First scientific support plan for a sustainable development policy (SPSD I)

Programme "Sustainable management of the North Sea"

Evaluation of the quality of turbot fry on the stock enhancement success in the North Sea

Summary of the research

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The restrictions of the fish populations became only clear in the nineties, when the yearly production decreased continuously with 2.5%. Clearly, the aquaculture production cannot compensate this decrease, although the share of the cultured fish increases exponentially (13% in 1994). In addition, the estimations of the future demand for fish and crustaceans expect a raise of 50% by the year 2025. The effects of insufficient natural renewal of the fish populations can be rectified by letting the population increase.

In this study special attention was given to the quality of the produced fish, next to crucial biological aspects in connection with the survival, growth and physiological condition (in other words specific quality characteristics) of the fish that will be put out in the sea.

Special attention was also given to the influence of turbot culture on the environment. No antibiotics were used during the larval phase, as the dangers that come with the use of antibiotics are well known. All experiments were conducted in recirculation systems, which results in a better control of the affluent and the effluent is reduced to a minimum.

Turbot larvae were cultured in 3 different systems. The growth, survival and pigmentation were monitored the first 11 days post-hatching. The first system has a batch phase and afterwards, from day 8 onwards, every tanks is connected to a separate biofilter. The second system is a recirculation system. The water passes through a protein skimmer with ozone injection and a biofilter. The third system is again a recirculation system where the larvae are kept in a cage. The water recirculates over a separate submerged biofilter.

High mortality in the beginning of the experiment made clear cut conclusions impossible. The flow rate in both recirculation systems caused the larvae too much stress. The use of probionts should offer an alternative for antibiotics in the future.

In the following experiments it was tried to protect the fish larvae by adding selected bacteria with a positive influence to the culture medium and/or to the food of the fish larvae. Survival and growth of the larvae were monitored.

Five different bacteria were used: *Vibrio proteolyticus, V. mediterranei, Aeromonas hydrophila, Glucanobacter sp* and a not identifiable Cluster A.

From day 5 onwards, there was a significant difference in the consumption of rotifers between the control and the treatments with probionts. The bacterial inoculation of the culture water has a positive effect on the first colonization of the gut of fish larvae, but this only became clear after 3 days. The bacteria that were added to the rotifer culture

were not recovered in the rotifers themselves. From these data it can be concluded that the addition of bacteria before start feeding has the biggest effect.

Cluster A, *V. proteolyticus* and *Glucanobacter sp.* have a positive effect on the survival on day 5 compared to the controle: 95%, 93%, 93% and 74%, respectively. *A. aeromonas* as well as *V. medditteranei* had no significant effect on larval survival.

Cluster A, *V. proteolyticus* and *Glucanobacter sp.*are potential probionts that need to be tested on larger scale.

Next to the experiments on alternatives for antibiotics, high quality tarbot juveniles needed to be produced.

The effect of addition of Vitamine C and E to a diet supplemented with fish oil on the quality of the produced turbot larvae was studied.

The turbot juveniles were kept in a recirculation system with 3 tanks (2 m^3 each) connected to a drum filter and a biological trikling filter. The filtered water is pumped back to the tanks through UV-sterilisers. 650 juveniles were put in each tanks and fed 3 times a day with 1) a standard granule (Provimi Turbot Starter), 2) the standard granule coated with 9% fish oli (DHA/EPA = 4%), 10% Vit C and 3) cfr. 2 with addition of 1000 ppm Vit E.

Survival and growth were monitored during the experiment and the length was measured just before release in the sea. The quality was determined using a modified salinity stress test.

When the fishes were released into the sea, there was a significant size difference. The fishes fed with diet 3 were bigger (16.04 ± 1.52) than the control group (15.17 ± 1.53). The fishes fed diet 2 had an intermediate size (15.62 ± 1.49). The survival at the end of the experiment was 85%, 92%, 90%, respectively for the 3 diets.

