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The External Abnormalities of Hatchery-Reared Black Sea Flounder (*Platichthys flesus luscus* Pallas, 1814) in Turkey

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Abstract

This study was designed to describe the occurence of external abnormalities in Black Sea flounder (*Platichthys flesus luscus*) reared in captivity. In the present study, a total of 2187 juvenile fish were examined. Eight hundred eighty eight *Platichthys flesus luscus* had one or more of the following conditions: reversed (sinistral), malpigmentation, malformed jaw, incomplete eye migration. Average total length and body weight of abnormal juveniles were significantly lower than that of normal juveniles ($P < 0.01$). During the intensive rearing of flounder, upper jaw deformities (55%) were the most commonly observed types of deformation. Aproximately 1.9% of deformed juveniles had incomplete eye migration which acquired the lowest total length and body weight. More than half (54.9%) of the individuals analyzed showed more than one deformity.

Keywords: Flounder, *Platichthys flesus luscus*, abnormality, malpigmentation.

Türkiye'de Kuluçkahanede Yetiştilen Karadeniz Pisi Balığında (*Platichthys flesus luscus* Pallas, 1814) Görülen Dış Anormallikler

Özet

Bu çalışma kuluçkahanede yetiştirilen Karadeniz pisi balığında (*Platichthys flesus luscus* Pallas, 1811) görülen dış anormallikleri tanımlamak için yapılmıştır. Çalışmada toplam 2187 genç birey incelendi. Aşağıda belirtilen anormalliklerden bir veya birden fazlası 888 bireyde saptanmıştır; ters (sinistral), kusurlu çene, tamamlanmamış göz göçü, renk bozukluğu. Ortalama toplam boy ve ağırlık değerleri anormal bireylerde normal bireylerden oldukça düşük gerçekleşmiştir (P<0,01). Pisi balığının yoğun yetiştiriciliğinde üst çene deformasyonları (%55) en yaygın anormallik olarak gözlenmiştir. Anormal bireylerin %1,9'unu oluşturan tamamlanmamış göz göçü anormalliğine sahip bireyler en düşük boy ve ağırlığa ulaşmıştır. İncelenen bireylerin yarıdan fazlasında (%54,9) birden fazla deformasyon görülmüştür.

Anahtar Kelimeler: Pisi balığı, *Platichthys flesus luscus*, anormallik, renk kusuru.

Introduction

The flounder (*Platichthys flesus luscus*) is a marine species recently adapted to aquaculture with high commercial value in the west of Turkey and in the Mediterranean basin. In Turkey, the first attempts to reproduce flounder under controlled conditions started in 1998 (Şahin, 2000) and since then considerable progress in induced reproduction has been made in recent years (Şahin *et al*., 2008; Aydin *et al*., 2011), larval rearing techniques are not yet well developed and the juvenile may show numerous anatomical defects such as skeletal deformities (jaw and opercular complex), impaired eye migration and malpigmentation.

In aquaculture, deformities are main problems, as they reduce the market value of the produced fish by affecting their external morphology, growth and survival (Kitajima *et al*., 1994; Koumoundouros *et al*., 1997, Boglione *et al*., 2001;Gavaia *et al*., 2002; Haga *et al*., 2002; Imsland *et al*., 2006; Le Vay *et al*., 2007; Puvanendran *et al*., 2009) and they lower the performances of hatchery-reared fish such as swimming ability, feed consumption, conversion index, and susceptibility to pathogens (Boglione *et al*., 2001; Imsland *et al*., 2006). Fish deformities have been resulted from nutritional deficiencies, environmental factors, rearing conditions and genetic factors (Estevez and Kanazawa, 1995; Divanach *et al*., 1997; Haaparanta *et al*., 1997; Dedi *et al*., 1998; Favaloro and Mazzola, 2000; Koumoundouros *et al*., 2001; Gavaia *et al*., 2002; Lewis *et al*., 2004).

Abnormalities in some fish species are reduced with farming practices such as the implementation of appropriate diets and optimized environmental condition (Fjelldal *et al*., 2005, 2006; Helland *et al*., 2009; Haga *et al*., 2011).

According to Bruno and Fraser (1988) reversal, an unusual rotation of the eyes, is extremely rare in the dextral flounder family *Pleuronectidae* and regarded as an abnormality in most species. Lacking data on external abnormalities in *Platichthys flesus luscus*, the purpose of this survey was to identify and describe the externally visible skin, eye and skeletal disorders in reared black sea flounder juveniles.

