Environmental stimuli improve learning capability in striped knifejaw juveniles: the stage-specific effect of environmental enrichment and the comparison between wild and hatchery-reared fish

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Abstract

Hatchery-reared fish often show different behavioral traits from their wild counterparts possibly due to the lack of environmental stimuli. Here, we aimed to reveal the stage-specific effect of environmental stimuli on the development of learning capability in striped knifejaw *Oplegnathus fasciatus.* The fish were raised for 15 days (50‑65 days post hatch) or 30 days (50–80 or 90–120 days post hatch) in either conventional rearing tanks (control) or in a structurally-enriched tank containing bricks, artificial sea grass, and plastic pipes (enriched environment), and were examined for learning capability using Y-maze reward conditioning. The learning capability of wild juveniles was also examined and their scores were compared with those of hatchery-reared fish (which we previously reported). Only fish in the 50–80 days post hatch enriched-rearing group showed significantly better scores than those in the control group, and wild fish performed better than hatchery-reared ones. The present results indicate that, although the learning capability of hatchery-reared fish is inferior to that of wild fish, exposure to a highly-structured environment at an appropriate stage promotes the development of learning capability. Such environmental enrichment can potentially improve the viability of hatchery-reared fish when they are released into the wild.

KEY WORDS: behavioral ontogeny, critical period, environmental enrichment, habitat complexity, *Oplegnathus fasciatus*, reward conditioning, stock enhancement, Y-maze.

Introduction

Animals are always challenged to adapt to the environment in which they find themselves. Physiological tolerance, as well as reflex, taxis, and other instinctive behaviors, are the main mechanisms used by the lower taxa of animals to adapt to the environment, whereas behavioral adaptability, or learning, may play a role in any taxa of animals possessing a central nervous system. Although there should always be a phylogenetic limitation on learning capability in each taxa, species that are often faced with a high requirement of behavioral adaptation are likely to have a high capability of learning.

The striped knifejaw *Oplegnathus fasciatus* is an ideal material for studying the learning capability of lower vertebrates. In our previous paper we studied the ontogeny of learning capability in this species and found that they learn fastest at about 70 mm in standard length (SL) under Y-maze reward conditioning [1]. Because striped knifejaw recruit from offshore to coastal reef habitat at 30–80 mm SL [2], we have suggested that they have the highest learning capability at the peak of requirement to adapt to a changeable environment, as coastal reef habitats tend to have a wide variety of site-specific prey items. Other than this species, both Pacific threadfin *Polydactylus sexfilis* and jack mackerel *Trachurus japonicus* show a high learning capability corresponding with a major habitat shift from offshore to coastal waters [3, 4]. In these studies the development of learning capability is assumed to be intrinsic. However, the learning capability itself can be modified in their developmental process by environmental stimuli, as has been shown in rodents [5, 6] and cephalopods [7].

Environmental enrichment, which is defined as a deliberate increase in environmental complexity with the aim of reducing maladaptive and aberrant traits [8], has been particularly in focus in aquatic animals for the last decade. Atlantic salmon *Salmo salar* reared in enriched environments have lower plasma cortisol levels and show more frequent shelter-seeking than those in a standard condition [9]. Enrichment via the substrate also reduces the aggressive behavior in gilthead seabream *Sparus aurata* [10]. In an enriched environment, the tropical octopus *Callistoctopus aspilosomatis* is more exploratory in the tank and more responsive to stimuli than those in a plain tank [11]. Furthermore, there are at least four published studies evaluating the effectiveness of environmental enrichment on learning in fishes, with either a positive effect in Atlantic cod *Gadus morhua* [12], zebrafish *Danio rerio* [13], and Atlantic salmon [14] or a neutral effect in three-spined stickleback *Gasterosteus aculeatus* [15]. Brydges and Braithwaite [15] suggested that genetic factors, rather than the experienced environment, might be more important in species under high predation pressure, such as the stickleback. However, none of the above-mentioned studies tested the efficacy of environmental enrichment on learning in more than one developmental stage of a target fish species.

The goal of the present research was to elucidate a stage-specific effect of environmental enrichment on the development of learning capability in fish. Two experiments were conducted for this purpose. In the first experiment, striped knifejaw were raised in either an enriched or plain environment: the former having submerged complex structures in a rearing tank around which the fish can play, and the latter being a conventional rearing tank with minimum physical structures. The treatment was applied at three different stages (or durations) of development in hatchery-reared juveniles. Second, the learning capability of wild-collected striped knifejaw juveniles was examined and their scores were compared to those of hatchery-reared juveniles, which we had reported in our previous paper [1]. As wild fish generally experience a more structurally-complex environment than hatchery-reared fish, we expected to observe higher learning capability in the wild fish.

Materials and methods

Experiment I. Environmental enrichment in rearing tanks

Twenty-six striped knifejaw juveniles were used in the experiment. They were hatched and reared from two lots of naturally spawned eggs (spawned on June 3, 2005 and June 25, 2005) from broodstock kept in the Maizuru Fisheries Research Station (MFRS) of Kyoto University. Each lot were reared in two 500 l polycarbonate tanks and were fed with rotifers *Brachionus plicatilis*, *Artemia* sp.

nauplii, wild-collected copepods, defrosted krill *Euphausia pacifica* and pellets in accordance with their growth. Due to a reduced number of fish in the second lot because of disease, the juveniles from the two tanks were mixed into one 500 l tank.

The enriched tank consisted of a 200 l polycarbonate tank with bricks (four bricks of 10 × 21 × 6 cm, two bricks of 6 × 21 × 6 cm, and a brick of 22 × 22 × 4 cm), four pieces of artificial seagrass (30 cm × 2 and 40 cm × 2, Porimon 180 mm diameter; Tanaka Sanjiro, Inc.), and two tripod structures made of 20 cm and 40 cm PVC pipes (15 mm diameter; Kubota CI). The control tank was the same but without any submerged structures, except for a draining pipe and an air stone (25 mm diameter) (Fig. 1). The walls of both tanks were covered with blue semi-transparent plastic sheets and the tops were covered with a blue net. The water was exchanged at a rate of 45 l per hour and aerated.

