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RESEARCH ARTICLE

EFFECT OF EARLY REARING AND HATCHERY ENRICHMENT ON ROUTE LEARNING ABILITY OF CLIMBING PERCH

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Abstract

Housing conditions have significant effects on the behaviour and physiology of captive animals. Enriching barren hatchery environments by providing structural complexity or companionship are generally considered beneficial as they can decrease the occurrence of abnormal behaviours and physiological responses. Effects of enrichment of rearing environments on behaviour are studied in juvenile climbing perch. Induced bred juveniles were reared under three different conditions i.e., in barren (normal), enriched and super enriched aquariums and tested their route learning capacity and memory retention ability in a maze. The results indicate that the spatial learning ability is enhanced in fish that had been reared in enriched environments. Among different rearing groups, juveniles reared in super enriched tanks show greater spatial learning ability than the other two rearing groups. The study reveals that experience during early larval life is critical in spatial learning ability of climbing perch.

Introduction

It is well known that the environment in which an animal lives has a compelling impact on the development and evolution of behavioral patterns, morphology, and life-history traits. Researchers have become interested in the possibility that learning and memory are likewise adaptively specialized for the requirements of a species' way of life (Balda *et al.,* 1998; Dukas, 1998).The terms learning capacity and learning potential are used to mean the ability to change one's abilities or knowledge through experience. An ability to move around an environment in a directed fashion, either in search of food and mates or to avoid hazards such as predators is a fundamental requirement of virtually all animals (Brown, 2003). A general capacity for spatial learning and memory can, therefore, be regarded as an advantageous skill, enabling animals to move efficiently within their environment and in some instances to plan their routes (Giraldeau, 1997; Healy, 1998). Furthermore, there is no evidence to suggest that specific spatial skills are required to track different objects in space (predators or prey); therefore, a generalized aptitude for spatial learning is likely to be applicable in all contexts.

Animals can improve their foraging ability and refine their social behaviour by learning from previous experiences (Pitcher *et al.*, 1982, Suboski 1989, Sackett *et al.*, 1999, Braithwaite & Salvanes 2005, Laland & Janik 2006). Animal development and the evolution of behaviour patterns, morphology and life-history traits are also strongly influenced by the environment (Magurran 2005). In mammals and birds, complex environments stimulate the development of neural tissue, cognitive performance and flexible behavior (Hunter *et al.*, 2002, Kempermann *et al.*, 2002, Bredy *et al.*, 2003, Rabin 2003).Fish reared and released for the purpose of rehabilitating populations have been housed in predator-free conventional hatchery environments with plenty of food. Such an environment allows better survival through the earliest life stages than nature does. However, these early rearing experiences do little to prepare the fish for the natural environment. To survive they need basic behavioural skills that allow them to find suitable food and avoid predation. Current evidence suggests that a hatchery

environment does little to promote these behavioural abilities (Braithwaite and Salvanes, 2005). Similarly, we should consider how issues relating to escapes from fish farming are associated with those of restocking. This could highlight which basic behavioural skills are necessary for survival and growth. Quantitative traits in natural populations depend on individual behaviours, and behaviours vary as do life history traits in a complex interaction between the population's genotype and the environment (Huntingford and Wright, 1989, 1992, 1993; Fuiman and Magurran, 1994; Carroll and Corneli, 1999; Salvanes *et al.*, 2004).

There are a variety of ways in which experience shapes behaviour, including environmental effects on the development of the neuro-endocrine and sensory systems and specific learning effects (Bateson and Martin, 1999; Huntingford, 2004).These types of processes are at work during development of fish (Huntingford, 2004; Braithwaite, in press). For example, there are studies illustrating how simple enrichment of the rearing environment affects learning ability, foraging skills, social behaviour, predator avoidance, aggression, and reproductive success (Fleming *et al.*, 1997; Berejikian *et al.*, 2000; Brown *et al.*, 2003; Braithwaite and Salvanes, 2005; Salvanes and Braithwaite, 2005). Since hatchery and wild fish grow up in very different environments, differential experience is likely to generate behavioural differences. Behaviours achieved early in life are likely to influence behaviour at later stages. Hence, deficiencies generated in early life are likely to affect later success.

