Turkish Journal of Fisheries and Aquatic Sciences 2: 23-27 (2002)

Problems and Achievements in Seed Production of the Black Sea Turbot in Russia

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Abstract

This paper summarizes the research done at the Russian Federal Research Institute of Fisheries and Oceanography to develop hatchery technology for the Black Sea turbot *Scophthalmus maeoticus* and release the juveniles produced into the sea. Quality of eggs and larvae, nutritional value of the live food organisms, and bacterial contamination of rearing tanks were the major factors responsible for the highly variable survival of turbot larvae during the first month of life, and the unpredictable hatchery production. Control of the timing of egg stripping and fertilization and removal of nonviable embryos during incubation helped improve the quality of eggs and larvae. Methods were developed to improve the quality of live food and the rearing conditions. Improved cultivation of live foods, enrichment of rotifers with algae mix, enrichment of *Artemia* metanauplii alternately with artificial diets and algae, and delaying the transition to feeding on *Artemia* nauplii helped improve the survival of turbot larvae. Only 5-7% of larvae had abnormal pigmentation. Survival was 80% at the end of the first month of life and 40-65% at the end of two months. Hatchery operation and production were thus stabilized. Regular release of hatchery-reared juvenile turbot into the sea can be an effective method of increasing the turbot population in the Black Sea.

Key Words: Black Sea turbot, *Scophthalmus maeoticus*, larvae culture.

Introduction

Development of hatchery technologies for the Black Sea turbot *Scophthalmus maeoticus* was started in 1990 at the Russian Federal Research Institute of Fisheries and Oceanography (VNIRO). The ultimate objective of the hatchery is to make possible the regular release of juvenile turbot to the sea to increase the stocks and thereby sustain the turbot fishery in the Black Sea.

The Black Sea turbot is a close relative of the Atlantic turbot *Scophthalmus maximus*. Therefore our studies were based on much of the experience in farming Atlantic turbot (Jones *et al*., 1974; Kuhlmann *et al*., 1981; Person-Le Ruyet, 1989) but taking into account the ecological peculiarities of the Black Sea turbot. Larvae were reared in green water, rotifers were used as first food, then *Artemia* nauplii and metanauplii, then artificial feed by the end of the first month of life.

Black Sea turbot was similar to the Atlantic turbot in the pattern of larval mortality during the first month of rearing. Three critical periods were seen. The first peak of mortality occurred on days 3-4 after hatching just before the larvae start feeding. Most of the larvae that died at this time had developmental abnormalities, i.e., the quality of eggs and larvae was the most important factor. Increased mortality rates were also observed on days 7-9 (second peak) and on days 13-16 (third peak) after hatching. The majority of larvae that died during these periods had no visible defects but had failed to feed. For Atlantic turbot, it is commonly supposed that the mortalities during the analogous critical periods are due to unsatisfactory quality of live food organisms and bacterial contamination of rearing tanks (Witt *et al*., 1984; Nicolas *et al*., 1989; Person-Le Ruyet, 1989; Person-Le Ruyet *et al*., 1991).

Thus, the survival of Black Sea turbot larvae is determined by the quality of eggs and larvae, adequacy of larval nutrition, and the hygiene and water quality in the rearing tanks. Studies at VNIRO were aimed at increasing larval survival and stabilizing the hatchery production. The development of hatchery technology for the Black Sea turbot can be subdivided into two stages. The first stage (1990-1994) concentrated on increasing the nutritional value of live food organisms, and maintaining favorable microbiological conditions in the rearing tanks to increase the larval survival. The second stage (1995-1997) focused on methods to standardize the quality of eggs and larvae.

