Turkish J. Marine Sciences 6:263-270 (2000)

Larval Rearing of Flounder, *Pleuronectes flesus luscus*, under Laboratory Conditions

Laboratuar Koşullarında Pisi Balığı, *Pleuronectes flesus luscus*, Yavru Üretimi

Temel Şahin

Central Fisheries Research Institute, Trabzon-Turkey

Abstract

To establish a seed production technique for flounder *Pleuronectes flesus luscus* the eggs and larvae were observed under artificial rearing conditions.
Larvae were obtained from eggs spawned artificially by tank-held broodstock. The egg fertilization rate varied from 35.3 to 59.2%. The fertilized eggs were approximately 1.15 mm in diameter, spherical, and without oil globule. From initial length of 2.7-3.1 mm on day 0, larvae grew to 10.4-14.0 mm on day 60. The feeding regime consisted of *Nannochloropsis, Brachionus, Artemia*, and granule feed. With this regime, survival rate were 9.6% at day 60.
Larval mortalities considerably increased within two days after hatching and during later part of the larval rearing period between day 8 and day 12 and between day 35 and day 40
The present study demonstrates that adult flounder can be obtained from wild and broodstock management and artificial spawning in captivity, and larval rearing can be achieved successfully.

Key words: Flounder, *Pleuronectes flesus luscus*, survival rate, Black Sea, larval rearing, mortality.

Introduction

As pollution and over-fishing have decimated both coastal and deep sea fish, the industry has increasingly turned to aquaculture and fish farming.

Fish culture activities began in Turkey with attempts to culture exotic species such as rainbow trout in the late 1960s, native species such as sea bass and sea bream in 1985. There are several native species, especially in the Black Sea, that may have considerable potential for commercial culture. Some of these species include flounder, turbot, sea trout, blue fish and red mullet (Çelikkale *et al.*, 1998) which are commonly found along the Black Sea coastline, and broodstock can be easily captured throughout the year.

Although other species belonging to *pleuronectidae* are commonly cultured in other countries, including species such as *P. olivaceus, P. californicus, P. microps, P. tropicus, P. woolmani* (Rosas *et al.*, 1999), available research on *Pleuronectes flesus luscus*, is limited by its population characteristics (Özdamar *et al.*, 1995; Güneş, 1997),

P. flesus luscus is one of the commercially important species frequently occurring along the near shore sandy bed area on the Black Sea coasts. It is also widely distributed in the northern and eastern part of Adriatic, and in the western parts of the Aegean Sea. Individuals of the species can reach 60 cm in total length (Fischer *et al.*, 1987), and previous studies have shown that there is a reproductive peak between December and January (Güneş, 1997).

The present work describes the results of research at Trabzon Central Fisheries Research Institute on aspects of larval rearing of the flounder.

Materials and Methods

Larvae were obtained from eggs spawned artificially by tank-held broodstock which were caught with a bottom trawl at the east of Trabzon (40°57'30" N, 39°51'42" E) from December 9 to 30, 1998.

Eggs were incubated at an average density of 250 eggs/l in 50 l polycarbonate tanks with water temperature of 13°C and constant aeration. One hour after stocking, aeration was withdrawn for a few minutes, the dead eggs allowed to settle and then subsequently removed. The density of hatching was estimated from four water samples taken from different sections of the tank using 500 ml beaker. Larvae were counted with naked eye, and estimated larval count (ELC) was computed using the formula

ELC=(Number of larvae counted/Total volume of water samples)*Volume of hatching tank.

Newly hatched (day 0) larvae were reared indoors in 500 l circular polyethylene tanks until metamorphosis at a stocking density of 20 larvae/l. Temperature, dissolved oxygen and pH values was measured twice a day. The water in the rearing tank was exchanged continuously and aerated with an air stone at a moderate rate.

Brachionus were introduced on day 3 and maintained at a density of 8-10 rot./ml until day 16. *Brachionus* density in the tank was monitored at 9.00 and 14.00 h and the amount adjusted to meet required density to be maintained. The amount was reduced to 3-5 rot./ml on day 17 upon addition of newly hatched *Artemia* nauplii until day 30. Increasing sizes of *Artemia* were given 0.5-1.0 ind./ml daily until day 42. On day 36, larvae were gradually weaned over to granule feed (400 μm) which contained about 55% crude protein, 10% crude fat, 13% crude ash, and 3% crude fiber on a dry basis. During the *Brachionus*-feeding days, *Nannochloropsis* were added daily 5-10x10^5 cells/ml as food for *Brachionus* and as water conditioner. This feeding scheme is summarised in Table 1.

Larvae were sampled periodically from the larval rearing tank, and were anaesthetised with 50 ppm ethylene glycol monophenyl ether for morphological observations and measurements.

