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Effect of Temperature on Reversed Asymmetry in Hatchery-Reared Flounder (*Platichthys flesus luscus* Pallas, 1811)

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Abstract

The effects of four different rearing temperatures (15, 18, 21 and 24ºC) on reversed asymmetry and larval development in the hatchery-reared larvae of the flounder, *Platichthys flesus luscus*, were investigated in this study. Incidence of reversed asymmetry (sinistral forms) were found to be 13.0, 10.4, 11.6 and 21.3% at 15, 18, 21 and 24ºC, respectively. The sinistrality rate was not affected by temperature between 15°C and 21°C, while this incidence was significantly higher at 24°C ($P<0.05$). At completion of the metamorphosis (day 60), juvenile final total length and survival rates were 18.4, 19.9, 18.8, 15.7 mm and 13.5, 9.0, 10.6, 9.2% at 15, 18, 21 and 24°C, respectively. The results of the present study strongly suggest that rearing temperature not only influences growth and survival during the larval development but also reversed asymmetry in the flounder at the metamorphosis, especially at high temperatures.

Keywords: Flounder, *Platichthys flesus luscus*, temperature, reversed asymmetry.

Kültür Pisi Balıklarında (Platichthys flesus luscus Pallas, 1811) Ters Asimetri (Sola Bakışlılık Oranı) Üzerine Sıcaklığın Etkisi

Özet

Bu çalışmada, kuluçkahane üretimi pisi balığı (Platichthys flesus luscus) larvalarının larval gelişimi ve sola bakışlılığı (ters asimetri) üzerine dört farklı sıcaklığın (15°C, 18°C, 21°C, 24°C) etkisi araştırılmıştır. Sola bakış oranı 15°C, 18°C, 21°C ve 24°C sıcaklıklar için sırasıyla %13.0, %10.4, %11.6 ve %21.3 bulunmuştur. Sola bakış oranı 24°C'de önemli derecede yüksek ($P<0.05$) iken 15°C, 18°C ve 21°C sıcaklıklardan etkilenmemiştir. Metamorfoz tamamlandığında (60. gün) juvenil total boyu ile yaşama oranları 15°C, 18°C, 21°C ve 24°C sıcaklıklar için sırasıyla 18.4 mm 19.9 mm, 18.8 mm, 15.7 mm ve %13.5, %9.0, % 10.6, %9.2 olarak belirlenmiştir. Çalışmanın sonuçları güçlü bir şekilde göstermiştir ki yetiştiricilik sıcaklığı sadece larval gelişim boyunca büyüme ve yaşama oranını değil özellikle yüksek sıcaklıklarda metamorfoz evresindeki pisinin sola bakışlılık durumunu da etkiler.

Anahtar Kelimeler: Pisi, Platichthys flesus luscus, sıcaklık, ters asimetri.

Introduction

Mariculture in Turkey has expanded in recent years, with seabass, seabream and rainbow trout being commercially grown, and research on marine fish species is being intensified. Several other fish species have been under consideration for culture purposes and among them, the flounder, *Platichthys flesus luscus*, appears to have good aquaculture potential. This species recently adapted to aquaculture conditions 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), while considerable progress in induced reproduction has been made in recent years (Şahin *et al*., 2008). However, larval rearing methods are still unreliable, with highly variable malformation (i.e. deformed jaw and opercula), impaired eye migration, abnormal pigmentation and improper reversal of juveniles.

Flatfish may sometimes display a phenomenon called reversal and that such individuals posses eye on the side which is usually eyeless and unpigmented (Díaz de Astaloa, 1997). In sinistral forms, the right eye migrates to the left side, whilst in dextral forms, the left eye migrates to the right side (Ahlstrom *et al*.,

1984). Many species are either right-sided (dextral) or left-sided (sinistral), but in a few species, reversed specimens occur in varying numbers (Díaz de Astaloa, 1997). 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.

There are no reports of temperature effects on the reversed asymmetry in hatchery-reared flounder *Platichthys flesus luscus* and thus, the present study aimed at investigating temperature effects on larval development and reversed asymmetry in this flatfish species under culture.

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. The eggs were incubated at a density of 100 eggs L^{-1} in a 50-L fiberglass tank with water at 18‰ salinity and 14ºC. The incubation tank was moderately aerated and water exchange rate was about 10 times per day.

Larval rearing temperatures were maintained at 15, 18, 21 and 24ºC with triplication by using 1000 watt thermostatically controlled aquarium heaters. Rearing temperatures were adjusted to the target temperatures at the mouth opening stage of the larvae at an acclimation rate of 1ºC per 12 h. The rearing experiment was terminated when all the larvae completed metamorphosis at around day 60.

