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Feeding behaviour of hatchery-reared juveniles of the Japanese flounder following a period of starvation

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Abstract

The effect of starvation on feeding behaviour was studied in hatchery-reared juveniles of the Japanese flounder in the context of improving stock enhancement strategies. Fish (54.0 ± 5.9 mm in total length) were deprived of food (mysids) for 1, 2, or 4 days, and their subsequent feeding behaviour was observed using a video camera. The phase of feeding action, the foraging pattern, the off-bottom duration, and the swimming speed were defined and analyzed. The feeding action was divided into four phases: aim, creep, attack, and return. The foraging patterns were classified into four types. The typical foraging pattern, with a single attack and a quick return to the initial position (type A), became less common as the fish were starved, while there was a greater incidence of slower returns (type B), returns to different positions (type C), and multiple feeding (type D). The frequency of the feeding action and the duration of off-bottom swimming increased as the food deprivation period increased. The swimming speed for creeping to the mysid prey increased, but that of the return decreased as the fish were starved. These changes in feeding behaviour under starvation may prolong the time flounder juveniles spend in the water column and may result in a higher risk of predation. In the stock enhancement program of Japanese flounder,

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starvation should be avoided to reduce predation and improve survival. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Stock enhancement, through the release of hatchery-reared juveniles, is one of the methods by which coastal fishery resources are being rebuilt. In natural waters, fish larvae have a high mortality rate because of their poor tolerance of starvation (O'Connell, 1976; Theilacker, 1986), and they are vulnerable to predation because of their poor swimming ability and their low level of visual accuracy (Bailey and Batty, 1984; Folkvord and Hunter, 1986; Rice et al., 1987; Fuiman, 1989). In modern stock enhancement, larvae are raised under artificial conditions to avoid starvation and predation, and only juveniles that have passed through the "critical period" are released.

In Japan, 20 million hatchery-reared juveniles of the Japanese flounder, *Paralichthys olivaceus*, are released every year, representing one of the most important species of marine stock enhancement in this country (Masuda and Tsukamoto, 1998; Tanaka et al., 1998). In some areas, the results of stocking have been remarkable (Fujita et al., 1993), but in most cases, they have been lower than expected.

Based on their morphological and physiological characteristics, juveniles originating from hatcheries do not seem to be inferior to their wild counterparts. Although hatchery-reared juveniles have a tendency toward albinism, they have well-developed sensory organs (Kawamura et al., 1989) and swimming ability (Miyazaki et al., unpublished data). However, field research suggests that mortality due to predation is higher in hatchery-reared juveniles than in wild juveniles (Furuta, 1996, 1998).

The potential predators of released juvenile flounder during the daytime are piscivorous fish such as 1+ year flounder and bartail flathead, *Platycephalus indicus*. Both wild and cultured flounder juveniles bury themselves into the sand in the daytime (Miyazaki et al., 1997); therefore, we assume that partial albinism of cultured fish would not be the critical factor of predation. Furuta (1991, 1996, 1998) has shown that predation tends to occur when the feeding behaviour of juveniles involves swimming up into the water column from the bottom. He concluded that one of the reasons for the high predation rate for hatchery-reared juveniles is their longer stay in the water column during feeding. In a rearing pond, starved flounder juveniles have been observed to swim up in the water column, even in the absence of food (Furuta, 1996). The predation of released juveniles increases in the season when mysid, *Acanthomysis robusta*, abundance decreases (Furuta, 1996; Furuta et al., 1997). This correlation also supports the strong relationship between foraging behaviour and predation.

Based on these observations, we hypothesized that starvation just after release may induce the potentially lethal swim-up behaviour of flounder juveniles. In this study, we observed the feeding behaviour of hatchery-reared flounder juveniles in detail. The effects of starvation on their foraging patterns, their off-bottom duration, and swimming

speed were analyzed to test our hypothesis. The potential of using behavioural approaches to improve stock enhancement programs is discussed.

2. Materials and methods

2.1. Fish husbandry

Juveniles of Japanese flounder used in the experiment were reared at the Tottori Prefectural Fisheries Experimental Station, Japan, in 1995. Broodstock were obtained from commercial fishermen and maintained in rearing tanks. Broodstock spawned spontaneously and the resultant fertilized eggs were maintained in a 500-l polycarbonate tank at 17°C. Larvae hatched out 48 h after fertilization and were fed with rotifers, *Brachionus plicatilis* and *Artemia salina*, from days 2 to 40 after hatching. They were fed with a formulated diet after they had attained ca. 30 mm or larger. Water temperature in the rearing tanks ranged from 17°C to 22°C.

