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Larval fish behavior can be a predictable indicator for the quality of Japanese flounder seedlings for release

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Abstract

In the Japanese flounder (*Paralichthys olivaceus*), a typical shivering behavior in the metamorphosing larvae called the Ω (Ohm)-posture is often observed; it disappears after the transition from the larval to juvenile stage, coinciding with the onset of aggressive behavior. From previous studies, I hypothesized that there is a positive correlation between the Ω-posture and aggressive behavior. A rearing experiment using individual otolith markings by ALC (Alizarin complexone) was conducted. On day 21 after hatching (metamorphosing stage), 200 fish showing Ω-posture (Ω fish) were labeled with ALC and another 200 fish (non-Ω fish) were not labeled before being transferred into the same tank and reared until day 58 (juvenile stage). Reverse sets of 200 otolith-labeled non-Ω fish and 200 otolith-unlabeled Ω fish were reared in the same manner. From behavioral observation of a total of 100 juveniles, I found a social rank with three categories: dominants, intermediates and subordinates, with the body sizes of the former being the largest. There was a positive correlation between Ω-posture and aggressive behavior as was revealed by checking the otolith label. Therefore, the Ω-posture is defined as a precursor behavior of aggression in the metamorphosing stage, indicating that we can predict the aggression of juveniles in this species by their behavior in the metamorphosing stage.
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Keywords: Larval quality; Social rank; Behavior; Otolith; Metamorphosis; *Paralichthys olivaceus*

1. Introduction

The concept of fish quality for release is clearly defined by Tsukamoto et al. (1999). Great attention has been paid to physiological and morphological problems of the seedlings; solving these physiological and morphological problems should be the fundamental condition for ensuring the quality of fish: that is, 'fish health' must be the prerequisite of the seedlings for release. However, fish health does not always satisfy the quality of fish for release, which is directly connected to the stocking effectiveness represented by the recapture rate. Therefore, 'fish quality' for release is defined as aptitude for release: how many fish survive in the field after release and how much they yield to stocking effectiveness. Since many fish species develop anti-predator and social behaviors in their early life stages and these behaviors have significant biological and ecological roles for survival (Noakes, 1978; Noakes and Godin, 1988; Huntingford, 1993; Fuiman and Magurran, 1994), it has been pointed out that studies on fish behavior are of practical importance to improve the quality of reared fish for stock enhancement (Masuda,

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2004; Olla et al., 1994). Fish quality of seedlings for release has been estimated using an index that directly reflects stocking effectiveness, such as growth, survival and recapture rate in the field. Most of these studies, however, are focused on the juvenile stage of fish to be released; in terms of judging fish quality for release, little attention has been paid to the relationship between larval behavior and juvenile behavior in the process of larviculture.

In this study, the relationship between larval and juvenile behavior was investigated in the Japanese flounder (*Paralichthys olivaceus*), which is one of the major target species for stock enhancement in Japan. Although more than 20 million juveniles are produced artificially and released every year, many studies show no increase of the flounder population around the coastal waters because of the high mortality of seedlings after release (Masuda and Tsukamoto, 1998; Tanaka et al., 1998). Defects in feeding behavior of the artificially reared seedlings (Miyazaki et al., 2000) and predation including cannibalism by wild stock (Furuta, 1996) are recognized as the major reasons for this unsatisfactory output from the stock enhancement of this species. In the wild, predation of newly settled juveniles by earlier settled ones may also occur (Tanaka et al., 1989). Thus, it would be useful if we could find a specific index that could predict and/or estimate aggressive behavior in early-stage juveniles of this species. It would also provide practical information for improving larviculture methods. A previous study demonstrated that the Japanese flounder exhibits a typical shivering behavior namely "Ohm (Ω)-posture" in the metamorphosing stage, and that the onset of aggressive behavior occurs from the juvenile stage when fish are completely settled (Sakakura and Tsukamoto, 2002). Moreover, "Ω fish," or those which show Ω-posture frequently in the metamorphosing stage, have been found to show significantly higher growth performance in the juvenile stage (Sakakura et al., 2004). Since aggressive behavior in Japanese flounder is known to be strongly size-dependent (Dou et al., 2000a, 2004), I hypothesized that Ω fish in the metamorphosing stage will show aggressive behavior more frequently in the juvenile stage than non-Ω fish.

2. Materials and methods

Naturally spawned eggs were transferred from Miyako Stock Enhancement Center of the National Center for Stock Enhancement, Fisheries Research Agency of Japan (formerly Japan Sea Farming Association; JASFA) to the Fisheries Experimental Station of Kyoto University. Approximately 7000 newly hatched larvae (day 0) were obtained on 10 July 1999. Larvae were kept in a 500 l (liter) transparent rearing tank with a filter system using specially formed ceramic beads for fish rearing (M10, Norra Co., Ltd., Kyoto, Japan). Enriched L-type rotifers (*Brachionus plicatilis* complex) were fed at a density of 10 individuals/ml from day 3 to day 18; enriched *Artemia franciscana* nauplii were supplied from day 12 at a density of 0.1–5.0 individuals/ml according to the growth. Water temperature was kept at 18–20 °C and light condition was natural.

Day 21 fish were sampled randomly from the rearing tank using a white plastic beaker. Following the method of Sakakura et al. (2004), fish showing the Ω-posture (Ω fish) were identified based on a 1-min observation, removed using a large glass pipette, and transferred into a tank (10 l, 18 °C) with aeration. Other fish (non-Ω fish) were transferred into another tank. This treatment was repeated until the total number of Ω fish reached 400. Non-Ω fish were also collected until 400. Following the same procedure, 30 Ω fish and 30 non-Ω fish were selected and anesthetized with MS222 (Tricaine, SIGMA, St. Louis, USA), then fixed in a 5% formalin solution. The standard lengths (SL, mm) of the fish were measured with a microscope. Developmental stages of the fish were determined following the definition by Minami (1982).

Following the procedure of Tsukamoto (1988), 200 Ω fish were labeled in 100 ppm of ALC (Alizarin complexone, Wako, Tokyo, Japan) for 24 h. After labeling, the labeled Ω fish and 200 non-labeled non-Ω fish were placed in a 50 l rectangular glass aquarium with the same filter system as the rearing tank (Tank A) and were reared until day 58, when fish had completely entered the juvenile stage and often showed aggressive behavior. They were fed with sufficient *Artemia* 3 times a day; artificial diets (A-400 and B-700, Kyowa-hakko Co. Ltd., Tokyo, Japan) were also supplied from day 27 in the same manner. Reverse sets of 200 otolith-labeled non-Ω fish and 200 otolith-unlabeled Ω fish were reared in the same manner (Tank B).

Twenty white observation tanks, each 30 cm in diameter containing 5 l of seawater (7 cm depth), were kept in a temperature-controlled room at 20 °C. The light intensity was maintained at 2000 lux during the experiment. Both water temperature and light intensity were adjusted to match those at noon in fine weather of the rearing tank. Fish used for behavioral observations were sampled from Tanks A and B with a hand net. A total of 50 fish were used for observation from Tank A. Ten groups consisting of 5 fish each (1 fish l^{-1}) were introduced into each of 10 observation tanks using a hand net. Fish were acclimated for 30 min before observations. The behavior of fish in each tank was recorded

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