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Rearing of Larvae and Juveniles of Black Sea Turbot, *Psetta maxima,* in Turkey

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Abstract

Rearing techniques for the larvae of the Black Sea turbot *Psetta maxima* have been developed at the Central Fisheries Research Institute (CFRI) in Trabzon since 1997. Research programs have been focused on finding out optimum rearing conditions regarding to larvae and juvenile rearing technology. Black Sea turbot were kept in indoor flow-through tanks and fed live food, rotifers and *Artemia*, for 45 days from hatching. The number of juveniles (100 mm TL) produced at CFRI increased from 8,000 in 1998 to 27,000 in 2000 but dropped to 14,000 in 2001. Production failed in 1999 because of trouble with the water intake system. For the future, CFRI aims to (i) establish spawning techniques for hatchery-bred broodstock, (ii) improve larval survival, and (iii) formulate a high-quality artificial feed. A number of private companies in Turkey intend to invest in commercial production of turbot by the techniques developed at CFRI.

Key Words: turbot, *Psetta maxima*, rearing temperature, mortality, stocking density, deformities, Black Sea,

Introduction

The total marine catches from most of the main fishing areas in the Atlantic and Pacific Oceans and from the Mediterranean and Black Seas reached their maximum production levels one or two decades ago and are now stagnating, declining, or depleted. In the last 15 years, progress in science and technology has led to new ways of producing seawater species. Many fish farms have been established in the Mediterranean to produce sea bass *Dicentrarchus labrax*, gilthead sea bream *Sparus aurata*, and turbot *Scophthalmus maximus* or *Psetta maxima*. France, Spain and Portugal are all producers of these species, both from wild fisheries and through various forms of cultivation. Turbot are rarely exploited as the primary target species the whole year in any fishing area, hence they retain a high market value. Total production of turbot amounted to nearly 11,700 metric tons in 1999, with 35% coming from aquaculture. The contribution of aquaculture can be increased through cost-effective improvements in broodstock management and husbandry practices.

Marine fish hatcheries have been in Turkey since 1980 and produced sea bream and sea bass. Turbot was chosen for farming because of its market potential, and production economics. Research to determine the optimum breeding and larval rearing conditions for turbot has been initiated and coordinated by CFRI in the Black Sea. Two main problems had to be solved: (i) low larval survival and (ii) abnormalities in color and in the skeletal system. This paper summarizes the results of the larval and juvenile rearing experiments on the Black Sea turbot.

Larval Rearing

Eggs and larvae for stocking

Turbot eggs and larvae for hatchery operations were obtained by stripping and artificial fertilization of eggs from newly caught wild broodstock during the spawning season from April to May.

Incubation of eggs

The temperatures in the incubation tank was maintained at 15°C. Eggs usually hatch after 110 h at water temperatures of 15°C.

Stocking of larvae

Hatched larvae were transferred from the incubators to the rearing tanks. After stocking of larvae, the water temperature in the rearing tank was gradually increased from 15°C to 18 or 21°C in four days. Initial larval density in the rearing tanks ranged from 20,000 to 30,000/m^3.

Tanks and tank management

Larvae were reared in 2 m^3 oval or 3 m^3 rectangular tanks. After stocking of larvae, the water temperature in the tank was gradually increased from 15°C to 18 or 21°C in 4 days. Water was not changed during the first 3 days after hatching. On day 4, 30% of the water was changed and water exchange rate increased from 30% to 1.500% a day as the larvae grew over the next 45 days. The tank bottom was cleaned daily starting on day 5 to remove the dead and uneaten food, fecal matter, and other detritus. A skimmer was set up at the water surface to remove debris such as rotifer shells, oil, and proteinaceous waste.

The larval population was estimated at night or in the dark, when larvae were homogenously distributed in the rearing water. Samples of larvae were collected with a modified column sampler made of PVC pipe 1.5 m long and 50 mm in diameter at bottom. Larval survival was calculated from the number of fish in the samples and the volume of water, compared with those at stocking.

Feeding of larvae

Three food items were used for the larvae: enriched rotifer *Brachionus plicatilis*, enriched *Artemia* nauplii, and an artificial larval diet (Kyouwa Hakko Kogyo Co. Ltd.). Rotifers were enriched by feeding them *Nannochloropsis* sp. and Micro oil (Kyouwa Hakko Kogyo Co. Ltd.), rich in highly unsaturated fatty acids or HUFA. *Artemia* was enriched with Micro oil before feeding them to the turbot larvae. Larvae were fed rotifers twice a day, *Artemia* 1–2 times a day, and artificial diet 2–5 times a day. *Nannnochloropsis* sp. was added to the rearing tank twice a day to maintain a density of 0.5 x 106 cells/*ml* (Figure 1).

