningsprocent for de 3 grupper er henholdsvis for gruppe 1 30%, for gruppe 2 44% og for gruppe 3 40%.

Befrugtningsprocenten afhænger i forsøget ikke af antallet af hanner. Gruppe 3 med færrest hanner (5) og hunner (19) havde den næstbedste befrugtningsprocent (40%).

Det er muligt, at den ret høje og fluktuerende temperatur 6-10°C i den første 2/3 af forsøgsperioden har haft en negativ effekt på resultatet. Stress kan også forårsage dårlig befrugtning og ujævn gyderytme, hvilket kan medføre, at æggene i højere grad holdes tilbage i ovariet og derfor bliver overmodne, hvilket nedsætter befrugtningsevnen (Kjesbu, O.S., 1989); (Holm, J.C., 1991).

2.4 Startfodringsforsøg 1993

På grund af de dårlige erfaringer i 1992 med brugen af klækkeriet til klækning af torskeæg blev selve startfodringsanlægget, udover at blive brugt til startfodring, også brugt til at klække æg i. Anlægget består af følgende elementer og har en volumen på ialt 18 m3:

Sandfilter 1,5 m3
Biofilterenhed; 1 rislefilter 0,6 m2, 2 neddykkede filtre á 1,2 m3
Køleenhed + køleresservoir (pumpesump)
UV-sterilisation
Beluftertårn
10 koniske startfodringstanke á 600 l
Hvirvelseperator

Fordelen ved at inkubere æggene i systemet er, at en stor mængde æg kan inkuberes ad gangen. Op til 500 ml eller 120.000 æg pr. kar. Samtidig er der minimal turbulens i karrene. Vandet tilsættes langs karvæggen nær bunden og cirkler til afløbet, som er centralt placeret ved vandoverfladen. Æggene roteres hermed på en meget skånsom måde rundt i karrene uden at blive udsat for turbulens - selv ved et højt vandflow, hvorved iltforholdene kan holdes optimale. Igennem alle klækkeforsøgene lå iltmætningsgraden på mellem 80-95%. Ulempen ved klækkemetoden er, at der ikke effektivt kan opsamles døde æg, da karrene er ca. 1 m dybe. Det er derfor ikke muligt at foretage et præcist estimat af dødelighedsforløbet.

På grund af karrenes store volumen er det heller ikke muligt at foretage et estimat af antallet af nyklækkede larver, da de ikke fordeler sig homogent. At foretage en homogen opblanding af larverne i et volumen på 600 l vil kræve, at de udsættes for en volsom turbulens. Torskelarver er meget følsomme overfor håndtering (Thomsen, B.M., 1981).

Strategien for startfodringsforsøgene var at tilbyde torskelarverne en bred vifte af fødeemner for på den måde at opnå størst mulighed for at opfylde deres krav til ernæring; et krav som ikke kendes for Østersø torskelarver. I

forbindelse med startfodringsforsøgene blev algerne Rhodomonas sp., Isochrysis galbana, Paulova lutheri og Skeletonema costatum produceret og brugt som startfoder fra dag 1. Flere forfattere har påvist, at torskelarver optager phytoplankton umiddelbart efter klækning (Homme, J.M., 1991);(Ellertsen, B. et al., 1976);(Meeren, T. van Der, 1991);(Eilertsen, H.C., 1992);(Tilseth, S. et al., 1987);(Pedersen, T et al., 1989);(Tilseth, S. 1990).

Umættede fedtsyrer er essentielle for overlevelse og vækst af marine fiskelarver, specielt vigtige er de to umættede fedtsyrer 20:5 (n-3) og 22: (n-3) (Watanabe, T. et al., 1983), og disse udgør op til 42,0-50,5% af indholdet af polære lipider hos torskelarver (Pedersen, T. et al., 1989).

Marint phytoplankton har et højt indhold af umættede fedtsyrer og er derfor velegnet som startfoder til marine fiskelarver enten som direkte føde eller sekundært ved berigning af andre fødeorganismer, som f.eks. hjuldyr og Artemia, hvorved disse får et højere indhold af de essentielle umættede fedtsyrer (Watanabe, T. et al., 1983);(Lubzens, E., 1987).

Nordsø torskelarver er med succes blevet startfodret ved brug af alger samt algeberigede hjuldyr og Artemia (Huse, J. et al., 1983). (Howell, B.R., 1984) opnåede med brug af denne teknik en overlevelse på 10% frem til efter metamoforsen, hvorefter dødeligheden var ubetydelig.

Opdrætsforsøg med pighvarre, rødspætter og skrubber har dog vist, at brugen af laboratoriefremstillede byttedyr ofte medfører, at en vis procentdel af fiskene bliver fejlpigmenterede. Ved supplement af en vis del naturligt zooplankton kan dette problem udgås i opdræt af skrubber (Seikai, T., 1985).

Udover alger blev der til startfodring brugt hjuldyr, Acartia tonsa og Artemia.

Produktionen af alger foregik i et klimarum ved 16°C i 30 l store plastikposer. Algerne blev belyst af et lyspanel bestående af 40 lysstofrør. Vandet til fortynding af kulturerne var sterilfiltreret opsaltet 20-22%o havvand fra startfordringsanlægget. Vandet blev akklimatiseret i klimarummet inden opspædning. Berigning af algerne foregik direkte i algeposerne.

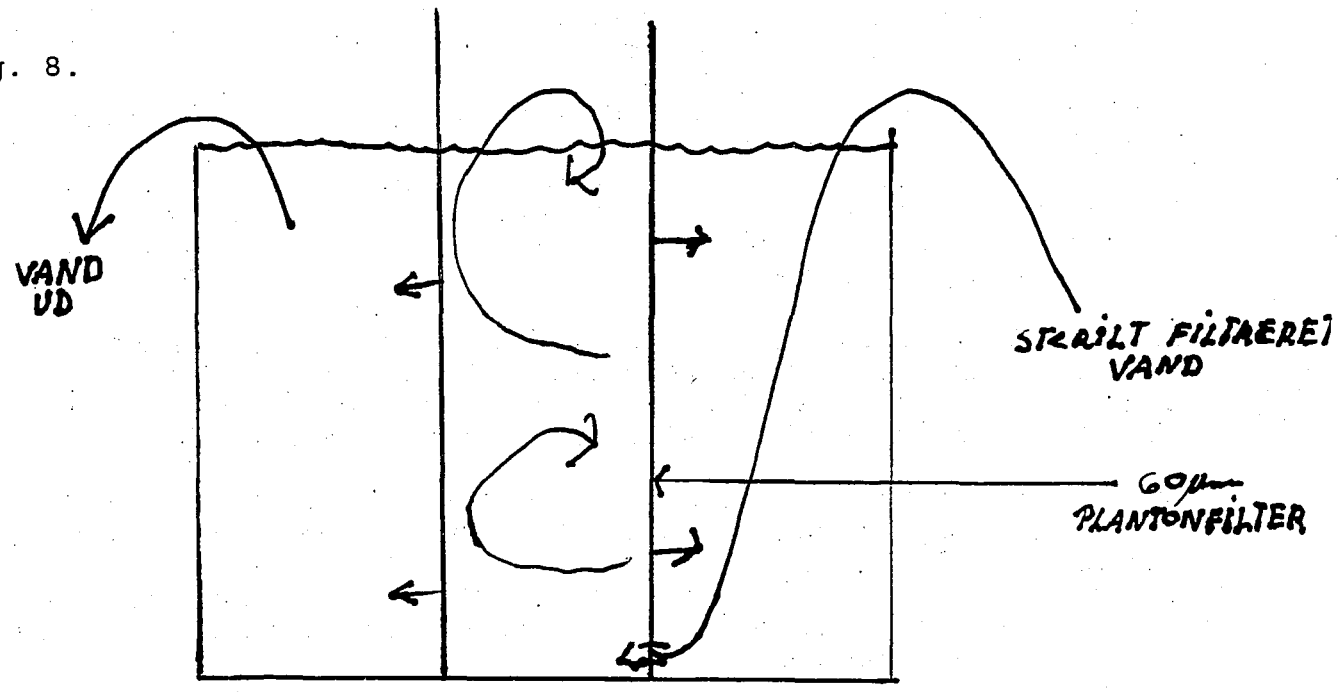
Produktionen af hjuldyr foregik i 6 koniske kar af 300 l ved 25°C i 22%o sterilfiltreret opsaltet havvand. Hjuldyrene, som blev produceret, tilhørte S-typen, den mindste af de to hovedtyper med en voksenlængde på 100-200 um. Den anden type har en voksenlængde på 130-340 um. Den mindste af de to typer er på grund af sin mindre størrelse mest velegnet som startfoder til små marine fiskelarver (Fukusho, K., 1989). Berigningen foregik ved tilsætning af algerne Rhodomonas og Isocrysis 50 l/dag/kar plus et kommercielt berigningsmedie, Culture Selco, efter firmaets foreskrifter. Berigning af

hjuldyrene blev foretaget hver 6. time døgnet rundt.

Inden udfodring af hjuldyrene blev de filtreret over et standard 60 um filter. Det viste sig dog hurtigt, at metoden var uegnet til filtrering af store mængder dyr, idet filtret klokkede til på grund af store mængder opslemmede algepartikler i vandet fra produktionstankene.

Der blev derfor konstrueret et cylindrisk modtryksfilter på 30 l, se fig. 8.

Fig. 8.



Fordelen ved filtret er, at det har en meget stor filteroverflade samt, at den kontinuerlige tilsætning af det sterilfiltrede vand skaber en rotation af filtratet, hvorved sekundært materiale effektivt frafiltreres. Vandet i balgen, som filtret er nedsænket i, skaber et modtryk, der bevirker en skånsom filtrering af hjuldyrene.

Efter filtrering blev hjuldyrene tappet på 5 l dunke og henlagt i hvirvelsperatorerne til temperaturakklimatisering inden udfodring.

Artemia æg blev klækket i 4 stk. koniske kar á 300 l ved 25°C i 35%o opsaltet sterilfiltreret havvand. Inden klækningen af æggene, som under disse forhold tog 24 timer, blev de decapsuleret, dvs. den yderste del af de to skaller blev fjernet. Proceduren består i:

1. Hydrering af cysterne.
2. Behandling i en hypokloritopløsning.
3. Afvaskning og deaktivering af klorrester.
4. Klækning.

Teknikken er detaljeret beskrevet i (McVey, J.P., 1983).

Fordelen ved decapsulering er, at i den efterfølgende klækkeproces øges klækkeprocenten, da det bliver nemmere for nauplierne at bryde ud af skallen. Samtidig virker behandlingen med hypoklorit som en desinfektion af cysterne (Campton, D.E., 1989).

Efter klækning blev nauplierne filtreret over et 125 um filter efter samme princip som beskrevet i fig. 8. Filtret var dog et kommercielt fremstillet standardfilter.

Akklimatiseringen af nyklækkede nauplier foregik i koniske beholdere på 1 l nedsænket i hvirvelseperatoren og gennemkoblet med luft. På denne måde kunne de opbevares i op til 24 timer med en koncentration på 15.000 nauplier/ml. Forsøg har vist, at de kan opbevares på denne måde ved 0-4°C i 48 timer uden nævneværdig energetisk tab (McVey, J.P., 1983).

På senere stadier i startfodringsfasen blev de nyklækkede nauplier beriget i enten 24 eller 48 timer afhængig af, hvor store de skulle være. Berigningen foregik med Super Celco (kommercielt produkt) og algerne Isocrysis og Rhodomonas.

Acartia tonsa æg blev produceret på HØI i Charlottenlund, konserveret og sendt til Bornholm, hvor æggene blev klækket i koniske beholdere på 1 l gennemkoblet med luft. Klækningen foregik i 16%o opsaltet havvand ved 20°C. Nauplierne blev akklimatiseret på samme måde som Artemia nauplierne og desuden fodret med Rhodomonas.

I startfodringsanlægget blev der ialt i perioden 18. april - 30. juni inkuberet 4.045.000 befrugtede æg med korrekt celledeling på aflæsningstidspunktet. Der blev i perioden observeret en meget variabel klækkesucces. I visse ægbatch var der op imod 100% dødelighed, i andre var dødeligheden meget lille, således kunne der kun observeres få døde æg på bunden. Svenske undersøgelser med Østersø torsk viser også, at kvaliteten er meget variabel (Pickova, J. et al., 1992). Udover de variable klækkeprocenter var det karakteristisk, at jo dårlige klækkeresultatet var, desto mere inficeret med svamp var batchene. I visse ægbatch med høj klækkeprocent blev der overhovedet ikke observeret svamp. Det menes, at svampen opstod som noget sekundært, når æggene var af dårlig kvalitet. I forsøg på at forhindre angreb af svamp blev nogle få ægbatch dypbadet i oxytetracyclin, men der kunne ikke spores nogen effekt.

Forsøg året før havde indikeret, at ved højere salinitet kunne svamp undgås (Buchmann, K. et al., 1992). Derfor blev æggene inkuberet i 20-24%o opsaltet havvand. Bassinvandet i anlægget

var 14%o Østersø vand oppumpet fra ca. 70 m dybde. Opsaltningen foregik med Instant Ocean (syntetisk salt) samme salt, som blev brugt til opsaltning af vandet til foderdyrsproduktionen. Saliniteten i anlægget matchede den i moderfiskeanlægget bortset fra mindre udsving.

Den valgte salinitet havde tilsyneladende ingen indvirkning på udbrud af svamp, men undersøgelser har vist, at spermatosomobiliteten hos Østersø torsk er optimal ved 20-26%o (Westin, L. et al., 1991). Ud fra den betragtning syntes strategien at være fornuftig.

Udfodring af byttedyr foregik som udgangspunkt efter en fodertabel for startfodring af havbars larver efter princippet

Larvealder (dage)	Antal hjuldyr/ l/dag	Antal Artemia/ l/dag
1-3 dage	1000	
4-6 dage	2000	
7-9 dage	3000	
10-11 dage	4000	
12-13 dage	7000	400 nyklækkede
14-17 dage	9000	1250-1750 nyklækkede
18-20 dage	4000	2000-3000 nyklækkede
21-24 dage		2500 nykl. + 1500 berig.
24-30 dage		7000-9000 berigede

Derudover blev algerne Isocrysis, Skeletonema og Paulova tilsat op til 5 l/dag. I hjuldyrsfasen blev der desuden tilsat op til 1000 stk Acartia/l/dag. Algerne blev forsøgt tilsat fra dag 1 og 3 til 14 dage frem. Der blev i visse forsøg tilsat mindre mængder foder end tabellen foreskriver; i andre forsøg mere. Temperaturen blev holdt fra 5,5-6,5°C i visse forsøg; i andre blev temperaturen i løbet af startfodringsfasen hævet fra 5,5°C til 8,5°C i løbet af en periode på 8 dage startende efter blommesæksabsorbtionsfasen. Forsøgene blev afsluttet efter maksimum 2 uger pånær de sidste batch. Efter 2 uger var der i alle tilfælde ingen eller kun få larver tilbage.

