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.

Key Words: larval quality, social rank, behavior, otolith, metamorphosis, Paralichthys olivaceus.

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, 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; Dou et al., 2004), I hypothesized that Ω fish in the metamorphosing stage will show aggressive behavior more frequently in the juvenile stage than non-Ω fish.

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 Tank 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 minutes before observations. The behavior of fish in each tank was recorded from above by an 8 mm video camera (Handycam CCD, SONY Co., Ltd., Tokyo, Japan) for 2 hours. Observations were conducted between 12:00 and 15:00. A total of 50 fish from Tank B were observed in the same manner. After the video recordings, all fish were anesthetized with MS222 and preserved in a 90 % ethanol solution. All individuals of each observation tank in the video records were discriminated by the video image analysis system (LA525, PIAS Co. Ltd., Tokyo, Japan), which recognized fish from the background color by thresholding of brightness (Sakakura and Tsukamoto, 1998). In this system, both body size and agonistic interactions (Nip: a fish attacks and bites at the tail or body of another fish) could be measured for each fish on every consecutive frame of the video record. The SLs of the preserved fish were measured using a caliper and checked against the individuals in the video record. Sagittal otoliths of each fish were also extracted and the ALC label in the otolith was examined under a UV-light microscope (Tsukamoto et al., 1989). The remaining fish in each tank were anesthetized with MS222 and preserved in a 90% ethanol solution for the preliminary observation of growth history analysis in the previous study (Sakakura et al., 2004).

Between-group comparisons of SLs and behavioral differences were undertaken using the Student's *t*-test for 2 groups and the Tukey-Kramer test after a one-way analysis of variance (ANOVA) among 3 groups. The *G*-test (equivalent to the Chi-square test) for independence (Sokal and Rohlf, 1995) was carried out to determine the survival rate between Ω fish and non-Ω fish, and the correlation between the distribution of Ω fish and dominant fish

Results

The initial SL of Ω fish (average ± standard deviation; 7.9 ± 0.4 mm, n = 30) was the same as that of non-Ω fish (8.0 ± 0.4 mm, n = 30; *t*-test, $P > 0.05$). The developmental stage of both Ω and non-Ω fish were all F-stage, the beginning of metamorphosis (Minami, 1982). When 200 Ω fish were collected, the number of non-Ω fish was 475, indicating that the composition of Ω fish in the larvae at F-stage was about 30 %.

At the end of the rearing experiment on day 58, the survival rate of both Tanks A and B was about 70 %. For the otolith analysis of the specimens used for the behavioral analysis, we could observe the ALC label clearly in the otolith and discriminate Ω and non-Ω fish; 17 Ω fish (34 %) and 33 non-Ω fish (66 %) for Tank A, and 31 Ω fish (62 %) and 19 non-Ω fish (38 %) for Tank B, respectively. The survival of Ω fish and non-Ω fish in the same tank were judged equal by the *G*-test ($G^2 = 2.64$, $P = 0.1042$ for Tank A, $G^2 = 1.465$, $P = 0.262$ for Tank B), indicating that ALC labeling and the behavioral characteristics in the metamorphosing stage did not affect the survival of the fish. Therefore, the data of behavioral analysis was pooled from Tank A and B for further analysis.

With slight modification of Sakakura and Tsukamoto (1999), fish were classified into three categories based on 2 hours of behavioral observation as follows: when fish A was nipping fish B, fish A was classified as 'dominant' and fish B as 'subordinate'; fish showing no agonistic interactions within 2 hours were classified as 'intermediate'. Aggressive behavior was observed from dominants (4.4 ± 6.6 nip/hour) solely toward subordinates and not intermediates (Tukey-Kramer test, df = 2, F = 14.735, P<0.05). The SL of dominants was significantly greater than those of intermediates and subordinates (Fig. 1.; Tukey-Kramer test, df = 2, F = 55.612, P<0.05). Comparing the distribution to a Chi-square table (Table 1), the dominant fish coincided significantly with the Ω fish, indicating the possibility that fish formerly showing strong

Ω-posture become aggressive fish as they grow (G-test, G = 7.655, $P < 0.05$).