Results

Water quality parameters were fairly constant during egg incubation and larval rearing period. Sea water temperature observed in egg incubation tank was 13°C and in the larval rearing tank it was 9.2 -12.6°C. Dissolved oxygen and pH values in the larval rearing tank varied 7.24-9.30 mg/l, and 7.75-8.37, respectively.

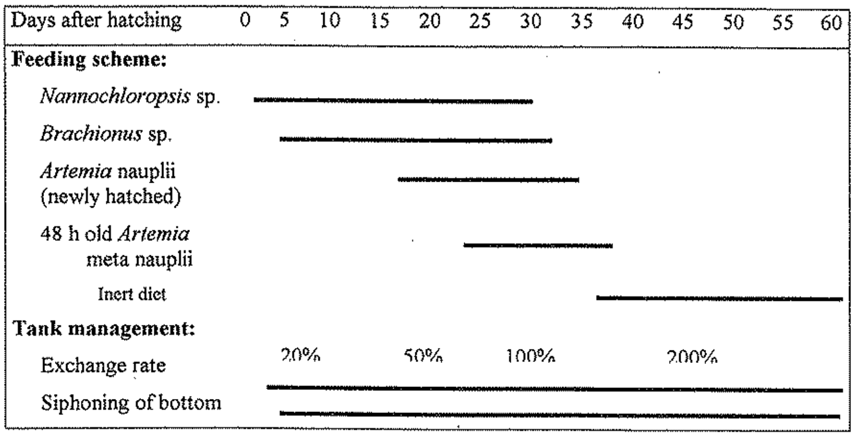

Table 1. Feeding and water management scheme in rearing *Pleuronectes flesus luscus* larvae.

The egg fertilization rate varied from 35.3 to 59.2%. The fertilized eggs were approximately 1.15 mm in diameter, spherical, and without oil globule. The egg development until hatching lasted for 6 days. After hatching, larvae were stocked at a density of 20 larvae/l in circular 500 l polyethylene tanks. The newly hatched larval size ranged from 2.7-3.2 mm in total length. The average size of yolk sac was 1.266 mm. The eyes were unpigmented, and the mouth and anus were not open until 4 days after hatching. L-type rotifers *Brachionus* sp. were introduced into larval rearing tanks at day 3 after hatching. By first feeding fish have increased in length to 4.0±0.1 mm on day 4 and larvae accepted rotifer, *Brachionus* sp. as first prey. The mouth width of flounder varied 152-173x10^{-3} mm at onset of feeding. On day 5 the yolk disappeared and feeding rate was 11%. The feeding rate reached 75% of total larvae number on day 9. Larvae were fed with newly hatched brine shrimp (*Artemia*) starting from day 17 to 42 and micro-artificial commercial diet from day 36 to the end of rearing period.

Larval mortalities considerably increased within two days after hatching and during later part of the larval rearing period between day 8 and day 12 and between day 35 and day 40 when the larvae were gradually weaned from feeding with *Artemia* metanauplii to inert formulated feed particles (Fig. 1).

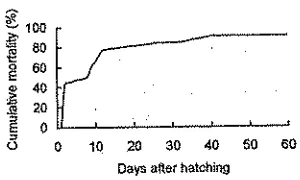

Fig. 1. Cumulative mortality of *Pleuronectes flesus luscus* larvae during 60 days of culture.

The growth was slow in the early stages, however, it accelerated after day 10 (Fig. 2). The total length reached to 10.4-14.0 mm and the larval survival was 9.6% on day 60.

The length-weight relation during the larval growth period was non-linear (Fig. 3) showing that the larvae gained weight during the later part of their larval stages.

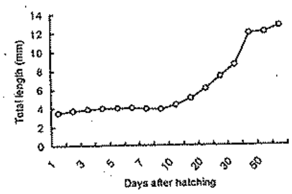

Fig. 2. Average growth in length *Pleuronectes flesus luscus* larvae during 60 days of culture.

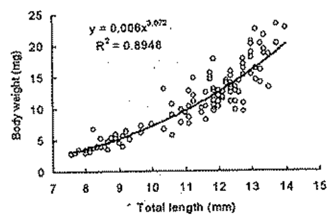

Fig. 3. Length-weight relationship of *Pleuronectes flesus luscus* larvae.

During the early stages, larvae were positively phototactic and aggregated in the bright areas of the larval rearing tank whenever the algal cell densities decreased in the tank due to active feeding of rotifers on *Nannochloropsis*. Maintenance of algal densities of 5-10x10^6 cells/ml was effective to reduce the phototactic behaviour and disperse the larvae in the tank.