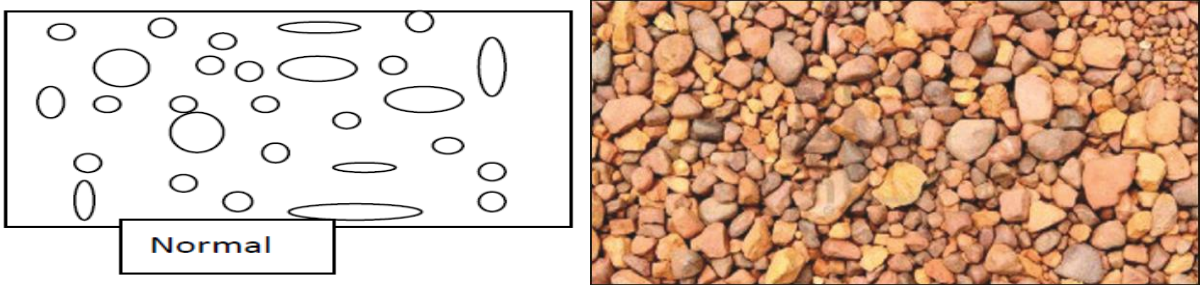
Material and Methods

2.1 Hatchery Rearing Environment

Healthy adult breeders were collected from a pond in Irinjalakuda (10°25' 10° 18' 47"**N** latitude 76°17'19 76°12'48"E longitude) located in Kerala during April-March 2010, and housed in large cement tanks for acclimatizing with the laboratory conditions. Fish feed pellets (Marvel aquarium fish feeds India Ltd.) were provided *ad libitum* for a two-weeks settling period and water was changed twice in a week. In June 2010, at onset of monsoon one milt oozing male (8cm) and one female (10cm) with bulged belly were selected from each population and induced to breed using Ovaprim (0.5ml/kg body weight). Within 12 hr of hatching of the eggs, breeders were removed from the tank and about thousand larvae of climbing perch were housed in four tanks (95 x 95 x 60 cm).

Sixty individuals were then randomly collected and reared as groups of twenty individuals in three different environments. One of the rearing environments was plain, i.e., a glass tank (95 x 95 cm and 60 cm) with sand substratum and no other additional cue. The second tank contained complex spatial cues (pebbles and a plastic model of ball, square, cone, and tube) kept in a fixed position to create a homogeneous environment (enriched). The third one contained spatial cues (pebbles and a plastic model of ball, square, cone, and tube) double the number that is provided in the first tank and these were moved around the tanks once in a week to create a heterogeneous environment (super enriched). Figure.1

(a)



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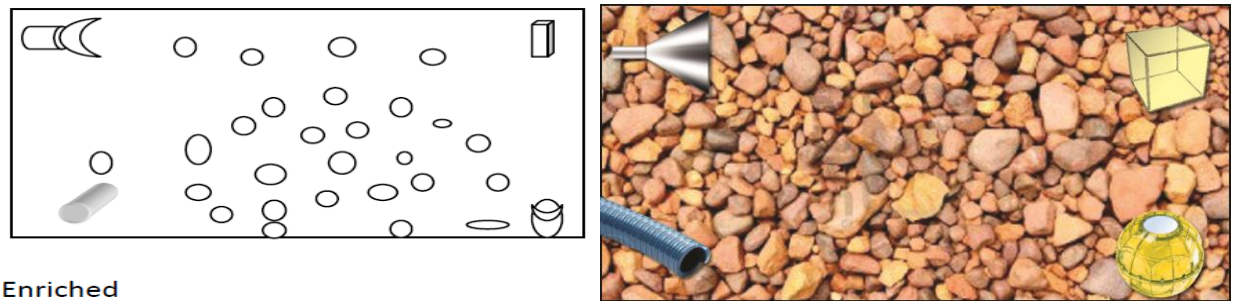