The fish were raised either in the enriched tank or in the control tank for three different periods as follows. Fish used for Group A originated from the second lot of the spawning, while those for Groups B and C originated from the first lot. Group A: When the fish reached 50 days post hatch (dph) (mean SL = 18.2 mm), ten fish, randomly selected from the holding tank, were transferred to the enriched tank, and another ten fish were transferred to the control tank. Testing was initiated 15 days after moving the fish into the enriched and control tanks. Six fish, three from each rearing condition, were selected randomly and were examined for learning capability. Group B: When the fish reached 50 dph (mean SL = 16.5 mm), ten fish were transferred from the holding tanks to the enriched tank, and another ten fish were transferred to the control tank. Testing was started 30 days after moving the fish into the enriched and control tanks. Ten fish, five from each rearing condition, were selected randomly and were examined for learning capability. Group C: When the fish reached 90 dph (mean SL = 55.4 mm), seven fish were transferred from the holding tanks to the enriched tank, and another seven fish were transferred to the control tank. Testing began 30 days after the transference. Ten fish, five from each rearing condition, were selected randomly and were examined for learning capability.

Measurement of learning capability

We used four sets of Y-maze experimental tanks (58 × 28 × 35 cm, Fig. 2). The far end of each tank was separated by a partition (a 30 × 30 cm gray acrylic board). Two light emitting diode lamps (LED; red and yellow, 3–6 V, EUPA) were set on the outside of the end of each arm. The color of the LED lamps was controlled by a switch. The wall of the tank was covered by a black sheet to minimize any effects of the observer. The experiment was conducted from August 2005 to October 2005 in the MFRS. Each fish was used only once in the conditioning experiment.

Applying the same size of tank for the whole size range of fish could have biased the learning score especially in larger individuals. In our previous paper cautiousness index was defined as the percentage of trials in which the fish fled back to the start area by being frightened of the drop of the reward pellet, and the index had no correlation with SL or learning score [1]. Therefore we consider that the size of experimental tanks had little effect on the learning score when we apply this size of tank for striped knifejaw ranging 20–100 mm SL. Besides our focus was on the comparison between enriched and control fish with a matching size, and so the bias from the tank size should have been minimum.

Striped knifejaw individuals were transported from the rearing tanks to the Y-mazes, with one fish in each Y-maze tank. First, the fish was driven into the start area. Then the two LED lamps, red on the right side and yellow on the left side, were turned on. The fish was rewarded with food pellets (Kyowa-B700, Kyowa Hakko Co., Ltd., or Otohime-S, Marubeni Nisshin Feed Co., Ltd.) that were provided using a pipette with sea water (about 3 ml) when the fish swam to the correct arm after the door was opened. After the fish was fed with the pellets, the LED lamps were turned off and the fish was driven back to the start area. When the fish swam to the incorrect arm, the LED lamps were turned off immediately and the fish was driven back into the start area without being provided with pellets. For more details on the procedure, see Makino et al. [1]. One session was composed of 10 trials and each individual fish took part in 12 sessions (120 trials).

The reward arm was consistently kept on either the right side (or the left side) during each session and illuminated by the red LED during all trials in the 12 sessions. With this procedure fish can use both the color of LED lamps and the direction in the maze as a cue for the correct arm; a procedure using only the color cue and randomizing the direction in each trial substantially reduced efficiency of learning (Masuda R, unpubl. data, 2004). When the fish visited the correct arm at a rate of seven or more out of 10 trials for three consecutive sessions, we considered that the fish had learned the conditioning, as this could only happen through random movements with a probability of less than 1% [16]. As each fish reached this criterion of learning in the original problem (R_0) (correct arm: right arm, illuminated by the red LED lamp), the reward arm were reversed, and the choice of the left arm illuminated by the red LED lamp was rewarded. When a fish reached the criterion of learning in the first reversal conditioning (R_1), the rewarded side was reversed again (R_2). The reversal of the rewarded side was conducted up to three times (i.e., to R_3), depending on their capability of learning. Based on the score of both the original and reversal problems, the individual score was calculated as the sum of the average percentage of correct choices in R_0, R_1, R_2 and R_3.

Experiment II. Wild fish

Sixteen wild individuals (26–100 mm SL) were used in the experiment. Fourteen of them were collected with drifting algae from a research vessel in Wakasa Bay from May 2006 to July 2006. One fish was captured with a hand net at a rocky reef by snorkeling, and another one was collected by angling, both in September 2006. The fish were kept in a 200 l polycarbonate tank and were fed with defrosted krill and pellets. They were given 7 days for the diet shift to artificial pellets after they had been collected. After the complete shift to the pellets, they were examined for learning capability. The experimental apparatus and learning test procedure were both the same as in Experiment I.

Data analysis

As a learning score tends to have a linear relation with SL within a limited size range [1, 4], the effect of rearing condition was tested using analysis of covariance (ANCOVA) with SL as a covariate for each enrichment period. The scores of the wild fish were compared with those of the hatchery-reared individuals presented in the previous paper [1]; the latter originated from three independent natural spawning events from the same broodstock as in Experiment I and were reared in 500 l tanks with a plain environment. Because we collected only two wild individuals larger than 70 mm SL, the comparison of wild and hatchery-reared fish was conducted within the range of matching size, that is, 22–65 mm (n = 14 and n = 15 for wild and hatchery-reared fish, respectively), using ANCOVA. Scores of fish were also compared among four treatments, i.e., enriched and control from the experiment I and the wild and the hatchery-reared from the experiment II. For this analysis data were either fitting to a quadratic curve using whole the size range of fish, or using ANCOVA using scores of 65 mm or smaller. All statistical analyses were conducted using R version 2.15.2 (R Development Core Team 2012).