Growth

Figure 2 shows the growth of hatchery-reared Black Sea turbot from hatching to day 45 in a 2 m^3 oval tank at water temperature of 21°C (achieved with a heating system). Larvae grew to mean total lengths of 4.5 mm at day 10, 7.8 mm at day 20, 11.3 mm at day 30, and 21.9 mm TL at day 45.

Relation of larval survival to water temperature

To know the optimum water temperature for turbot larvae from hatching to day 10, rearing trials were done at 15, 18, and 21°C. Newly hatched larvae were stocked 300 in each 30-liter tank immersed in a water bath with electronically controlled water temperature. Larvae were fed enriched rotifers (density 5/*ml*) on days 3-10, and 50% of the water was changed every day starting day 4. The experiment showed that the larval survival at 21°C was

Days	3-5	6-11	12-15	16-17	18-20	21	22-23	24-25	26-29	30-40	n+1
Nanno (cells/*ml*)				0.5 x 10^6							
Enriched rotifer (ind./*ml*)	2	5	5	5	5	4	3	2			
Artemia nauplii (ind./*ml*)			0.2	0.1							
Enriched *Artemia* (ind./*ml*)				0.1	0.4	0.4	0.4	0.4	0.4	0.4	
			Particle size (µm)				250	400	700		
Artificial feed			Quantity (g/10x10^3 larvae)				10	15	18		

Figure 1. Feeding regime for Black Sea turbot larvae reared in the hatchery.

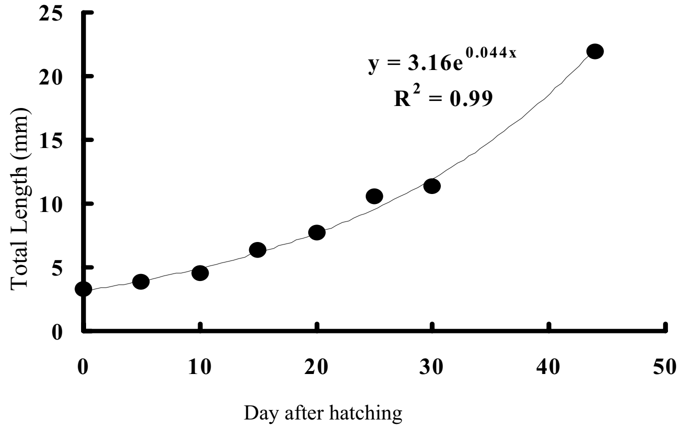

Figure 2. Growth of Black Sea turbot larvae from hatching to D-45 in a 2 m^3 tank.

significantly higher ($p<0.05$) than at the lower temperatures (Figure 3); thus the optimum rearing temperature for turbot larvae may be 21°C. Studies must also be done to determine the relation between water temperature and the incidence of deformities in larvae.

Larval survival for production

The larval rearing experiments on Black Sea turbot were carried out from 1998 to 2001 and juveniles about 20 mm total length during metamorphosis were harvested at day 40 . The survival of larvae to 20 mm averaged 4.6% (n=6) in 1998, 7.9% (n=3) in 1999, 7.3% (n=10) in 2000, and 4.0% (n=5) in 2001.

Results of the larval rearing runs in 2000 are shown in Table 1. Survival rates varied from 1.2 to 20.6% and only 3 of 10 trials achieved survival rates more than 10%. There were three typical mortality patterns during the experiments (Figure 4). One pattern (Type A) involved high mortality from day 2 wherein the larvae all died or only a small percentage survived until day 10. This pattern was seen in 52.8% of all rearing trials. In the second pattern (Type B), high mortality started after day 5 and lasted till day 10. This pattern resulted in very poor survival rates in 19.4% of the rearing trials. In the Type C pattern, the survival was stable until day 10 and larvae started to die after day 10 and until day 25. In this case, survival of larvae to 20 mm TL was usually good.