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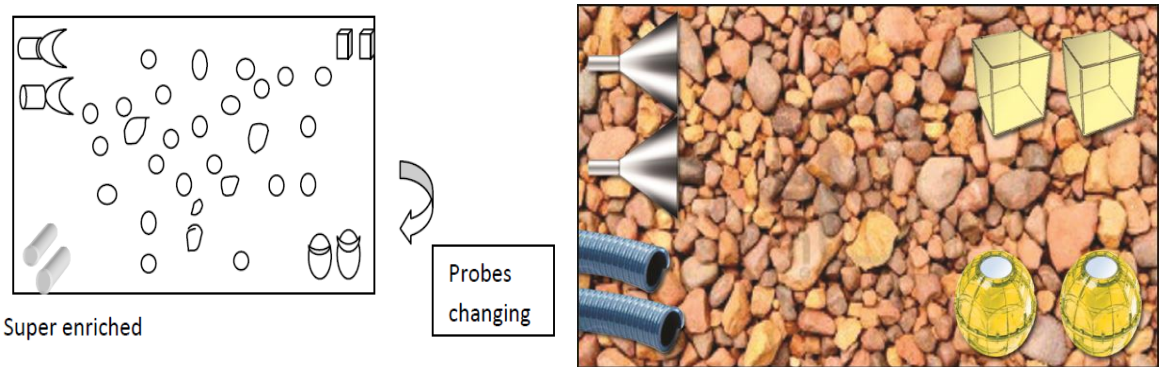
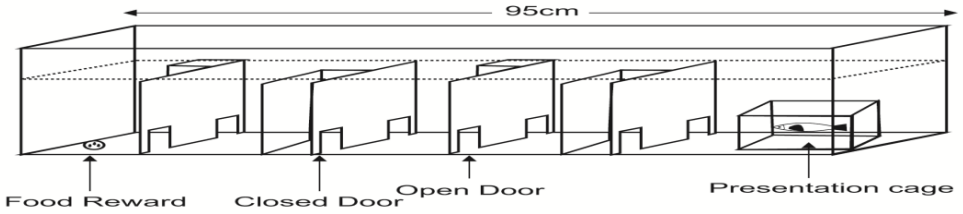


Figure: 1 Hatchery rearing environment (a) Normal (b) enriched (c) Super enriched

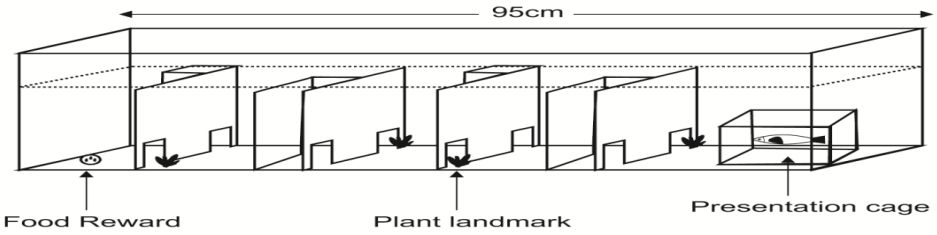
There were four replicate sets for each of the groups. The rearing tanks (95 x 95 x 60 cm) were supplied with aerated, water at 40 cm height of the tank, and the room was maintained on a 12:12 light: dark cycle photoperiod with day-light fluorescent tubes positioned 1.5 m above the centre of each tank. The tanks were placed side by side on a table. At the start of the rearing, we randomized which tank received which rearing environment and the distribution of individuals among the various replicate tanks. Once the fish had reached 10 months of age, 30 fish were removed from the holding tank (here designated an 'enriched and super enriched and improvised' environment) and transferred to three glass tanks for the experiment.

2.3 Testing Apparatus

The experimental apparatus design was similar to that reported in Girvan and Braithwaite (1998). A line maze was set up in a 95 x 30 x 30 cm aquarium in 24 cm depth of water. To reduce the use of extra-maze cues, the tank was surrounded by black paper from three sides. On one side, a black screen was put enabling the observer to watch the fish through a small slit in the black screen without disturbing the fish. Lighting was provided by one fluorescent lamp (14 W), above the experimental tank. The tank was divided across its width by three plain partitions made from Plexiglas, 15 cm apart, each with two evenly spaced doors (6 x 3 cm). At one end of the tank, there was a 15 x 30 x 30 cm release area with a presentation cage, made of transparent, perforated acrylic sheets (15cm x 10cm x 27cm) with a sliding door on the top, which could be raised using a pulley system with minimum disturbance to the fish. To begin a trial, a single fish was carefully dip-netted from its holding tank and allowed to settle in the release area for two minutes after which the presentation cage was raised. Six minutes were provided for exploration of the experimental setup. At the other end of the tank, three earthworm pieces in a Petri dish was placed as a reward to encourage the fish to swim through the maze. Each group was divided into two groups: one group was tested in a plain maze, the second group in a maze containing visual landmarks (Fig. 2).



