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# Physical Abnormalities of Olive Flounder (*Paralichthys olivaceus*) Juvenile Found During Sorting Process in Anmyeon, South Korea

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## Abstract

Olive flounder (*Paralichthys olivaceus*) is the most popular consumption fish in South Korea. Korean consumes flounder as sashimi, spicy soup, stew and cutlet. Due to its popularity; Korean considers Olive flounder as the nation's raw fish. Despite advanced aquaculture technique, modern facilities and massive production, fingerling size grading and abnormal sorting are conducted by traditional method. This study was conducted by observation. A group of 5 to 6 person worked meticulously to sort out abnormal fingerling and juvenile. Sorting is based on physical traits such as the shape and structure of head, jaw, operculum, fin, body; body coloration, pigmentation and length. Due to the exclusiveness and technicality of this profession, information and standard in grading and sorting has been lacking. This research provides the information about sorting criteria of Olive flounder juvenile and detail description of physical abnormalities being sorted out.

## INTRODUCTION

Olive flounder (*Paralichthys olivaceus*) is one of the most popular and the most important finfish aquaculture species in South Korea (NFRDI, 2006; NFRDI, 2016; NSO, 2020). Olive flounder, popularly known as *nubchi* (South Korea), *hirame* (Japan), bastard halibut or Japanese flounder, is best consumed as raw food (*hoe* in Korea or sashimi in Japan). Korean people believed that consuming flounder can build up strength, revive energy and increase appetite. People with diabetes and liver disease, someone who is recovering from illness are encouraged to consume this fish (NFRDI, 2016).

Olive flounder is part of left-eyed flounder group that lives along the coast of Korea, Japan, and China. Pelagic larvae swim upright but at the end of pelagic stage undergo metamorphosis; the right eye migrates to the opposite side. During metamorphosis, they gradually swim closer to the bottom and finally lay on their left side at the bottom. The ocular side, the side with both eyes, is covered by ctenoid scale while the blind side, the side without eyes, is covered by cycloid scale. Flounder spend their juvenile and adult stage as benthic fish (NFRDI, 2006). Flounder hatchery and grow-out farm spread adjacent to Korean coastline, from the southernmost part, Jeju Island (Jung *et al.*, 2020; Park *et al.*, 2012), the northwestern most part, Taean County (Yoo *et al.*, 2020), to the easternmost part, Pohang (Park *et al.*, 2012). Other than for commercial purpose, flounder hatchery raised flounder larvae for stock enhancement (Seo *et al*., 2010; Yoo *et al*., 2020).

Korean government developed two flounder strains: aquaculture strain for commercial purpose and wild strain for stock enhancement purpose. Aquaculture strain is specifically bred for consumption while wild strain is specifically bred for release to water adjacent to Korean coastline (Kang *et al*., 2011).

In 2018, capture production of flounder in South Korean reached 4.762 tonnes while in Japan reached 6.600 tonnes. South Korea produced 37.250 tonnes of flounder by aquaculture, decreased by 9.6% from previous year, compared to 2.200 tonnes of aquaculture production by Japan. The total value of aquaculture production from both of this area is USD 513.4 million (FAO, 2020).

Olive flounder is produced at a relatively stable quantity. Olive flounder production quantity is twice bigger than Korean rockfish production quantity. In 2019, flounder aquaculture production improved up to 16.3% after it dropped 9.6% in 2018 (Table 1). The closest competitor, Korean rockfish, generated 20.348 tonnes while another flatfish, sole, only generated 3.669 tonnes. The market value of flounder aquaculture production is almost triple the market value of Korean rockfish and more than 10 times the market value of sole, another type of flatfish (NSO, 2020).