Results

Experiment I. Effect of environmental enrichment

The fish reared in the enriched tank usually stayed around, and often passed through, the tunnels made of bricks or pipes except for feeding time, when they darted to the food pellets and then hid behind the structures. The fish in the control condition did not show such behavior. The average SL ± SD of the enriched groups A, B, and C given the learning tests were 33.7 ± 4.2, 58.8 ± 3.0, and 80.6 ± 6.4 mm respectively, and that of the control groups were 33.3 ± 1.5, 56.0 ± 5.3, and 80.2 ± 2.3 mm respectively. There were no significant size differences between the enriched and control condition fish in each age group (Welch's t-test, P = 0.91, 0.63, and 0.91, and N = 6, 10, and 10, in groups A, B, and C, respectively). At the start of the experiments some individuals fled back to the start area without feeding when the food pellets were provided. However, this did not occur after 10 to 40 trials at the latest.

A typical individual from group A cleared the original problem ("R_0") in five sessions. The correct answer rate then decreased because it was not able to adapt to the reversal of the reward arm instantly and took six sessions to clear the reversal problem ("R_1"; Fig. 3a). In contrast, a typical individual from group B required fewer trials to learn R_0 as well as R_1 (Fig. 3b). Fish from group C typically showed a learning curve between those from groups A and B (Fig. 3c).

All of the 26 fish (A-enriched: n = 3; A-control: n = 3; B-enriched: n = 5; B-control: n = 5; C-enriched: n = 5; C-control: n = 5) demonstrated a learning capability, clearing at least R_0. Fish in the B-enriched group scored more highly than those in the B-control group, whereas no significant difference was observed between the enriched and the control fish in groups A and C (ANCOVA, $F_{1, 2}$ = 0.001, P = 0.98, $F_{1, 6}$ = 23.5, P = 0.0029, and $F_{1, 6}$ = 1.1, P = 0.34 in groups A, B and C, respectively) (Fig. 4). The average scores of the enriched groups A, B, and C were 148.2, 303.5, and 263.5 points, and those of the control groups were 145.8, 224.2 and 236.9 points, respectively.

Experiment II. Wild versus hatchery-reared fish

All of the fish collected in the wild cleared at least the original problem (R_0). The relationship between SL (mm) and individual score was approximated by a quadratic curve ($y = -0.1066(x - 68.32)^2 + 341.6$, $R^2 = 0.6192$; x: SL, y: score) with the maximum score at 68 mm SL (Fig. 5). The scores of the wild fish within the size range of 22–65 mm SL were approximated to the regression line as follows:

$y = 4.531x + 62.74$, $R^2 = 0.5102$, $n = 14$

Those of the hatchery-reared were approximated as follows:

$y = 5.354x - 11.45$, $R^2 = 0.8889$, $n = 15$

The score of the wild fish was higher than that of the hatchery-reared fish as compared within this size range (ANCOVA, $F_{1, 25}$ = 5.0, P = 0.034).

Analysis including whole the size range of fish revealed that there were no significant differences among the four treatments of enriched, poor, wild, and hatchery-reared ($P > 0.05$). When compared in fish at 65 mm SL or smaller, scores of control fish in the experiment I were lower than the wild (ANCOVA, $F_{1, 18} = 14.0$, $P = 0.0014$), the hatchery-reared ($F_{1, 19} = 11.4$, $P = 0.0030$), or enriched treatment fish ($F_{1, 12} = 8.68$, $P = 0.011$), whereas that of enriched fish was not significantly different from the wild ($F_{1, 18} = 3.75$, $P = 0.068$) or the hatchery-reared fish ($F_{1, 19} = 0.67$, $P = 0.42$).

Discussion

The critical period for environmental enrichment to improve learning capability

In this study, the fish reared in the enriched environment from 50 to 80 dph showed a better learning capability than the control fish, indicating that a rearing condition with submerged structures enhanced the development of learning capability. There was no difference in the learning capability between the test and control fish in group A. We suggest that 15 days of experience in an enriched environment from 50 to 65 dph was either too short or did not occur at an appropriate time in their development to influence their learning capability. The average score of the group reared in the enriched condition from 90 to 120 dph (group C) tended to be higher than that of the control group, although there was no significant difference. These results imply that striped knifejaw have a period during which they are sensitive for the development of learning capability. The size ranges exposed to enrichment were 18.2–33.7, 16.5–58.8, and 55.4–80.6 mm SL in groups A, B, and C, respectively. Striped knifejaw is reported to recruit to shallow artificial reefs at the size range of 30–80 mm with the average of 59 mm SL [2]. We therefore suggest that this species has a high sensitivity to a structurally rich environment at the early stage of coastal recruitment.

Fitting to a quadratic curve resulted in non-significant differences among the four treatments of the enriched, the control, the wild and the hatchery-reared. This was probably because scores of fish in group A and C masked the effect of enrichment in the experiment I, and the lack of data at the size range between 65 and 92 mm in the wild fish reduced the power of analysis in the experiment II. Here we can emphasize the importance of considering developmental stage when we study the learning capability of fish.

Comparison within the matching size range revealed that the control fish in the experiment I had lower score than the hatchery-reared fish in the experiment II. Although the hatchery tanks lacked structures, they were larger in size and had more individuals than the control tanks; these factors could have facilitated the development of learning capability. In contrast the enrichment fish did not differ either from the wild or the hatchery-reared fish, suggesting that structural enrichment compensated for the negative effect of small tank size or fewer number of fish.