(a)



(b)

Figure: 2 Experimental setup: a) without plant land mark b) with plant land mark

2.4 Pre-training

During the pre-training period, thirty fish from each group were familiarized with the set-up by transferring single fish from the holding tank to the release area. During this stage two doors of each partition were opened so that fish get maximum acclimatization with experimental setup. After two minutes of settling period in the presentation cage, they were released, allowing them to swim the length of the tank to obtain a food reward twice in a day. For group 1, the maze had no landmarks present (NoLM). For group 2, landmarks (small identical plastic plants) were placed next to the all doors (LM). Fish was returned to their holding tank five minutes after their first contact with the food reward. Food was withdrawn from the test fish for 24 hours in their holding tanks to maintain a reasonable level of feeding motivation in all subjects. It is observed that after five pre-training trials, the fish learn to swim through the doors and directly obtain the food reward in the 5^{th} chamber.

2.5 Training Phase and Test

During training phase, maze was modified in such a way that one door on each wall was opened other lead to a dead end. The fish had to swim through a zig-zag route to pass through the maze efficiently. Group 1 (NoLM) remained without landmarks, whereas for group 2 (LM) a landmark (Plastic plant) was positioned next to the open doors only (figure 1). Fish were able to locate the food reward in less than 6minutes after four trials. Four-day test trial was given so that each fish get eight trials in the modified apparatus (i.e. two trials per fish each day). During test, on the fifth day food was withdrawn for 24 hours to maintain a reasonable level of feeding motivation in all subjects. Experiment was conducted on the sixth day and we recorded number of trials and time taken by each fish to reach the reward, starting from the point the fish leave the release area (i.e. from presentation cage), until the caudal fin passes through the rewarded door.

In the second experiment the open and closed doors were reversed, in such a way that previously open doors now led to dead-ends, and vice versa. For group 2 (LM), the landmarks were moved to the new open doors, thereby remaining reliable indicators of the route through the maze. Fish were given only one test trial in the modified maze. After one day memory was tested in the modified maze. Next day, the learning ability was tested in the modified maze. The number of trials, time taken and errors committed by the fish in finding the reward were recorded.

Results

Two-way ANOVA with enrichments as first factor and type of maze as second factor was done for comparing the number of trials taken by the fish to reach the reward. The results show that F-value for comparing the interaction between type of maze used and rearing environment was found to be non significant [$F(2, 84) = 0.026$, $P=0.974$]. F-value for comparison between performance of larvae reared under different enrichments [$F(2, 84) = 45.852$, $P<0.001$] is found to be significant indicating that there exists significant difference in the number of trials taken by the fish reared under different rearing conditions. Similarly significant F-value in the case of Type of maze indicates that there exists significant difference in the number of trials between performance of the fish tested in No-land mark maze and land-mark maze. Post hoc analysis of LSD was done for comparing the different pairs of enrichments and the results are given. (Fig.3)

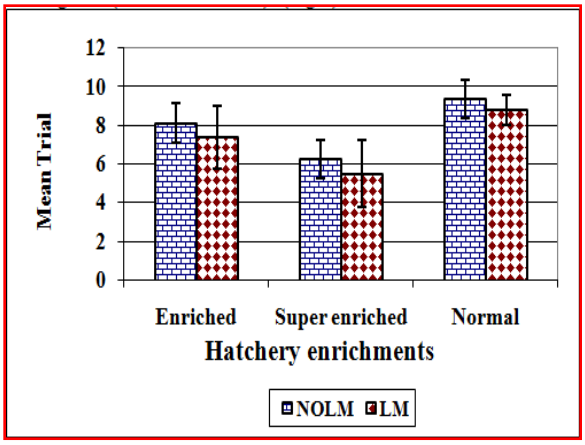


Figure: 3 Mean no. of trials taken to reach the reward by hatchery reared climbing (in landmark and No land mark maze).