The flounder larvae were nearly colourless and quite transparent. Approximately half of the larvae completed their transformation up to 60 days after hatching.

Discussion

The rates of fertilization, hatching, and larval survival at first feeding were low in the experiment. The low fertilization rate is thought to be due to the quality of the egg and/or sperm (Koya *et al.*, 1994). The low hatching and larval survival also indicated the quality of eggs obtained from the experimental broodstock was not good (Fernandéz-Palacios *et al.*, 1995).

Occurrences of high mortality during the intensive culture of flounder indicated that modifications are needed in several phases of the culture system. During the initial phase of culture, high mortality occurred within two days after just hatching. Larval mortality that occurred prior to absorption of yolk could not be due to starvation, but may be due to developmental problems related to egg quality. Induction of ovulation, stripping of gametes and artificial fertilization would be expected to result in inferior quality material compared to material collected from naturally spawning broodstock. Olsen *et al.* (1999) reported that initial larval mortality in indoor rearing systems is at times severe (>90%) during the first 2-3 days of feeding. Mortality is the most pronounced during the first week after initiating feeding. Further mortality beyond weeks 3-4 is in most cases related to inadequate nutritional or microbial condition (Olsen *et al.*, 1999). Successful first feeding, in which larvae make the transition from endogenous to exogenous nutrient supply, is critical to survival. Mortality after the larvae become competent to feed may be related to developmental problems (some morphological abnormalities was observed) or starvation due to unfavourable feeding conditions such as the size of prey provided (Burke *et al.*, 1999). The mouth width of flounder (152-173x10^{-3} mm) at onset of feeding was slightly smaller than turbot (200-230x10^{-3} mm: Moteki, 1999) and grouper (225-268x10^{-3} mm: Ordonio-Aguilar *et al.*, 1995). This suggests that the L-type rotifer may not be an optimal size prey item for flounder at the first feeding. An alternative first feed is the S-type *Brachionus*, which is smaller in size. Other factors such as temperature, the amount of turbulence, light and addition of algae to culture tanks (Oie *et al*., 1997) could affect the extent of success at first feeding.

Earlier larval rearing trials of other flatfish species to metamorphosis had limited success and survival was low and inconsistent. For turbot, *Scopthalmus maximus*, Robin (1998) reported survival of 7.5-8.3-20.2% (using three different enrichment materials) on day 27. Estévez *et al*. (1999) obtained mean survival of 30% on day 45. Smigielski (1975) found that in summer flounder, *Paralichthys dentatus* L., 90-95% of mortalities occurred within one week after hatching. Olsen *et al*. (1999) claimed that overall final survival for flatfish species after metamorphosis and weaning (e.g., day 80) is normally in the range of 0-10%. In this study, mortality during first week of hatching occurred 51% and the final survival of the flounder is within normal range (9.6% on day 60).

Bengtson (1999) showed that summer flounder undergoes the transition from a bilaterally symmetrical pelagic larva to an asymmetric settled juvenile beginning at about 35 days after hatching. The settlement of the flounder in this study has started on day 40, but not completed up to 60 days after hatching. Completion of transformation for other larvae may take at least two weeks more. The usual duration of settlement is about one month. Because settlement is very size-dependent (Schreiber and Specker, 1998), the extended settlement period is related to size differences among individuals. In addition, sea water temperature affects morphological development of larvae and consequently, their settlement period.

In summary, the present study demonstrated that adult flounder could be obtained from wild and broodstock management and artificial spawning in captivity could be achieved successfully. This experimental-scale information will be the key to the further development of this species for aquaculture. However, additional research is needed to identify the performance of different life stages in different rearing systems and to identify optimal and acceptable environmental conditions and satisfactory diets.

Özet

Pisi balığı *P. flesus luscus* yavru üretim tekniğini geliştirmek için yumurta ve larvalar yapay koşullar altında incelendi.
Larvalar tanklarda tutulan anaçlardan alınan yumurtalardan elde edildi. Yumurta döllenme oranı 35.3-59.2% arasında değişti. Döllenmiş yumurtalar yaklaşık olarak 1.15 mm, küresel ve yağ damlacığı yoktu. Yumurtadan çıktıktan sonra larvalar ilk boyları 2.7-3.1 mm'den 60. günde 10.4-14.0 mm'ye ulaştı. *Nannochloropsis, Brachionus, Artemia,* ve granül yemeden oluşan yemleme sistemi ile yaşama oranı 60. günde %9.6 olarak gerçekleşti.
Larval ölümler yumurtadan çıkıştan sonraki iki gün içinde, larval üretimin sonraki periyotları 8-12. günler ve 35-40. günler arasında oldukça yükseldi.
Bu çalışma, yetişkin pisi balığının doğadan temin edilebileceğini ve anaç bakımı, yapay yumurta alımı ve yavru üretiminin başarılı bir şekilde gerçekleştirilebileceğini göstermiştir.