Live Food Organisms and Bacterial Contamination in Rearing Tanks

These two problems are interconnected since live foods are a major source of bacterial contamination in rearing tanks. The most effective way to reduce bacterial load in rearing systems is to produce live prey with minimal microflora and to provide them to turbot larvae in the appropriate way. Bacterial contamination of live food depends on the duration of cultivation and the enrichment method used to increase their nutritional value (Nicolas *et al.*, 1989; Minkoff and Broadhurst, 1994). It is best to feed zooplankton with phytoplankton that meets the nutritional requirements of marine fish larvae – microalgae such as *Monochrysis*, *Isochrysis*, *Pseudoisochrysis* and some other species.

Based on the comparison of feeding intensity of the Black Sea turbot larvae and their survival at various degrees of microflora development, the maximum allowable concentration of microbial cells in the rearing water was determined. The total microbial number (TMN) should not exceed 1000-1500/ml. The maximum allowable values of TMN of live foods harvest are estimated based on the ratio between the volume of each type of applied foods and the volume of a rearing tank. They are as follows: for algae, rotifers and *Artemia* nauplii - not more than 50, 100 and 500 microbial cells per ml, respectively.

A cultivation regime for algae and rotifers was developed to obtain harvests that fulfilled the TMN requirements. The duration of cultivation did not exceed 5-7 days. The nutritional value of rotifers was increased directly in the larval rearing tanks by daily addition of several species of algae (*Monochrysis*, *Isochrysis*, *Pseudoisochrysis*) maintained at 0.3-0.5×10^6 cells/ml. The less the volume of live food added, the lower the risk of bacterial contamination of rearing tanks.

Artemia eggs and nauplii were disinfected. The metanauplii were enriched with either artificial nutritional mixture or algae. The differently enriched metanauplii were added into larval rearing tanks in turns.

Larval rearing tanks were prepared 5-6 days before stocking of larvae. First, algae were grown, then rotifers were added. By the time larvae began feeding 3-4 days after hatching, the rotifer concentration had become 10/ml. When larvae were actively feeding, mixtures of several microalgae were daily added into the tanks. Rotifers were also added when the concentration fell to 3/ml. *Artemia* nauplii were added into the tanks along with rotifers when the larvae were 12-13 days of age at water temperature of 18±0.5°C and able to assimilate them. *Artemia* metanauplii enriched with the nutritional mixture were included in the larval diet on day 14-15. The larvae were weaned to artificial diet at age 25-28 days. Under this feeding regime, the survival rate of larvae was improved but the growth rate was retarded. Survival reached 40% or more by the end of the first month and only 5-7% of the larvae had abnormal pigmentation.

However, extremely high mortality up to 100% occurred rather frequently when larvae were 16-20 days old. Bacterial contamination and the nutritional inadequacy of the live food could not explain all unsuccessful rearing of the Black Sea turbot larvae. One probable explanation for the instability of hatchery production is the variability of larval survival by the time of first feeding and the resulting actual stocking density of the larvae during the rest of the rearing period. The balance between the larvae and the rotifers is disturbed and the rearing system is destabilized. Moreover, the mortality of Black Sea turbot larvae at the final stage of the endogenous feeding correlates well with the percentage of larvae with deformities.

Quality of Eggs and Larvae

The quality of eggs and larvae was estimated by the percentage of fertilization and hatching. Larvae obtained from batches of eggs with a high fertilization and hatching rates (not less than 75%) were considered to be suitable for rearing. The percentage of abnormal embryos and larvae was also an indicator of quality, when the type of abnormality and their probable causes are considered. Mortality in Black Sea turbot larvae during the first stages of rearing was determined by the conditions of obtaining and incubating the eggs.

Stripping of gametes

Gametes were obtained from both wild turbot during the spawning season and from captive broodstock. Spontaneous spawning of wild spawners in the tanks occurred very seldom, but the broodstock spawned regularly. During the spawning season, each captive female can spawn up to 10 times at an interval of 3-7 days, but the maturation periodicity of consecutive egg portions has not been established yet. Females and males were put in different tanks to prevent natural spawning. Timely egg stripping was difficult to guarantee and a long stay in the body cavity had a negative effect on the quality of ovulated eggs (McEvoy, 1984; 1989; Kjorsvik *et al.*, 1990). Turbot eggs stripped 24 hours after ovulation all died unfertilized (Figure 1). Stripping within the first 6 hours after ovulation did not significantly reduce the fertilization rate, but the number of defective eggs increased sharply with the time lapsed. Thus, fertilization rate by itself was not a reliable criterion of egg quality.