Materials and Methods

The broodstock of fish used during this investigation were collected from the estuarine waters of the Black Sea coast near Trabzon using a bottom trawl. Eggs from one female flounder were fertilized with the pooled sperm from two males. Approximately 50,000 eggs were incubated at a density of 100 eggs/l in ten 50 l fiberglass tanks with water at 18‰ salinity and 14ºC. The incubation tank was moderately aerated and the water was allowed to flow to achieve about a 1000% daily water exchange.

A total of 30,000 newly hatched larvae (74,7% hatching rate) were stocked in three 500 l capacity fiberglass indoor hatchery larval rearing tanks at a stocking rate of 20 larvae/l. After stocking, the water temperature was gradually increased from 14°C to 19°C in 10 days. It was keept at 19°C for larval and juvenile stage. The seawater used in the hatchery was pre-treated using pressurized sand filters and a UV sterilization system. The rearing was carried out in the same tanks throughout the rearing period of 130 days. Photoperiod was applied at 12L:12D with 300 lux light intensity. Moderate aeration and green algae, *Nannochloropsis oculata*, were provided in the larval rearing tanks 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* was introduced on day 4 when the larvae partly absorbed their yolk. The rotifer density in the larval rearing was maintained at 5 rotifer/ml from day 4 to day 25 the larval rearing period. During the rotifer feeding days, *Nannochloropsis* was added daily 0.5 million cells/ml as food for *Brachionus* and as water conditioner. From day 13 onwards, newly hatched *Artemia* nauplii were introduced into the tank. The number of *Artemia* nauplii was 0.2 ind./ml from day 13 to day 33 of the culture period. Artemia metanauplii enriched with 'Red Pepper' (Bern Aqua, Belgium) were used 0.2 individual/ml from day 20 to day 45 in the larval rearing tank. On day 25, larvae were gradually weaned over to granule feed (Caviar, Bern Aqua, Belgium). 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 15 and continued throughout the larval rearing period. Metamorphosis started 30 days post hatching.

On day 130, a total of 2187 juveniles (7,29% final survival rate) were sampled from the rearing tank, anesthetized with 50 ppm ethylene glycol monophenyl ether for morphological observations and measurements. All juveniles were assessed for malformation of operculum and jaw, abnormal pigmentation, incompleted eye-migration and reversed eye migration for flounder *Platichthys flesus luscus.*

Data were tested for normal distribution using Kolmogorov-Smirnov. Homogeneity of variances was tested using Levene's F-test (15). Growth data were compared with parametric analysis using a Student t-test. Relationship among deformities was evaluated with a Spearman Rank Order test. Statistical analyses were carried out by Statistica 7 (Stat Soft. Inc. Tulsa, Oklahoma, USA)

Results

A total of 2187 individuals were measured, observed and photographed on 130 day post hatching. The frequency of the different types of abnormalities observed during the juvenile rearing season 2008 were presented in Table 1 and Figure 1. A large number of deformities were observed in the individuals used for this study. The deformities were externally apparent and were identified as fallows:

<u>Normal:</u> juvenile completes eye migration from the left to the right side of the body (dextral). The pigmentation pattern of the ocular side was similar to that of the adults (the upper surface was usually dull brown or greenish dark brown, in colour with reddish-brown blotches (Figure 1a) and the blind side was white, (Figure 1b)).

<u>Sinistral form (reversed juvenile)</u>: the right eye migrates to the left side (Figure 1c).

<u>Ocular side malpigmentation (OSMP)</u>**:** Irregular patches of pigment and discrete dark areas develop on the ocular side (Figure 1d). No pigmentation (Figure 1e) and malpigmentation (Figure 1f) was observed in the ocular side of the body.

<u>Blind side pigmentation (BSP)</u>**:** Present of pigmentation in the ocular side of the body (Figure 1 g).

<u>Malforme upper jaw (MUJ)</u>: The upper jaw was shortened along with a reduction or deformation of the dentary, maxillary and palatine (Figure 1h).

<u>Ocular side operculum (OSO)</u>**:** Shortening of the distal part of the operculum in the ocular side of the body (Figure 1i).

