

Larval Rearing of the Black Sea Turbot, *Scophthalmus maximus* (Linnaeus, 1758), under Laboratory Conditions

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Abstract: To establish a seed production technique for Black Sea turbot, *Scophthalmus maximus*, eggs and larvae were observed under artificial rearing conditions.

Larvae were obtained artificially from eggs of tank-held broodstock. The egg fertilization was 27.6% and the fertilized eggs were 1.213±0.063 mm in diameter, were spherical pelagic and had one oil globule. From an initial length of 3.12±0.14 mm on day 0, the larvae grown to 167.28±15.32 mm in the normal group and to 159.98±12.25 mm in the abnormal group on day 246. The feeding regime consisted of *Nannochloropsis oculata*, *Brachionus plicatilis*, *Artemia*, and granule feed. The survival rates were 5.2% on day 60, and 4.59% on day 246. High mortality of larvae occurred within 15 days of hatching, during the transition from endogenous to exogenous feeding and from rotifer to *Artemia*-feeding.

The present study demonstrated that adult Black Sea turbot can be obtained from the wild, and from broodstock management and artificial spawning in captivity, and larval rearing can be achieved successfully.

Key Words: Black Sea turbot, *Scophthalmus maximus*, Survival Rate, Larval Rearing, Mortality

Laboratuar Koşullarında Karadeniz Kalkan Balığı, *Scophthalmus maximus* (Linnaeus, 1758), Larva Yetiştiriciliği

Özet: Karadenizdeki kalkan balığı, *Scophthalmus maximus*, yavru üretim tekniğini geliştirmek için yumurta ve larvalar yapay koşullar altında incelendi.

Larvalar tanklarda tutulan anaçlardan alınan yumurtalardan elde edildi. Yumurta döllenme oranı 27.6%, döllenmiş yumurtalar 1.213±0.063 mm çaplı, bir yağ damlacıklı, küresel ve pelajik özellikte idi. Yumurtadan çıktıktan sonra larvalar; ilk boyları 3.12±0.14 mm'den 246. günde normal grupta 167.28±15.32 mm, anormal grupta 159.98±12.25 mm'ye ulaştı. *Nannochloropsis oculata*, *Brachionus plicatilis*, *Artemia*, ve granül yemeden oluşan yemleme sistemi ile yaşama oranı 60. günde %5.20, 246. günde %4.59 olarak gerçekleşti.

Larval ölümler yumurtadan çıkıştan sonraki 15 gün içinde, iç beslenmeden dış beslenmeye ve rotifer ile beslenmeden *Artemia* ile beslenmeye geçiş sürecinde oldukça yükseldi.

Bu çalışma, yetişkin Karadeniz kalkanının doğadan temin edilebileceğini ve anaç bakımı, yapay yumurta alımı ve yavru üretiminin başarılı bir şekilde gerçekleştirilebileceğini göstermiştir.

Anahtar Sözcükler: Karadenizdeki kalkan balığı, *Scophthalmus maximus*, Yaşama Oranı, Larval Üretim, Ölüm Oranı

Introduction

Aquaculture in Turkey is restricted to the production of a small number of marine fish species (gilthead sea bream, sea bass, Atlantic salmon and rainbow trout), diversification is one of today's greatest challenges for further aquaculture development. This interest in diversification focuses on only a few species, such as turbot, flounder, mullet and blue fish (1) which play an important role in production.

Black Sea turbot, *Scophthalmus maximus*, which is

one of the most expensive table fish in Turkey, occurs in coastal waters in the Black Sea and grows up to 100 cm in total length, and 20 kg in body weight (2). Fish farmers wish to develop *S. maximus* culture, but artificial seed production techniques are not yet established.

In an attempt to establish a seed production technique for *S. maximus*, the author observed larval development and successfully produced a few thousands seeds with a size 16.7 cm in total length for the first time under tank conditions in Turkey.

Materials and Methods

Egg incubation

The broodstock of fish used during this investigation were collected from the estuarine waters (18‰ salinity) of the Black Sea coast near Trabzon using a bottom trawl during 1997 and 1998. Eggs from one female turbot were fertilized with the pooled sperm from two males on April 7, 1998. The eggs were incubated at a density of 300 eggs/l in a 350 l fiberglass cylindroconical tank with water at 18 ppt salinity and 15°C. The incubation tank was moderately aerated and the water was allowed to flow to achieve about a 200% daily water exchange. The density of hatchlings was estimated from five water samples taken from different sections of the tank using a 500 ml beaker. Larvae were counted with the naked eye, and estimated larval counts (ELC) were computed using the formula (3) $ELC = (\text{Number of larvae counted} / \text{Total volume of water samples}) * \text{Volume of hatching tank}$.

