Morphological Specificity in Cultured Starry Flounder
*Platichthys stellatus* Reared in Artificial Facility

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Abstract

The starry flounder *Platichthys stellatus*, like all flatfish, exhibits conspicuous lateral asymmetry in numerous traits, most obvious of which is the migration of one eye to the other side of the head during metamorphosis. Additional changes related to eye migration include asymmetrical pigmentation, and a behavioral shift from larvae that exhibit upright, open-water swimming to juveniles and adults that lie on the ocean floor, eye side up. However, the morphology of these juveniles has been quite plastic in recent years, a phenomenon which is thought to be related to a diverse suite of semi-intensive and intensive larviculture methods. The cause of morphological abnormalities in the farmed flatfish is poorly understood. In the present study, we observe the features of morphological specificity and abnormality of immature fish (mean total length 23 cm) and survey the occurrence frequency of the specificity and abnormality of juvenile (mean total length 6.70 cm) in artificial culture facility. We find 2 types of abnormality (e.g., albino in ocular side and hypermelanosis in blind side) and 1 type of specificity (e.g., lateral polymorphism). These considerably differ from normal individuals (has sinistral eye and pigmented on only one side) by several characteristics (dextral eye, ocular side albinism, blind side hypermelanosis). The incidences of albinism, hypermelanosis, and body reversal are 10.1 ± 2.56%, 91.7 ± 1.7%, and 13.1 ± 1.1%, respectively. These suggest that these morphometric and morphological differences occur more in artificial environment during and just after metamorphosis.

Key words: *Platichthys stellatus*, Starry flounder, Abnormality, Body reversal, Albinism, Hypermelanosis

Introduction

The pleuronectiforms are bilaterally symmetrical after hatching, but, after metamorphosis to the juvenile stage, they become laterally asymmetrical. During metamorphosis, one side eye migrates to the other side and occupies a position beside the opposite side eye while their epidermal skin is asymmetrically pigmented on the ocular side. Venizelos and Benetti (1999) report that this fish has larval-type melanophores symmetrically distributed on both epidermal sides before metamorphosis. However, on the blind side, a consistent cytolysis of chromatoblasts inhibits pigmentation at the peak of metamorphosis. Simultaneously, three types of pigment cells (adult type of melanophore, xanthophore, and iridophore) change rapidly on the ocular side. The resulting morphology is asymmetrical. The ocular side exhibits varied epidermal colors, but the blind side is white (Bolker and Hill, 2000).

Starry flounder *P. stellatus* (Pleuronectiformes: Pleuronectidae) is a cold water marine fish found in the North Pacific, spanning Korea, Japan, Sea of Okhotsk, Bering Sea, Alaska to California. Because of this specific development, the flatfish also belongs to Pleuronectiforme that is a heterosomatic and laterally asymmetrical flatfish, which moves one of its dextral eyes to the sinistral side of its body during metamor-
hatchery, the juvenile (total length [TL] 2.7-6.7 cm) and immature abnormalities of starry flounder produced in a commercial facility and then released to the wild for increasing the coastal fish populations have staining on the blind side (Jeong and Jeon, 2008). Therefore, if this trait is hereditary, the released ambicolored flounders could change the innate characteristics of wild flounder.

However, any scientific researcher has not gotten information yet about the traits of these morphological abnormalities and the frequency of their occurrence in the commercial production of starry flounders. Therefore, we need to understand these abnormal phenomena through studies on biological feature, genetic impact, and the cause and mechanism of the morphological abnormalities. We study the types and numerical traits of morphological specificity of the body and skin pigment abnormalities during artificial seed production in starry flounder.

**Materials and Methods**

**Experimental animals and morphological observation**

To examine the traits and frequency of these morphological abnormalities of starry flounder produced in a commercial hatchery, the juvenile (total length [TL] 2.7-6.7 cm) and immature fishes (mean TL: 23 cm) hatchery produced from the eggs of 480 wild starry flounder brood fish captured in East Sea of Korea are used as experimental animals. The experimental fishes are reared with gentle aeration and filtered seawater (1- to 5-fold water exchange rate per day, depending on biomass) at a natural water temperature (WT; mean 19.9°C ± 4.2°C), salinity (30.9 ± 0.3 psu), dissolved oxygen (7.3 ± 0.8 mg/L) and artificial lighting that simulates the natural photoperiod. The fishes are fed twice a day (10:00 and 16:00) to satiation using a commercial pellet diet, except on the sampling days.