Dickel and his colleagues showed that the rearing environment has a considerable effect on the ontogeny of learning and memory in cuttlefish *Sepia officinalis* [7]. They reared the cuttlefish juveniles either individually in a plain condition without any structures, or in an enriched condition with other individuals, substrate, and structures. They found that the acquisition and retention of a learning task in the cuttlefish reared in the enriched condition were significantly better than those of the impoverished group. They further confirmed that the rearing environment during the 2nd and/or 3rd months of cuttlefish life is crucial for the development of memory. The sensitive period for the development of behavioral flexibility has been reported in invertebrates, mammals, and birds [6]. Rosenzweig and Bennet concluded that the use and experience of the nervous system is necessary to induce plasticity in behavior, and is necessary for the full development of species-specific brain characteristics and behavioral potential, and environmental enrichments are especially effective early in the life history [6]. We observed that fish in the environmentally enriched tank often swam through the brick tunnels and other structures. Such spontaneous behavior might well have enhanced the development of their central nervous system, resulting in the better score of learning capability.

Indeed Kihslinger and Nevitt revealed that yolk-sac larvae of stealhead salmon *Oncorhynchus mykiss* reared in an enriched environment with stones grew brains with significantly larger cerebella than those reared in conventional tanks [17]. Furthermore, such an effect of enrichment on the brain is effective only in the early stage of life history in the case of the Atlantic salmon *Salmo salar*, as juveniles reared in a plain environment catch up in brain growth with those in an enriched environment [18]. It is most likely that the sensitive stage for environmental stimuli is species-specific corresponding with its life history strategy.

Environmental enrichment-enhanced growth has been observed in gilthead seabream [10] and cuttlefish [7]. In contrast, in our experiment, the growth of striped knifejaw in the enriched environment did not differ from that of the control fish. This discrepancy may be due to our relatively short rearing period, or perhaps the environment first influences behavioral characteristics, such as social behavior and learning capability, which then induce later differences in growth. Such a proposal is consistent with the case of steelhead salmon juveniles reared in an enriched environment that showed a higher social rank in a rearing tank than those kept in a plain environment, but without any growth difference, and then had better growth after release into a quasi-natural stream [19].

The superiority of wild fish to hatchery-reared fish

We revealed that wild juveniles of the striped knifejaw had better learning capability compared to the hatchery-reared juveniles. Their superiority in learning capability can be attributable to the following factors: (1) a wild environment is rich in various stimuli and thus improves their learning capability; (2) wild juveniles receive better quality of prey and thus can better develop central nervous system; and (3) only the individuals with a relatively high learning capability can survive in the wild.

Although we assume that the first factor is most likely as it was supported by the results of Experiment I, the other two factors are also possible. Typical wild preys, such as copepods and decapods, contain a high amount of docosahexaenoic acid (DHA). DHA is the major component of brain membrane phospholipids, and dietary DHA is used to compose the central nervous system [20]. The size of the brain is indeed reported to be larger in wild than in hatchery-reared individuals in rainbow trout *Oncorhynchus mykiss* [21] and guppy *Poecilia reticulata* [22].

In our previous paper, we reported that there was a strong correlation between the growth rate and individual score in hatchery-reared striped knifejaw juveniles [1]. If there is a variation in learning capability in the wild at a relatively early stage, those with higher learning capability are likely to grow faster and survive better. However, in the present study, it was revealed that wild fish still have a high variability in scores (Fig. 5), which may represent both genetic variation and variation in experience between individuals. It is noteworthy that wild-caught guppies have more variation in brain size than their offspring do in captivity [22].

Fish personality could affect the learning score; for instance, bold rainbow trout can learn a task more quickly than shy individuals [23]. This is probably not the case in striped knifejaw, because our previous work revealed that learning score had no correlation with a cautiousness index [1]. Therefore, the superiority of the score in the wild fish is more likely to represent their learning capability rather than boldness or other personality traits.

Implications for marine stock enhancement and perspectives

The high mortality of hatchery-reared fish after release has been a major problem in stock enhancement projects, and this is partly due to the behavioral inferiority of hatchery-reared fish compared to wild ones [24–26]. For example, in Japanese flounder *Paralichthys olivaceus*, hatchery-reared juveniles spend a longer time off the bottom than their wild counterparts, which is suggested to be a cause of high mortality of the released seedlings [27]. Such a maladaptive behavior of flounder can be mitigated by a simple training procedure of bottom feeding and net chasing [28].

Pre-release training and/or environmental enrichment has been reported to improve post-release performance in tuskfish *Choerodon schoenleinii* [29], white seabream *Diplodus sargus* [30], and Atlantic salmon [31], but not in the case of stealhead [19] or other fishes [8]. Overall, this is an area which requires further research. We suggest that the ontogenetic critical period is particularly important to consider when evaluating the impact of environmental enrichment, both for the fundamental understanding of the development of learning and its application for stock enhancement and conservation.

Present study also revealed that striped knifejaw has a peak of learning score at approximately 70 mm SL both in the wild and hatchery reared individuals (Fig. 4, 5), implying that this species has an innate peak of learning capability at this size. Masuda and Ziemann [3] also showed that hatchery-reared Pacific threadfin juveniles at 50–90 mm were better at learning compared to smaller or larger conspecifics, and suggested that fish with this developmental stage of high learning capability would be adaptable to a new environment when released into the wild. Such an idea is certainly applicable to the striped knifejaw, and probably other species targeted for stock enhancement.

Studies into environmental enrichment have been conducted vigorously using higher vertebrates in zoo, farm, and experimental model animals, particularly in the context of animal welfare [32]. Some of the knowledge from such studies, such as that environmental enrichment improves animal longevity and reproduction, may well be applicable to the field of fisheries and aquaculture. In return, some marine fishes can be good models for understanding the efficacy of environmental factors on behavioral ontogeny.

In conclusion, we found that environmental enrichment at an appropriate developmental timing improves learning capability in striped knifejaw juveniles. We also confirmed that wild individuals of this species have better learning capability compared to the hatchery-reared counterparts. A future research subject in this field would be the effect of dietary condition on their learning capability and concurrent developmental changes in the brain. Interspecific comparison among different reef fishes using a Y-maze is also a promising research field.