I sidste forsøgsserie som startede den 30. juni og varede frem til 10. august blev der produceret 192 stk. torsk, 30-40 mm lange ud af 7 batch. Torskene var velpigmenterede og uden synlige skeletdeformiteter. Disse gennemgik et temperaturforløb som følger:

30. juni - 7. juli:	6,5°C
8. juli - 17. juli:	7,5°C
19. juli - 3. aug.:	8,5°C
4. aug. - 10. aug.:	10,0°C

Saliniteten blev i samme periode sænket fra 22%o til 13%o.

I perioden 27. maj til 19. juni svingede indholdet af totalammonium fra 0,2-0,5 mg/l. Efter den 19. juni - 10. august svingede den mellem 0,1-0,3 mg/l. pH lå igennem hele

forsøgsperioden på 8,3-8,4.

Der kan ikke gives nogen forklaring på hverken de meget variable klækkeresultater eller på den ringe larveoverlevelse. Karakteristisk for startfodringsforsøgene var, at larvedødeligheden startede allerede fra dag 1 og efter 10-12 dage var der i alle forsøgende kun meget få larver tilbage. Normalt er første kritiske stadie omkring first feeding og næste omkring matamorphosen (Thorisson, K., 1992). I forsøgene var det ikke noget problem at få larverne til at optage føde. Efter 4. dagen kunne de første larver med føde i maven iagttages. Men da de fleste døde inden 10-12 dage, nåede de aldrig frem til metamorphosen. Blandt de larver, som blev ældre end 12 dage, kunne et besynderligt adfærdsmønster hyppigt iagttages. Larverne forsøgte at svømme op til overfladen og spandt samtidig omkring deres egen akse; disse forsøg blev foretaget under voldsomme energiudbrud. Efter en sådan aktiv fase blev larverne fuldstændigt passive og dalede ned gennem vandsøglen for derefter igen at forsøge at nå overfladen under kraftig energiudfoldelse. Mønstret gentog sig ofte flere gange og endte altid med, at larverne til sidst lå passivt hen på karbunden. Der er ingen tvivl om, at sådanne larver var ved at dø. Det kunne også iagttages, at laverne var meget mørkt farvet og havde ingen eller kun ganske lidt føde i tarmen. Årsagen til dette adfærdsmønster, som af medarbejderne på anlægget blev benævnt "dødspiruetten", kendes ikke.

3. Opdræt 1994

Formålet i 1994 var at kombinere de to opdrætsmetoder; semiintensiv- og intensivmetoden ved at udnytte filterteknikken fra det førstnævnte til at filtrere naturligt zooplankton fra havet og bruge disse til startfodring af torskelarverne under kontrollerede forhold i intensivanlægget for på den måde at udelukke fejlernæring som årsag til larvedødelighederne. Naturligt zooplankton, som larverne lever af på opvækstpladserne, bør være det mest næringsrigtige foder. Om artssammensætningen af det kystnære zooplankton samt indholdet af essentielle stoffer matcher planktonet i opvækstområderne vides ikke.

Der blev foretaget en kvantitativ og kvalitativ monitering af æggene fra de to grupper moderfisk; gruppe A med en gennemsnitsvægt på 2,4 kg og gruppe B med en gennemsnitsvægt på 3,3 kg, altså 2 forskellige årgange.

For at klarlægge, om der er en størrelsesforskel mellem æggene i forhold til gydetidspunkt samt evt. størrelsesforskelle mellem æg gydt af forskellig størrelse hunner, blev der foretaget tørstofbestemmelse og diametermåling af æggene igennem gydeperioden.

For at teste, om der skulle være en korrelation mellem klækkeprocent og indhold af visse miljøgifte blev der udtaget

prøver af de enkelte ægbatch, som indgik i forsøgene til senere analyse. Der blev testet for 9 PCB-congener samt DDD, DDE og DDT.

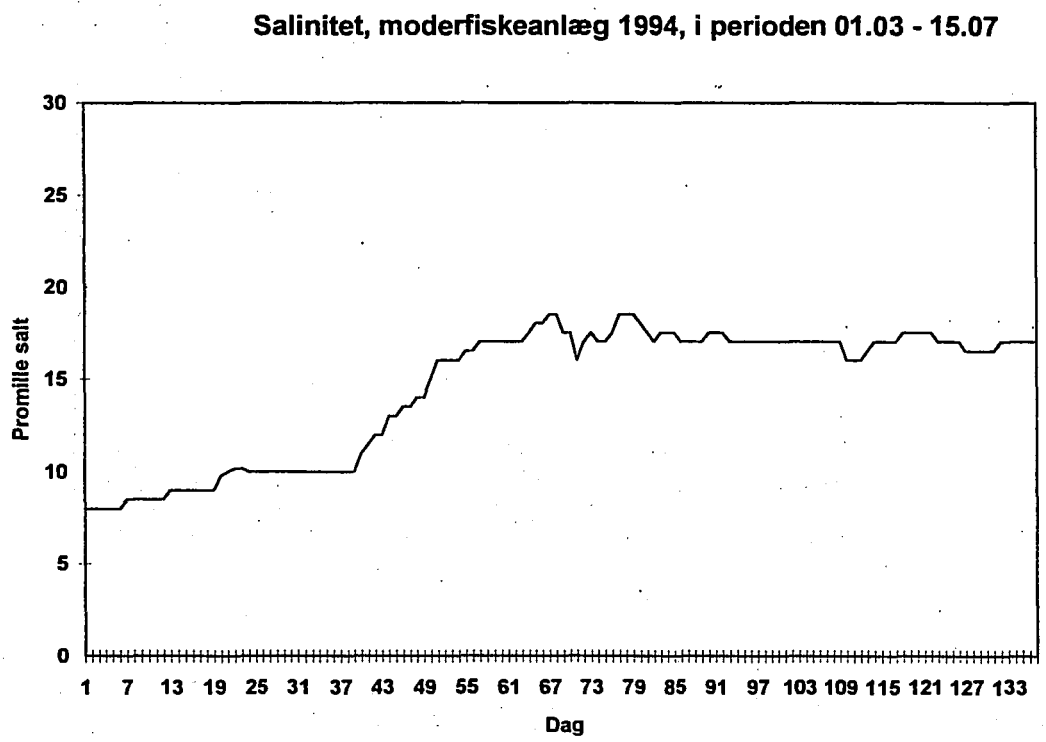
3.1 Moderfiskehold

Moderfiskeanlægget blev i 1994 forbedret ved at biofilterenheden blev forøget med 3 m3. Fiskene til forsøgene blev ligesom i 1993 fanget ud for Snogebæk i februar måned. Fansten foregik denne gang med langliner på 10-20 m dybde, torskene blev ilandbragt i tanke med vand og overført til bassinerne i anlægget, som var fyldt med 7-8%o havvand fra Østersøen.

3.2 Fysiske parametre i moderfiskeanlægget

Salinitet

Fig. 9.



Saliniteten i anlægget blev over en periode på 2 måneder hævet fra 7-8%o til 16-17%o. Forsøgene året før havde vist, at svamp ikke kunne undgås ved en saltholdighed på 22%o samt, at problemet var meget variabelt. Derfor blev det besluttet at arbejde med en for Østersø torskene naturlig salinitet. Østersø torskeæg har en variabel flydeevne og flyder inden for 12,3-16,9%o, afhængig dels af hunnen, som producerer æggene,

dels af batch nummeret (Nissling, A., 1991).

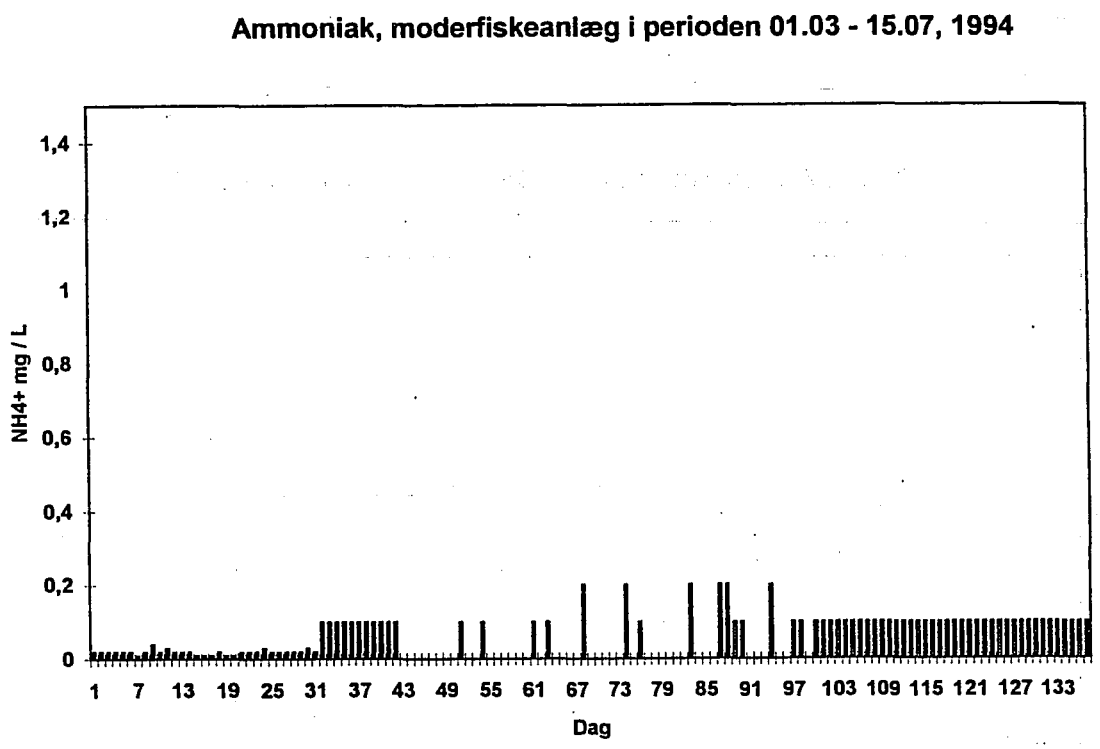
Opsaltningen foregik ved tilsætning af Instant Ocean (syntetisk salt).

Saliniteten er temmelig konstant igennem forsøgsperioden. De mindre udsving skyldes udskiftningen af en del af vandet i anlægget. Spædevandet, som blev tilsat, var 14‰ Østersø vand oppumpet fra ca. 70 m dybde.

Ammoniak

Ammoniakindholdet i moderfiskeanlægget er vist i fig. 10 og blev målt ved brug af samme metode som året før.

Fig. 10.

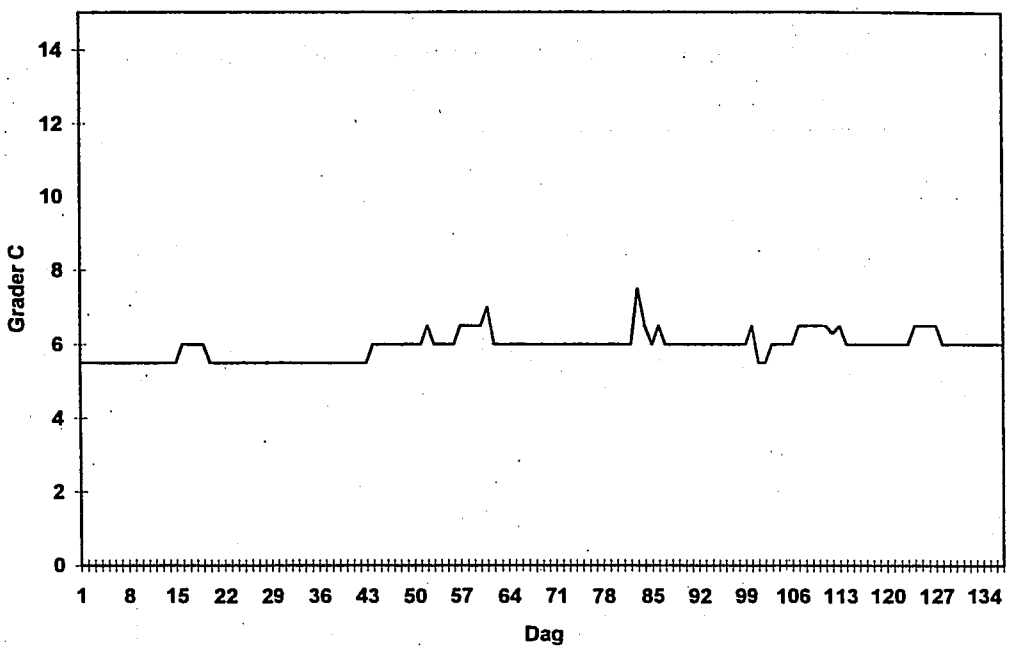


Af figuren ses det, at koncentrationen er væsentlig lavere end året før, fra 0-0,2 mg/l med et gennemsnit på under 0,1 mg/l. Årsagen skyldes dels den forøgede biofilterkapacitet samt, at filtrene fra forsøgets start blev podet med Nitrosomonas og Nitrobacter bakterier specialiseret til at danne ammoniak og nitrit til nitrat.

Temperatur

Fig. 11 viser temperaturforløbet igennem sæsonen. Den har således ligget på mellem 5,5-6,5°C med en enkelt top på 7°C i nogle få dage. Temperaturforløbet var således væsentligt mere stabilt og lå på et mere optimalt niveau end året før. Det skyldes, at kølingen var slået til igennem hele perioden.

Fig. 11. **Temperatur, moderfiskeanlæg i perioden 01.03 - 15.07, 1994**

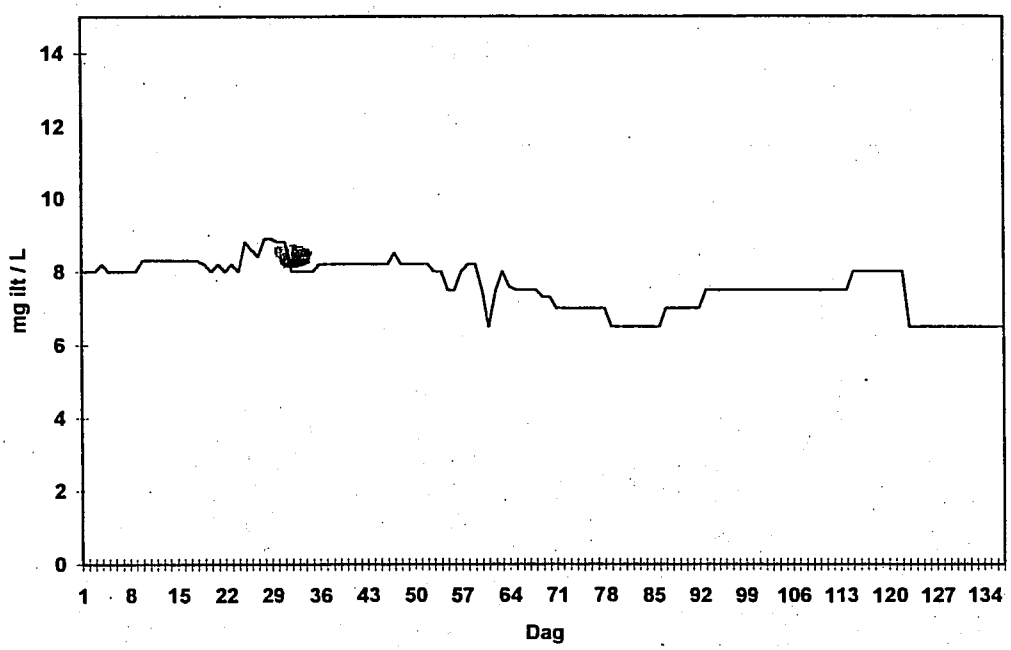


Ilt

Iltforholdene i anlægget igennem forsøgsperioden fremgår af fig. 12 og ligger mellem 7-9 mg/l og har dermed været tilfredsstillende.