2.2. Experimental protocol and analysis

Experiments were conducted in May 1995. Approximately 100 juveniles with the least amount of albinism were collected using a hand net and stocked into three 500-l polycarbonate tanks. Fish used for experiments were 90 days after hatching and their total length was 54.0 ± 5.9 mm (mean \pm S.D.). Preceding the experiments, juveniles were acclimated for 5 days to light and thermal conditions that were the same as those of the experimental tanks. During this acclimation period, the fish were fed with live mysids once a day.

Three acrylic tanks ($60 \times 45 \times 40$ cm³) were used for the experiment and were set up indoors under natural light conditions. Sand (grain size of 0.2–0.7 mm) was spread on the bottom to a thickness of 5 cm, and filtered sea water was added to a depth of 40 cm. The water in the tank was exchanged at four times per hour. Ten fish of similar size were distributed to each experimental tank. This density allowed for the feeding behaviour of all fish to be observed independently. The tanks were covered with a blue vinyl sheet to separate the tanks from observers.

Three different levels of starvation were tested. The control fish were deprived of food for 1 day, and the 2D and 4D starvation fish were deprived of food for 2 and 4 days, respectively. The longest starvation period in this study was based on the fact that most juvenile flounder have empty stomachs soon after release and gradually start feeding on the third day after release (Yamada et al., 1989). After these conditions were established, approximately 50 mysids were put into the tanks using a “feeding pipe” (25 mm diameter PVC pipe set at a depth of 5 cm from the surface). Fish feeding behaviour was recorded for 30 min through holes in the vinyl sheet using a video camera (Sony Handycom-8). The water temperature was kept at about 20°C during the experiment.

The video recordings were analyzed to determine the effects of starvation on feeding behaviour. We focused on the following three factors. (i) Phase and pattern: the

movement of the fish was traced by handplotting the fish's snout position on a transparent sheet placed on the video monitor. The hand trace was conducted at 1/3 s intervals, with a frame-by-frame analysis and the course was then divided into phases. Based on the difference in phases, each foraging action was identified as one of four specific patterns (A, B, C, and D) as: fish returned close (ca. 1 TL or less) to the initial position (A); fish returned in the same direction but not close to the initial position (B); fish returned in a different direction after the capture (C); and fish attacked prey several times in a single off-bottom behavior (D). The incidents of attacks observed in 5 min were counted from five different scenes in each tank, and their average was calculated. (ii) Off-bottom duration: time spent swimming in the water column was measured for all the feeding actions 5 min after putting the mysids into the experimental tank. Differences in the off-bottom duration in the three experimental tanks were compared by the Kruskal–Wallis test followed by Dunn's test (Zar, 1996). (iii) Swimming speed: swimming speed for each feeding action was measured based on plots of 1/3 s intervals. The swimming speeds from the three tanks were compared by ANOVA followed by Bonferroni's test (Howell, 1997).

3. Results

3.1. Action phase and foraging pattern

The typical feeding behaviour of juvenile Japanese flounder was divided into four phases: aim, creep, attack, and return (Fig. 1). First, a fish slowly raised its head while other parts of the body remained on the bottom (aim). The fish then slowly moved into the water column toward a mysid (creep). At a distance of about 5 mm, the fish accelerated rapidly and ingested the prey (attack). After the attack, the fish immediately changed swimming direction, swam downward through the water column, landed near its initial position, and buried itself in the sand (return).

Four patterns of feeding behaviour were observed and were defined as types A, B, C, and D (Fig. 2). In type A, the fish wiggled during the creep phase and the attack began

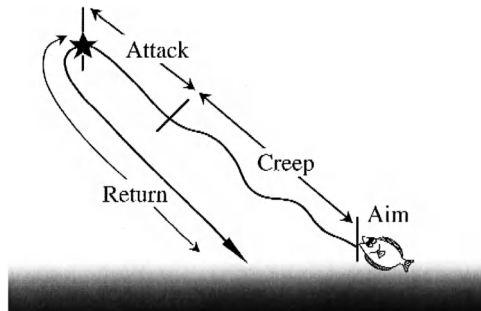


Fig. 1. The feeding action of juveniles of Japanese flounder was typically composed of four phases: aim, creep, attack and return. (★) The position at which the flounder bite a mysid.