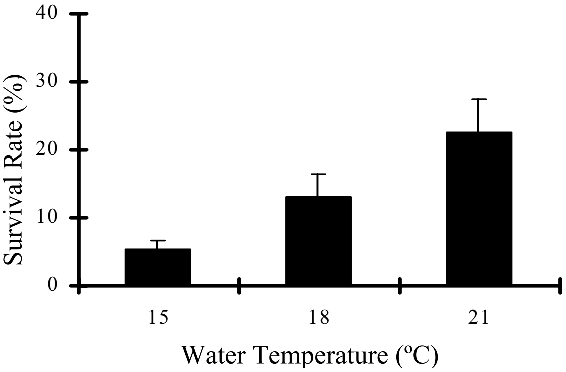

Figure 3. Relation between the temperature and the survival rates (%) of Black Sea turbot larvae from hatching to Day 10. Vertical bars on the graph indicate standard error of mean survival rate (n = 4). Different letters on the standard errors differ significantly ($p<0.05$).

Table 1. Results of larval rearing trials for Black Sea turbot in 2000.

Trial No.	No. of larvae stocked (x 10^3 ind.)	Tank capacity (m^3)	Rearing water temperature (°C)	No. of larvae harvested at 20 mmTL	Survival (%)
1	66.5	2.0	21.0	820	1.2
2	44.0	2.0	21.0	7,800	17.7
3	35.0	2.0	21.0	1,250	3.6
4	35.0	2.0	21.0	3,750	10.7
5	35.0	2.0	18.0	1,250	3.6
6	35.0	2.0	18.0	7,200	20.6
7	29.0	3.0	21.0	2,400	8.3
8	22.5	2.0	21.0	380	1.7
9	47.0	3.0	21.0	630	1.3
10	35.0	2.0	21.0	1,500	4.3
Total	384.0			26,980	7.0

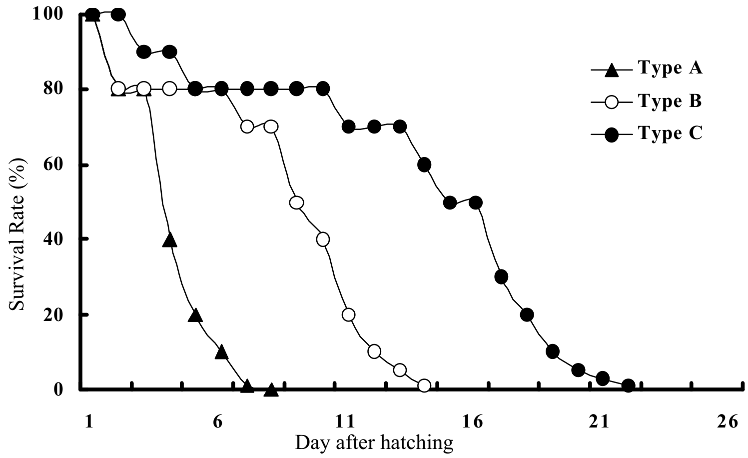

Figure 4. Three typical mortality patterns of larval Black Sea turbot during the hatchery operations in 2000 – 2001.

Resolving the main reasons for high larval mortality during the critical stages will increase hatchery output. According to Minkoff and Broadhurst (1994), egg and larval quality is the important factor that affects survival up to day 9. However, our hatchery trials included one with a low fertilization rate of only 19.0% but with the second highest larval survival of 17.7%. Therefore, egg quality is not a definitive factor for larval mortality but must be studied further. Survival certainly depends on the successful transition to exogenous feeding during the first week. After day 9 onwards, mortalities are generally associated with live food nutrition, hygiene, and physico-chemical conditions of the rearing water. The critical periods seen in larval turbot over the first 25 days of life correspond to the transition from the primitive basic modes to the stable more functional modes of swimming and feeding (Kohno *et al*., 2001).

Nursery of Juveniles

Stocking density experiment

To know the optimum stocking density for juvenile turbot, a 45-day growth experiment was conducted in 200-liter tanks with surface area 0.4 m^2. Turbot of about 50 mm TL were stocked in triplicate at four densities: 100, 188, 275 and 364 fish/m^2 . Artificial diet with particle size 2-3 mm diameter and 48% crude protein (Ecobio Co. Ltd) was fed to the turbot 3-4 times a day. Water exchange was 2.000% of the rearing volume every day. The experiment showed that feed intake declined significantly with stocking density. Significantly higher feed efficiency was obtained at stocking densities of 275 and 364 fish/m^2, and significantly higher specific growth rate at 188 and 275 fish/m^2 (Table 2). From polynomial analysis, the optimum stocking density in terms of feed efficiency was 250 fish/m^2, but in terms of growth, 350 fish/m^2 was still reasonable.

