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EFFECT OF AGE AND REARING ENVIRONMENT IN ROUTE LEARNING OF CLIMBING PERCH, (*ANABAS TESTUDINEUS* BLOCH, 1792)

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ABSTRACT-*This study examines spatial learning ability of lab reared climbing perch in a complex environment and effect of critical age on learning. We reared climbing perch larvae in four different environments varying in complexity and tested their spatial learning ability at juvenile and adult stage (ieat 2nd and 10th month of rearing). Climbing perch reared in native tanks with wire mesh barrier at the bottom, middle or upper level of water column showed marked difference in route learning ability. The larvae reared in tanks with wire mesh barriers at the middle of the water column exhibited highest learning capacity, as compared to fish from other three rearing groups. This suggests that a single change in rearing environment can trigger differential cognitive abilities, probably for the lifetime. The present study also supports the idea that spatial learning ability shifts during ontogeny i.e. as the age increases learning ability also increases, possibly due to life experiences and enhanced neurogensis. Thus spatial learning ability may vary considerably in response to varying environment experienced by the fish during early life. The possible role of native habitat complexity in the route learning ability, which is critical for location based navigation of fish, is discussed. The results highlight the need to consider enrichment strategies when designing captive rearing of fish for conservation and replenishing of diminishing fish stock.*

KEYWORDS*: Anabas testudineus; age; climbing perch; rearing environment; Route learning; spatial learning; memory*

INTRODUCTION-Learning plays a pivotal role in the behavioural development of all vertebrates, and fish are no exception. Behavioural biologists have identified a variety of ways in which experience shapes behaviour (Bateson and Martin, 1999) such as general environmental effects on the development of the neuro-endocrine and sensory systems, as well as specific learning effects. Early experience of change and heterogeneity can help to promote the capacity to learn faster and change behaviour (Laviola and Terranova, 1998). Experience dependent differences are commonly identified by rearing fishes of the same genetic background in hatchery or in wild conditions.

Rearing environment of fish has an enormous impact on later behaviour and that it affects the normal development of the young in different ways (Huntingford, 2004). Learning during an early phase of development can shape many behavioural patterns later in life and is an important component in the acquisition of many behaviour patterns(Brown *et al.,* 2007; Moretz*et al.,* 2007; Salvane s*et al.,* 2007; Chapman *et al.,* 2008). Animals that undergo habitat shift face a number of challenges as they move between habitats; for example, they may encounter new predator species and may be vulnerable as they adapt to their new surroundings. An ability to adapt quickly to the new environment is likely to influence post-transition survival, and an understanding the influence of early rearing of hatchery reared climbing perch on spatial learning is important in this context.

The genetic, developmental, and environmental factors directly influence an individual's learning capacity. Any observed differences in learning of fishes are usually ascribed to developmental effects and differences in early rearing environment. Spatial cognition modifies the processes of recruitment, competition and foraging migration in fishes. Spatial memory capabilities can evolve and are predicted to vary with the spatial demands of the environment (Sherry, 2006). In fish, spatial memory can enhance foraging rate (Hughes and Blight, 1999), territory defense (Lamanna and Eason, 2003), and predator avoidance (Markel, 1994). Fitness of an organism is thus, positively correlated with foraging success and growth rate .Fish may invest much into spatial learning capacity as their ecology and lifestyle demand. The foraging skills of fishes become fine-tuned to prevailing ecological conditions through learning (Hughes *et al.,*1992; Warburton, 2003). Fish learn to recognize the prey, how to handle them and to locate where they are likely to be, through life experience (Brown *et al.,*2003). Studies in mammals and birds show that the hippocampus plays a crucial role in spatial memory (Healy *et al.,*2004). Similar to the mammalian and avian hippocampus, and the reptilian medial cortex, the lateral telence phalic pallium of the teleost fish are selectively involved in spatial memory (Rodríguezet al., 1994; Lópezet al., 1999, 2000).Habitat remains the most compelling ecological factor that can account for differences in route learning and use of visual cues by pond and channel climbing perch (Sheenaja and Thomas, 2011). The spatial learning ability may be hampered by rearing in unnatural captive environments, since environmental cues that are available early in life in nature, but possibly not in captivity, may be necessary to guide individuals towards learning or otherwise developing behaviour that will be adaptive in the future (Kieffer and Colgan, 1992; Snyder *et al.,*1996). Complex spatial rearing environment can increase behavioural repertoire and improve learning in a number of animal taxa (Brown *et al.,*2003; Freire and Cheng, 2004). Salvanes*et al.,*(2007) have demonstrated that, sticklebacks (*Gasteroste usaculeatus*) exposed to spatial heterogeneity during rearing are bolder; they were also faster at reaching the shelter than fish reared without spatial cues.