Larval rearing

The newly hatched larvae, 1 or 2 hours old, were stocked in an 8 m³ capacity raceway fiberglass indoor hatchery larval rearing tank at a stocking rate of 20 larvae/l. The tank bottom was plain and was not covered with sand or any other substrate. The seawater used in the hatchery was pre-treated using pressurized sand filters and a UV sterilization system. The larval rearing was carried out in the same tank throughout the larval rearing period of 60 days. Moderate aeration and green algae, *Nannochloropsis oculata*, were provided in the larval rearing tank after stocking with larvae (day 0). Water was changed initially on the fourth day and every day thereafter with an initial exchange rate of 50%/day. *Brachionus plicatilis* were introduced on day 2 when the larvae partly absorbed their yolk. The rotifer density in the larval rearing was maintained at 8-10 rot./ml from day 2 to day 14 and 3-5 rot./ml from day 15 to day 30 of the larval rearing period. *Brachionus plicatilis* density in the tank was monitored at 9.00 and 14.00 h and the amount adjusted to meet the required density to be maintained. During the *Brachionus*-feeding days, *Nannochloropsis* was added daily 5-10x10⁵ cells/ml as food for *Brachionus* and as water conditioner. From day 10 onwards, newly hatched *Artemia* nauplii were introduced into the tank. The number of *Artemia* nauplii was increased from 0.3 ind./ml on day 10 to 1.0 ind./ml on day 30 of the culture period. From day 30 onwards,

48-h-old *Artemia* metanauplii treated with 'Super Selco' were used. The amount of *Artemia* metanauplii in the larval rearing tank was gradually increased from 0.5 ind./ml on day 30 to 1.0 ind./ml on day 45. On day 25, larvae were gradually weaned over to granule feed (Ø 400 µm, Fry Feed KyowaB, Kyowa Hakko Co. Ltd., Tokyo, Japan), which contained about 55% crude protein, 10% crude fat, 13% crude ash, and 3% crude fiber on a dry basis. From day 25 onwards, the larvae were hand-fed with inert feed every 3 h to satiation. During this period, water exchange was increased to approximately 300%/day and then to 500%/day. Siphoning of the tank bottom to clean the sediments was initiated on day 4 and continued throughout the larval rearing period. From day 4, a triangular oil skimmer was used to skim the surface scum in the larval rearing tank. This feeding scheme and tank management are summarized in the Table.

Juvenile rearing

After all larvae had completely metamorphosed, on day 60, a total of 4840 juveniles were graded into two groups (normal fish and fish with color abnormalities) and replaced in the raceway fiberglass tanks of 8 m³ capacity, with an operating depth of 30 cm and bottom area of 8 m². The water flow rate was manually controlled to levels not exceeding 2.5 l/min. The juveniles were hand-fed to satiation four times during the light period and daily food consumption was recorded for each tank. The feed size was gradually increased from 0.3-0.5 mm to 0.8-1.2 mm and to 2.0 mm depending on juvenile size. Growth, expressed as specific growth rate (SGR, %/day), and feeding rate, as a percentage of body weight (F, %/day), over intervals between each weighing and for the entire experiment, were calculated using the formulas

$$SGR = 100 * [(\ln(W_t) - \ln(W_i)) / t],$$

$F = 100 * [(F_0 / (B_i + B_f) / 2) * t]$, where $\ln(W_t)$ and $\ln(W_i)$ are the natural logarithms of weight at time t and of the initial weight, respectively; t is the time intervals in days, F_0 food supply (g), and B_i and B_f initial and final biomass in the tank, respectively.

Larvae and juveniles were sampled periodically from the larval rearing tank, being anesthetized with 50 ppm ethylene glycol monophenyl ether for morphological observations and measurements. Values are given as mean ± standard deviation.

Days after hatching	0	5	10	15	20	25	30	35	40	45	50	55	60
Feeding scheme:													
Nannochloropsis sp.	—————												
Brachionus sp.	—————												
Artemia nauplii (newly hatched)	—————												
48 h old Artemia meta nauplii	—————												
Inert diet	—————→												
Tank management :													
Exchange rate	← 50% ← 100% ← 300% ← 500% →												
Siphoning of bottom	—————→												
Setting of oil skimmer	—————												

Table

Feeding and water management scheme in rearing *Scophthalmus maximus*.

Results

Egg incubation

Seawater temperature was constant in the egg incubation tank at 15°C. The egg fertilization rate was 27.6% and the fertilized eggs were 1.213 ± 0.063 mm in diameter, were spherical and pelagic and had one oil globule. Hatching occurred 5 days after spawning with a hatching rate of 74.5%.

Larval rearing

Water quality was fairly constant during the larval rearing period. Seawater temperature was between 17.2 and 23.3°C. Dissolved oxygen and pH values in the larval rearing tank were 6.34-8.37 mg/l and 7.75-8.37, respectively.