Discussion

A behavioral transition in Japanese flounder from the larval to juvenile stage was demonstrated in this study. Larvae showing Ω-posture in the metamorphosing stage tended to become dominant, showing aggressive behavior in the juvenile stage. Since the social rank of this species is size-dependent (Dou et al., 2000a; Dou et al., 2004) and Ω-posture in the metamorphosing stage is significantly and positively related to high growth in the juvenile stage (Sakakura et al., 2004), the findings in this study can be considered reasonable.

Formerly, the Ω-posture of the Japanese flounder larvae had been recognized as a feeding behavior, since it looks like an S-spike posture, which is a common attacking posture toward food items in fish larvae (Fukuhara, 1986; Dou et al., 2000b). However, Sakakura and Tsukamoto (2002) demonstrated that the Ω-posture was observed at the same frequency regardless of the presence or absence of food, and that no feeding behavior was observed in the absence of food. The duration of left-sided bending in the Ω-posture was significantly longer than that of right-sided bending in the beginning of metamorphosing stage (F-stage), and the left side is the one to which the eye migrates after metamorphosis. Furthermore, the Ω-posture is positively and significantly related to growth (Sakakura et al., 2004) and aggression in the juvenile stage (Table 1). Similar behavioral correlation from the larval to juvenile stage is reported in the yellowtail Seriola quinqueradiata (Sakakura ant Tsukamoto, 1999). In yellowtail larvae, a J-posture, which is a shivering behavior similar to the Ω-posture in Japanese flounder, is frequently observed, and it disappears after the onset of aggressive behavior when yellowtails become juveniles (Sakakura and Tsukamoto, 1996). It is confirmed that the J-posture is a precursor behavior of aggressive behavior because larvae showing the J-posture strongly became aggressive and dominant in schools at the juvenile stage (Sakakura and Tsukamoto, 1999). Synthesizing those findings, I propose that the Ω-posture in Japanese flounder larvae should be a good index for predicting the behavior and growth in juvenile stage because this behavior can be easily observed in both rearing and experimental tanks. Our research group has started to investigate whether there are Ω-posture like characteristics in other metamorphosing flatfishes and whether these behavioral correlations are common in flatfishes.

Agonistic interactions and cannibalism can cause economic losses in aquaculture and larviculture (Smith and Reay, 1991; Howell et al., 1998). Therefore, grading and sorting aggressive individuals in a rearing tank is essential to avoid the mortality from cannibalism; indeed, the size-grading method has been developed for certain species as amberjack Seriola dumerili (Shiozawa et al., 2003). On the other hand, aggressive dominants in one batch of the Japanese flounder can be biologically active fish and have a better potential for survival in the wild condition after release. It seems very informative to estimate and/or predict the behavioral characteristics (aggressive behavior) of the Japanese flounder as early as possible both for aquaculture and seedling production for release because larviculture is costly and laborious. Little attention has been paid to how larval issues are related to fish quality for release (Masuda and Tsukamoto, 1999; Masuda et al., 2002; Nakayama et al., 2003). However, the approach in this study, investigating behavioral correlations in the early life stages of marine fishes, not only provides basic biological information but also suggests a novel approach for evaluating fish quality for release. Further study will examine the ecological and biological meanings of such behavioral transitions, and to evaluate the stocking effectiveness from the index behavior, as the index behavior presented here occurred under experimental conditions. Thus, we have to carry out a field study using these index behaviors and determine the stocking effectiveness. Then, we will be able to sort the fish with behavioral fitness for release.

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Figure caption

Fig. 1. Standard length in relation to social rank: ‘Dominant’ = aggressive fish; ‘Subordinate’ = attacked by aggressive one; and ‘Intermediate’ = no agonistic interactions. Vertical bars represent standard deviations, and different alphabets indicate significant differences (a>b, $P < 0.05$; Tukey-Kramer test)

Table 1.

Chi-square table to determine the correlation between aggressive dominance (juvenile stage) and prior tendency of Ω-posture (metamorphosing stage).

Social rank in juvenile stage	N	Behavior in metamorphosing stage	
		Ohm	Non-Ohm
Dominant	35	23*	12
Intermediate	27	10	17
Subordinate	38	17	24*

Asterisks indicate significant difference (*G*-test, $P < 0.05$)

Fig.1. (Sakakura, 2006)