The results of the modified stress test showed that the fish fed diet 2 had the highest quality. The addition of vitamins, especially Vit E, and addition of HUFA's enhances the quality of the juveniles and should be recommended in restocking programs and for commercial purposes.

An experiment was conducted to evaluate the effect of oxidation of the oil present in the feed on the need of Vit E in juvenile turbot. Diets with different Vit E (0 or 200 ppm) in combination with either oxidized (60 meq peroxide/Kg) or unoxidized (7 meq peroxide/Kg) triglyceride oil. A standard ICES weaning diet was included as a control.

Wet and dry weight of the whole body fish and the liver, specific growth rate, hepasomatic index and levels of vitamin C and E in the liver were measured.

At the end of the experiment, the whole body weight and the specific growth of the fish fed the diet containing oxidized oil, without additional Vit E were significantly lower than of the other fish. A two-way analysis of variance showed a significant effect of the oxidation, but not of the dietary Vit E level on the weight and on the specific growth rate of the fish. The liver weight and the hepatosomatic index on the other hand were affected by the vitamin E level. Diets without Vit E resulted in the highest liver weight and hepatosomatic index.

Already after 36 days, the tocopherol level in the diets was reflected in the one in the liver.

Several methods to determine the quality of turbot larvae and juveniles were used and/or made perfect. It is of utter most importance that the released fish have a high quality to guarantee a high survival rate.

The quality of the turbot larvae was determined by 3 different reproducible tests: the salinity stress test, the Cellular Energy Allocation method and the determination of the phagocytosis capacity.

In the first test, the respiration speed was the stress indicator. A higher respiration speed is caused by a stressful situation and a worse physiological condition of the experimental organisms. The CEA-concept is a good alternative for the conventional 'Scope of Growth' method, which is too labour intensive to be used routinely. The energy reserve is quantified via the determination of the fat, sugar and proteine content of the test organism. A spectrophotometer is used for the measuring the levels. The difference between the energy reserve and the energy consumption is expressed in mJ per organism per hour and reflects the energy available for growth and reproduction. The phagocytosis capacity is a measure for the quality of the fish.

The modified salinity stress test gives significant differences according to the different diets used without killing the organisms. Therefore, this test can be used to determined the quality of the turbot juveniles before they are released into the sea.

The determination of the energy reserves through the sugars and proteins did not give any problems. The protocols had to be adjusted according to the size of the fish larvae. It is impossible to use separate organs in small fish. If the other components (energy reserve and the use of lipids and energy) could be determined, then the CEA test is convenient to determine the stress resistance and the quality of turbot in the future.

Through the determination of the phagocytosis capacity, one is able to define the diet used before, even 8 weeks after the fish have been given fish trash.

The release of farmed animals into natural ecosystems has genetic implications. It is therefore necessary on the one hand not to introduce genetically foreign organisms on the place of release. For this reason, one should use brood stock originating from the population present at the site of the release of the cultured juveniles. Therefore, it is important to document the structure of the populations of the target species within its range of distribution. Genetic analysis of turbot originating from different locations within the natural range of distribution has shown that the animals coming from the Irish Sea can be considered as a separate stock. There are also indications that this species can be further divided into a (sub)population English Channel – Bay of Biscay and a (sub)population North sea- Celtic Sea.

On the other hand, it is necessary to enlarge the range of genetic diversity of the released animals, by minimizing the inbreeding, domestication and genetic drift. As the parental genetic effect also determines the stress resistance condition and the survival, the genetic diversity of the juveniles and the released fish will as well be determined by the parental genetic effect, namely by genetic loss.

According to the technical feasibility study, the water quality at the Belgian coast is too low to allow turbot larviculture. For that reason, a complete closed recirculation system is the only possible culture method. Next to the advantages of the control of the inlet, you have also less effluent flowing back to the natural environment. It is very difficult, economically spoken, to draw conclusions on the feasibility of the turbot culture, as the variation of the market prize is not predictable.