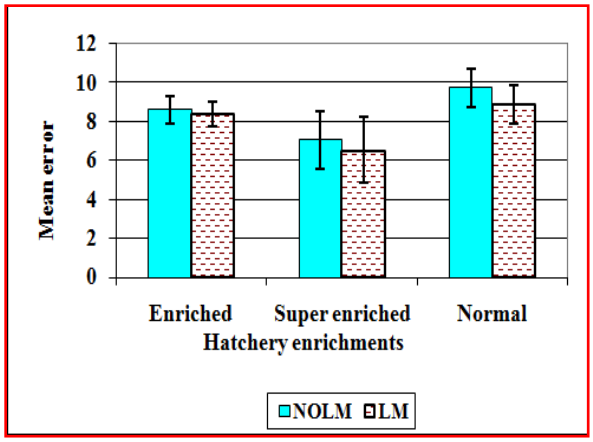


Figure: 4 Mean no. of Errors committed by hatchery reared climbing perch in reversed landmark and No land mark maze.

Number of trails taken by fish to reach the reward is significantly different among fish reared under different enriched environments. Fish reared in enriched and super enriched environments took less number of trials to reach the reward compared with larvae reared in normal tanks, in land mark maze than in No-land mark maze.

Two-way ANOVA with enrichments as first factor and type of maze as second factor was done for comparing the number of errors committed by larvae in the reversal maze experiment of climbing perch induced bred from channel population and reared in enriched tanks. F-value for comparing errors committed by fish reared enriched and super enriched tank [F (2, 84) =37.162, P<0.001] was found to be significant compared with larvae reared in normal tanks. Similarly significant F-value in the case of Type of maze indicates that there exists significant difference in the number of errors committed by fish tested in No-land mark maze and land mark maze [F F (1, 84) =4.864, P<0.030]. Post hoc analysis of LSD was done for comparing the different pairs of enrichment tank and the results are given in Table 5 and 6 (Fig. 4).

Number of errors committed by fish under different enriched rearing environment is significantly different from each other. Fish from super enriched condition committed significantly less number of errors to reach the reward than fish from normal and enriched hatchery rearing conditions. Fish reared in enriched environment committed lesser number of errors in reaching the reward in land mark maze, as compared to those tested in No-land mark maze (Fig.4).

Discussion

Attempts in this paper is to study the environmental enrichment in rearing tank by the provision of probes or other stimuli having functional relevance to the climbing perch. This study reveals two key observations. First, the behavior of induced bred juvenile climbing perch exposed to spatial cues in the nursery environment over 10 months is different to that observed climbing perch from the same genetic background, but reared in a plain nursery environment. Differences were observed in spatial learning ability in the relation use of cue, shelter, homing and feeding behavior. Our results are consistent with earlier findings demonstrating that enrichment improves the way animals respond to new situations. The data showed that rearing background influenced learning and how fish responded to spatial stimuli. Enrichment in rearing tank, fish show higher learning ability than plain fish. The finding that enrichment in the early rearing environment promotes learning abilities in fish supports and adds to our previous findings that environmental complexity in the rearing environment enhances fish behavioural repertoire (Braithwaite & Salvanes 2005, Salvanes & Braithwaite 2005, Salvanes *et al.*, 2007). It is interesting that fish can learn and remember land marks faster when reared in enriched environment. Thus here we find novel effect enrichment on spatial learning. These behavioural skills are likely to be important for growth and survival in nature. In this paper, environmental enrichment is defined as an improvement in the biological functioning of captive animals resulting from modifications to their environment. Evidence of improved biological functioning could include increased lifetime reproductive success, increased inclusive fitness or

a correlate of these such as improved health. However, specifying an appropriate endpoint is problematic, especially for domestic animals. Here in this study the quality of the external environment within the animals' sensory range also deserves greater attention.