AIM-Even though, the role of topographical features at the bottom of the water body in shaping spatial cognition is discussed widely little is known about how the complexity in the water column influence learning differences among siblings. Many species of fish have been shown to be not only capable of spatial learning and memory, but of using multiple methods to form cognitive maps of their environment depending on the type and complexity of the environment (Odling- Smee and Braithwaite 2003; Hughes and Blight, 2000; de Perera 2004; 2003; Braithwaite and de Perera, 2006). However, very little is known about how the complexity in the water column influences spatial learning differences among siblings. We focused on how spatial complexity at different levels of water column of rearing environment influence spatial learning ability in fish. Future implications of early rearing environment was assessed by comparing route learning ability of hatchery reared climbing perch under different complexity at juvenile and adult stage.

MATERIALS AND METHOD

Induced breeding and rearing of larvae

Healthy adult breeders were collected from a pond located in Avittathur(10°18' 47'' N,76°17' 19'' E) during April-March 2009, and housed in large cement tanks for acclimatizing the laboratory conditions. Fish feed pellets (Marvel aquarium fish feeds India Ltd.) were provided *ad libitum* for two-week settling period and water was changed twice in a week. In June 2009, at onset of monsoon one milt oozing male (8cm) and one female (10cm) with bulged belly were induced to breed using Ovaprim (0.5ml per one kilogram of its body weight injected intramuscularly). Within 12 hr of hatching of the eggs, breeders were removed from their breeding tank and larvae were reared in groups under one of the four different rearing environments.

Initially the larvae were reared in 500 ml beakers up to one month and as they grew, they were transferred to larger tanks. In order to test how complex rearing conditions affect later learning capacity of fish four different rearing conditions were provided for the developing larvae. The water column of beakers were provided with wire net barriers with mesh size of 1cm^2,cut into circular shape so as to fit tightly in 500ml beakers and aluminium wires of 1inch length, fixed at the corners of each mesh acted as poles (Figure1). All rearing tanks were covered with black paper in order to isolate the fish from ambient cues. Since Climbing perchis an obligatory air gulping fish, intermittently it emerges to the surface of the water column for gulping the air. Barriers fixed at the bottom or middle or upper region of the water column, offered hindrance for the upward air gulping movements of the fish larvae as they have to cross the barrier by swimming through the mesh of the net.

The four rearing environments were:

1. 'Barren hatchery'—this was identical to a normal hatchery tank with barren environment and gravel at the bottom of the tank (Figure1-A).
2. 'Upper complexity'— Barrier was attached about 10 cm. just below the surface of the water column of the rearing vessel, `so that the poles attached to the net project downwards facing the bottom (Figure1-B).
3. 'Bottom complexity '— Barrier was fixed about 10 cm above the bottom of the rearing tank; poles projecting upward from net (Figure1-C).
4. 'Middle complexity'— Barrier was fixed at middle of the water column; with poles projecting downward and upward from the net. (Figure1-D).

We reared 20 larvae (2±2 mm) in a 500 ml beaker for one month and 20 replicas of the treatment regimen were kept for further use in case of mortality of the larvae and they were provided Artimianaupliii*ad libitum*. After one month they were transferred to rectangular aquarium with appropriate wire net barrier with mesh size (3cm^2 and pole=2inch) for next six months. Then the juveniles were transferred to 500 litre cement tanks with barrier made of larger mesh size (6 cm^2 and pole: 3inch). Water was changed once in a week with properly aerated pond water. After two months they were acclimatized with pellet feed (Marvel feeds; Aquarium Systems, India) and before one week of the experiment, they were given chance to feed on live earthworms, which acted as the reward during later experiments. One week before the experiment all individuals are brought back to a normal glass aquarium (24 x 24 x 38 cm) without barriers.