Table 1. Total length and weight of normal and abnormal flounder juveniles *P. flesus luscus* on 130 DAH (DAH: Days after hatching, OSMP: Ocular side malpigmentation, BSP: Blind side pigmentation, MUJ: Malformed upper jaw, OSO: Ocular side operculum, BSO: Blind side operculum, IEM: Incomplete eye migration, TL: Total length, W: Body weight, SD: Standart deviation)

Type of juveniles	n	%	TL±SD	W±SD
Normal (dextral)	1299	59.4	56.8 ± 0.33^a	2581.7 ± 52.43^a
Abnormal	888	40.6	48.7 ± 0.37^b	1675.9 ± 42.54^b
Sinistral	263	29.6	58.3 ± 0.38	2910.2 ± 67.42
OSMP	244	27.5	49.1 ± 0.71	1793.4 ± 88.89
BSP	69	7.8	46.7 ± 1.19	1554.5 ± 129.54
MUJ	488	55.0	48.3 ± 0.50	1598.8 ± 58.20
OSO	156	17.6	48.1 ± 0.89	1607.7 ± 106.38
BSO	188	21.2	49.6 ± 0.76	1744.4 ± 87.83
IEM	17	1.9	45.7 ± 0.85	1535.8 ± 93.41

Blind side operculum (BSO)**:** Shortening of the distal part of the operculum in the blind side of the body (Figure 1j).

Incomplete eye migration (IEM)**:** The left eye were detected from the right side of the body. The eye migration route over the skull was unfilled, leading to a strong hooking of the anterior part of the dorsal fin (Figure 1k).

While a total of 1299 (59,4%) juveniles had normally external formation, 888 specimens (40.6%) showed different types of deformities. Average total length and body weight of abnormal juveniles were significantly lower than that of normal juveniles ($P<0.01$) (Table 1). 263 (29.6%) juveniles had eyes on the left side of the body (sinistral form). About 488 (55.0%) of the deformed fish had upper jaw deformities which was the highest occurence of malformation. Approximately, 1.9% of deformed juveniles had incomplete eye migration which acquired the lowest total length and body weight (Table 1).

Number of deformities ranged from one to six in one fish. More than half (54.9%) of the observed individuals showed more than one deformity while 45.1% of the individuals presented only one deformity. Less than one percent (0.3%) of fish showed six different tipe anomalies (Figure 2).

Relationships between abnormalities in hatchery-reared flounder assessed with the Spearman Rank Order test were exhibited in Table 2. There was a positive correlation between abnormality in ocular side pigmentation and incomplete eye migration ($r = 0.18$), but a negative correlation between ocular and blind side pigmentation ($r = -0.11$), and upper jaw malformation ($r = -0.38$). Incomplete eye migration showed a positive correlation with ocular ($r = 0.18$) and blind side pigmentation ($r = 0.23$), but a negative correlation with upper jaw malformation ($r = -0.15$), ocular ($r = -0.13$) and blind side operculum ($r = -0.13$).

Discussion

Deformities are observed commonly in hatchery-reared flatfishes and are believed to develop during metamorphosis (Seikai *et al*., 1987). In this study, results showed an overall incidence of deformities of 40,6% in hatchery-reared fish. The average total length and body weight of abnormal juveniles were significantly lower than that of normal juveniles, probably because of low feed intake. In the present study, several types of abnormalities including malpigmentation, failure of eye migration, abnormal jaws and deforme operculum were observed.

Reversal asymmetry is common in reared either dextral or sinistral flatfish. Bisbal and Bengtson (1993) found that 4.4% of the 1295 laboratory-reared summer flounder exhibited the reversed condition. In some pleuronectid species, such as *Pleuronectes herzensteini*, *Pleuronectes yokohamae*, *Pleuronichthys cornutus* and *Verasper variegatus*, reversals have been frequently observed in hatchery-reared individuals, although rarely seen in wild fishes (Aritaki and Seikai, 2004). In the left-eyed flounder *P. lethostigma*, abnormal eye migration constituted 0.5-10% in fingerling reductions (Benetti *et al*., 2001). The flounder *P. flesus luscus* is a right-eyed (dextral) meaning that the right side is normally the ocular side after metamorphosis. Sinistral form of *P. flesus luscus* juveniles represented as 29,6% in this research. The causes of reverse phenomenon are not known (López *et al*., 2009).