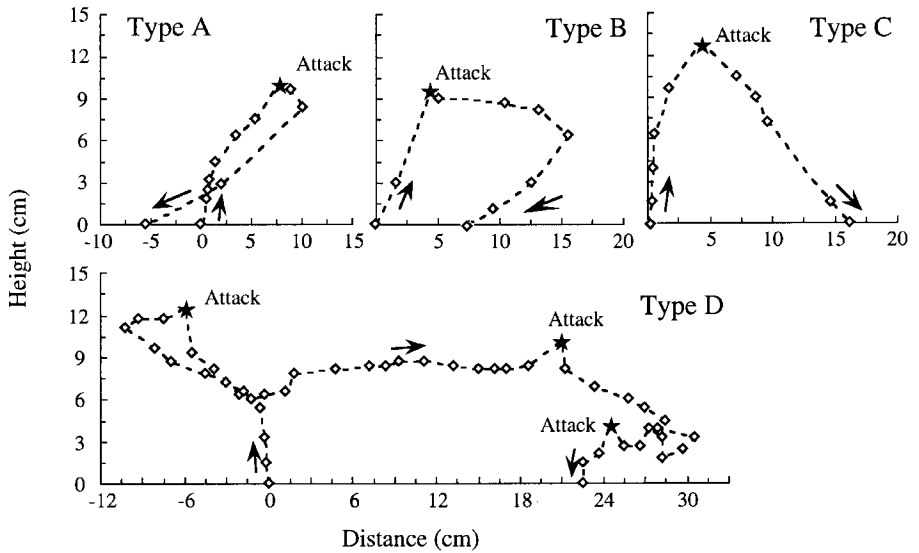


Fig. 2. Four types of foraging patterns observed in flounder juveniles. (◇) Position of fish every 1/3 s. (★) The position at which the fish bite a mysid. (→) Swimming direction of fish.

when the fish was approximately 7–8 cm away from the prey. During the return phase, the fish turned aside adroitly, changing the swimming direction and diving quickly, and the settling position was almost the same as where the feeding action was initiated. In type B, the creep was straight and the speed of the fish was faster than that in type A. After capture of the prey, the fish did not turn immediately, floating in the water column for about 1 s. When returning, the swimming speed was slower than for type A. In type C, the turning behaviour was absent, and the fish moved downward and obliquely through the water column after the attack, landing at a different site from the initial position. These three patterns of feeding action were the same as those described in

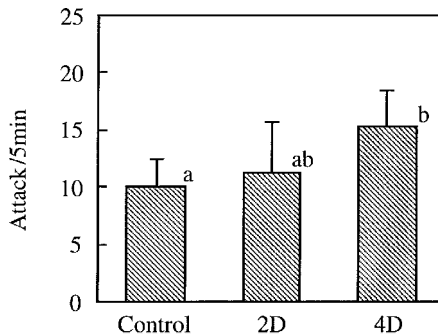


Fig. 3. Mean (±S.D.) number of feeding actions observed in 5 min (number of counts = 5). Vertical bar indicates standard deviation of five time observations, and letters give significant difference.

Furuta (1991, 1998). In the present experiment, another pattern, type D, was observed. In this pattern, the fish carry out several attacks during one feeding action. Fish swam around off the bottom for 10 s or more and attacked all mysids encountered. We defined this feeding behaviour as multiple feeding.

3.2. Effects of starvation

Thirty minutes of observation prior to the addition of mysids revealed that fish never swam off the bottom when food was not present. After addition of mysids, the frequency of attack increased as the food deprivation period increased, with the mean number of attacks \pm S.D. being 10.0 ± 2.5 , 11.2 ± 4.5 and 15.4 ± 3.1 ($n = 5$) for the control, 2D and 4D groups, respectively (Fig. 3). A significant difference was observed only between the control and 4D groups ($P < 0.02$, $F = 9.5$, Bonferroni's test).