Fig. 12.

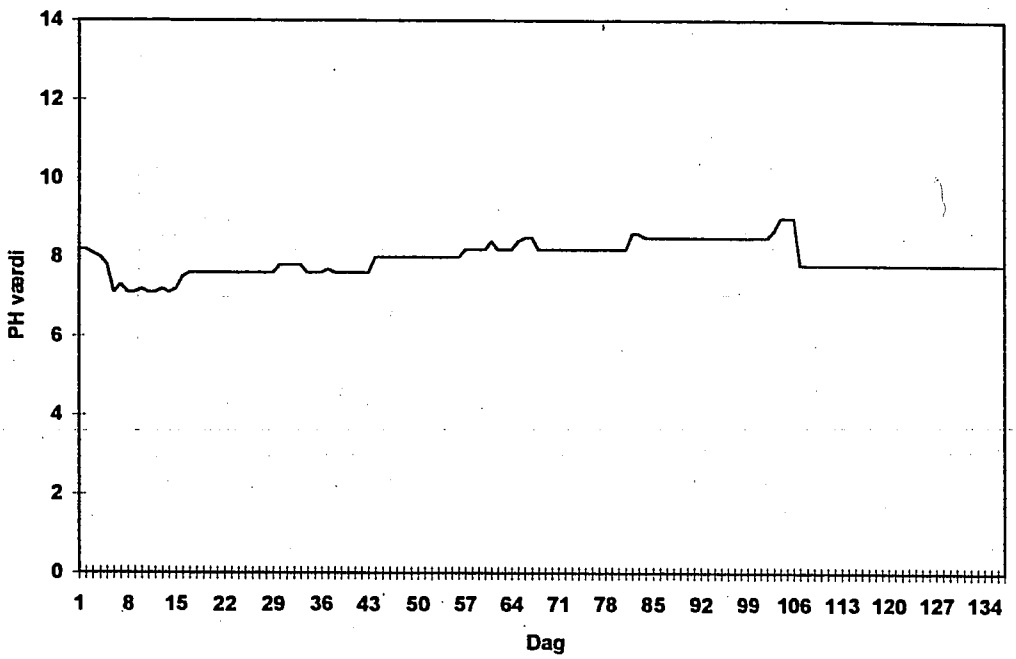
Iltindhold i vandet i perioden 01.03 - 15.07, moderfisk gruppe B, 1994



pH

Ph forholdene gennem forsøgsperioden, fig. 13 lå som året før på omkring 8.

Fig. 13. **PH forhold, moderfiskeanlæg i perioden 01.03 - 15.07, 1994**



3.3 Moderfiskenes gydebiologi

Produceret ægmængde

To grupper moderfisk indgik i forsøgene; gruppe A med en gennemsnitsvægt på 2,4 kg og gruppe B med en gennemsnitsvægt på 3,3 kg. Mængden af æg som blev produceret af de to grupper er vist i fig. 14.

Daglige ægmængde igennem gydeperioden. Moderfisk gruppe A. (14 hunner, 11 hanner)

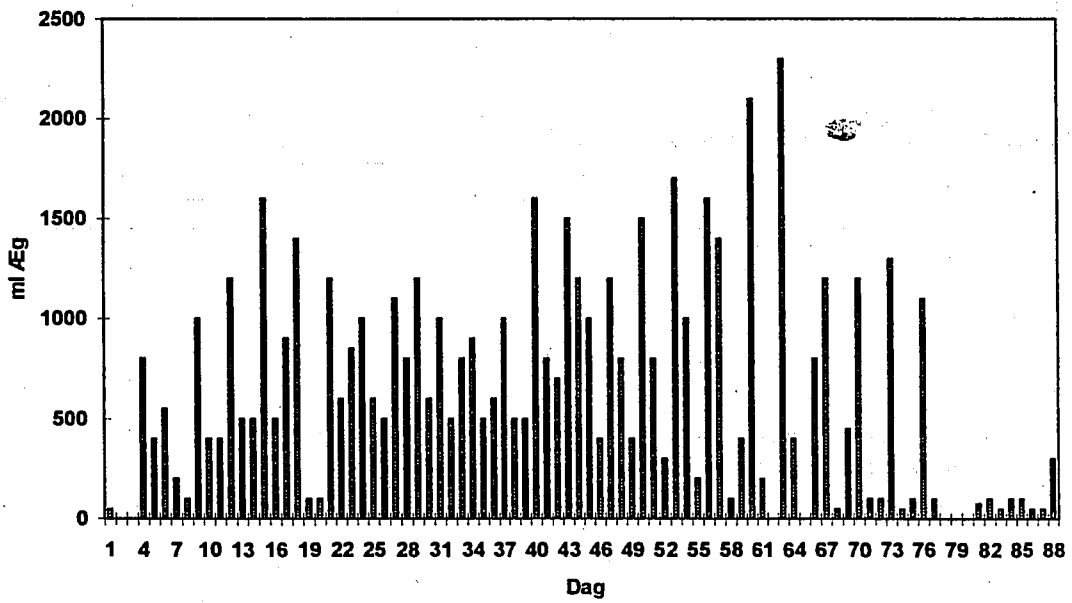
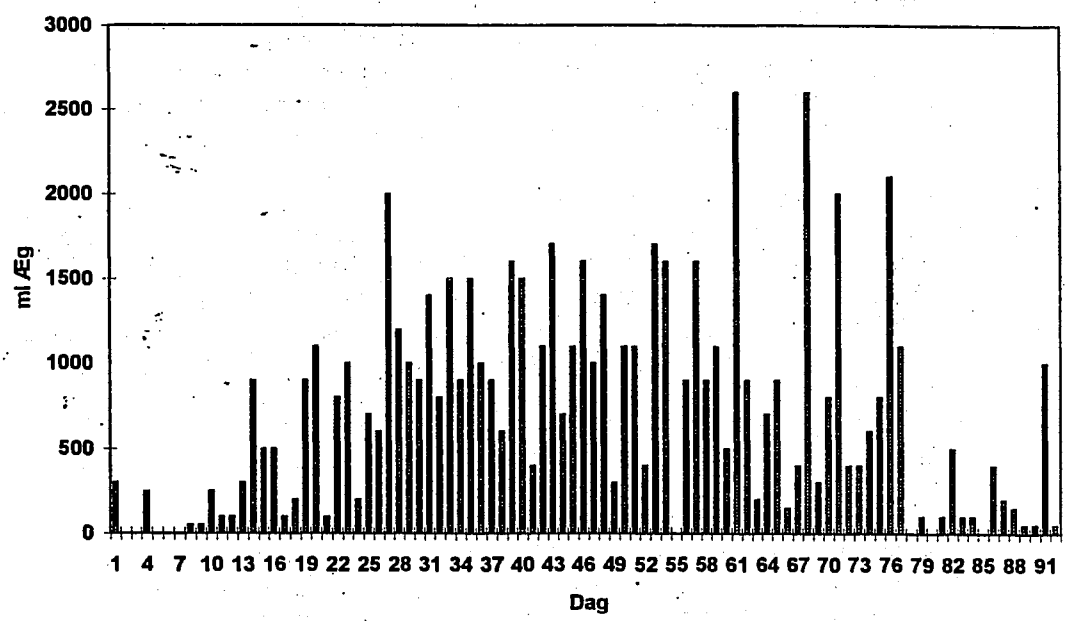


Fig. 14.

Daglige ægmængde igennem gydeperioden. Moderfisk gruppe B. (8 hunner, 8 hanner)



Gruppe A gød ialt 56.425 l æg gennem sæsonen, som varede ca. 90 dage. Dette svarer til ialt 13.542.000 æg eller 1,881 l æg = 451.400 æg pr. kg hun. Gruppen bestod af ialt 14 hunner og 11 hanner ved gydeperiodens afslutning. Der døde ingen hunner i løbet af gydesæsonen.

Gruppe B gød ialt 65,751 l æg gennem sæsonen, som varede ca. 90 dage. Dette svarer til ialt 15.780.000 æg eller 2,481 l æg = 595.440 æg pr. kg hun. Gruppen bestod af ialt 8 hunner og 8 hanner ved gydeperiodens afslutning. En hun døde den 6. juli, dvs. mængden af gydte æg pr. kg hun er i realiteten lidt mindre.

For begge grupper var der et svagt toppunkt for gydningen i første halvdel af gydeperioden. (Kjesbu, O.S., 1989) fandt, at toppunktet for en enkelt Nordsø torskehun lå i sidste halvdel af gydesæsonen.

I 1994-sæsonen var der en positiv korrelation mellem moderfiskenes størrelse og mængden af gydte æg ligesom tidligere beskrevet for både Nordsø og Østersø torsk. Mængden af gydte æg pr. kg. hun var væsentlig større i 1994 end i 1993.

Mængden af gydte æg er ikke et helt korrekt estimat af, hvad en Østersø torsk på henholdsvis 2,4 og 3,3 kg kan præstere men et gennemsnitstal. Der var således en størrelsesvariation blandt moderfiskene; gruppe A's mindste fisk var 1,291 kg og største 3,354 kg og for gruppe B var mindste fisk 1,804 kg og største 4,583 kg.

Befrugtningsprocent

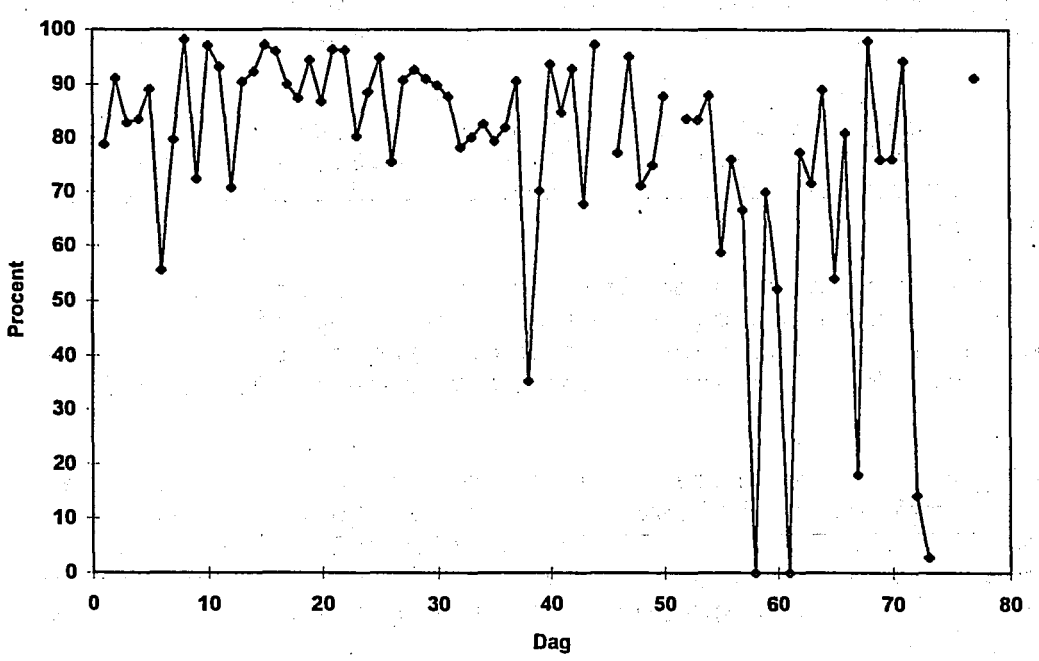
Den daglige befrugtningsprocent blev bestemt fra gydesæsonens start 1. maj til 15. juli. Samme metode som året før blev brugt. Det ses af fig. 15, at trods en variation fra dag til dag, er variationen langt mindre end året før. Størst variation er der i gruppe A i slutningen af sæsonen; i gruppe B i begyndelsen og slutningen af sæsonen. Den gennemsnitlige befrugtningsprocent for hele sæsonen var for gruppe A 79% og for gruppe B 72%, altså væsentlig højere end året før.

Forskellen i resultaterne mellem de to år skyldes formodentlig dels håndteringen af fiskene under indfangningen, som første år foregik med garn. Andet år blev fiskene fanget på kroge af medarbejderne på projektet, der gjorde sig stor umage. Når krogene således blev rygtet, blev det gjort meget langsomt for ikke at udsætte fiskene for trykforandringer på for kort tid, dels de fysiske parametre i anlægget, som i 1994 var langt mere stabile og optimale. Specielt temperaturen var temmelig høj i 1993, mens den i 1994 lå tæt på det optimale, 5,5-6,5°C.

Det er værd at bemærke, at gydesæsonen i 1993 startede 15. april, mens den startede 1. maj i 1994, 15 dage senere. Den højere temperatur kan meget vel have fremskyndet sæsonen, således at fiskene har forceret gydningen, hvilket måske har bevirket en ringere ægkvalitet og dermed en ringere befrugtningsprocent.

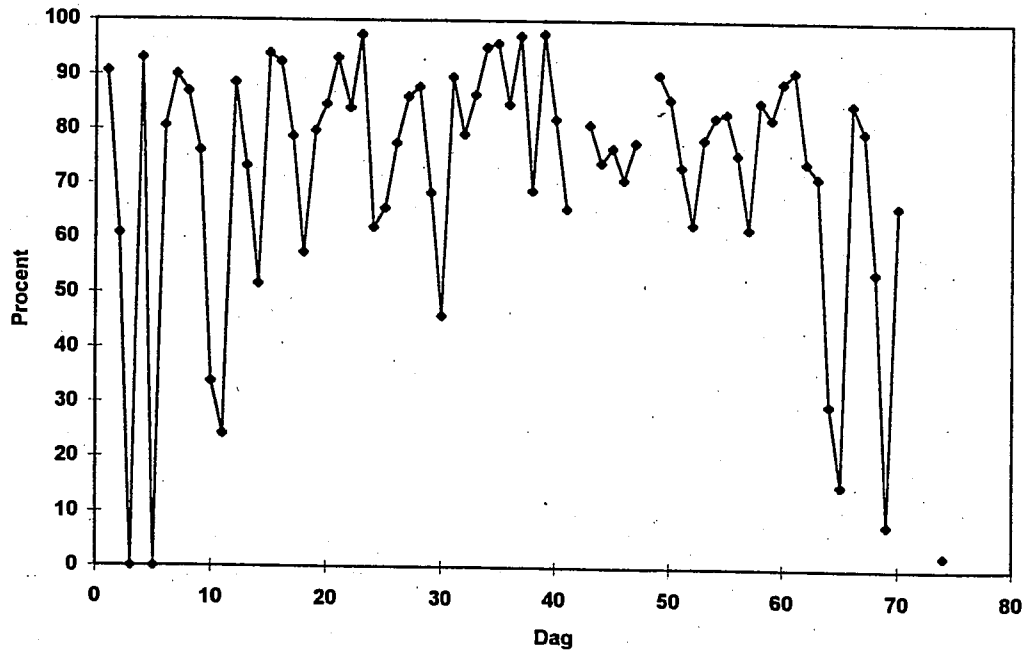
Fig. 15.