Table 1. Aquaculture finfish production in South Korea (NSO, 2020).

| Finfish type | Parameter | Period | | |
|---|---|---|---|---|
| | | 2017 | 2018 | 2019 |
| Olive flounder | Quantity[a] | 41.207 | 37.241 | 43.320 |
| | Value[b] | 584.138 | 495.391 | 430.685 |
| Korean rockfish | Quantity | 22.344 | 22.702 | 20.348 |
| | Value | 171.191 | 192.508 | 155.473 |
| Flathead mullet | Quantity | 6.828 | 6.382 | 6.644 |
| | Value | 54.319 | 54.223 | 51.222 |
| Red seabream | Quantity | 6.806 | 5.103 | 5.502 |
| | Value | 77.090 | 68.102 | 60.911 |
| Sole | Quantity | 2.564 | 3.373 | 3.669 |
| | Value | 2.8554 | 34.651 | 37.600 |

[a] Quantity (tonnes)
[b] Value (million KRW)

One of the problems in hatchery-reared flounder is high incidence of abnormalities, sometimes can goes up to 19.1% in 50-d post hatch juvenile of Olive flounder (Sawada *et al*., 2001) and 54.9% in 130-d post hatch juvenile of Black Sea flounder (Aydin, 2012). One abnormal physical trait such as shortened upper jaw was discovered as low as 1.9% (Sawada *et al*., 2001) or as high as 55.0% (Aydin, 2012). Other than shortened upper jaws, there are other abnormal traits such as body malpigmentation, skeletal anomalies, incomplete eye migration and shortening of operculum (Sawada *et al*., 2001; Aydin, 2012; Kang *et al*., 2012).

Fish with abnormal physical traits must be sorted and removed from rearing tank. Currently, there is no technology or machine to identify and remove all those unwanted fish. In Korea, especially South Chungcheong Province, groups of highly experienced middle-aged women were employed intermittently to solve the sorting problem (H.O. Kwon, personal communication, May 3, 2018). They meticulously observe and identify abnormal fish with unwanted physical traits. Due to the scarcity of this profession and the experience involved in the sorting process, their skill is always in demand and they must work in tight schedules and travel to hatcheries that are in various counties. This condition resulted in sorting delays, especially in the spring, when demand for flounder juvenile is high. This

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paper described the process and timing of sorting in Olive flounder juvenile and provides detailed description of abnormalities and unwanted physical traits. This research can be used for inexperienced sorters as the basis for identifying unwanted physical traits in Olive flounder juveniles.

## METHODOLOGY

### Place and Time

Fertilized eggs were collected from Genetics and Breeding Research Center and were transferred to Jungang Fisheries hatchery on January 16th, 2018. Hatchery was in Anmyeon, Taean County, South Chungcheong, South Korea..

### Research Materials

The materials used in this experiment were Olive flounder egg, larva and juvenile, calcium hypochlorite $Ca(OCl_2)$ (Niclon 70-G, Tosoh Corporation, Japan), rotifer (feed for larva), marine *Chlorella*, and granule feed (Otohime Hirame B2, Marubeni Nisshin Feed Co. Ltd., Japan),

The equipments used in this research were PVC tank with 6 m diameter, 10 m x 10 m rectangular tank, thermostat, UV sterilizer, 5.5 mm stainless steel mesh, 14.5 mm mesh grader, and circulation pump.

### Research Design

The experimental design being used in this research was case study.

### Work Procedure

Fertilized eggs were sterilized, acclimatized before evenly distributed and incubated in PVC tanks. Water in incubation tank was gently aerated and temperature was set at 17 °C. The shape of incubation-rearing tank was circular with diameter of 6 m while water depth was set at 80 cm. Water temperature was gradually increased to 19 °C at a rate of 0.5 °C after hatching. Seawater was pre-treated with pressurized sand filter, transferred to temporary reservoir tank and treated with 50 ppm calcium hypochlorite $Ca(OCl_2)$.

After seven days, water was transferred to final reservoir tank and sterilized with UV sterilization system. Larvae were fed with rotifer for 14 days since 1 dph (day post hatching) and artemia for 35 days since 3 dph. In order to reduce light penetration and set up green water culture, marine chlorella was added to rearing tank every four days from 0 dph to 21 dph. Larvae were fed with granule feed, from day 19 onwards. Uneaten feed and dead larvae were siphoned out at 22 dph. Larva started swimming closer to the bottom and lying on tank bottom at 26 dph. After the completion of metamorphosis stage at 35 dph, 50% of rearing water was replaced and tank was filled with new water. In order to reduce density, floating larvae were transferred to another tank.