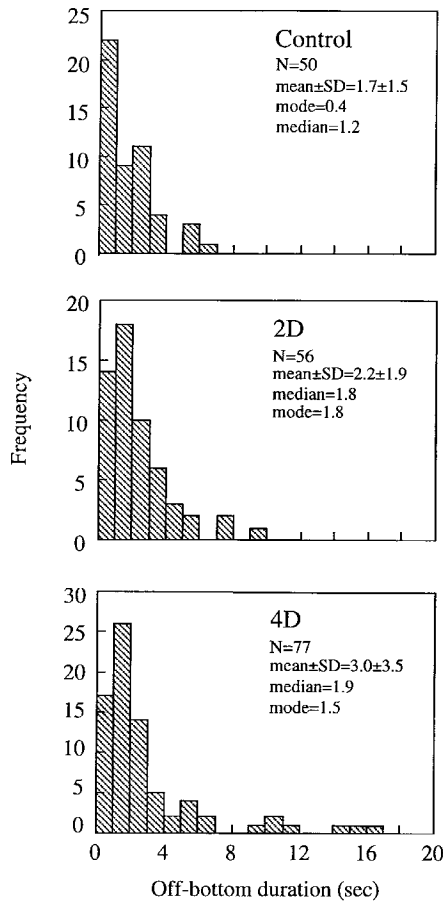


Fig. 4. Frequency distribution of the off-bottom duration of each feeding action during a 5-min observation period.

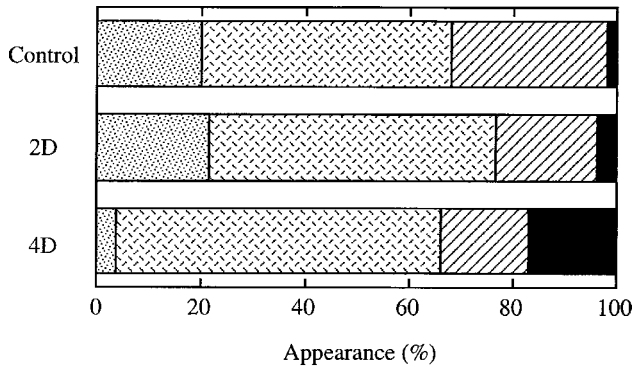


Fig. 5. Composition of the feeding patterns observed in 5 min. Mean observations for fish starved for 1 day (control), 2 days (2D) and 4 days (4D). (▤) Type A. (▥) Type B. (▧) Type C. (■) Type D. See explanation of feeding types in the text.

The duration of off-bottom feeding actions also increased in accordance with food deprivation (Fig. 4; $P < 0.01$, between control and 2D; $P > 0.25$, between 2D and 4D; $P < 0.0005$, between control and 4D, Dunn's test). In the control group, feeding action with an off-bottom duration of 1 s or less accounted for about 50% of all feeding actions. The percentage of quick feeding actions decreased with increases in the deprivation period to below 25%. The longest action was 6 s in the control group, and 9 s in the 2D group. In the 4D group, multiple feedings were frequently observed, with the off-bottom duration being 5 s or longer, representing 20% of all feeding actions. The longest feeding action took 16 s.

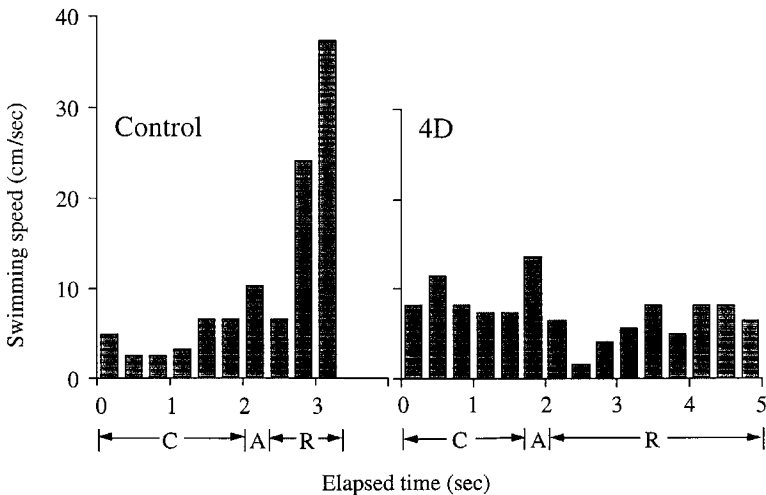


Fig. 6. Change of the swimming speed for type A was compared between the control and 4D groups. In the 4D fish, the swimming speed of the creep (C) phase was faster, while the return (R) phase was slower than in the control groups.

Table 1

Swimming speeds (cm/s) of the creep, attack, and return phases of feeding action type A were compared between the control and 4D groups

Phase	Starvation		Probability
	Control	4D	
Creep	4.6 ± 2.1	6.0 ± 2.6	$P = 0.051$
Attack	17.1 ± 9.0	17.4 ± 7.1	$P = 0.954$
Return	20.2 ± 9.0	8.7 ± 6.5	$P < 0.001$

Five feeding actions in each food deprivation period were analyzed for this comparison. The 2D was not significantly different in any of phase ($n = 5$).