Daglige befrugtningsprocent igennem gydeperioden. Moderfisk gruppe A



Daglige befrugtningsprocent igennem gydeperioden. Moderfisk gruppe B

Fig. 15.



Ægdiameter og ægtørstofbestemmelse i relation til sæson og moderfiskestørrelse

Ægdiameteren blev målt igennem et måleokular med 25 stregenheder = 1 mm.

Ægtørstofmålingerne blev foretaget i en Me termostatstyret ovn ved 57,5°C +/- 0,1°C i 24 timer.

Fig 16 viser relationen mellem ægdiameter og gydetidspunkt. Hvert punkt er et gennemsnit af 20 målinger.

Fig. 16.

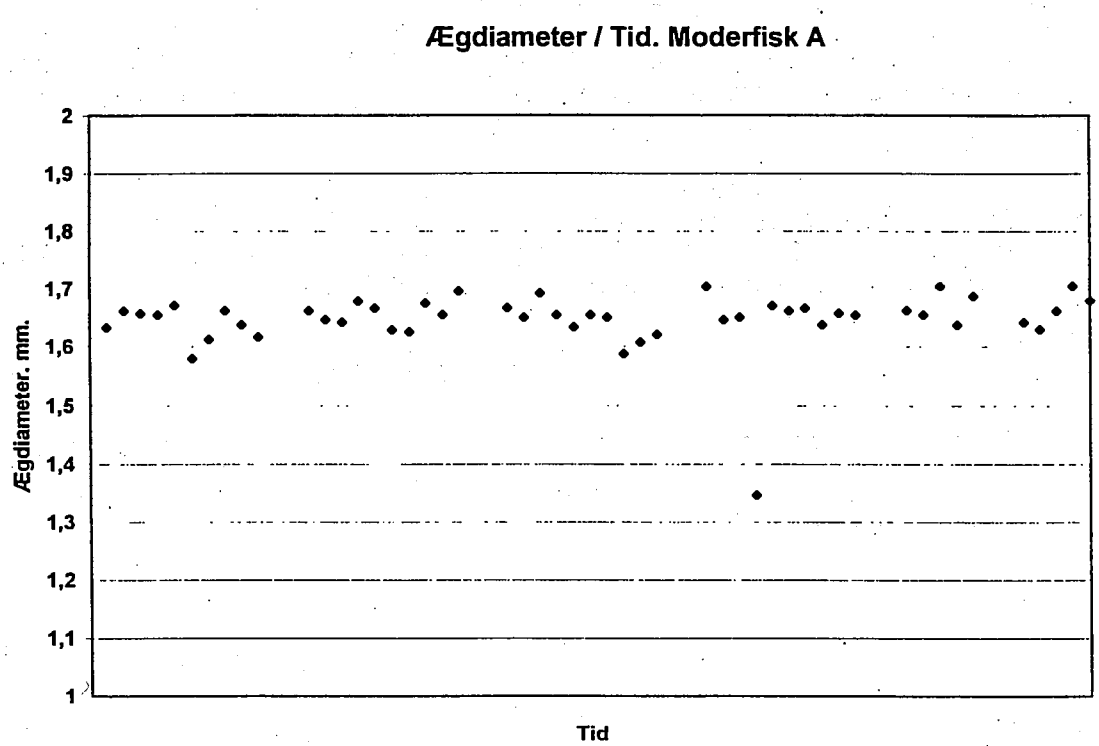
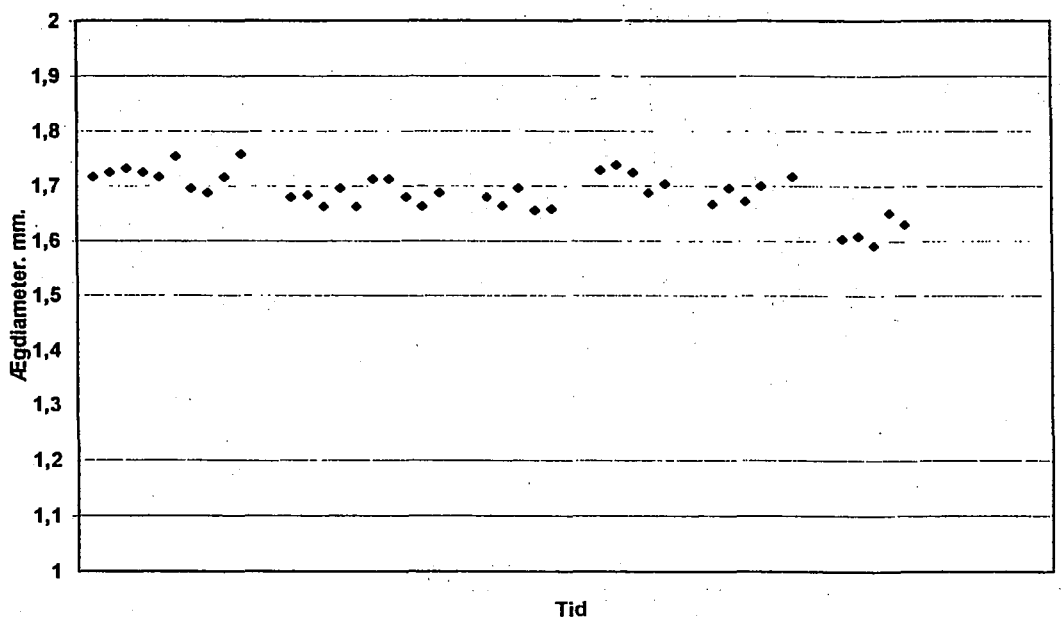


Fig. 16.



Det kan ses af figuren, at ægdiameteren er konstant igennem gydesæsonen samt, at der er en positiv korrelation imellem ægdiameter og moderfiskestørrelse. For gruppe A er gennemsnitsdiameteren 1,65 mm, mens den for gruppe B er 1,72 mm. De individuelle variationer, der kan ses imellem punkterne på figuren kan meget vel skyldes, at de to grupper moderfisk ikke var homogent sammensat med hensyn til størrelsen af hunnerne.

(Kjesbu, O.S., 1989) fandt at, der var en sæsonvariation af ægdiameteren hos Nordsø torsk med et toppunkt i første trediedel af sæsonen med en diameter på ca. 1,38 mm faldende til 1,26 mm sidst på sæsonen. Æggene var gydt af en hun på 4,200 kg. Ikke alene var der en sæsonvariation, men ægdiameteren var væsentlig mindre end for Østersø torsk på trods af fiskens størrelse. (Hislaj, J.R.G. et al, 1987) fandt en størrelsesvariation hos Nordsø torskeæg indsamlet i felten på 1,16-1,60 mm.

Ægtørstofdata for samme æg er præsenteret i fig 17. Der er samme liniære tendens med hensyn til vægt i relation til sæson. Det kan af figuren yderligere ses, at der er en positv sammenhæng imellem ægdiameter og tørstofindhold.

Fig. 17.

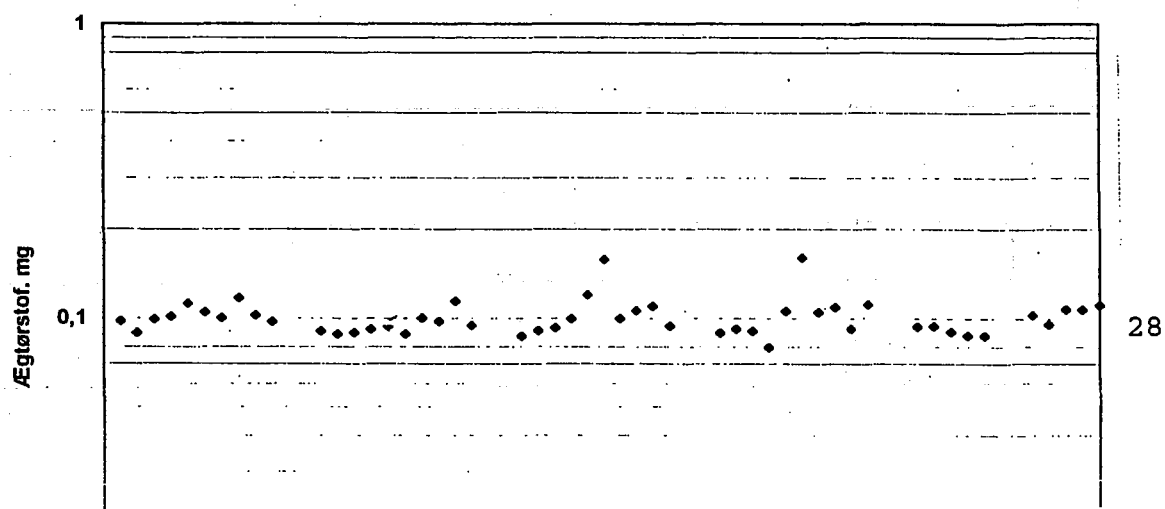
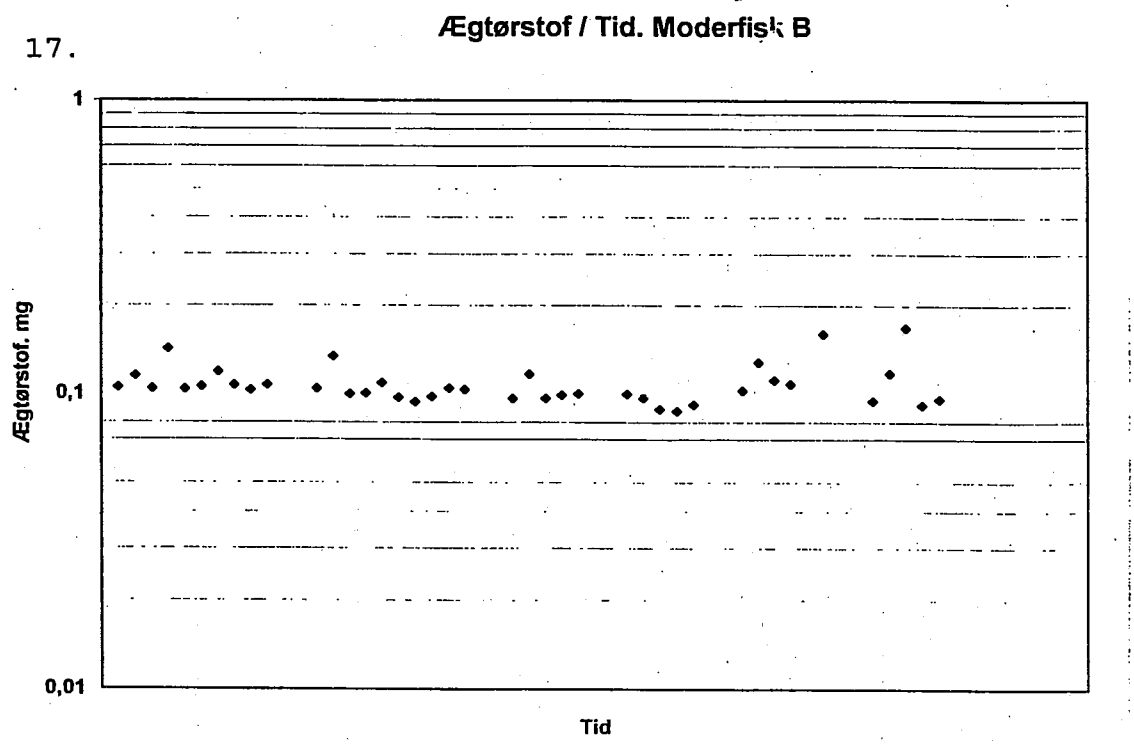


Fig. 17.



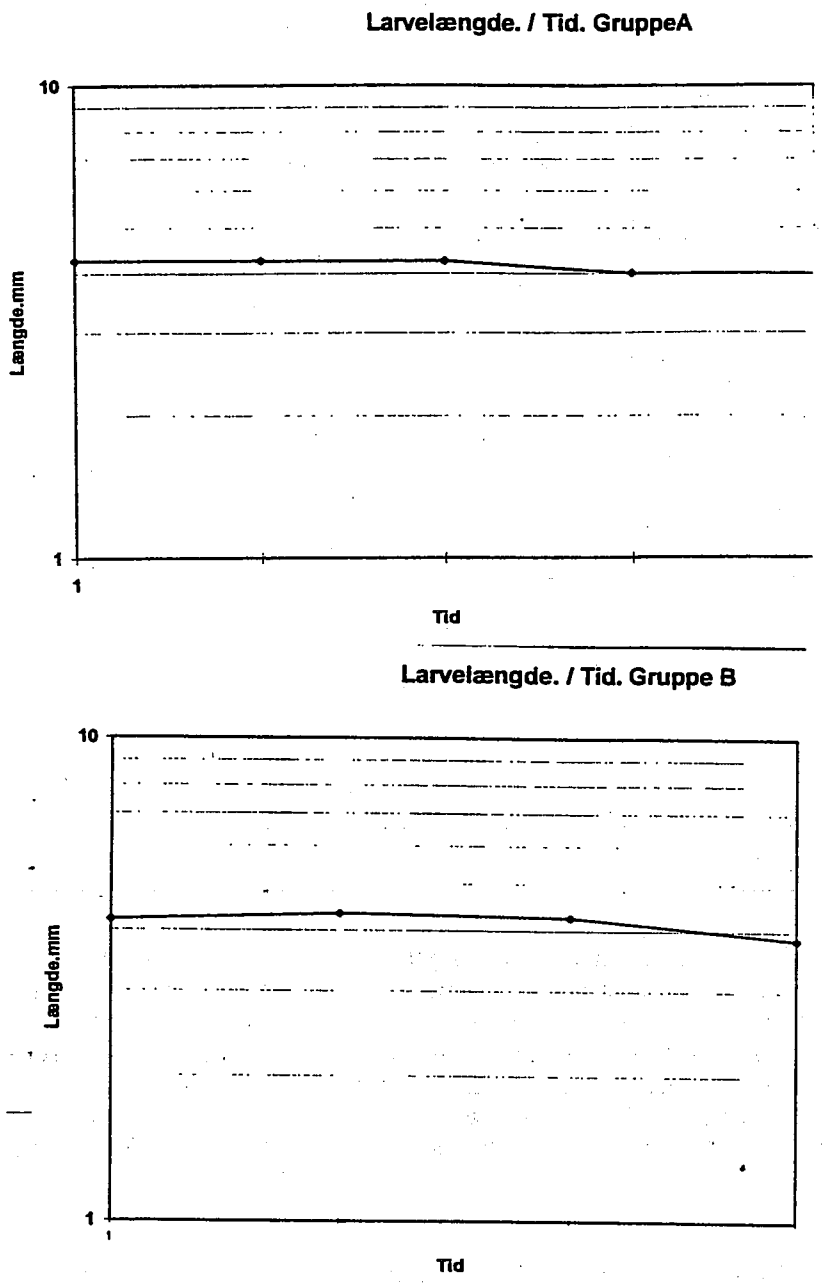
Ægtørstofindholdet i gruppe A ligger omkring 0,100 mg; i gruppe B omkring 0,120 mg. Nordsø torskeæg på 1,4 mm har et tørstofindhold på 0,110 mg (Knutsen, G.M. et al, 1985) og er dermed tungere end Østersø torskeæg i forhold til diameter. Det skyldes Østersø torskens tilpasning til de specielle hydrografiske forhold i Østersøen, som lav salinitet. Stor ægdiameter og en lille massefylde giver en god flydeevne.