The first size sorting was conducted at 40 dph using 5.5 mm stainless steel mesh. Smaller fish escaped through the mesh and remained in PVC tank while larger fish were transferred to 10 m x 10 m concrete rectangular tank. From this day onward, water in PVC tank was circulated with circulation pump (In Line circulation pump A-055, 150 W, 85 l $minute^{-1}$) while water in concrete tank was circulated with 2 circulation pumps (Hanil PA 280, 580W, 6000 l $minute^{-1}$). Sorting was conducted every week using larger mesh size.

On day 75, flounder juvenile was sorted with 14.5 mm mesh grader and fish was examined for abnormalities. Larger fish were transferred to sorting bench and a group of examiner checks abnormal physical traits. Types of abnormal physical traits are grouped based on the altered part of the body (Divanach *et al*., 1996). Abnormal fish were discarded while normal fish were returned to rearing tanks.

### Data Analysis

Data for the experiment was based on qualitative data such as photographs of flounder juvenile, interviews, observations, and notes.

## RESULTS AND DISCUSSION

Both eyes of normal Olive flounder were located on the left side. During pelagic stage, larvae swim upright and look like normal larvae. Right after the onset of metamorphosis, the right eye moves to the opposite side (sinistral side) and stays above the left eye. Gradually, larvae swim closer to the bottom and lay on their right side. In normal fish, the right side (blind side) of the body lacks pigmentation and surface texture is smooth while the ocular side is pigmented and rough (NFRDI, 2006). There are various noticeable abnormal physical traits, most of them are clearly visible to novice observer. Abnormal physical traits can be grouped into several categories: body shape, body pigmentation and skeletal deformities (Divanach *et al*., 1996).

### Shape

Shape is the most important visual condition that affects market value of a fish even if the fish is marketed for consumption (Divanach *et al*., 1996). Farmed fish, especially in high density culture, may develop various abnormal shapes (Kang *et al*., 2012). The most common is dwarfism and stunted growth. In this study, we discover dwarf juvenile flounder with short and deep body. Normal fish is in oval shape and bilaterally symmetrical. Lateral line connects the tip of mouth at the anterior end and the tip of caudal fin at the posterior end. In case of dwarfism, the posterior part is normal while the tip of anterior part is directed upward, not forward. Head was shrunk to the point that there was a concave line from the dorsal part to head part instead of the normal convex line. Body shape is bilaterally asymmetrical and lateral line is located higher and closer to dorsal rather than at the center of body (Figure 1).

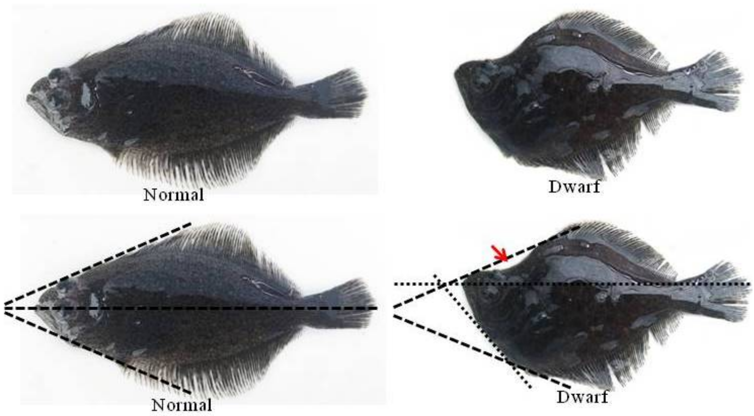


Figure 1. Features of shape abnormalities in flounder juvenile. Dashed line indicated normal shape while dotted line indicated abnormality.

Shape is an important basis in sorting juvenile flounder and influences price when fish reach market size. Olive flounder has been domesticated in South Korea since 1980 and decades of inbreeding resulted in the change of body shape (NFRDI, 2006). Farmed flounder is classified as high-body while wild flounder is classified as low-body. In order to improve body shape and genetic diversity, Genetics and Breeding Research Center conduct artificial fertilization between farmed male flounder and wild female flounder in 2004. The result was promising and there was improvement in growth and body shape (Park *et al*., 2012).