After hatching, the larvae were transferred to an 8.0 m³ raceway fiberglass tank at a density of 20 larvae/l. The newly hatched larval size ranged from 2.63 to 3.33 mm in total length with a mean of 3.12 ± 0.140 mm. The average size of the yolk sac was 926 µm with a 243 µm diameter oil globule. At hatching the larvae were inactive, and they lacked a functional mouth, gut and eyes. The eyes were unpigmented and the mouth and anus were not open until 3 days after hatching. During the embryonic phase the larvae were planktonic and were found approximately 10 cm from the water surface. The yolk and oil globule disappeared on day 4 after hatching. L-type rotifer *Brachionus plicatilis* sp. were introduced into the larval rearing tank on day 2 after hatching. By the first feeding, fish increased in length to 3.44 ± 0.262 mm

on day 3 and at the onset of exogenous feeding the gut was distinguishable as a straight tube and the larvae accepted the rotifer *Brachionus plicatilis* sp as first prey. The active feeding of larvae on rotifers increased from day 4 and the feeding rate reached 45% on day 4, and 100% on day 8. Swim bladder inflation occurred from day 5 to 8. More than 95% of the larvae had their swim bladder inflation by day 8. Larvae were fed with newly hatched and 48-h-old brine shrimp (*Artemia*) starting from day 10 to 45, and 19% of the larvae fed on *Artemia* on day 14, and micro-artificial commercial diet was given from day 25 to the end of rearing period.

Metamorphosis occurred from day 17 onwards when the larvae reached 7.58 ± 1.07 mm in length and all the larvae completed metamorphosis by day 60. During metamorphosis larvae became progressively flat and asymmetric. The migration of the right eye began 30 days after hatching. After 40 days after hatching, the right eye was on the top of the head and the larvae started to descend to the bottom of the tank.

Dead larvae, floating at the surface, were observed at the time of mouth opening and during the following few days. High mortality of larvae occurred 12 days after mouth opening, and during the transition from endogenous to exogenous-feeding and from rotifer to *Artemia*-feeding. The larval survival was approximately 64.52% at the onset of first feeding on day 5 and 12.47% at the transition from rotifer to *Artemia*-feeding on day 15. The survival rate decreased to 5.48% at the onset of the weaning period and became almost stable

from day 25 onwards. Larval survival was 5.2% at the end of larval rearing period on day 60 (Fig. 1). All larvae completed their transformation up to 60 days after hatching.

Juvenile rearing

Seawater temperature, dissolved oxygen and pH values in the juvenile rearing tanks were 12.3-23.8°C, 6.09-10.9 mg/l and 8.09-8.28, respectively. 52.3% of juveniles were normally pigmented, and the others (47.7%) had color abnormalities. From a total length of 33.38±4.62 mm on day 60, juveniles grew to 167.28±15.32 mm in the normal group, and to 159.98±12.25 mm in abnormal group (Fig. 2), and the mean wet weight increased from 0.55±0.30 g on day 60 to 86.35±26.84 g in the normal group, and to 70.17±18.91 g in abnormal group on day 246.

The length-weight relation during the rearing period was curvilinear (Fig. 3), showing that the Black sea turbot gain weight during the later part of their larval and juvenile stages.

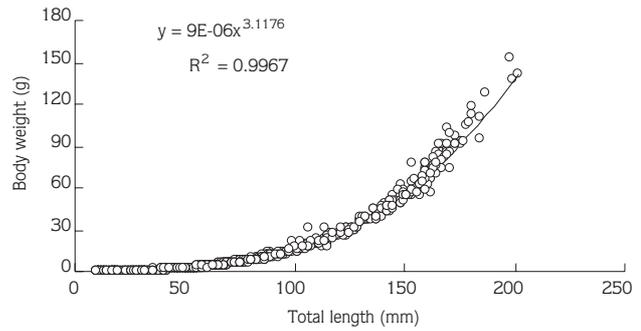


Fig. 3. Length-weight relationship of *Scophthalmus maximus*.

The mean specific growth rates (SGR) and feeding rates as a percentage of body weight (F) in normal and abnormal groups were 3.7% and 3.6% and 5.5% and 5.7%, respectively. The survival rate during the juvenile rearing period was almost stable. Only 0.61% of overall mortality occurred during this stage and the final survival rate was 4.59% on day 246.

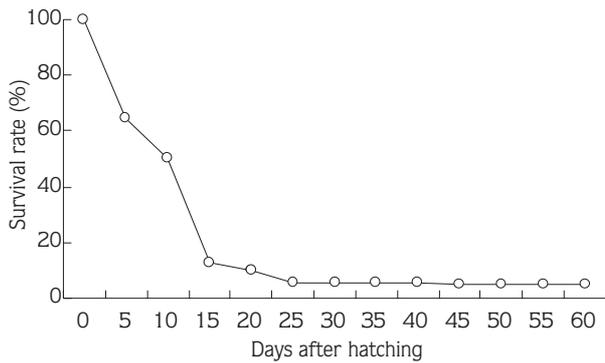


Fig. 1. Survival rate of *Scophthalmus maximus* during culture period.