Længdebestemmelse af nyklækkede larver i relation til sæson og moderfiskestørrelse

Længdebestemmelserne af de nyklækkede larver blev målt igennem et okular med 25 stregenheder = 1 mm.

Fig. 18 viser sammenhængen imellem længdebestemmelse og sæson. Hvert punkt er et gennemsnit af 50 målinger. Figuren viser en ikke helt liniær sammenhæng. Således er der en faldende tendens sidst på sæsonen. Det kan evt. skyldes en prøveudtagningsfejl. Klækningen af æggene er ikke synkronisk, men strækker sig over op til 2 døgn. Tendensen er iøvrigt, at larverne fra gruppe B er længere, bortset fra sidste måling.

Fig. 18.



3.4 Klækkeforsøg

På baggrund af de i 1993 meget variable klækkeresultater blev der i 1994 foretaget en monitering på dødeligheden under inkubationsfasen.

Klækkeriet fra 1992 blev lavet om. Der blev konstrueret 10 stk. cylindriske beholdere, 30 cm i diameter, 50 cm lange med bund af 500 um planktonnet til opsamling af døde æg. Cylindrene var fæstnet direkte i køleresservoiret og havde hver deres seperate neddykkede vandindtag. Vandet i klækkeriet blev filtreret igennem et kulfilter, UV-behandlet, kølet og sterilfiltreret igennem en serie patronfiltre på henholdsvis 5 um, 2 um og 0,2 um, beluftet over et beluftertårn, inden det returneredes til inkubatorerne. Det totale vandvolumen i klækkeriet, 3,5 m2, blev sterilfiltreret 1 gang pr. time. Vandforbruget til klækkeforsøgene var væsentligt mindre end filterkapaciteten, hvilket bevirkede, at vandet tilsat

inkubatorerne blev sterilfiltreret og beluftet flere gange inden brug. Forholdene burde således være optimale.

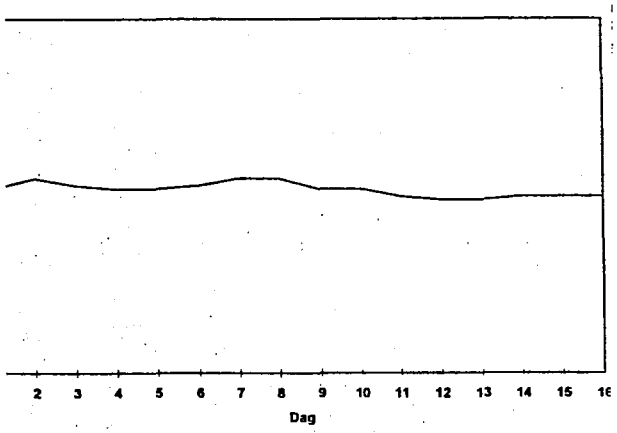
Der blev foretaget 5 klækkeforsøg med æg fra moderfiskegruppe A og 4 klækkeforsøg med æg fra moderfiskegruppe B.

Råvandet som blev brugt til forsøgene var 7-8%o Østersøvand filtreret over et 60 um filter og opsaltet til 17%o ved iblanding af syntestisk salt, Instant Ocean. Efter hver forsøgsrunde blev klækkeriet tømt for vand og desinficeret med ferskvand tilsat NAOH så pH blev hævet til 14. Klækkeprocenterne er et gennemsnit af i forsøg A1, 5 parallelforsøg; A2, 4 parallelforsøg; A3, 4 parallelforsøg, A4, 2 parallelforsøg, A5, 2 parallelforsøg; B1, 4 parallelforsøg; B2, 2 parallelforsøg; B3, 2 parallelforsøg og i forsøg B4, 2 parallelforsøg.

Fig 19. viser klækkeprocenterne i de enkelte forsøg samt temperaturforløbet. Den reelle klækkeprocent refererer til, at der er korrigeret for, at befrugtningsprocenten i ingen tilfælde var 100%. De døde æg blev opsamlet hver anden dag og antallet estimeret ved optælling af 10 ml pr. 50 ml. æg.

I forsøg A2 og A3 er dødeligheden størst i første halvdel af inkubationsfasen. I de andre forsøg i serie A er der en jævn dødelighed igennem hele fasen.

Fig. 19.



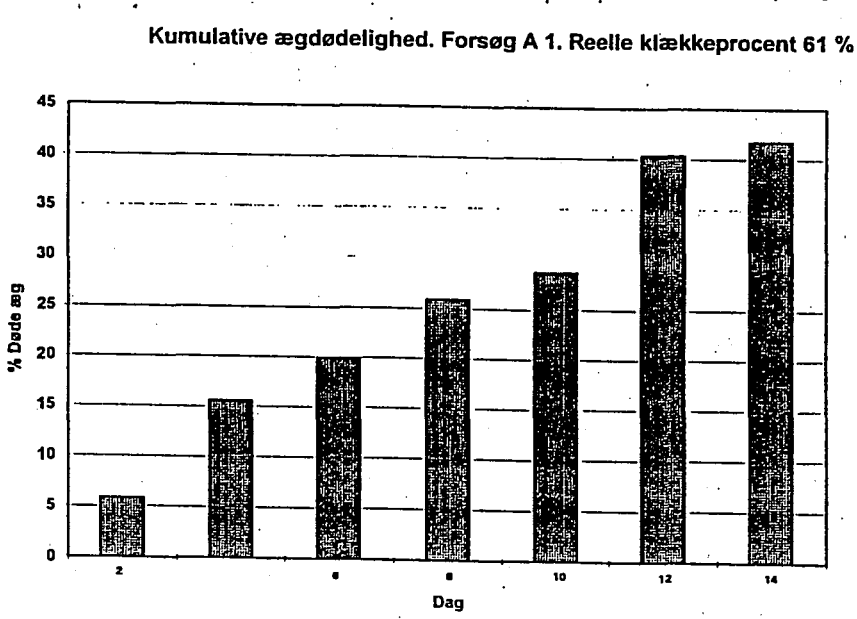
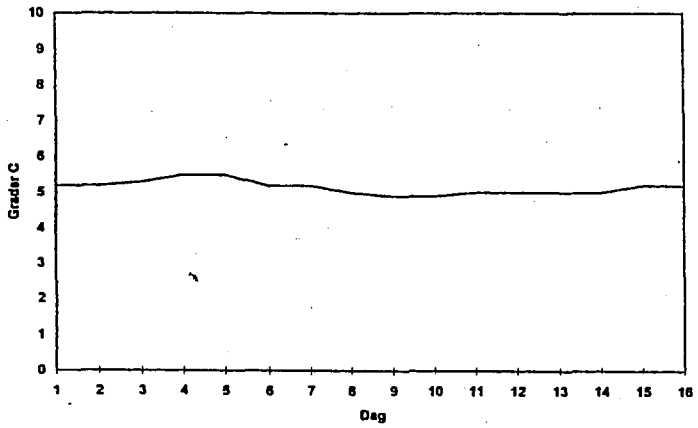
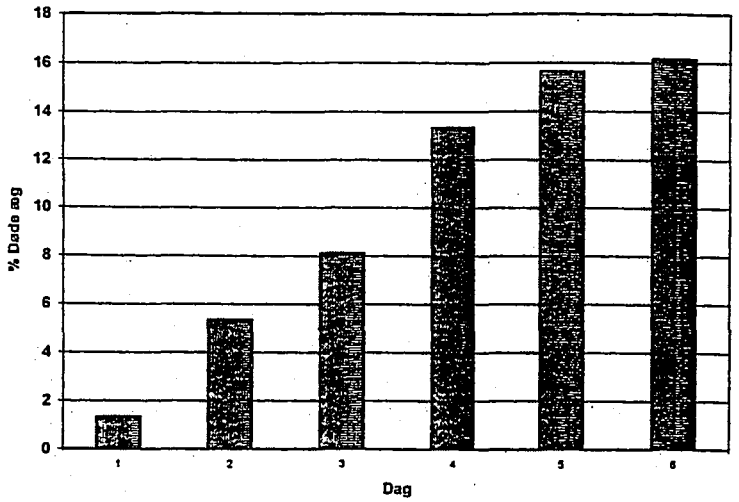


Fig. 19.

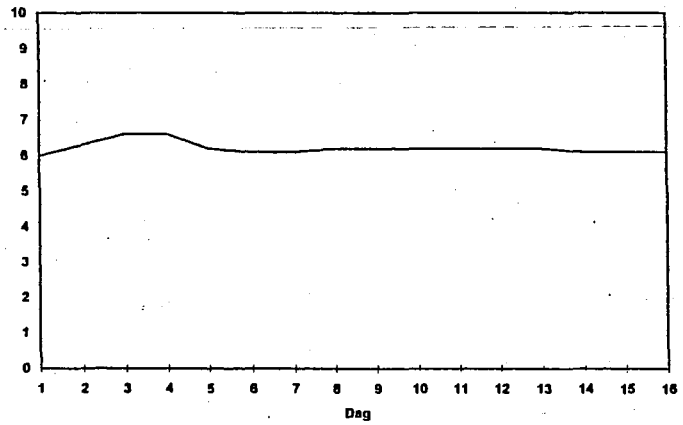
Temperatur, klækkeri, forsøg A2



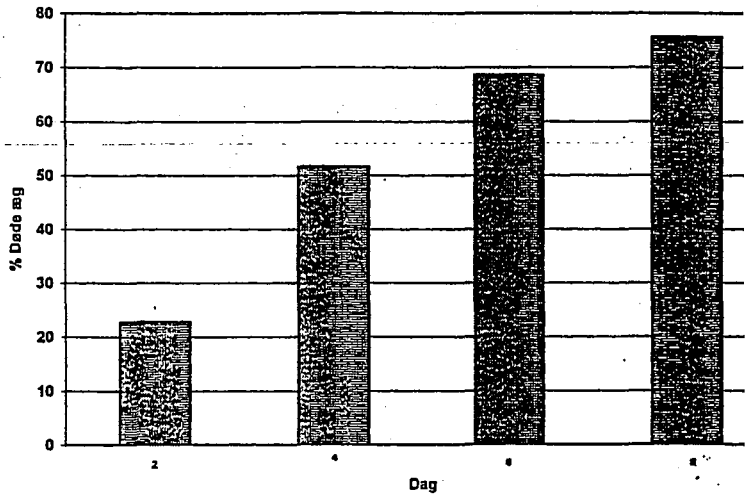
Kumulative ægdødelighed. Forsøg A2. Reelle Klækkeprocent 84



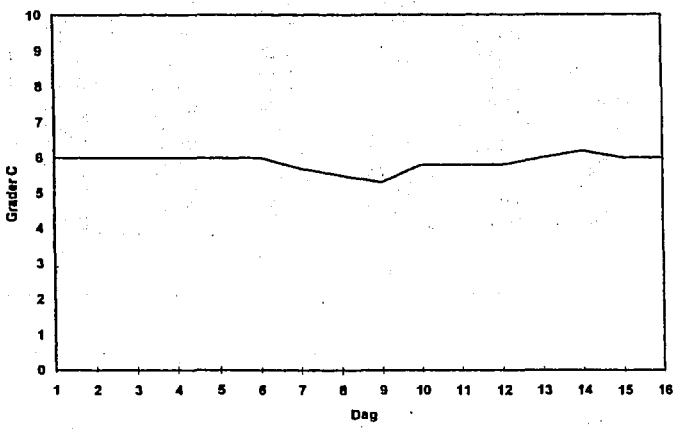
Temperatur, klækkeri, forsøg A3



Kumulative ægdødelighed. Forsøg A3. Reelle Klækkeprocent 24



Temperatur, klækkeri, forsøg A4



Kumulative ægdødelighed. Forsøg A4. Reelle Klækkeproce

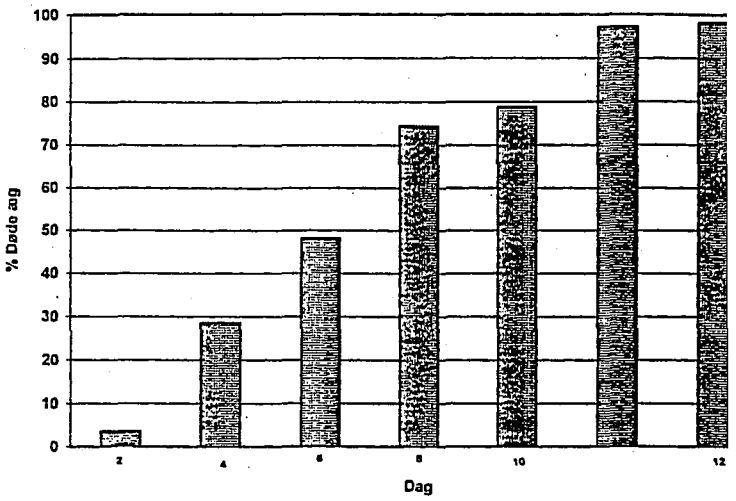
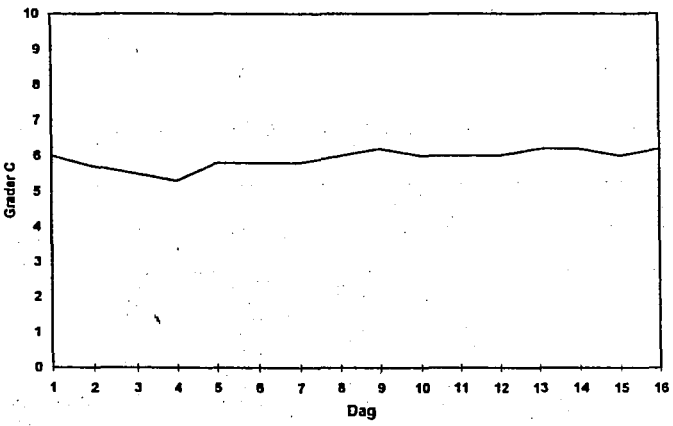
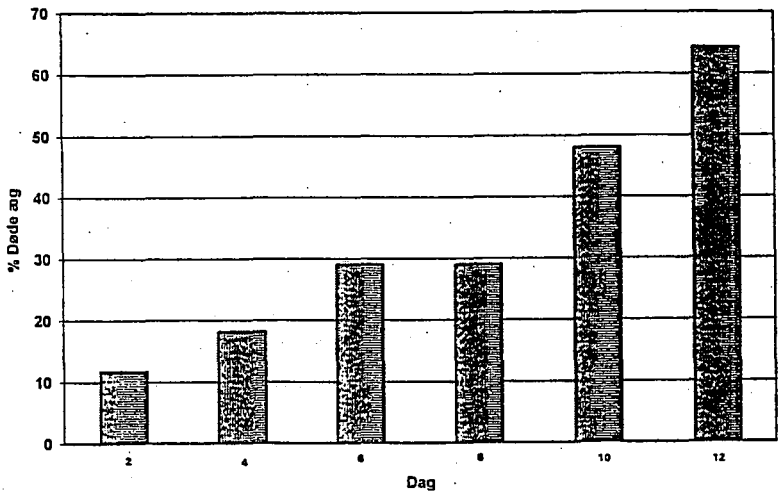


Fig. 19.