Acknowledgments The authors thank Drs. Barry Berejikian (Northwest Fisheries Science Center, National Oceanic and Atmospheric Administration, USA), Takashi Yokota (Fisheries Research

Agency), Kohji Takahashi (Nagasaki University), Sanshiro Kaneko (MFRS) for their constructive comments on earlier drafts of the manuscript. Dr. Keiichi Fukaya (The Institute of Statistical Mathematics) gave an excellent advice for the statistical analysis. Comments from Dr. Yutaka Kurita and two anonymous reviewers are also greatly acknowledged. This work was supported in part by a Grant-in-Aid for Scientific Research (15780133) given to R. M.

References

1. Makino H, Masuda R, Tanaka M (2006) Ontogenetic changes of learning capability under reward conditioning in striped knifejaw *Oplegnathus fasciatus* juveniles. Fish Sci 72:1177–1182
2. Masuda R, Shiba M, Yamashita Y, Ueno M, Kai Y, Nakanishi A, Torikoshi M, Tanaka M (2010) Fish assemblages associated with three types of artificial reefs: density of assemblages and possible impacts on adjacent fish abundance. Fish Bull 108:162–173
3. Masuda R, Ziemann DA (2000) Ontogenetic changes of learning capability and stress recovery in Pacific threadfin juveniles. J Fish Biol 56:1239–1247
4. Takahashi K, Masuda R, Yamashita Y (2010) Ontogenetic changes in the spatial learning capability of jack mackerel *Trachurus japinicus*. J Fish Biol 77:2315–2325
5. Kempermann G, Kuhn HG, Gage FH (1998) Experience-induced neurogenesis in the senescent dentate gyrus. J Neurosci 18:3206–3212
6. Rosenzweig MR, Bennet EL (1996) Psychobiology of plasticity: effects of training and experience on brain and behaviour. Behav Brain Res 78:57–65
7. Dickel L, Boal JG, Budelmann BU (2000) The effect of early experience on learning and memory in cuttlefish. Develop Psychol 36:101–110
8. Näslund J, Johnsson JI (2014) Environmental enrichment for fish in captive environments: effects of physical structures and substrates. Fish Fisheries DOI: 10.1111/faf.12088

9. Näslund J, Rosengren M, Villar DD, Gansel L, Norrgård JR, Persson L, Winkowski JJ, Kvingedal E (2013) Hatchery tank enrichment affects cortisol levels and shelter-seeking in Atlantic salmon (*Salmo salar*). Can J Fish Aquat Sci 70:585–590

10. Batzina A, Dalla C, Papadopoulou-Daifoti Z, Karakatsouli N (2014) Effects of environmental enrichment on growth, aggressive behaviour and brain monoamines of gilthead seabream *Sparus aurata* reared under different social conditions. Comp Biochem Physiol A 169:25–32

11. Yasumuro H, Ikeda Y (2011) Effects of environmental enrichment on the behavior of the tropical octopus *Callistoctopus aspilosomatis*. Mar Fresh Behav Physiol 44:143–157

12. Strand DA, Utne-Palm AC, Jakobsen PJ, Braithweite VA, Jensen KH, Salvanes AGV (2010) Enrichment promotes learning in fish. Mar Ecol Prog Ser 412:273–282

13. Spence R, Magurran AE, Smith C (2011) Spatial cognition in zebrafish: the role of strain and rearing environment. Anim Cogn 14:607–612

14. Salvanes AGV, Moberg O, Ebbesson LOE, Nilsen TO, Jensen KH, Braithwaite VA (2013) Environmental enrichment promotes neural plasticity and cognitive ability in fish. Proc Royal Soc B 280:20131331

15. Brydges NM, Braithwaite VA (2009) Does environmental enrichment affect the behaviour of fish commonly used in laboratory work? Appl Anim Behav Sci 118:137–143

16. Kawamura G, Shimowada T (1983) Discrimination of striped shape by Japanese parrotfish. Nippon Suisan Gakkaishi 49:55–60 (in Japanese with English abstract)

17. Kihslinger RL, Nevitt GA (2006) Early rearing environment impacts cerebellar growth in juvenile salmon. J Exp Biol 209:504–509

18. Näslund J, Aarestrup K, Thomassen ST, Johnsson JI (2012) Early enrichment effects on brain development in hatchery-reared Atlantic salmon (*salmo salar*): no evidence for a critical period. Can J Fish Aquat Sci 69:1481–1490

19. Berejikian BA, Tezak EP, Flagg TA, Larae AL, Kummerow E, Mahnken CVW (2000) Social

dominance, growth, and habitat use of age-0 steelhead (*Oncorhynchus mykiss*) grown in enriched and conventional hatchery rearing environment. Can J Fish Aquat Sci 57:628–636

20. Masuda R, Takeuchi T, Tsukamoto K, Sato H, Shimizu K, Imaizumi K (1999) Incorporation of dietary docosahexaenoic acid into the central nervous system of the yellowtail *Seriola quinqueradiata*. Brain Behav Evol 53:173–179

21. Marchetti MP, Nevitt GA (2003) Effects of hatchery rearing on brain structures of rainbow trout, *Oncorhynchus mykiss*. Env Biol Fish 66:9–14

22. Burns JG, Saravanan A, Rodd FH (2009) Rearing environment affects the brain size of guppies: lab-reared guppies have smaller brains than wild-caught guppies. Ethology 115:123–133

23. Sneddon LU (2003) The bold and the shy: individual differences in rainbow trout. J Fish Biol 62:971–975

24. Tanaka M, Seikai T, Yamamoto E, Furuta S (1998) Significance of larval and juvenile ecophysiology for stock enhancement of the Japanese flounder, *Paralichthys olivaceus*. Bull Mar Sci 62:551–571

25. Masuda R, Tsukamoto K (1998) Stock enhancement in Japan: review and perspective. Bull Mar Sci 62:337–358

26. Burke JS, Masuda R (2010) Behavioral quality of flatfish for stock enhancement. In: Practical flatfish culture and stock enhancement. Wiley-Blackwell, Iowa, pp 303–322