Hatchery reared climbing perch with enrichment in tank achieved reward significantly greater spatial learning ability than climbing perch reared in a conventional hatchery environment. Overall, the results suggest that super enriched hatchery environments influence the spatial learning ability in larvae from both pond and channel hatchery reared population (Fig 3). Our data show that enrichment in the early rearing environment had a significant effect in route learning capacity of climbing perch. Fish reared in enrichment in the tank showed faster learning ability than fish from normal hatchery. These fish show less number of trials to reach the reward as compared with the Normal. Among the three different enrichment environments, fish reared in tanks fitted with Super enrichment took least number of trials to reach the reward. It may be due to the fact that super enrichment provides unpredictable changing environment comparing with enriched and normal. As the fish is an obligatory air gulping species, climbing perch has to moves through the probes in water column several times. In this situation fish has to move through the small holes in probes by struggling. These maneuvers need higher dexterity, flexibility of the body and accuracy of visual sense. It is possible that this early experience might have provided them faster cognitive ability in later life (Figure 3). Experiments with cod, in which juveniles are reared for most of their nursery period in enriched conditions (an environment characterized by variability), have shown Juvenile cod reared in enriched tanks develop more flexible behaviour than fish reared in standard hatchery conditions (Braithwaite and Salvanes, 2005; Salvanes and Braithwaite, 2005). This suggests that experiencing enrichment and variability has an impact on adult behavioural phenotype as they develop. This emphasizes the importance of the quality of early life environment in shaping fish behavior.

Behaviours that fish learn early in life are likely to influence their skills later. Recently, it was shown that enrichment in rearing tanks generates fish with more flexible behaviour than conventional rearing tanks. These fish have the potential for greater survival if released for restocking as enriched environments may generate more suitable feeding behaviour, anti-predator responses, and social behaviour for the wild. However, it remains to be determined how well this increased behavioural flexibility improves post-release survival. Our results are in line with those reported by Berejikian *et al.,* (2000, 2001) who studied steelhead juveniles and Metcalfe *et al.,* (2003) studying Atlantic salmon. Berejikian and co-authors showed that fry reared in an enriched hatchery environment achieved a higher social dominance rank than size-matched fry reared in conventional hatchery environments.

Reversed maze experiment juveniles reared enriched hatchery environment up to sub adult stage made significantly more mistakes in the reversed plain maze than the land mark maze (Fig 4). Number of mistakes being made was not that large in comparison with the enriched and super enriched rearing environment. Although the increased absolute number of mistakes in reversed maze experiment suggests that these fish may be confused with reversing the maze. Fish made fewer mistakes in the reversed landmark maze which strongly suggests all the hatchery reared fish used the visual landmarks to guide them through this maze. Our results suggest that they relied on the primary visual cue when conflicting situations of reversal maze experiment.

In a different comparison of spatial learning ability in benthic or limnetic fish, *G. aculeatus* from benthic populations were found to solve a maze task faster than limnetic populations (Odling-Smee *et al.*, 2008). Superior spatial learning and memory in the benthic fish presumably arises because these fish spend much of their time in a visually more complex environment on the lake floor. In contrast, the limnetic fish populations live in the water column where they will rarely encounter fixed landmarks that can be used for spatial reference. In addition to this, critical periods of development occur at key transitions from one life stage to the next, such as early postnatal development and puberty (McCormick and Green, 2012). These periods have heightened neural development that makes the brain even more plastic and receptive to change (Knudson, 2004). A fish's brain grows continuously throughout its lifetime, suggesting that brain growth may be impacted by the environmental conditions of fish early experiences. Such environmental feedback on brain development in teleost fishes is likely to influence individual behavior and habitat preferences into adult life (Zaunreiter *et al.*, 1991; Kotrschal and Palzenberger, 1992). Enrichment of the juvenile environment is a start, but the impact that different factors have at specific stages during early development and the effects these have on the animal's neural development would seem to be areas now in need of attention.Furthermore, we found differences in the learning propensities of these different groups in terms early rearing environment. These results suggest that relatively simple manipulations of the

uniform hatchery environment generate fish with advanced learning capacities.

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