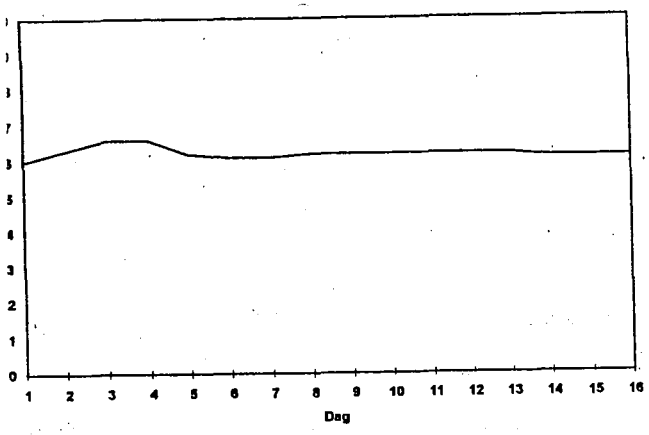
Temperatur, klækkeri, forsøg A5



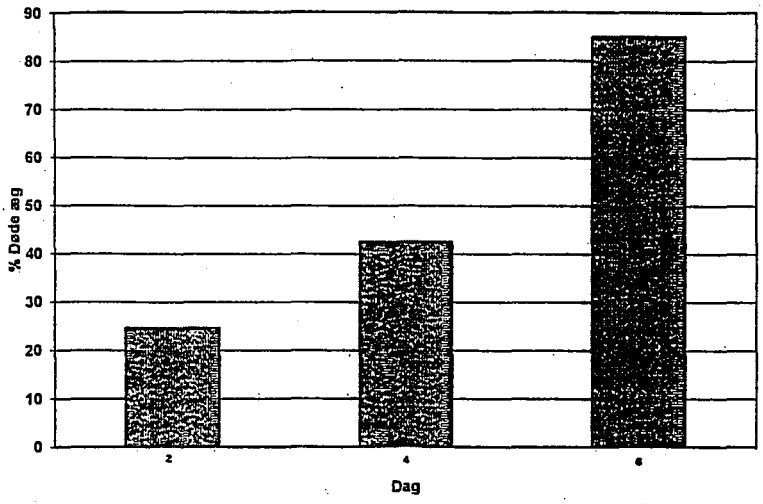
Kumulative ægdødelighed. Forsøg A5. Reelle Klækkeprocent 51 %



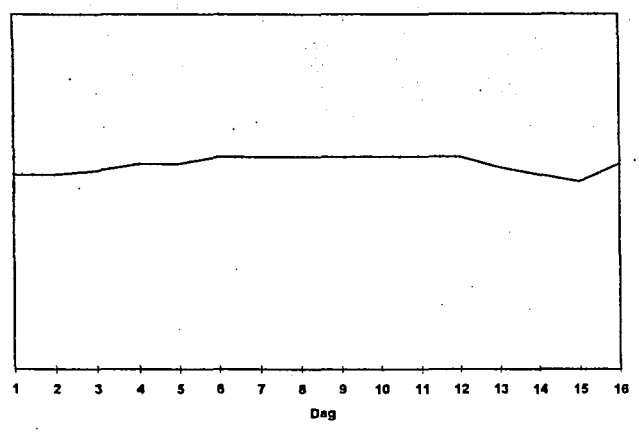
Temperatur, klækkeri, forsøg B1



Kumulative ægdødelighed. Forsøg B1. Reelle Klækkeprocent 18 %



Temperatur, klækkeri, forsøg B2



Kumulative ægdødelighed. Forsøg B2. Reelle Klækkeprocent 10 %

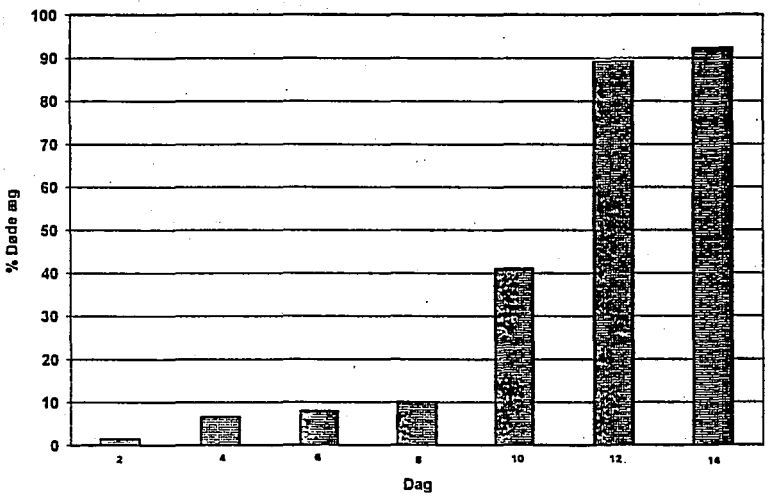
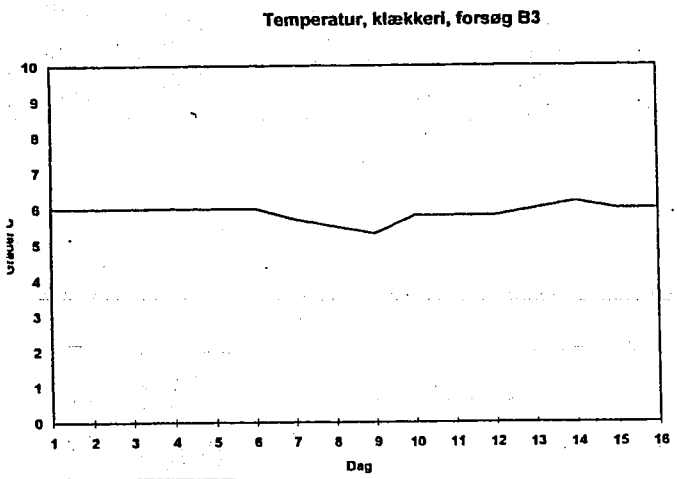
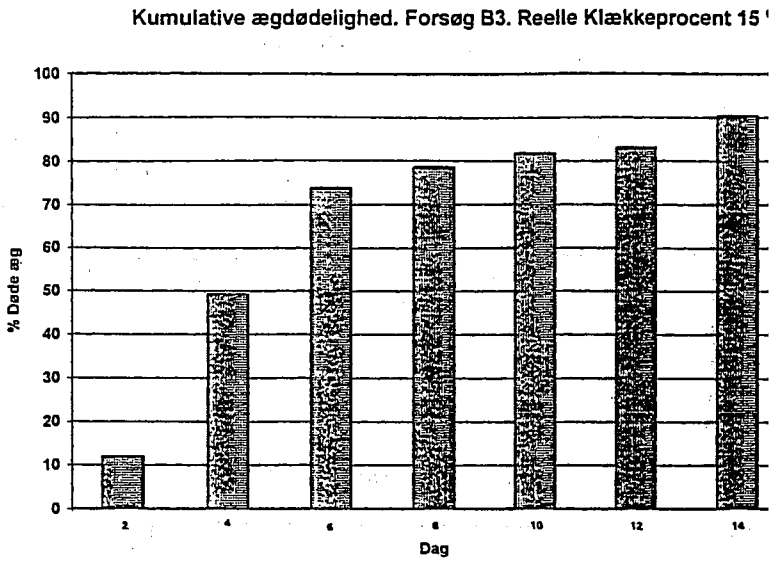
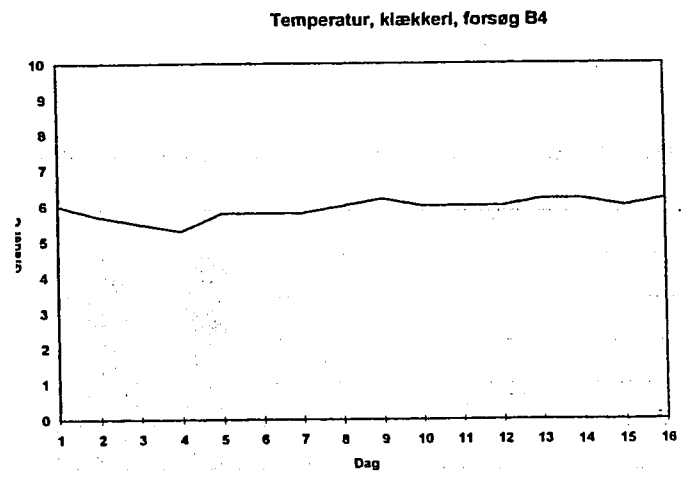
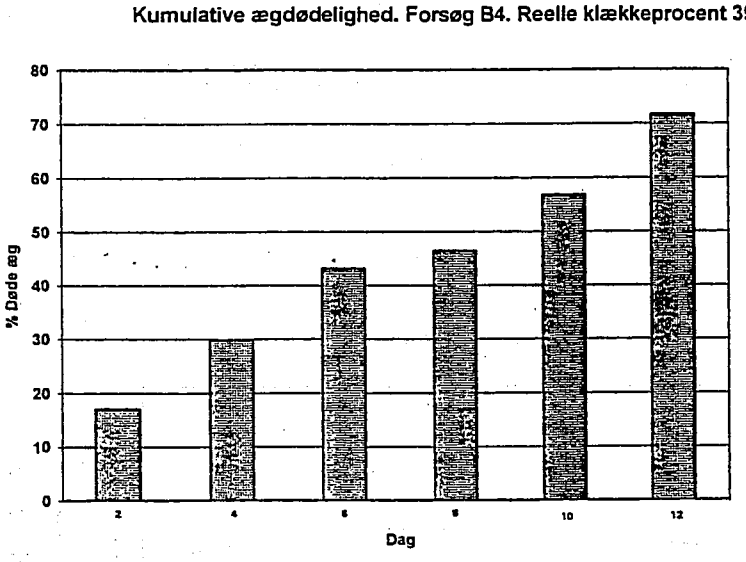


Fig. 19.


Temperatur, klækkeri, forsøg B3
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Dag


Kumulative ægdødelighed. Forsøg B3. Reelle Klækkeprocent 15
100
90
80
70
60
50
40
30
20
10
0
% Døde æg
2 4 6 8 10 12 14
Dag


Temperatur, klækkeri, forsøg B4
10
9
8
7
6
5
4
3
2
1
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Dag


Kumulative ægdødelighed. Forsøg B4. Reelle klækkeprocent 3
80
70
60
50
40
30
20
10
0
% Døde æg
2 4 6 8 10 12
Dag

I forsøgsserie B er dødeligheden i forsøg B1 og B3 størst i første halvdel; i forsøg B2 i sidste halvdel af forsøgsserien. I B4 er der et jævnt forløb.

Karakteristisk for klækkeforsøgene er der meget variable klækkeprocenter i begge serier. I serie B er klækkeprocenterne generelt lavere.

Der blev produceret ialt 239.700 larvr. Temperaturen har i alle forsøg ligget mellem 5-6,5°C med et maksimalt udsving på 0,5°C igennem inkubationsfasen.

I svenske forsøg på at klække Østersø torsk opnåede man ingen klækkeprocenter på over 60% (Pickova, J. et al., 1992). I klækkeforsøg med pighvarre fr den vestlige Østersø var bedste resultat 54% klækkede æg.

3.5 Analyseresultater

For at teste, om æggene skulle være kontaminerede med miljøgifte og disse påvirke klækkeresultatet blev en delprøve fra hvert ægbatch udtaget og sendt til analyse for DDD, DDE, DDT og PCB-congenerne CB28, CB52, CB101, CB105, CB118, CB138, CB153, CB170 og CB 180.

Analyseresultaterne er fremkommet ved enkeltanalyse og er dermed behæftet med en vis usikkerhed. Der var ikke æg nok til at foretage dobbeltanalyse. De ovenfor nævnte miljøgifte er bundet til lipider, og det er derfor æggenes lipider, der er analyseret. Torskeæg har ligesom andre pelagiske marine fiskeæg et meget lille indhold af lipider (Craik, J.G.A. et al., 1987);(Lönning, S. et al., 1988). Der skal derfor ret store mængder æg til for at muliggøre en ekstraktion af tilstrækkeligt meget lipid til dobbeltbestemmelse.

PCB-conger, hvoraf der findes 209 forskellige, er nedbrydningsprodukter fra PCB. De 9 congener, der her er analyseret for, udgør 70-80% af det totale antal og bruges derfor som markører.

Tabel 1 viser koncentrationen af DDD, DDE og DDT i de forskellige ægbatch.

Tabel 1.

Ægbatch nr.	p,p DDD	p,p DDE	p,p DDT	ug/kg rogn
A1	0,062	1,323	0,282	
A2	0,048	1,023	0,282	
A3	0,057	0,937	0,406	
A4	0,070	2,017	0,336	
A5	0,057	0,942	0,148	
B1	0,045	1,526	0,159	
B2	0,125	3,899	0,741	
B3	0,027	1,625	0,341	
B4	0,082	2,762	0,520	

Der er ingen positiv korrelation mellem klækkeprocenterne og indholdet af hverken DDD, DDE eller DDT, heller ikke hvis tallene summeres.

Et DDT indhold på 2,95 mg/kg rogn medfører stor ægdødelighed hos søørred samt efterfølgende dødelighed blandt blommesæksyngel. Koncentrationer på 2,67 mg/kg rogn eller mindre påvirker ikke klækkeprocenten eller dødeligheden hos blommesæksyngel (Burdick, G.E. et al., 1964).