**Quantitative pattern of body reversal and pigment abnormality**

1,024 flounders are randomly collected from rearing tank using a net. The fishes are anesthetized with 2-phenoxyethanol (1/1000 dilution, 0.3–0.4 mg/L) and rinsed with distilled water to remove salt. The total length (TL), body height (BH), and body mass (BM) of each animal are measured. Both sides of the sampled flounders were photographed with a digital camera (Dimage A2; Konica Minolta, Tokyo, Japan). The numbers of reversal (dextral), albino, and ambicolored individuals in each group were counted, and the abnormal fish ratio (no. abnormal fish/no. total fish × 100%) is calculated. Individuals with a stained area of more than 2% on the blind side or with an unstained area of more than 2% on the ocular side are considered ambicolored and albino. In an ambicolored fish, the stained area ratio of blind side (stained area of blind side/total area of blind side × 100%) is analyzed with a microimaging analysis system (Qwin 3.1; Leica, Wetzlar, Germany) using the photographs.

**Results**

**Body reversal**

Ordinary (sinistral) starry flounders have both eyes located on the left side of the body. The fish is bilaterally symmetrical after hatching but becomes left-laterally asymmetrical after metamorphosis. During metamorphosis, the right eye migrates to the left side and occupies a position beside the left eye. The left epidermal skin is asymmetrically pigmented by melanophores and xanthophores. However, on the right side, pigment cells were inhibited during metamorphosis and are subsequently consumed by melanophages in the skin. Only white iridophores are selectively and massively different, showing white color. However, in the present study, we find a few reversal (dextral) flounders showing a reversal lateralization in which left eye migrates to right side and occupies a position beside the right eye during metamorphosis. Therefore, the right side chromatophore features of a flounder generally resemble those on the left side of an ordinary flounder (Fig. 1). The ratio is 13.1 ± 1.1%.

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we observe two types of malpigmentation in cultured starry flounders. The first is pseudo-albinism (or hypomelanosis), which prevents the normal development of color on the ocular side (Fig. 2). The ratio is $10.1 \pm 2.5\%$ (Table 1). The second malpigmentation is hypermelanosis on the blind side (ambicoloration). Malpigmentation on the blind side stretches from the posterior head to the anterior caudal fin on the blind side along the above and bottom dorsal fins, although rarely, to the abdomen area (Fig 3). The numbers of ambicolored individuals and the ambicolored fish ratio are $91.67 \pm 1.67\%$ and $17.40 \pm 1.34\%$, respectively (Table 1).

**Discussions**

Through the shape observation upon the first generation of farmed starry flounders, which are produced through artificial insemination between sinistral male and female of wild starry flounders *P. stellatus*, we learn that starry flounder has symmetrical two body forms (sinistral and dextral). Sinistral fish has its body biased to the left side so that its eyes are located on the left side. Because of this specific development, the flounders have a specific feature of showing diverse color on the ocular side and achromatic color on the blind side (Fig. 1). However,
of the position of the eyes and the color of body from left to right side. The fish develops eyes and colors on the side that is expected to become the blind side (Okiyama and Tomi, 1970; Chen, 1980; Bi et al., 1987; Bruno and Fraser, 1988; Suzuki, 1994; Diaz de Astarloa, 1997; Ivankova, 1997; Guibord and Chapleau, 1999; Silva et al., 2007; Ivankova et al., 2008). The ratio of sinistral fish observed in the present study is 86.9%, and the ratio of dextral fish is 13.1%. This result suggesting that the percentage of dextral fish is meaningfully higher in farmed fish than in wild fish. This lateral polymorphism in the fish is rare from the evolutionary stand point, and is found in only a few types of the total 715 pleuronectiformes (Munro, 2005). Norman (1934), Ivankova and Ivankova (2006), and Ivankov et al. (2008) suggested that individuals with this abnormality have similar characteristics of the most primitive form of pleuronectiformes. For example, in Psettodes which is less evolved in eye bias, the same rate of sinistral and dextral fish has been observed. So, we assume that lateral polymorphism is a feature displayed during the early stage of differentiation of species in the evolution of pleuronectiformes. However, some suggest that this can be caused by intergeneric hybrids (Kosaka, 1980). Also, considering that this species lives in both the sea and the rivers, this lateral polymorphism may have emerged from the differences in traits between sea water living fish and fresh water living fish (Bergstrom, 2007). Further research will be required to learn the causes.

According to Bergstrom (2007), the starry flounder showed stronger geographical body patterns than any other species. Specifically speaking, this species, found across the Siberia to the mid-western coast of the United States, showed clear geographical patterns in terms of the direction of body asymmetry. Ratio of dextral (reversal) fishes decrease as they move from Central California through Alaska to North East Asia. Previous researches (Hubbs and Kuronuma, 1945; Orcutt, 1950; Forrester, 1969; Bergstrom, 2007) confirm that among the group of wild starry flounders living in the Pacific coast of North America, right side reversal takes up 30-50%, compared to 25% in Alaska and 3% or less in North East Asia (Russia, Japan and Korea). Although we assume that genetics play a role in body laterality and its direction (Policansky, 1982; Boklage, 1984; Hashimoto et al., 2002), the mechanism to maintain such geographical variance remains uncertain. In the present study, the dextral ratio of farmed fish produced from East-Asian wild stocks was extremely higher compared with that of their wild counterparts. The farmed flounders show 4 times higher ratio than the percentage found in other researches on wild fish. However, we were not able to find the exact causes whether it is the problem of credibility of the study or whether it is a deformation that occurs in specific farming conditions. We assume that the difference of living conditions and rearing environments in artificial farms and the wild might affected the development of the genes that determine lateral direction during metamorphosis and thus affected the ratio of right-side reversal. Because actual causes of high body reversal ratio in farmed starry flounders have not been identified, physiological environment and molecular biological research on this is needed.