Unusual pigmentation generally occures in juvenile flat fish culture. Ocular side malpigmentation have been observed from 24.5% to 100% in hatchery reared Japanese flounder (*Paralichthys olivaceus*) (Seikai et al., 1987) and from 1% to 35.6 in Atlantic halibut (*Hippoglossus hippoglossus*) (Næss and Lie, 1998) and from 1% to 5% in Chilean flounder, (*Paralichthys adspersus*) (Silva, 2001). Nearly 100% malpigmented fish have been observed in tank cultured *Paralichthys dentatus* and *Paralichthys lethostigma* (Stickney and White, 1975). Blind side pigmentation have been occured 95% in hatchery reared Japanese flounder (*Paralichthys olivaceus*) (Tominaga and Watanabe, 1998) and from 0.8% to 12.8% in Atlantic halibut (*Hippoglossus hippoglossus*) (Næss and Lie, 1998). In the present study, juveniles represented more than 35% OSMP and about 7% BSMP. Seikai (1998) and Takeuchi

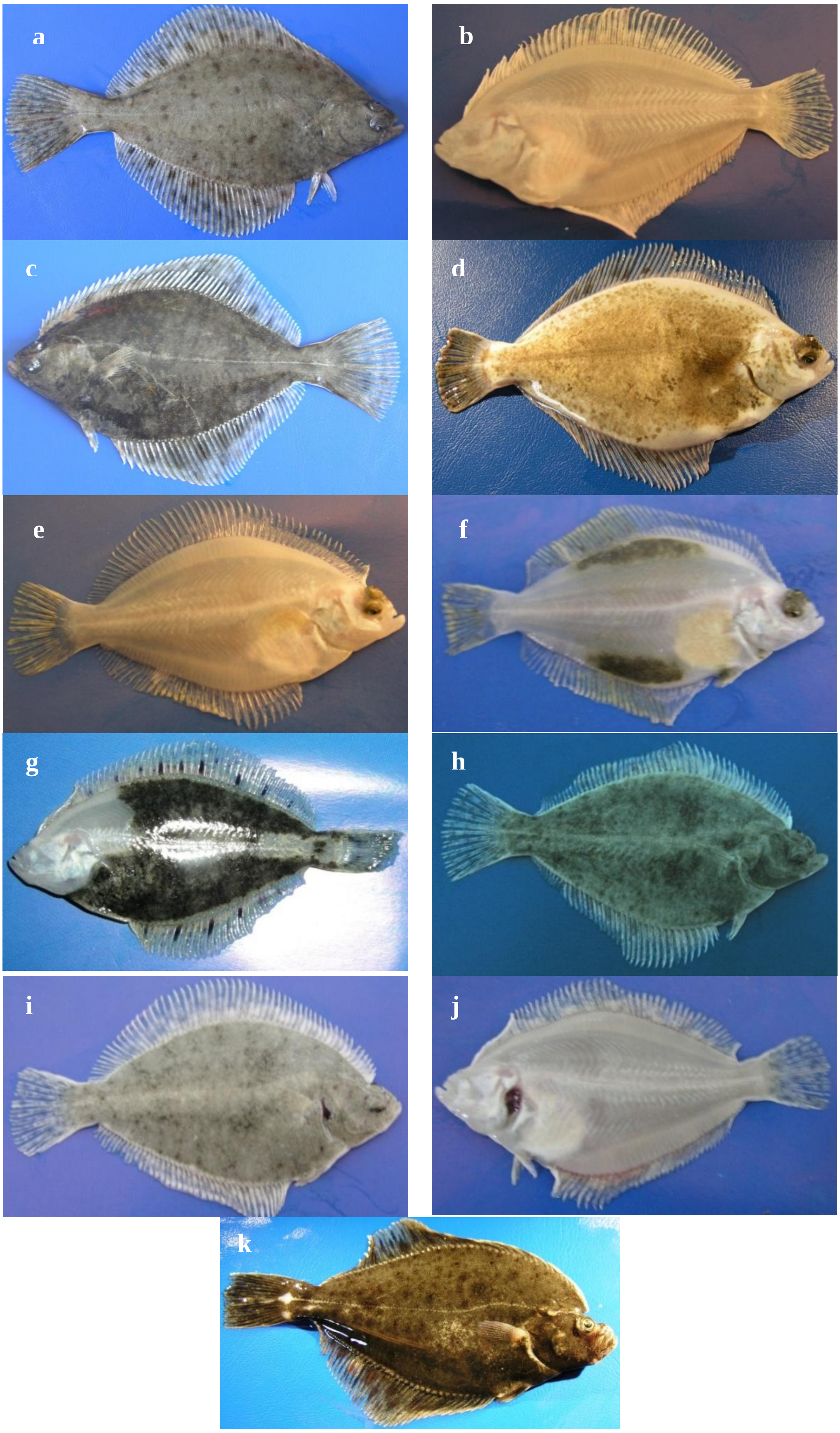

Figure 1. Types of abnormalities observed during 2008 juvenile production of *P. flesus luscus* a, b: normally pigmented juvenile (a: ocular side, b: blind side); c: Sinistral form (reversed juvenile); d, e, f: ocular side malpigmentation (OSMP); g: blind side pigmentation (BSP); h: malformed upper jaw (MUJ); i: ocular side operculum (OSO); j: blind side operculum; k: incomplete eye migration (IEM).