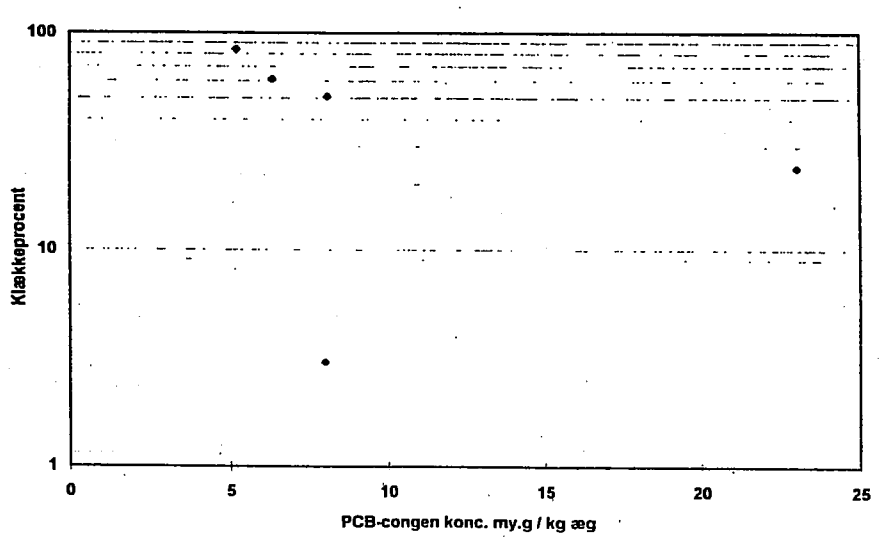
DDT koncentrationer på 1,09-2,76 mg/kg rogn af Coho laks i Lake Michigan medførte en dødelighed på 15-73% af ynglen i løbet af 8 uger, mens koncentrationer på 0,55-0,66 mg/kg rogn medførte en dødelighed på 1-5% i samme periode (Johnson, H.E., 1969).

DDE koncentrationer på 18 ng/g fedt eller højere påvirker klækkeprocenten hos sild fra den vestlige Østersø (Hansen, P.D. et al., 1985). De nævnte koncentrationer ligger væsentligt over de koncentrationer som blev fundet blandt de ægbatches, der indgik i forsøgene.

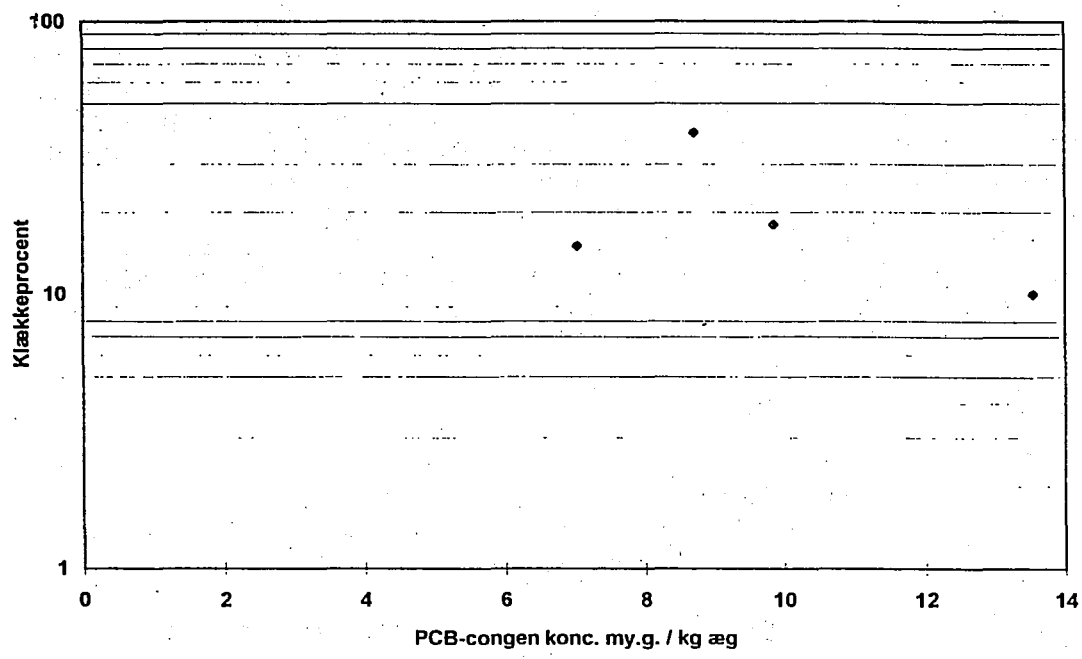
Fig 20 viser klækkeprocenten som funktion af PCB-congen indholdet for de to forsøgsserier A og B; koncentrationerne af de 9 congener er summeret.

Det ses af figuren, at der ikke er korrelation imellem klækkeprocenten og indholdet af PCB-congener.

Fig. 20. **Klækkeprocent i relation til PCB-congen indhold i æg. Moderfisk gruppe A**



Klækkeprocent i relation til PCB-congen indhold i æg. Moderfisk gruppe B



Korrelationskoefficienterne er således for forsøgsserie A: R2 = -0,480 og for forsøgsserie B: R2 = -0,421. PCB koncentrationer på 120 ng/g fedt har en negativ effekt på klækkeraten hos vestlige Østersø sild (Hansen, P.D. et al., 1985). De i klækkeforsøgene fundne koncentrationer ligger væsentligt under den nævnte værdi på 120 ng/1g fedt. Undersøgelser af Coho lakseyngel har vist, at PCB koncentrationer på 3,9-5,2 mg/kg har medført henholdsvis sygdomstegn og død. Flere undersøgelser har vist, at chlorinerede hydrocarbonforbindelser kan påvirke æg- og larvekvaliteten hos fisk i negativ retning (Bengtsson, B.E., 1978);(Karås, P. et al., 1991);(Sandström, O. et al., 1988);(Westernhagen, H.V. et al., 1988).

Larveforsøg

Der blev foretaget 2 startfodringsforsøg i 1994; et med

larverne fra klækkeforsøg A1, hvori indgik 85.400 larver og et med larver fra klækkeforsøg A2 med 115.300 larver. Forsøgene blev udført i 17%o opsaltet havvand ved 5,5-6,5°C. Larverne blev fodret efter samme skema som året før, men istedet for laboratoriefremstillet føde blev der brugt naturligt zooplankton frafiltreret naturligt havvand.

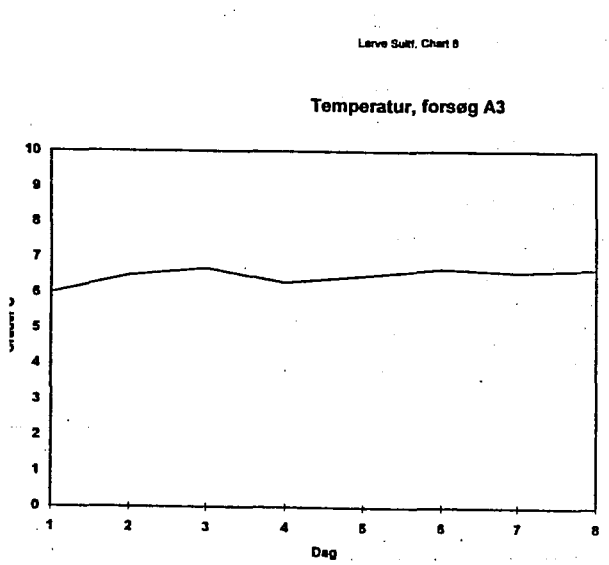
Zooplanktonet 60-150 um blev udfodret sammen med algerne Tetraselmis, Rhodomonas og Isocrysis. I forsøg A1 overlevede larverne 11 dage; i forsøg A2 i 12 dage. I forbindelse med tørstofanalyser blev det undersøgt, om larverne i forsøg A1 havde føde i maven. Dag 4 havde 40 ud af 50 spist; dag 6 havde 24 ud af 50 spist. Som året før døde larverne inden for de to første uger, selv om de blev fodret med naturligt zooplankton. Det kunne tyde på, at problemet ikke er af ernæringsmæssig karakter. Det bemærkelsesværdige er, at larverne villigt begyndte at æde for derefter for en stor dels vedkommende at holde op igen inden for en periode på 2 dage.

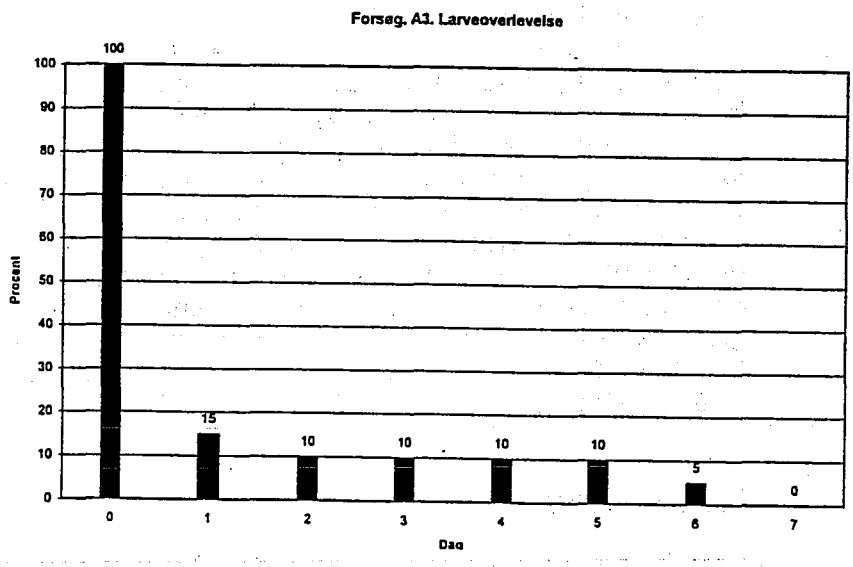
3.7 Sultforsøg med larver

For at teste larvernes overlevelsespotentiale blev der iværksat en serie sultforsøg. Larver fra ægbatch A3, A4, A5, B1, B2, B3 og B4 indgik i forsøgene.

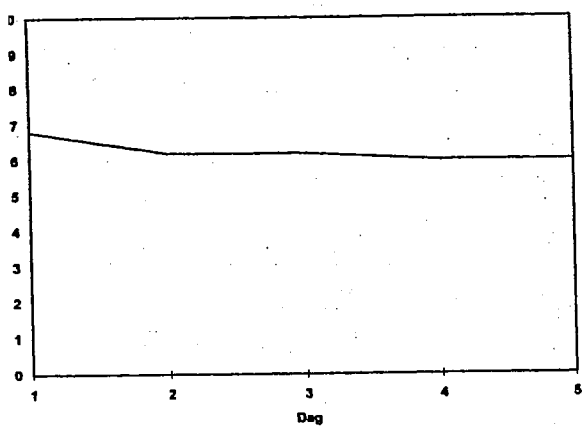
20 larver fra hver gruppe blev udlagt i cylindre med en diameter på 22 cm, en længde på 30 cm og med en bund bestående af 500 um planktonnet. Cylindrene var neddykkede i en tank fra produktionsanlægget og hver cylinder havde et seperat vandindtag. De overlevende larver blev talt 1 gang pr. døgn. Fig 21 viser larveoverlevelsen pr. dag samt temperaturforløbet.

Fig. 21.