Meanwhile, in the present study, other abnormalities significantly found in cultured starry flounders are hypopigmentation (pseudo-albinism) and hyperpigmentation (hypermelanosis) in the ocular and blind sides, respectively. Pleuronectiformes, which are heterosomatic and laterally asymmetrical flatfish, belong to a monophyletical order, moving one of their eyes to the other side of their body during metamorphosis. Because of this specific development, flatfish have a specific feature showing diverse color on the ocular side and achromatic color on the non-eyed side (Hensley, 1997). However, farming of pleuronectiformes creates individuals with skin malpigmentation in both sides in high rates. Such malpigmentation includes albinism and hypermelanosis on the ocular and blind sides, respectively. The malpigmentations have been found most commonly in artificial culture facility (Venizelos and Benetti, 1999; Bolker and Hill, 2000), while the features are rarely found in the wild (Díaz de Astarloa, 1995, 1997, 1998; Ivankov and Ivankova, 2002; Chaves et al., 2002; Carnikián et al., 2006; Macieira et al., 2006; Silva et al., 2007). The pseudo-albinism on the ocular side is a common feature among farmed pleuronectiformes (Nakamura et al., 1986; Purchase et al., 2002). From the economic perspective, this symptom undermines the commercial values of fish seeds (Seikai and Mat-
sumoto, 1994) and makes them vulnerable to predators when released to the sea because of the lack of cryptic coloration, thus making it less effective in increasing maritime resources (Seikai, 1985; Seikai and Matsumoto, 1994; Howell, 1994; Furuta, 1998; Furuta et al., 1998). Also, since Shikano (2005) has confirmed the genetic role of this symptom, one concern is that a releasing of albino flounder seedlings may change the original traits of wild flounder in the ecosystem.

What causes the pseudo-albinism then? There are diverse opinions. Ecologists believe that rearing environment is a primary cause for albinism in flatfish ( Fukusho et al., 1986; Seikai and Matsumoto, 1994), while nutritionists have cited critical trace elements, such as phospholipid, DHA, EPA and Vitamin A & D, as main causes ( Seikai, 1985; Nakamura et al., 1986; Seikai et al., 1987a,b; Yamamoto et al., 1992). On the other hand, genetic biochemists believe that biochemical problems due to genetic defect cause this symptom (Bolker and Hill, 2000).

Second malpigmentation is hypermelanosis on the blind side (ambicoloration). Causes and mechanisms for this symptom are still unknown, as well as the technologies to treat or prevent the symptom. Our research shows very high rates of hyperpigmentation on the blind side, similar to olive flounder Paralichthys olivaceus (Yamanome et al., 2007) and barfin flounder Verasper moseri (Yamanome et al., 2005). Researchers so far have suggested that the coloring on the blind side of pleuronectiformes is caused by inappropriate rearing conditions in artificial facilities, such as improper density (Takahashi, 1994), nutrition (Haga et al., 2004; Tarui et al., 2006), background color (Yamanome et al., 2005), and the lack of sand substratum for burrowing behavior (Ottesen and Strand, 1996; Iwata and Kikuchi, 1998). However, no research has identified the clear cause and reasons.

The blind-side hypermelanosis has been reported in some types of pleuronectiformes that belong to Bothidae and Pleuronectidae. The hyperpigmentation on the blind side can be categorized into partial and whole body malpigmentation. The body pigmentation is observed in limited types of flatfish only, such as Platicthys stellatus, Paralichthys olivaceus, Paralichthys californicus, Paralichthys patagonicus, Scophthalmus maximus, Rhombosolea tapirina, Hypsopsetta guttulata, Limanda aspera and Lepidopsetta polyxystra (Norman, 1934; Haaker and Lane, 1973; Maruyama, 1976; Ivanov and Ivankova, 2002; Ivankova and Ivankov, 2006; Diaz de Astarloa et al., 2006). Such body malpigmentation can be traced back to the origin of flatfish. Most researchers have suggested that pleuronectiformes have evolved from primitive fishes, Zeiformes, or Percociformes (Norman, 1934; Fridman, 2008).

We study morphological specificity in farmed starry flounders born from wild sinistral mother fish. The results demonstrate that there are one distinct lateral polymorphism and two of skin abnormality (ocular-side albinism and blind-side hypermelanosis), and they occur in meaningfully higher frequency than in their wild counterparts. However, why a higher percentage of morphological specificity and abnormality are occurred in the cultured starry flounder than in the wild starry flounder. So, further research will be needed in order to find the reason.

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