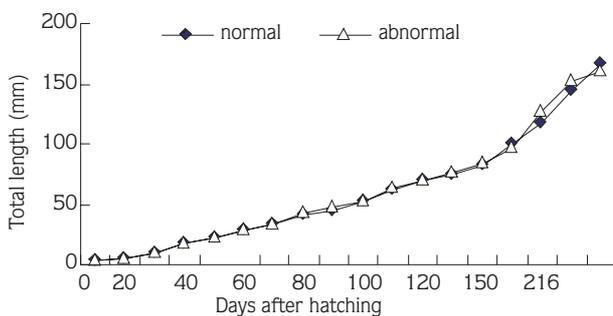


Fig. 2. Growth in length *Scophthalmus maximus* larvae and juveniles during culture period.

Discussion

In the experiment "green water" was used for rearing Black Sea turbot. The water was kept "green" with *Nannochloropsis* and larvae were fed enriched rotifers and *Artemia*. For turbot, green water improves the appetite, initial growth rate, survival and viability of the larvae/fry (3,4). Addition of algae might have multiple advantageous effects on tank environment which include: reduction in bacterial load in enrichment and culture (5), increased feeding due to turbidity (6), maintenance of rotifer nutritional value to the larvae (7), therapeutic properties (8).

Although fertilization was low, hatching rates and larval survival at first feeding (64.52% on day 5) were rather high in the experiment. Koya et al. (9) claimed that the quality of the egg and/or sperm affect the fertilization rate. The high hatching and larval survival that indicate the quality of eggs obtained from broodstock was good (10). Bromage et al. (11) suggest that three factors significantly affect egg quality: bacterial colonization of the egg surfaces, broodstock nutrition, and overripening of the egg.

Initial feeding is critical for successful rearing of marine fish. This phase of changeover from endogenous to exogenous energy sources is generally referred to as the critical period. During the initial phase of culture, high

mortality occurred within 15 days of hatching. Larval mortality that occurred prior to the absorption of the yolk could not be due to starvation but may be due to developmental problems related to egg quality (12). Mortality after larvae are competent to feed may be due to developmental problems or to starvation due to unfavorable feeding conditions such as the size of prey provided (12). Cunha and Planas (13) reported that the use of small rotifers significantly improves the initial feeding performance of turbot at the earlier developmental stages. The L-type rotifer used in this experiment may not be an optimally sized prey item for turbot at first feeding. Apart from prey size, as mentioned by Øie et al. (4), seawater temperature, the amount of turbulence and light, and the addition of algae to culture tanks also could affect the extent of success at first feeding.

The highest mortality peak in the hatchery phase was observed from day 10 to day 15, when the larvae started to feed on *Artemia*. The reasons for this mortality were multiple and resulted from inadequate nutrition as well as microbial infection (14). The source of bacterial infection can generally be traced back to the eggs and live food (15).

The survival of earlier larval rearing of turbot to metamorphosis was inconsistent. Robin (16) reported survival of 7.5-8.3-20.2% (using three different enrichment materials) on day 27, and Estévez et al. (17) obtained a mean survival of 30% on day 45. Olsen et al. (18) claimed that overall final survival for flatfish species after metamorphosis and weaning (e.g., day 80) is

normally in the range of 0-10%. According to Witt et al. (19), good average survival rates from hatching to weaning ranges between 5% and 10%. The present study yielded a survival rate of 5.20% on day 60, and 4.59% on day 246. These values are within normal ranges.

The assessment of fry quality has focused mainly on the appearance of malpigmentation in flatfish. Approximately half of the juveniles reared in this experiment had color abnormalities. Several authors (23-25) have reported that water exchange, aeration and illumination are among the culture factors that can induce malpigmentation in flatfish. For commercial and economic purposes, it is highly recommended to eliminate undesirable low quality larvae/juveniles. The occurrence of malpigmented juveniles can be reduced through the supplementation of prey enriched with fatty acids (22:6n-3; 20:4n-6), phospholipids and vitamin A (23).

From an economic point of view, the main variables in larval rearings are survival and growth. The growth rates obtained are probably not the maximum for Black Sea turbot. Higher feeding rates and rearing temperatures may result in higher growth rates of Black Sea turbot larvae and juveniles.

Although good progress was made concerning the larvae and juvenile culture of Black Sea turbot in this study, there are a number of areas that need further research, including establishment of optimum stocking density, environmental conditions, different rearing systems and satisfactory diets.

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