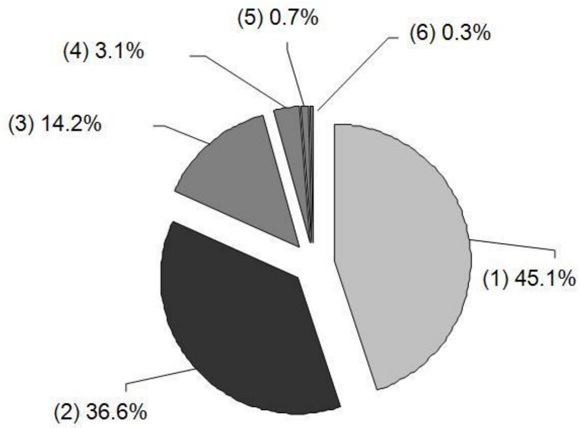

Figure 2. Incidence of abnormalities (in paranthesis) observed during 2008 juvenile production of *P. flesus luscus*. More than half (54.9%) of the individuals analyzed showed more than one deformity while 45.1% of the individuals presented only one deformity.

Table 2. Relationship between abnormalities seen in flounder juveniles *P. flesus luscus* on 130 DAH (DAH: Days after hatching, OSMP: Ocular side malpigmentation, BSP: Blind side pigmentation, MUJ: Malformed Upper Jaw, OSO: Ocular side operculum, BSO: Blind side operculum, IEM: Incomplete eye migration)

	Dextral	Sinistral	OSMP	BSP	MUJ	OSO	BSO	IEM
OSMP	-0.03	0.03	1.00	-0.11	-0.38	-0.09	-0.13	0.18
BSP	0.03	-0.03	-0.11	1.00	-0.13	-0.09	-0.11	0.23
MUJ	0.01	-0.01	-0.38	-0.13	1.00	-0.13	-0.13	-0.15
OSO	0.00	0.00	-0.09	-0.09	-0.13	1.00	0.04	-0.13
BSO	0.01	0.00	-0.13	-0.11	-0.13	0.04	1.00	-0.13
IEM	-0.01	0.01	0.18	0.23	-0.15	-0.13	-0.13	1.00

(2001) suggested the use of DHA and vitamin A in the enrichment of live prey and early weaning to artificial diets to prevent abnormal pigmentation in flatfish.

The upper jaw deformities were the highest occurence of malformation in this investigation. The frequency of jaw deformities increases in intensive aquaculture and in inbred populations. Jaw deformity could be caused by many factors such as mechanical injury, nutritional deficiencies, parasitism, teratogenic substances, and adverse environmental conditions or genetic aberration (Quigley 1995).

Poorly or incompletely formed gill opercula have been observed in a wide variety of teleosts (Brown and Núñez, 1987). In this study, 38.7% of abnormal juveniles exhibited some degree of gill opercula malformation both ocular and blind sides. Responsible factor for these deformities may be genetic (Chandrasekeran and Rao, 1981) or may be due to nutritional and environmental problems (Brown and Núñez, 1987).

During the intensive rearing of flat fish species, incomplete eye migration is frequently observed. Incomplete eye migration has been observed from 25% to 53% in hatchery reared yellowtail flounder (Copeman *et al.*, 2002) and from 0.8% to 12.8 in Japanese flounder (*Paralichthys olivaceus*) (Seikai *et al.*, 1987). In this study, incomplete eye migration was observed as the lowest type of deformation, and the individuals which had incomplete eye migration acquired the lowest total length and body weight. High levels of dietary arachidonic acid delayed eye migration in Senegalese sole (Villalta *et al*., 2005). Diet is a main factor effecting eye migration in flat fish (Seikai 1985; Copeman *et al*., 2002; Hamre *et al*., 2007).

The present study does not intend to discuss the causes of the abnormalities, but only to single out the fact that such malformations are registered in the hatchery-reared flounder *P. flesus luscus*. The present report is the first in the Black Sea region. The deformities may have been caused either by environmental disturbance, nutritional deficiency in the feeds, a genetic mutation, or a combination of the three factors. Inbreeding can also elicit such abnormalities in fish species. Therefore, more research is needed to exactly identify the factors causing such deformities.

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