### Pigmentation

In left eyed flounder such as Olive flounder, only the ocular side is pigmented while the blind side is achromatic. Unfortunately, malpigmentation, such as hypomelanosis, is quite common in hatchery-reared flounder (Bolker *et al.*, 2005). In case of hypomelanosis, pigment development is prevented, and narrow or broad areas of body lacks pigment (Figure 2).


Figure 2. Features of pigment abnormalities in flounder juvenile.

Malpigmentation is a general occurrence in hatchery-reared flounder and wild flounder. Different type of life feed supplied at different larval developmental stages can cause 24.6% to 100.0% albinism in Olive flounder larvae. The highest occurrence of normal coloration was achieved when zooplankton is supplied before the beginning of body compression stage. The onset of body compression is believed to be the most important stage in pigment formation. Continuous supply of life feed from body compression stage to early metamorphosis stage and quality or type of life feed are important factors in preventing color abnormality in ocular side (Seikai *et al.*, 1987).

Nutrient such as vitamin A, docosahexaenoic acid (DHA) and phospholipids hold important role in color abnormality. Nutrient deficiency prevents rhodopsin formation and visual transmission from eye to central nervous system. As a result, melanophore–stimulating hormone (MSH) production is lacking and production of pigment is arrested (Kanazawa, 1993). However, excess supply of vitamin A resulted in skeletal deformation (Haga *et al.*, 2011). Nutrition and rearing environment, rather than genetic, are the main cause behind malpigmentation (Seikai and Matsumoto, 1994).

## Spinal Column Deformity

Spinal column deformation is another type of abnormal physical trait in juvenile flounder. Spinal vertebra bent at several locations and affects the shape of fish. Scoliosis (lateral bent) and lordosis (bent in sagittal plane) are the most common spinal deformity (Figure 3). Juvenile suffering from spinal deformation is swimming awkwardly in water surface. Its size was significantly smaller than conspecific. Incidence of spinal column deformity ranged from 4.15 to 68.8% in hatchery-reared flounder (Lü *et al.*, 2015).

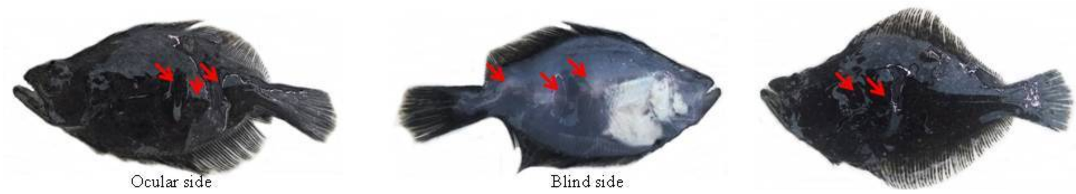


Figure 3. Features of spinal column deformities in flounder juvenile: Arrows indicate severe combination of scoliosis and lordosis in the same specimen.

Spinal column deformity is a common sight in hatchery-reared flounder but rarely found in wild caught flounder. In many cases, juvenile flounder has more than one deformed location in vertebrate. Disproportionate nutrition, genetics and adverse environmental factors such as temperature and pollution are the causative agents behind spinal column deformities (Divanach *et al.*, 1996; Shikano *et al.*, 2005; Haga *et al.*, 2011; Davidson *et al.*, 2011; Yang *et al.*, 2016).

Skeletal abnormalities affect fish growth and their market value and abnormal fish is discarded whenever found during sorting or transferring.

## Head Deformity

Head deformity is the most common abnormalities in Olive flounder juvenile and the hardest to notice by novice examiner. There are three different types of head deformities in this study: shortened upper jaw, gap under ocular side operculum, underdeveloped ocular side operculum (Figure 4). Shortened upper jaw is only visible after fish reach a certain age while underdeveloped ocular side operculum is easily noticeable. In severe cases, underdeveloped distal part of operculum expose gill arch while in minor cases expose only small part of gill. Shortened upper jaw occurs at a rate of 1.9-3.9% (Sawada *et al.*, 2001) while elongation of lower jaw occurs at a rate of 86.7% (Sawayama *et al.*, 2012) in Olive flounder juvenile.


Figure 4. Features of head abnormalities in Olive flounder juvenile: A shortened upper jaw, B gap under ocular side operculum C. underdeveloped ocular side operculum.