27. Furuta S (1998) Comparison of feeding behavior of wild and hatchery-reared Japanese flounder *Paralichthys olivaceus*, juveniles by laboratory experiments. Nippon Suisan Gakkaishi 64:393–397 (in Japanese with English abstract)

28. Takahashi K, Masuda R, Yamashita Y (2013) Bottom feeding and net chasing improve foraging behavior in hatchery-reared Japanese flounder *Paralichthys olivaceus* juveniles for release. Fish Sci 79:55–60

29. Kawabata Y, Asami K, Kobayashi M, Sato T, Okuzawa K, Yamada H, Yoseda K, Arai N (2011)

Effect of shelter acclimation on the post-release movement and putative predation mortality of hatchery-reared black-spot tuskfish *Choerodon schoenleinii*, determined by acoustic telemetry. Fish Sci 77:345–355

30. D'Anna G, Giacalone VM, Fernández TV, Vaccaro AM, Pipitone C, Mirto S, Mazzola S, Badalamenti F (2012) Effects of predator and shelter conditioning on hatchery-reared white seabream *Diplodus sargus* (L., 1758) released at sea. Aquaculture 356–357:91–97

31. Hyvärinen P, Rodewald P (2013) Enriched rearing improves survival of hatchery-reared Atlantic salmon smolts during migration in the River Tornionjoki. Can J Fish Aquat Sci 70:1386–1395

32. Young RJ (2003) Environmental enrichment for captive animals. Blackwell, Oxford

Figure captions

Fig. 1 Schematic drawings of (a) the enriched tank with bricks, plastic plants, and plastic pipes and (b) the control tank without any major structures

Fig. 2 Schematic drawing of the Y-maze tank used for conditioning striped knifejaw juveniles. LED = light emitting diode

Fig. 3 Typical learning process of individuals from each treatment group. Fish were reared in either an enriched (*closed triangles*) or plain (*open circles*) environment during the period of 50–65 (a), 50–80 (b), or 90–120 days post hatch (c). Note that the breaks in the lines represent reversals

Fig. 4 The learning performance represented as the individual scores of fish from the enriched (*closed triangles*) or control (*open circles*) treatment. A, B, and C above the arrows represent the treatment period of 50–65, 50–80, and 90–120 days post hatch, respectively

Fig. 5 The ontogenetic change in learning performance in wild (*closed circles*) and hatchery-reared (*open squares*) individuals. Data of hatchery-reared fish were redrawn from our previous study [1]; their rearing environment was equivalent to the control of the present study, although using a larger tank (500 l vs 200 l). The quadratic curve representing each group was imposed