The newly hatched larvae (1-2 hours old) were stocked into 500-L fiberglass larval rearing tanks at a stocking rate of 10 larvae L^{-1}. 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 tanks throughout the larval rearing period lasting 60 days. Water was changed for the first time on the fourth day and every day thereafter with an exchange rate of 50% day^{-1}. The rotifer (*Brachionus plicatilis*) and algae (*Nannochloropsis oculata*) were introduced as feed on day 4 when the larvae partly absorbed their yolksac. The rotifer density in the larval rearing tank was maintained at 5 rotifers mL^{-1} from day 4 to day 25. Throughout the rotifer-feeding period, the alga was added daily at 0.5 million cells mL^{-1} as food for the rotifer and as water conditioner. Newly hatched *Artemia* nauplii were introduced into the tanks at 0.2 nauplii mL^{-1} from day 13 to day 33. *Artemia* metanauplii enriched with 'Red Pepper' (Bern Aqua, Belgium) were used at 0.2 ind. mL^{-1} between day 20 and day 45. On day 25, the larvae were gradually weaned onto granulated feed (Caviar, Bern Aqua, Belgium) by hand-feeding every 3 h to satiation. During this period, water exchange rate was increased to 300% per day and then to 500% per day towards the end of the culturing period. Siphoning of the tank bottom to clean the sediments was initiated on day 15 and continued everyday throughout the larval rearing period.

After all the larvae completely metamorphosed (day 60), the fish were sampled from the rearing tank, anesthetized with 50-ppm ethylene glycol monophenyl ether for morphological observations such as reversed eye migration.

After the data were checked for normality (by Shapiro–Wilk's test) and homogeneity of variances (by Levene's test), an analysis of variance (one-way ANOVA) was used to compare the groups in SPSS software for Windows (version 17.0; IBM Corporation, Armonk, NY, USA). Scheffe's pair-wise comparison test was used to localize differences between the groups at $P<0.05$. Arcsine transformation was carried out for percentage data before further statistical analyses.

Results and Discussion

The eggs from hatchery-spawned flounder were hatched and the larvae were successfully reared until metamorphosis in captivity for duration of 60 days in this study. Final survival rates ranged from 9.0% to 13.5%, depending on water temperatures (Table 1). The final larval survival at 15°C water temperature was significantly higher ($P<0.05$) than those in other treatments. Yet, larval growth and development were inversely affected by low or high rearing temperatures. The lowest final total length (15.7 mm) was obtained at 24ºC ($P<0.05$) while the others sustained similar growths until the metamorphosis ($P<0.05$, Table 1). The present results confirms our previous findings (Aydın *et al*., 2012) in that optimal temperature for larval culture of the flounder originating in the Black Sea is around 20-21°C and temperatures above or below that level inversely affect growth performance of this species.

In addition, our experimental results also showed that survival rate and frequency occurrence of normal fish were especially poorer at 24ºC compared lower temperatures 15-21 °C (Table 1 and Figure 1). This clearly indicate that rearing temperature not only influenced larval growth and development, but also the occurrence of reversed asymmetry in juvenile flounders, which varied little among the fish grown at lower temperatures of between 15 and 21°C ($P>0.05$) in comparison to higher (24°C) ($P<0.05$). At completion of the metamorphosis (day 60), the sinistrality rates were found to be 13.0, 10.4, 11.6 and 21.3% at 15, 18, 21 and 24ºC, respectively (Figure 1). Our results agree with Aritaki and Seikai (2004), who stated that hatchery-reared brown sole *Pseudopleuronectes herzensteini* have a high probability of developing morphological metamorphosis-related abnormalities closely linked to rearing temperature. Reversed asymmetry has also been reported to be common in reared flatfish by López *et al*. (2009), although the causes of this

Table 1. Final length (mm) and survival (%) of juveniles of the flounder *Platichthys flesus luscus* (initial length 3.2 ± 0.03 mm) reared throughout the larval development in four different temperatures.

Temperatures (C°)	Final total length (mm)	Final survival rate (%)
15	18.4 ± 2.40 [a]	13.5 ± 1.14 [a]
18	19.9 ± 2.89 [a]	9.0 ± 0.56 [b]
21	18.8 ± 2.91[a]	10.6 ± 1.78 [b]
24	15.7 ± 2.17 [b]	9.2 ± 0.62 [b]

Each value represents a mean ± sd. (n=3). Means marked with different letters in the same column are significantly different from each other (P<0.05).