A quick test for overripe eggs was developed based on the change in permeability of egg envelopes with time after ovulation. The ovulated eggs are submerged in a dye solution and the quality of the eggs is estimated from the dyeing rates and intensity of coloration. This simple test makes it possible to determine within a few minutes the potential fertilization rate and egg viability and to choose the best egg batches for incubation.

Fertilization of eggs

Dry or semi-dry fertilization techniques were used for the eggs of both Black Sea and Atlantic

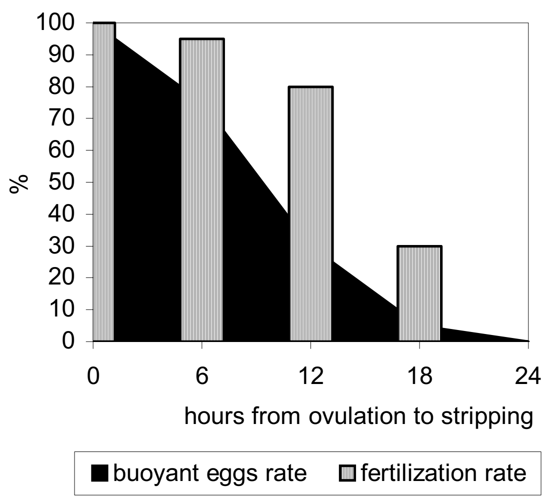

Figure 1. Effect of the delay between the ovulation and stripping time on the quality of the Black sea turbot eggs.

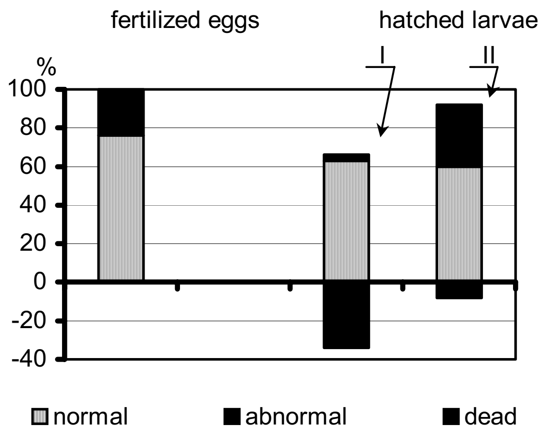

Figure 2. Incubation of the Black Sea turbot eggs in the incubation units of new (I) and previous (II) designs.

turbot: sperm slightly diluted with sea water was poured onto the stripped eggs, mixed, and then gradually diluted further with sea water (Person-LeRuyet, 1991; Fauvel *et al*., 1992). These techniques usually resulted in 90-95% fertilization. Before fertilization, Black Sea turbot eggs have weak envelopes easily deformed during mixing. When insufficient ovarian liquid was stripped with the eggs, the percentage of fertilized eggs with various defects was higher. The wet stripping technique could potentially reduce egg deformation. Comparative experiments on fertilization of six batches of Black Sea turbot eggs showed that semi-dry and wet fertilization techniques did not result in significantly different fertilization rates and hatching rates (Table 1). But the wet technique reduced considerably the number of nonviable larvae with defects in development.

Incubation of eggs

Successful incubation of Black Sea turbot eggs depends on the strict control of environmental variables within the optimum values. Incubation of eggs under a gradual temperature rise of 13 to 15°C, stable salinity of 17-18‰, and constant water exchange made it possible to reach up to 90-95% hatching. But on average 25-30% of hatched larvae had various developmental defects, which probably contributed to the mortality of larvae during the earlier days of rearing.