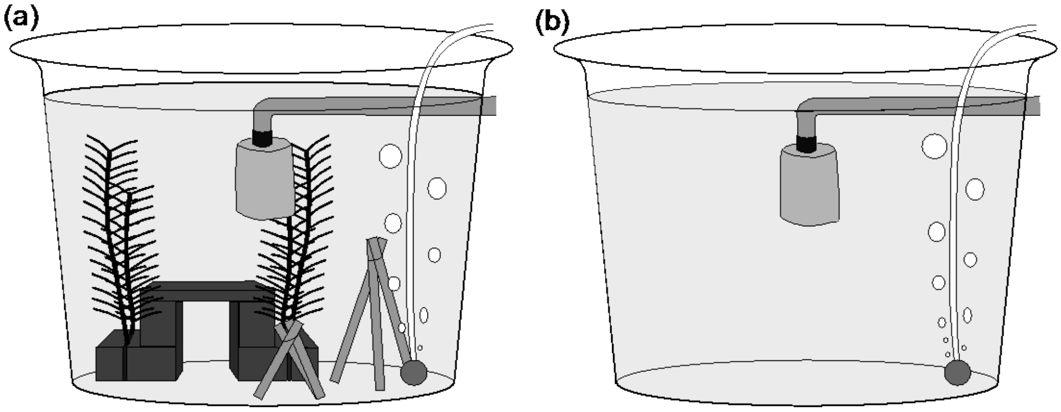


Fig. 1

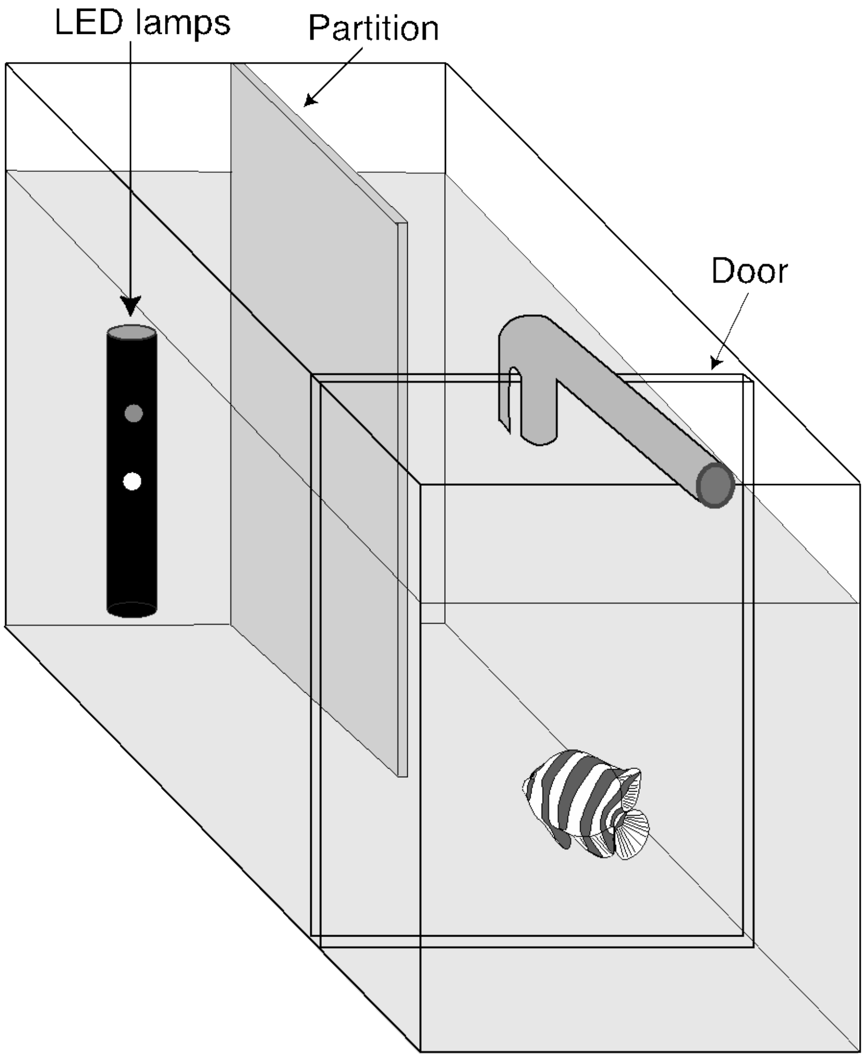


Fig. 2

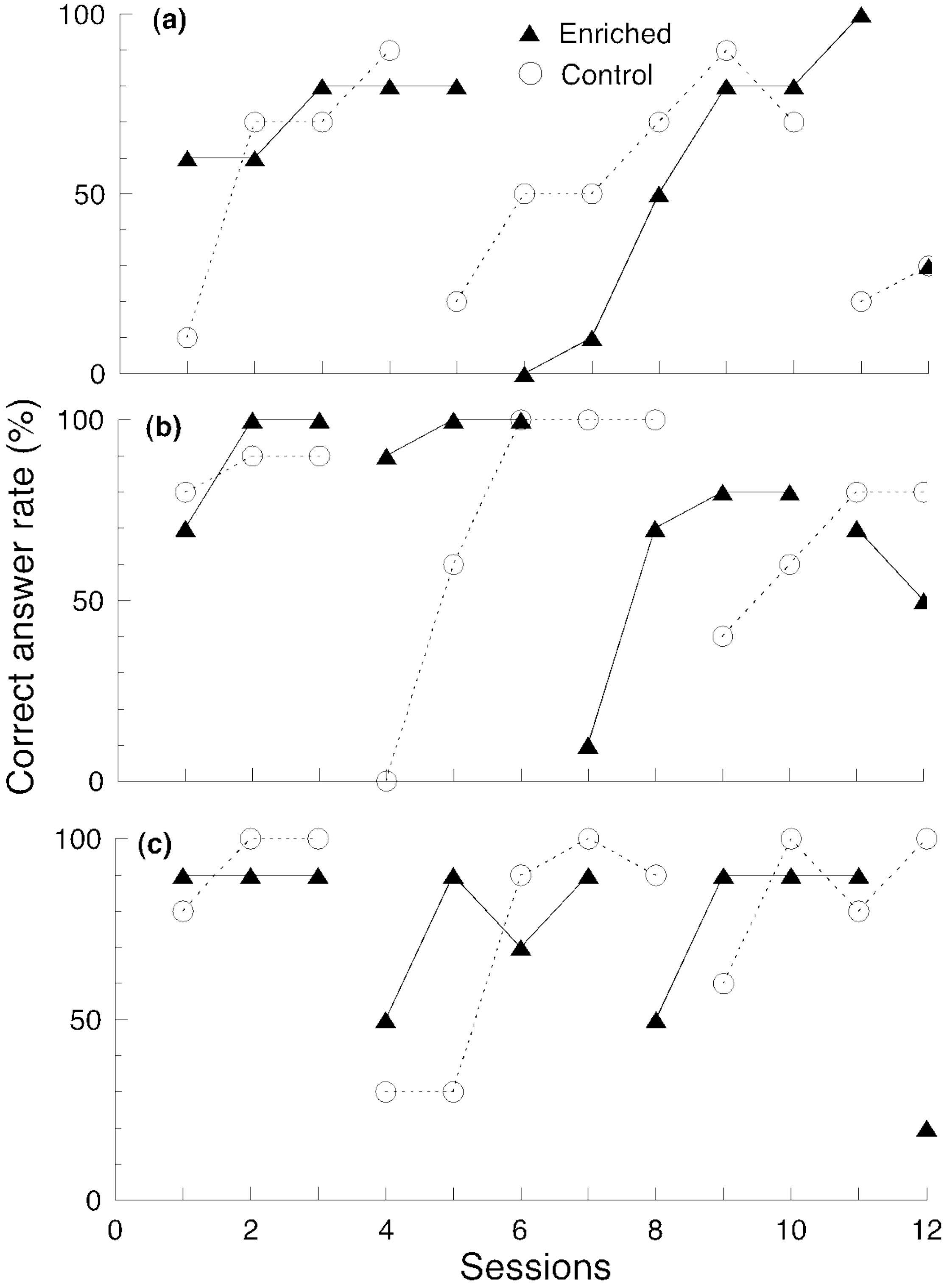
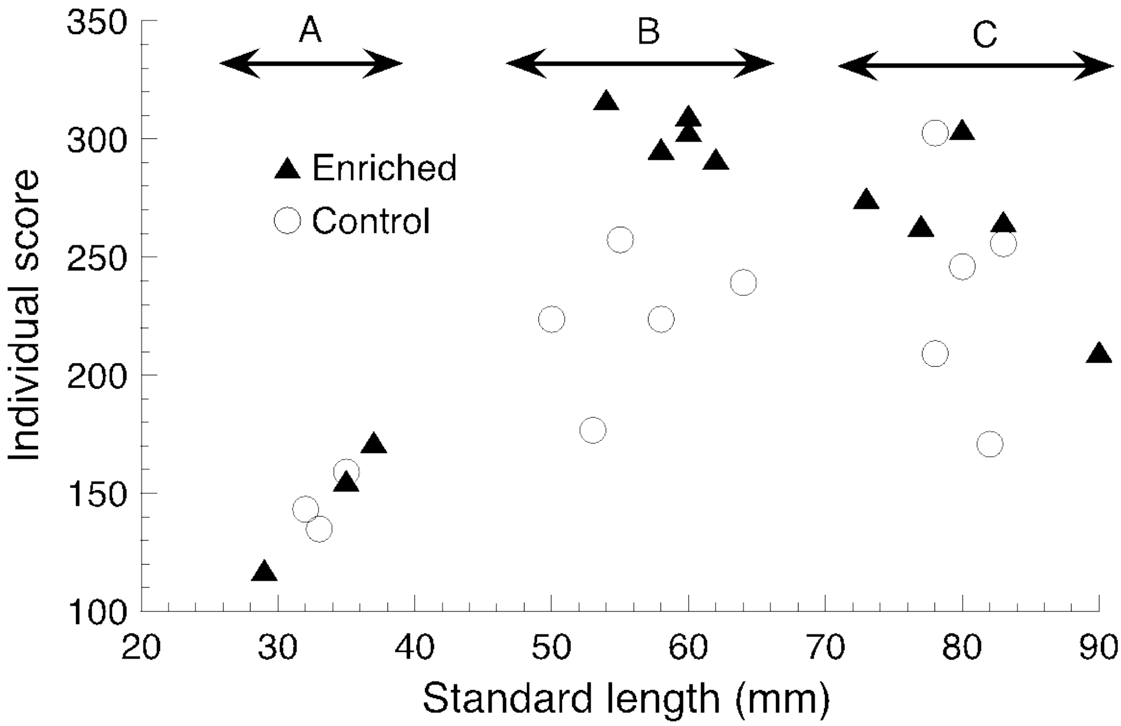