In the 4D group, the frequency of type A feeding behaviour was only 20% of that in the control group, while that of type D behaviour was five times more than in the control group (Fig. 5). Therefore, as starvation advances, feeding actions became slower and multiple feedings increased.

The swimming speed during type A feeding action was compared between the control and 4D group. In the control group, swimming speed was low during the creep phase and fast in the attack phase (5 and 10 cm/s, respectively; Fig. 6). During the return phase, although the fish slowed down when changing direction, they rapidly accelerated to 20–40 cm/s when diving down to the bottom. In the 4D group, the swimming speed of the creep phase was about 1.3 times faster than that of the control group (Table 1). In contrast, during the return phase, the speed decreased to 50% of the control group. The time the fish spent in the creep phase was slightly shorter in the 4D than in the control group, while that for the return was about three times longer in the 4D than in the control group. Significant differences in swimming speed were observed between the control and the 4D groups during the return (control, 20.1 ± 9.0 cm/s; 4D, 8.7 ± 6.6 cm/s, $P < 0.001$, $F = 20.2$; Bonferroni's test).

4. Discussion

4.1. Feeding behaviour and predation

Juveniles of Japanese flounder are considered to be susceptible to predation when they swim up from the bottom to feed (Tanaka et al., 1989; Furuta, 1996, 1998). In the present experiment, this swim-up feeding behaviour was divided into the following four phases: aim, creep, attack and return. During the "creep" phase, the swimming speed of juveniles is up to 25% slower than in other phases. As flounder eyes are on the upper body side, they may not be able to recognize bottom-dwelling predators during the creep phase. We therefore consider that flounder juveniles are most vulnerable to predation when they are in the creep phase of their feeding action.

Furuta (1996, 1998) reported that wild flounder juveniles show type A foraging patterns in 38% of all cases, while cultured fish tend to show type A patterns in only 11% of cases, with types B and C patterns occurring more frequently. Furuta (1992) has

shown that lower rearing density increases the occurrence of type A activity pattern in cultured juvenile flounder and, through a releasing experiment, that low-density rearing can reduce predation mortality. The type A rapid turning action may be important in avoiding predators.

Furthermore, time spent off-bottom for one feeding action is longer in cultured flounder than in wild animals (Furuta, 1996, 1998). The slower feeding action may be due to the feeding regime in the rearing facility. Flounder juveniles may have been conditioned to feed with slower movements and prolonged action by being fed commercial pellets that are motionless and floating. The lack of experience with regard to predation attack might be another cause of slower movement of the cultured flounder.

4.2. Effect of starvation on predation

Behaviour under starvation conditions has been reported for larvae, juveniles, and adults of several species (Beukema, 1968; Laurence, 1972; Rice et al., 1987; Croy and Hughes, 1991). Beukema (1968) has shown that the three-spined stickleback, *Gasterosteus aculeatus*, shows more encountering, grasping, and eating behaviour as food deprivation time is increased from 16 to 88 h. The increased feeding activity due to starvation coincides with our results in flounders. In the stickleback, increased feeding activity may not directly result in a risk of predation, while in flounder juveniles, the increase in off-bottom swimming may be a critical predation factor, since the fish are vulnerable when they are away from the sandy bottom.

According to field research in stock enhancement, about 70% of flounder juveniles has empty stomachs 1 week after their release, even when mysids are abundant at the release site (Yamada et al., 1989; Furuta et al., 1997). The “point of no return” (i.e. starvation period when fish become too weak to resume feeding; Blaxter and Hempel, 1963) in 57-mm flounder juveniles is reported to be 15 days (Yamada et al., 1989). Therefore, before mortality by starvation occurs, serious mortality might occur as a result of predation induced by abnormal feeding behaviour such as multiple feedings.

We can suggest several factors that might prevent fish from feeding soon after release: (i) handling stress when they are released is so strong that it takes them several days to recover; (ii) it takes time for them to adapt to environmental changes from hatchery to wild conditions; and (iii) hatchery juveniles lack the ability to effectively catch wild prey. Each of these factors should be studied by behavioural and field research to find a way to improve hatchery techniques and releasing strategies. We believe that such an approach is essential to resolve potential predation problems after release and thus to achieve greater success in stock enhancement.

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