Elimination of nonviable larvae during incubation was the most cost-effective way to counter losses of larvae during rearing, so new designs for incubation units were tested. In this new unit (Figure 2), the intense flow, turnover, and mixing of water (0.5-1 volume change/hour) and air (10-30 ml/min) agitated the eggs and destroyed non-viable embryos. The new incubation unit resulted in lower survival but of healthy viable embryos and larvae.

All the above methods of egg stripping, fertilization, and incubation made it possible to produce hatched larvae of high quality, to increase the survival during early rearing, and to stabilize the hatchery process as a whole (Figure 3). The larval survival by the end of the first month of life has approached the greatest possible (theoretical) value suggested by Person-Le Ruyet (1989).

Future Investigations

Survival of the Black Sea turbot fed different kinds of foods at different stages of development up to 2 months is shown in Figure 4. The increased mortality of turbot during the second month is caused by two factors: technical problems with water supply and water quality and lack of appropriate artificial foods (starting food for whitefishes or *Artemia*

Table 1. Parameters of fertilization and hatching of the Black Sea turbot.

Fertilization technique	Fertilized eggs (%)	Abnormal fertilized eggs (%)	Hatched larvae		
			Total (%)	With defects (%)	Normal (%)
Semi-dry	72-90	10-50	70-90	20-50	40-50
Wet	75-91	5-10	90-99	5-10	85-89

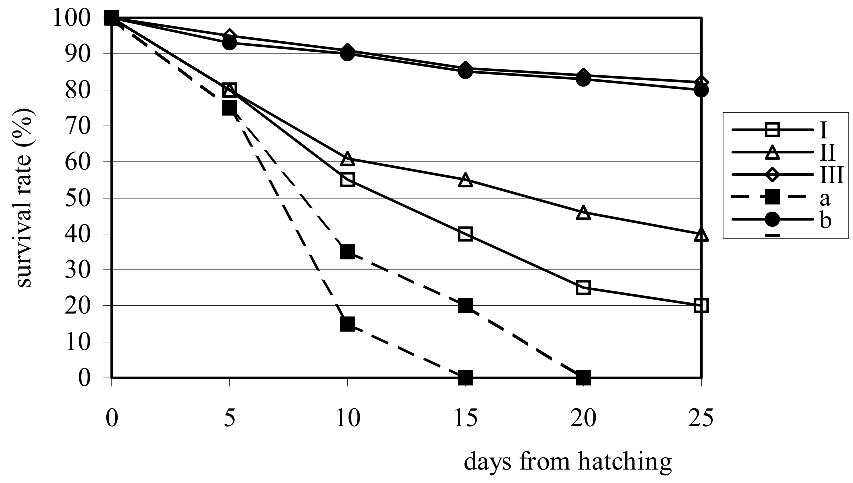

Figure 3. Survival curves of the Black sea turbot from hatching up to day 25:

I – survival rates for 1990-1991;
II - survival rates for 1992-1994;
III - survival rates for 1995-1997;
a - typical unsuccessful survival curves for 1990-1994.
b - the greatest possible survival (theoretical) rate of turbot (from Person-Le Ruyet, 1989).