(a)
(b)
(c)
Enriched
Control
Correct answer rate (%)
Sessions
100
50
0
0
2
4
6
8
10
12

Fig. 3



A
B
C
Enriched
Control
Individual score
350
300
250
200
150
100
20
30
40
50
60
70
80
90
Standard length (mm)

Fig. 4

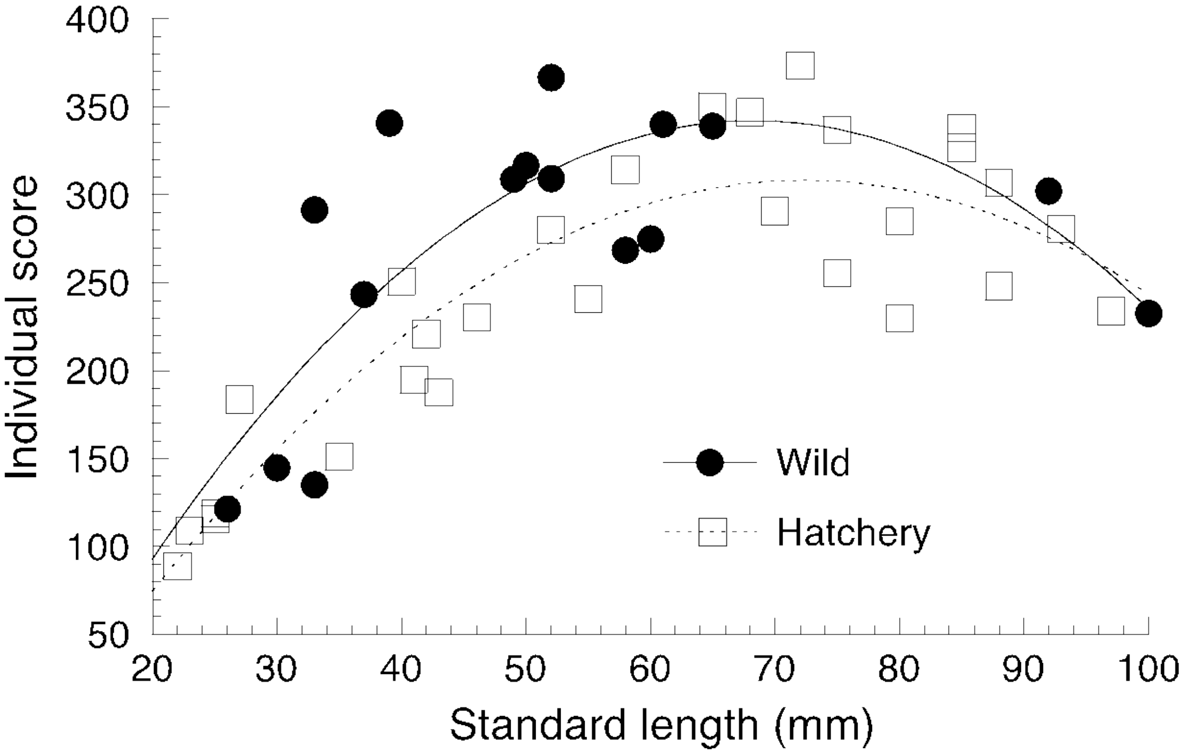

Individual score
400
350
300
250
200
150
100
50
20
30
40
50
60
70
80
90
100
Standard length (mm)
Wild
Hatchery

Fig. 5

環境刺激はイシダイの学習能力を向上させる：特定の成長段階において見られた環境エンリッチメントの効果および天然稚魚と人工稚魚の比較

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水槽中に構造物を設けたエンリッチ環境または通常の環境で、イシダイ稚魚を 50～65、50～80 または 90～120 日齢にわたり飼育したのち、Ｙ字型迷路の報酬訓練を用いて学習能力を調べた。また同方法で天然稚魚の学習能力も調べた。その結果、50～80 日齢にエンリッチ環境で育成した個体の成績のみ対照区よりも優れていた。また天然稚魚の学習能力は人工稚魚よりも高かった。水槽中に構造物の乏しい人工稚魚の学習能力は天然魚より劣るが、適切な時期にエンリッチ環境で育成することで学習能力の発達が促進される可能性がある。