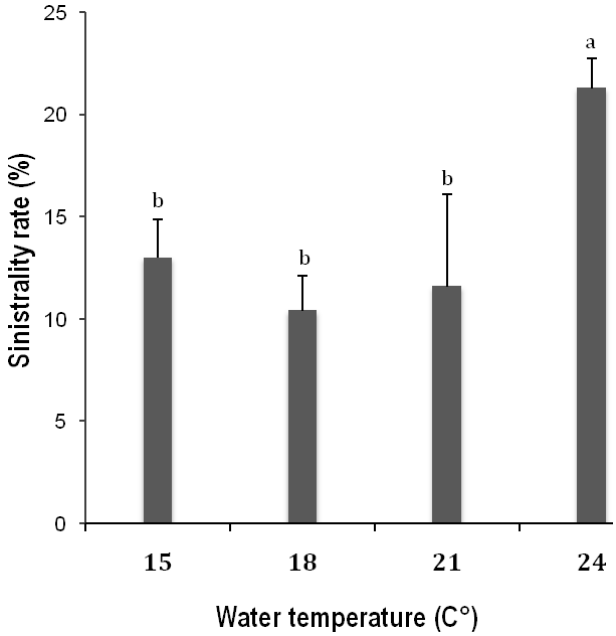

Figure 1. Sinistrality rate in juveniles of the flounder *Platichthys flesus luscus* reared in four different temperatures throughout the larval development. Each column represents a mean ± sd. (n=3). Means marked with different letters are significantly different from each other (P<0.05).

phenomenon are not known (Benetti *et al*., 2001). In some pleuronectid species, such as *P. herzensteini*, *P. yokohamae*, *P. cornutus* and *Verasper variegatus*, reversals have been frequently observed in hatchery-reared individuals, although rarely seen in wild fish (Hubbs and Hubbs, 1945; Goto, 2009). Bisbal and Bengston (1993) reported a 4.4% reversed asymmetry in the laboratory-raised summer flounder, *Paralichthys dentatus* at the metamorphosis. In our case, 24°C temperature has led to as high as 21% reversed asymmetry in the Black Sea flounder.

In conclusion, the results of the present study clearly suggest that rearing temperature not only influences growth and survival during the larval development but also reversed asymmetry at metamorphosis in the flounder *P. flesus luscus* originating in the Black Sea.

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References

Ahlstrom, E.H., Amaoka, K., Hensley, D.A., Moser, H.G., Sumida, B.Y. 1984. Pleuronectiformes: Development. In: Ontogeny and systematics of fishes. Spec Publ. 1, Am. Soc. Ichthyol. Herpetol. (Moser H.G., Richards W.J., Cohen D.M., Fahay M.P., Kendall A.W, Richardson S.L., eds.). Allen Press, Lawrence, KS, pp. 640-670.

Aritaki, M., Seikai, T. 2004. Temperature effects on early development and occurrence of metamorphosis-related morphological abnormalities in hatchery-reared brown sole *Pseudopleuronectes herzensteini*. Aquaculture 240, 517–530.

Aydın, İ., Ak, O., Küçük, E., Polat, H., Ceylan, B. 2012. Optimum temperature and growth performance of hatchery reared Black Sea flounder (*Platichthys flesus*

luscus Pallas, 1814). Turk. J. Vet. Anim. Sci. 36(2), 101-106.

Benetti, D.D., Grabe, S.W., Feeley, M.W., Stevens, O.M., Powell, T.M., Leingang, A.J., Main, K.L., 2001. Development of aquaculture methods for southern flounder, *Paralichthys lethostigma*: I. Spawning and larval culture. J. Appl. Aquacult. 11(1/2), 113-133.

Bisbal, G.A., Bengtson, D.A. 1993. Reversed asymmetry in laboratory-reared summer flounder. The Progressive Fish-Culturist 55, 106–108.

Bruno, D.W., Fraser, C.O. 1988. A case of reversal in the common dab, *Limanda limanda* (L.). Journal of Fish Biology 32, 483–484.

Díaz de Astarloa, J. M. 1997. A case of reversal in *Paralichthys orbignyanus* a shallow-water flounder from the south-western Atlantic. Journal of Fish Biology 50, 900–902.

Goto, T. 2009. Reversals in two dextral flounder species, *Microstomus achne* and *Cleisthenes pinetorum* (Pleuronectida; Teleostei), from Japan. Journal of Fish Biology 74, 669–673, doi:10.1111/j.1095-8649.2008.02132.x

Hubbs, C.L., Hubbs, L.C. 1945. Bilateral asymmetry and bilateral variation in fishes. Papers of the Michigan Academy of Sciences, Arts, and Letters 30, 229–310.

López, A.V., Müller, M.I., M. Radonić, M., Bambill, G.A., Boccanfuso, J.J., Bianca, F.A. 2009. Larval culture technique and quality control in juveniles of flounder *Paralichthys orbignyanus* (Valenciennes, 1839) in Argentina. Spanish Journal of Agricultural Research 7(1), 75-82.

Şahin T. 2000. Larval rearing of flounder, *Pleuronectes flesus luscus*, under laboratory conditions. Turkish J. Mar. Sci. 6, 263-270.

Şahin, T., Erdinç, G., Aydın, İ., Polat, H. 2008. Reproductive Characteristics and Egg Development in Flounder (*Pleuronectes flesus luscus*) in the Southern Black Sea. The Israeli Journal of Aquaculture – Bamidgeh 60(1), 20-26.