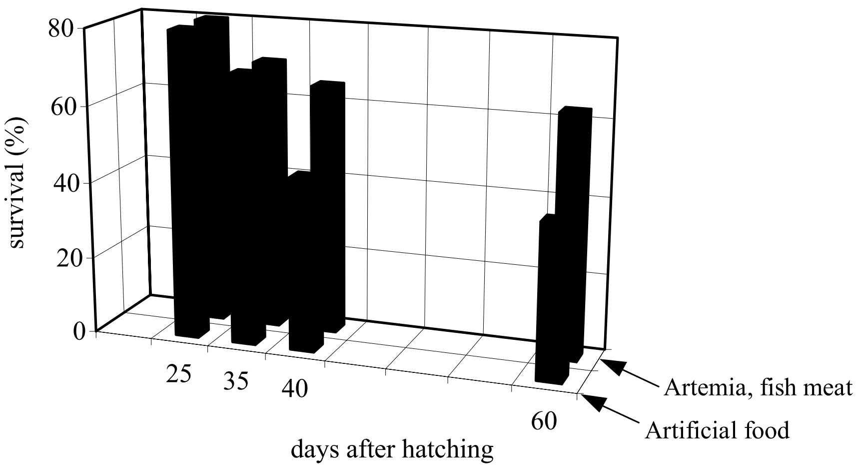

Figure 4. Survival rates of the Black Sea turbot from hatching to an age of 2 months using different kinds of foods during the second month:

- starting food for white fishes;
- artemia combined with fish meat.

combined with fish meat). These problems are supposed to be solved in a state turbot hatchery that is planned to be built in the Anapa district.

Practical Implementation

During the period 1992-1997, more than 165,000 juveniles of Black Sea turbot produced in the hatchery were released into the Black Sea, including 50,000 juveniles each year in 1995-1997. An increase in the number of juvenile turbot had been recorded in the Anapa bank area, the release site. Now the generations of 1993-1996 are estimated as abundant. It appeared that restoration of the Black Sea turbot population was possible through the release of hatchery-reared juvenile turbot into the sea.

References

Fauvel, C., Omnes, M.H., Suquet, M. and Normant, Y. 1992. Enhancement of the production of turbot, *Scophthalmus maximus* (L.) larvae by controlling overripening in mature females. Aquacult. Fish. Manage., 23: 209-216.

Jones, A., Alderson, R. and Howell, B.R. 1974. Progress toward the development of a successful rearing technique for larvae of the turbot, *Scophthalmus maximus* L. J.H.S. Blaxter (Ed.). The Early Life History of Fish. Springer-Verlag, Berlin. 731-737 pp.

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

Kuhlmann, D., Quantz, G. and Witt, U. 1981. Rearing of turbot (*Scophthalmus maximus* L.) on cultured food organisms and post metamorphosis growth on natural and artificial food. Aquaculture, 23: 183-196.

McEvoy, L.A. 1984. Ovulatory rhythms and overripening of eggs in cultivated turbot *Scophthalmus maximus* L. J. Fish. Biol., 24: 437-448.

McEvoy, L.A. 1989. Reproduction of turbot (*Scophthalmus maximus* L.) in captivity. Cuadernos de Area de Ciencias Marinas, Seminario de Estudios Galegos, 3: 9-28.

Minkoff, G. and Broadhurst, A.P. 1994. Intensive production of turbot, *Scophthalmus maximus*, fry. P. Lavens and R.A.M. Remmerswaal (Eds.). Turbot Culture: Problems and Prospects, European Aquaculture Society Special Publication, 22: 14-31.

Nicolas, J.L., Robic, E. and Asquer, D. 1989. Bacterial flora associated with a trophic chain consisting of microalgae, rotifers and turbot larvae: Influence of bacteria on larval survival. Aquaculture, 83: 237-248.

Person-Le Ruyet, J. 1989. The hatchery rearing of turbot larvae (*Scophthalmus maximus*). Cuadernos de Area de Ciencias Marinas, Seminario de Estudios Galegos, 3: 57-91.

Person-Le Ruyet, J., Baudin-Laurencin, F., Devauchelle, N., Metailler, R., Nicolas, J.L., Robin, J. and Guillaume, J. 1991. Culture of turbot (*Scophthalmus maximus*). L.A. McVey (Ed.). Handbook of Mariculture, Finfish Aquaculture, CRC Press Publication, Boston, Vol. II: 21-41.

Witt, U., Quantz, G. and Kuhlmann, D. 1984. Survival and growth of turbot larvae *Scophthalmus maximus* L. reared on different food organisms with special regard to long chain polyunsaturated fatty acids. Aquacult. Eng., 3: 177-190.