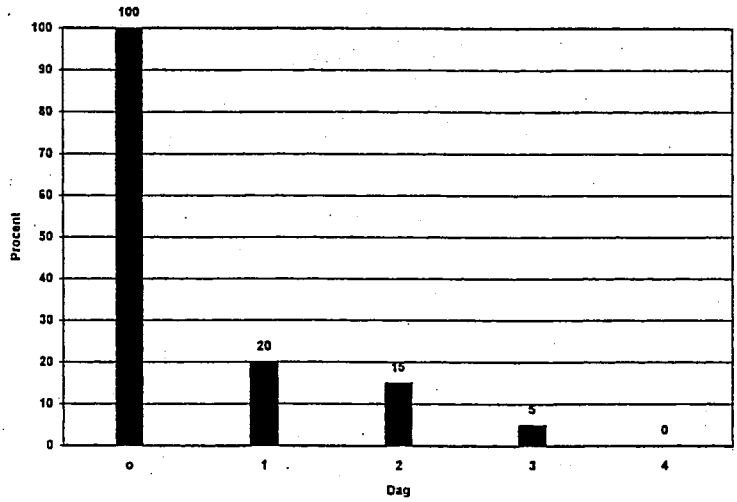




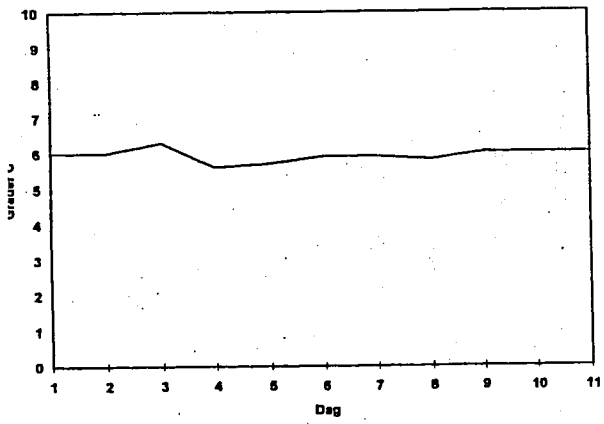
Temperatur, forsøg A4



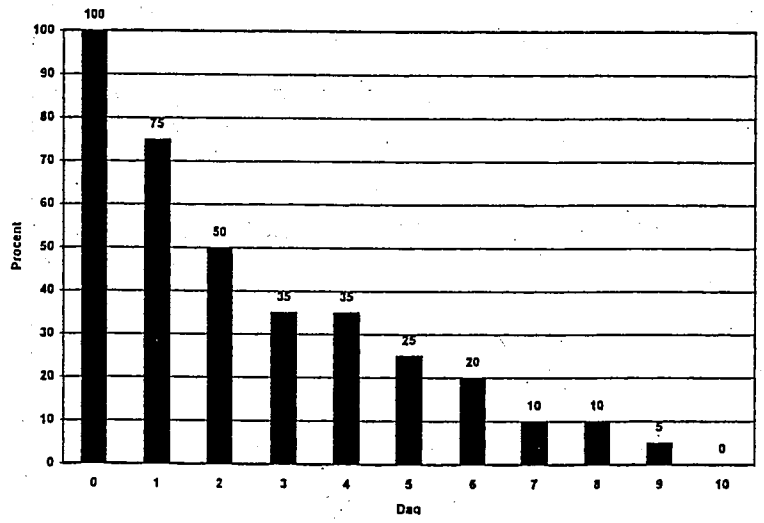
Forsøg. A4. Larveoverlevelse



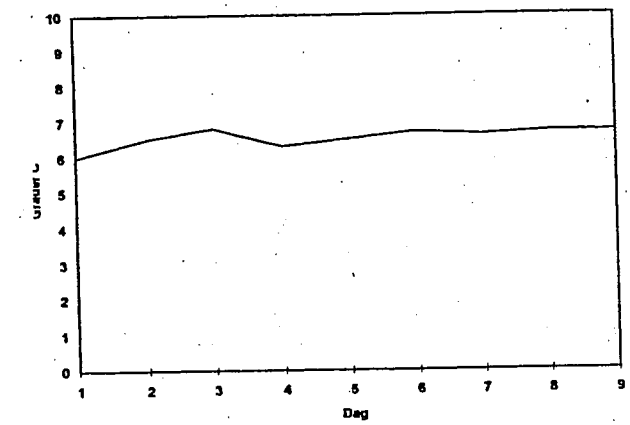
Temperatur, forsøg A5



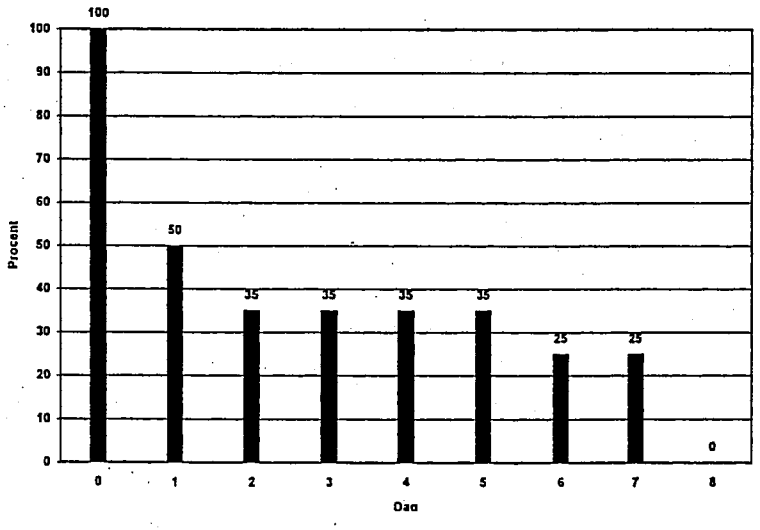
Forsøg. A5. Larveoverlevelse



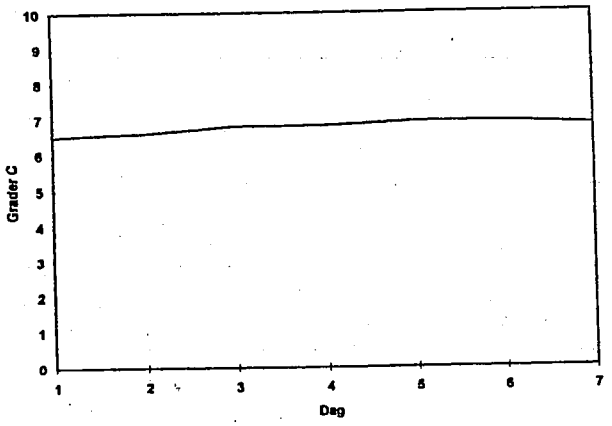
Temperatur, forsøg B1



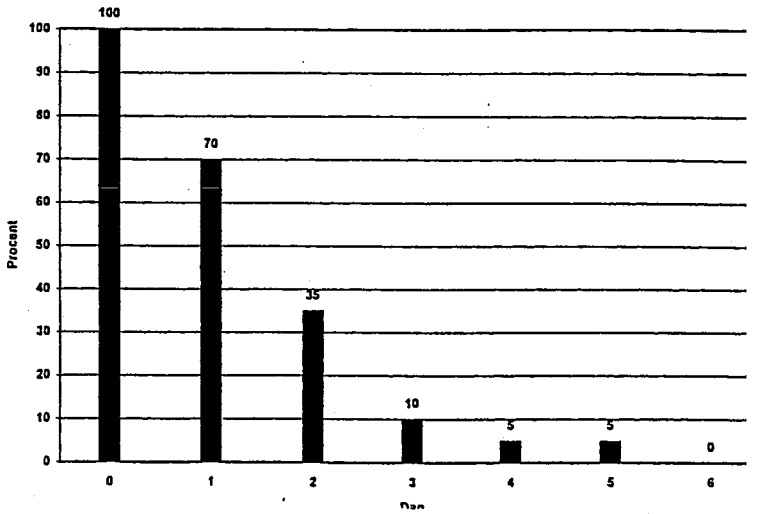
Forsøg. B1. Larveoverlevelse



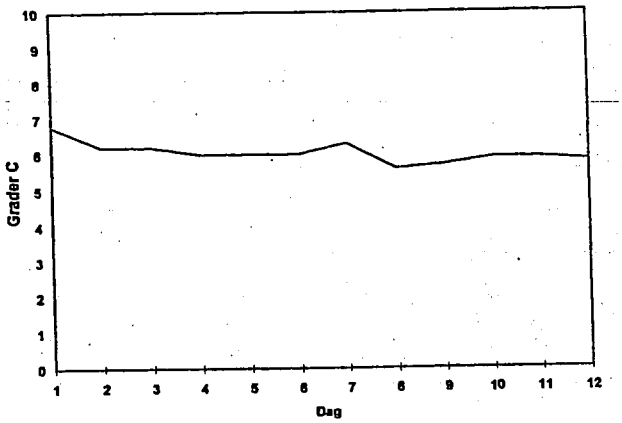
Temperatur, forsøg B2



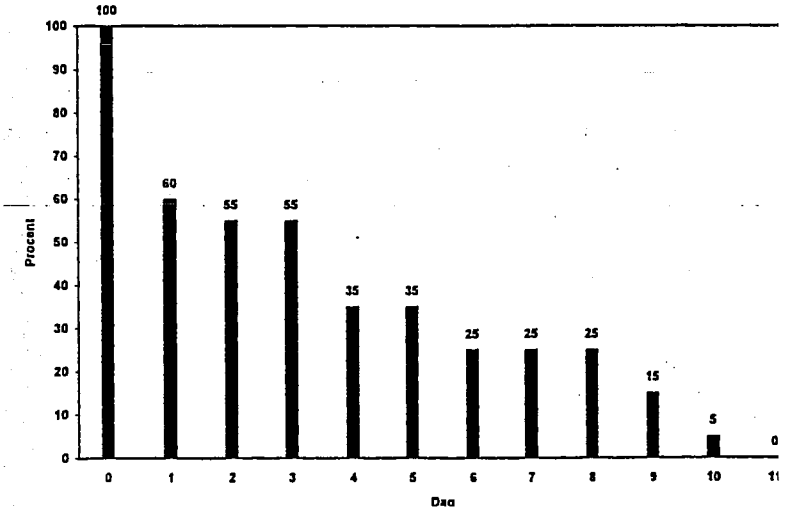
Forsøg. B2. Larveoverlevelse



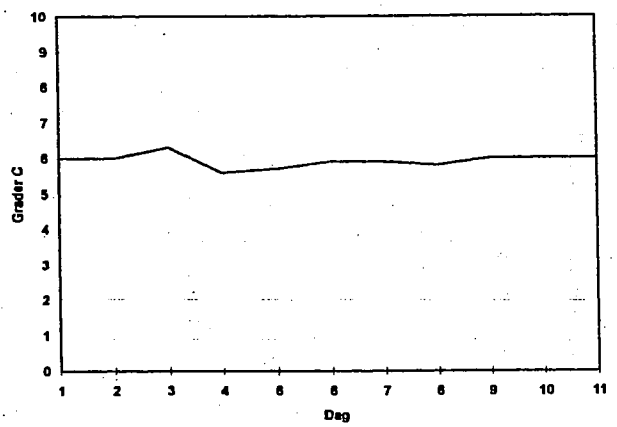
Temperatur, forsøg B3



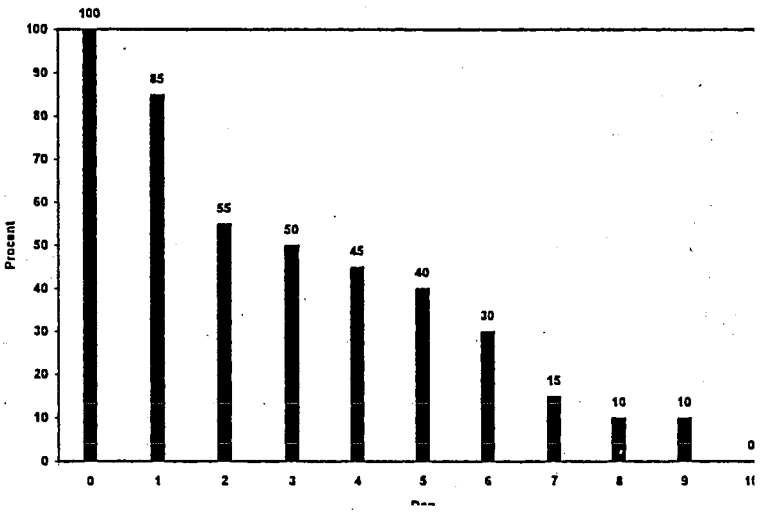
Forsøg. B3. Larveoverlevelse



Temperatur, forsøg B4



Forsøg. B4. Larveoverlevelse

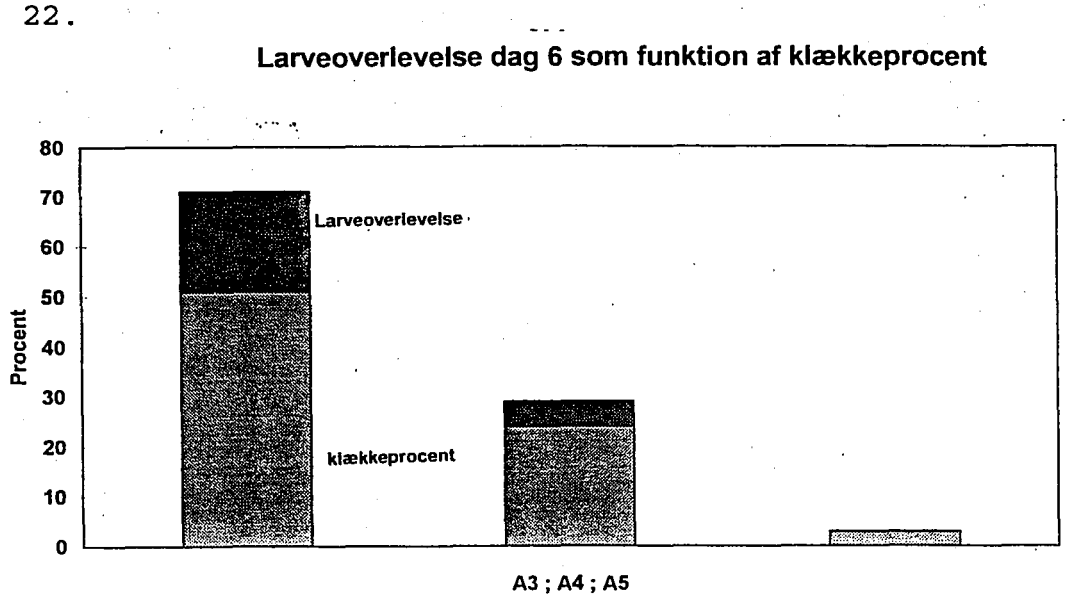


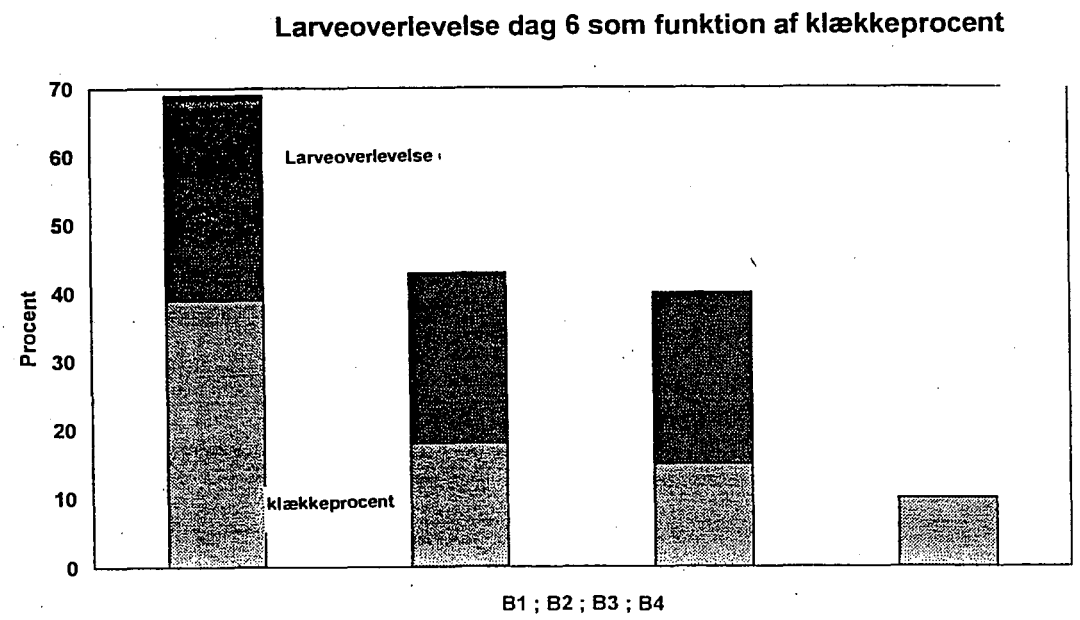
Karakteristisk er den høje dødelighed allerede fra dag 1. Ved blommesæksfasens afslutning dag 6 er der kun få larver tilbage i de fleste forsøg. Således forsøg A3: 5%, A4: 0%, A5: 20%; B1: 25%, B2: 0%, B3: 25%, B4: 30%. Forsøg med Nordsø torskelarver har vist, at de uden føde kan leve i op til 16 dage ved 6,9°C (Yin, M.C. et al., 1986). Dødeligheden starter først for alvor ved 11. dagen (Yin, M.C. et al, 1987).

Larverne i de fig. 21 viste forsøg levede i væsentlig kortere tid; den længste periode var 10 dage.

Fig. 22 viser larveoverlevelsen ved blommesæksfasens afslutning, dag 6, som funktion af klækkeprocenten. Som det ses af figuren, er der en sammenhæng mellem klækkeprocenten og larveoverlevelsen. Det kunne tyde på, at der en eller anden faktor, som påvirker klækkeprocenten og senere larveoverlevelsen.

Fig. 22.





KONKLUSION

Forsøgene igennem de 3 år har vist, at det er muligt at få Østersø torsk til at gyde med succes i fangenskab; at der kan produceres store mængder æg, men at klækkeprocenterne er meget variable.

Det kan lade sig gøre at producere larver i produktionsrelevant antal. Larverne spiser allerede fra dag 4, men har en meget høj dødelighed allerede umiddelbart efter klækning. Dødeligheden er næsten 100% efter 2 uger. Både i 1993 og i 1994 foretog Landbohøjskolen sygdoms monitering af æg og larver, uden at kunne relatere dødeligheden ti lpatogene infektioner.

Det kan lade sig gøre at producere yngel i lille antal uden synlige defekter. Larverne dør tilsyneladende ikke af fejlernæring. Der er en korrelation mellem klækkeprocenten og larveoverlevelsen.

Videre undersøgelser må klarlægge problemerne omkring æg- og larvedødelighed inden en større produktion af yngel kan gennemføres.

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