Abnormalities in Olive flounder are believed to occur during rotifer feeding stage (Sawayama *et al.*, 2012), artemia feeding stage (Dedi *et al.*, 1998) and metamorphosis stage (Okada *et al.*, 2005; Seikai *et al.*, 1987). Several external factors such as chemical (Suzuki *et al.*, 2000), life feed (Seikai *et al.*, 1987; Sawayama *et al.*, 2012), nutrition (Dedi *et al.*, 1998), stocking density (Kang *et al.*, 2012), hierarchies (Alvarez *et al.*, 2006), and internal factors such as endocrine (Okada *et al.*, 2005), genetic (Sawayama and Takagi, 2010; Sawayama *et al.*, 2012) contributed to various abnormalities in Olive flounder.

After yolk exhaustion, hatched larvae start exogenous feeding. Failure to supply feed, live feed or starter feed, resulted in starvation and malnutrition. Starvation in flounder larvae were indicated by head shrinkage. In severe case, the size of head became noticeably smaller and concave formation was developed between head and dorsal part of body. In starved larvae, head shrinkage was found as early as 5 dph. Olive flounder larvae able to withstand starvation for only two days before suffering from head shrinkage even if feeding is commenced after two days (Dou *et al.*, 2002).

Under normal rearing condition, pelagic larvae undergo metamorphosis spontaneously. Some larvae undergo metamorphosis at a much slower rate. Due to the difference in metamorphosis rate, earlier group completed metamorphosis at a much faster rate and attached to bottom earlier. This group occupies and dominates all available space at bottom of tank while slower group fails to find any space. Competition of space creates two separate hierarchical groups. Weaker group was attacked by dominant group and the group must swim continuously in water column. This condition leads elevation of stress hormone in weaker group. Body shape and body of dominant group and weaker group were visibly different. Weaker group has deformed head, narrow dorsal fin and pale color. However, when density is reduced and weaker group is transfer to empty tank, they returned to normal growth and normal color (Alvarez *et al.*, 2006).

Normal Olive flounder juvenile has longer upper jaw compared to the lower jaw. In abnormal fish, noticeable difference in length between lower and upper jaw is visible after 50 dph (Sawada *et al*., 2001; Sawayama *et al*., 2012). Abnormal fish has significantly smaller upper jaw index (UJI), significantly shorter upper jaw and significantly smaller standard length compared to normal fish (Sawayama and Takagi, 2016). Some bones in the upper jaw shrink and distorted, resulting in shorter upper jaw (Sawada *et al*., 2001). Genetic or inbreeding is one of the reasons behind shortened upper jaw but not the main reason (Sawayama *et al*., 2012). Shortened upper jaw also occurs when mating different parents from different families. Behavior can also result in deformities. Aggressive swimming while feeding caused the snout to bump against tank side or bottom and may result in bone fracture and growth inhibition (Sawayama and Takagi, 2016)

Nutrition also contributed to shortened upper jaw deformity. Larva fed rotifer enriched with old and preserved Nannochloropsis lead to jaw deformation. *Nannochloropsis* preservation caused the algae to flocculate. Feeding with preserved (refrigerated or frozen) *Nannochloropsis* resulted in rotifer's failure to consume the algae because of its large size. As a result, rotifer starved and lost its vitality. EPA and DHA content in rotifer were reduced by half. Supplying bad quality rotifer resulted in lower jaw elongation. Supplying bad quality rotifer resulted in jaw deformities (Sawayama *et al*., 2012)

Incidence of deformed operculum was observed in hatchery-reared Olive flounder and other flatfish (Aydin, 2012). The reason behind abnormal operculum development can be traced to lack of important nutrient such as vitamin C (Frischknecht *et al*., 1994). Metamorphosis in flatfish is influenced by thyroid hormone. Abnormality that resulted after metamorphosis is likely caused by thyroid hormone deficiency or thyroid gland malfunction (Okada *et al*., 2005).

## CONCLUSION

Abnormalities occur after the switch from endogenous feeding to exogenous feeding, especially during feeding with life feed. Failure to provide feed, especially feed with adequate amount of vitamins and fatty acids, will result in various types of abnormalities. Furthermore, abnormal physical traits can be grouped based on body shape, body pigmentation and skeletal deformities.

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