References

Bengtson, D. A. (1999). Aquaculture of summer flounder (*Paralichthys dentatus*): status of knowledge, current research and future research priorities. *Aquaculture* 176:39-39.

Burke, J. S., Seikai, T., Tanaka, Y., Tanaka, M. (1999). Experimental intensive culture of summer flounder, *Parlichthys dentatus*. *Aquaculture* 176:135-144.

Çelikkale, M. S., Okumuş, İ., Kurtoğlu, İ. Z., Başçınar, N. (1998). The Present state and potential of coastal aquaculture in the Black Sea. The Proceedings of the First International Symposium on Fisheries and Ecology, 2-4 September 1998, Karadeniz Technical University Faculty of Marine Science, Trabzon.

Estévez, A., McEvoy, L. A., Bell, J. G., Sargent, J. R. (1999). Growth, survival, lipid composition and pigmentation of turbot (*Scopthalmus maximus*) larvae fed live-prey enriched in Arachidonic and Eicosapentaenoic acids. *Aquaculture* 180:321-343.

Fernández-Palacios, H., Izquierdo, M. S., Robaina, L., Salhi, M., Vergara, J. M. (1995). Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead sea bream (*Sparus aurata* L.). *Aquaculture* 132:325-337.

Fischer, W., Schneider, M., Bauchot, M.-L. (1987). Fishes FAO d'identification des especes pour les besoins de la peche. (Revision I). Mediterranee et mer Noire. Zone de Pêche 37. Vol. II, Vertebres, FAO and EC Project GCP/INT/422/EEC, FAO, Rome, pp. 1241-1243.

Güneş, E. (1997). Trabzon Kıyılarında Pisi (*Platichtys flesus luscus*) Balıklarının Bazı Populasyon Özellikleri Üzerine Bir Araştırma. Yüksek

Lisans Tezi, KTÜ Fen Bilimleri Enstitüsü, Balıkçılık Tekn. Müh. Anabilim Dalı, Trabzon, 45 p.

Koya, Y., Matsubara, T., Nakagava, T. (1994). Effect artificial fertilization method based on the ovulation cycle in Barfin flounder *Verasperi moseri*. *Fisheries Science* 60(5):537-540.

Moteki, M. (1999). Summary of development of Black Sea turbot changeover of energy sources. JICA Fish Culture Development Project in the Black Sea, pp. 7, Technical Report, Central Fish. Research Ins., Trabzon.

Oie, G., Makridis, P., Reitan, K. I., Olsen, Y. (1997). Protein and carbon utilization of rotifers (*Brachionus plicatilis*) in first feeding turbot larvae (*Scopthalmus maximus* L.). *Aquaculture* 153:103-122.

Olsen, Y., Evjemo, J. O. and Olsen, A. (1999). Status of the cultivation technology for production of Atlantic halibut (*Hippoglossus hippoglossus*) juveniles in Norway/Europe. *Aquaculture* 176:3-13.

Ordonio-Aguilar, R., Kohno, H., Ohno, A., Moteki, M., Taki, Y. (1995). Development of grouper, *Epinephelus coioides*, larvae during changeover of energy sources. *J. Toyo Univ. Fish.* 82:103-108.

Özdamar, E., Samsun, O., Erkoyuncu, İ. (1995). Karadeniz demersal türlerinden pisi balığında *Platichtys flesusu luscus*, (Pallas 1811) 1994-1995 av sezonu için bazı populasyon parametrelerinin tahmini. Doğu Anadolu Bölgesi II. Su Ürünleri Sempozyumu, 14-16 Haziran 1995, Atatürk Üniversitesi, Erzurum.

Robin, J. H. (1998). Use of borage oil in rotifer production and Artemia enrichment: effect on growth and survival of turbot (*Scopthalmus maximus*) larvae. *Aquaculture* 161:323-331.

Rosas, J., Arana, D., Cabrera, T., Millán, J., Jory, D. (1999). The potential use of Caribbean flounder *Paralichthsu tropicus* as an aquaculture species. *Aquaculture* 176:51-54

Scheiber, A. M., Specker, J. L. (1998). Metamorphosis in the summer flounder (*Paralichthys dentatus*): influence of stage-specific thyroidal status on larval development and growth. *Gen. Comp. Endocrinol.* 111:156-166.

Smigielski, A. S. (1975). Hormone-induced spawning of the summer flounder and rearing of the larvae in the laboratory. *Prog. Fish. Cult.* 37(1):3-8.

Received 30.6.2000
Accepted 05.07.2000