Survival

Hatchery-reared turbot 20 mm TL and about 40 days old were nursed in 2 m^3 tanks until they reached 100 mm TL or age about 110 days. Artificial diet with particle size 2-3 mm diameter and 48% crude protein (Ecobio Co. Ltd) was given to the turbot at 2-4% of the fish weight daily. Water exchange rate was 2.000% per day. Survival rates from 20 mm TL to 100 mm TL averaged 35% in 1998, 82% in 2000, and 83% in 2001. Table 3 shows that survival of turbot in the nursery varied from 56.2 to 95.8% in 2000.

Table 2. Percentage survival, final body weight (FBW), feed efficiency (FE), feed intake rate (FIR) and specific growth rate (SGR) of juvenile Black Sea turbot reared with different stocking density for 45 days.

Stocking density (fish/m^2)	Survival (%)	FBW (g)	FE^1	FIR^2 (%)	SGR^3 (%)
100	100/NS	15.4b	166b	1.81a	3.64b
188	93	17.7a	187ab	1.69ab	3.94a
275	92	17.6a	208a	1.51b	3.93a
364*	94	16.7ab	207a	1.49b	3.81ab

Values are means of three replicate groups. Means with different letter in the same column differ significantly ($p<0.05$).

[1] Feed conversion efficiency = 100 x weight gain (g)/total feed intake in dry basis (g).

[2] Feed intake rate = 400 x total feed intake in dry basis / (W_0+Wt) (N_0+Nt) x 42.

w_0 : mean body weight at start, w_t : mean body at end.

N_0 : number of fish at start, N_t : number of fish at end.

[3] Specific growth rate = 100 x [ln (mean final weight) - ln (mean initial weight)]/42.

* Duplicate

Table 3. Results of juvenile rearing for Black Sea turbot in 2000.

Trial No.	No. of larvae stocked (20 mmTL)	No.of tanks used	No. of juveniles harvested (100 mmTL)	Survival (%)	Classification		
					Euplastic	Mal-pigmented	Deformed
1	820	1	744	90.7	39.5	31.2	29.3
2	7,800	6	5,967	76.5	57.3	28.4	14.3
3	1,250	1	749	59.9	59.0	37.5	3.5
4	3,750	4	3,137	83.7	69.7	28.6	1.7
5	1,250	1	807	64.6	85.8	11.8	2.4
6	7,200	7	6,897	95.8	69.7	28.6	1.7
7	2,400	2	1,968	82.0	83.3	16.0	0.7
8	380	1	333	87.6	89.5	7.2	3.3
9	630	1	354	56.2	91.8	2.5	5.6
10	1,500	1	1,129	75.3	84.0	16.0	0.0
Total	26,980		22,085	81.9	68.2	25.8	6.0

Deformities

Juvenile turbot of 100 mm TL harvested from the nursery included four distinguishable groups: euplastic fish , malpigmented fish, deformed fish. In the 2,000 nursery runs, euplastic fish made up 68.2% of the total, malpigmented fish, 25.8%, and deformed fish 6.0% (Table 4). Malpigmented and deformed fish included: fish pigmented on both sides, fish that were white on both sides, fish whose eyes migrated only partially or not at all, fish metamorphosing on to the right side rather than the left side, and skeletal deformities .

The main cause of malpigmentation is said to be nutritional factors during early larval rearing (Minkoff and Broadhurst, 1994). Most turbot hatcheries have trouble with pigmentation of the larvae during the initial years of production. This problem has been solved by enrichment of larval prey with the highly unsaturated fatty acid DHA (Docosa Hexaenic Acid), phospholipids, and vitamin A (Olsen, 1997; Planas and Cunha, 1999). In our trials, the rotifers and *Artemia* were enriched with HUFA-rich oil before feeding them to larvae. Studies on nutrition and other factors are necessary to solve the malpigmentation problem in turbot.

Conclusion

Development of larval rearing techniques in the hatchery and juvenile rearing techniques for Black Sea turbot was started in Turkey in 1997 because of the demand for alternative farmed species. Optimization of rearing conditions allows for more reliable and cost-effective production of turbot larvae. Further investigations will focus on: (i) establishing spawning techniques for hatchery-bred broodstock, (ii) improving larval survival, and (iii) developing a high-quality artificial feed.

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