Test apparatus and experimental procedures

The maze design was similar to that used by Odling-Smee and Braithwaite (2003) with a few modifications. A four-arm maze was constructed of 1.5mm Plexiglas, each arm being 35cm long, 15cm wide and 20cm high, which can easily converted into a T maze by inserting a partition at the junction. The maze was submerged in aerated water to a depth of 20cm in a circular tank with gravel substratum.

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). There is a sliding door which could be raised using a pulley system with minimum disturbance to the fish. To motivate the fish in maze food reward was provided by securing earthworms into a petridish placed at the end of rewarded arm. As climbing perch is a shoaling fish and prefer to join conspecifics (Binoy and Thomas, 2004) the focal fish was given an additional reward by placing two conspecifics in a transparent bottle, just outside the rewarded arm. The fish therefore received a double reward (food and conspefics) in the goal chamber. The climbing perch at 2nd month and 10th month after hatching were taken for the study. The conspecifics were visible to the fish only after it passes through the door in the partition and reach at the end of the T-maze (Figure 5.2).

Pre-training

The purpose of pre-training was to familiarize fish with the apparatus and procedure. During pre-training all trap doors were raised, allowing the fish free access through both exits in the partitions and to both ends of the T-maze. Fish were pre-trained in groups of 10. Ten fish from each treatment were netted from their holding tanks to the presentation cage. After 5 minutes, the door was raised remotely using pulley system and the fish were given free access to both arms of the T-maze. Food was withdrawn from the experimental fish for 24 hours to keep them at a reasonable level of motivation to feed on the food provided in the tank. After five pre-training sessions; the fish swam out of the start box and found food at the end of T-maze. Each group remained in the maze for 12 hours and 12 hour in their home tanks.

Training

Fish were given three trials a day for five days. In each trial two petridishes were placed in the arms of the T maze, only one of which contained food reward (five earthworm pieces) and other arm without reward. Two conspecific fish were also placed (second reward) in a bottle outside the end of the rewarded arm. Each test fish was introduced into the start box and left here for 5 min with the opaque cover in position. After 5 minutes, the door was raised and recorded the time and number of trials taken by the fish to leave the start box and to enter the rewarded arm, and which arm the fish chose first. Entry into the arm of the maze is assumed to be complete, when the base of the caudal fin of the fish had passed through the door of the partition. Each trial was terminated after the fish had fed, or 10 minutes after the release of the fish. Fish were left for 3 minutes after feeding, then, if they had left the rewarded end, we encouraged them to swim to non-rewarded end. The trap door was then lowered, and the apparatus modified in such a way that the previously rewarded end became the start box for the next trial. In this way, maze was rotated anticlockwise.

Test

During the test, on the fifth day before the test trials, food was withdrawn for 24 hour to maintain a reasonable level of feeding motivation in all subjects. Experiment was conducted on the sixth day and recorded time (with in 360 sec), and the number of trials taken by each fish to reach the reward, starting from the time fish leave the release area (i.e. from presentation cage), until the caudal fin passes through door of the rewarded arm. The time taken by the fish to reach the reward on the first trial that the fish actually reached the reward. Similarly, "number of trials", is that the number of trials until the first trail where the fish reach the reward. Testing was conducted in hatchery reared 2nd month (i.e. juvenile stage) and the same fish was tested with same procedure in 10th month (adult stage).

RESULT

Two-way Analysis of variance (ANOVA) was conducted using SSPS software,with number of trials taken by the fish to reach the criterion as the dependent variable; age and rearing treatment taken as factors. The interaction effect between age and rearing treatments was found to be non- significant (F (3, 112) = 0.36, P=0.779). F-value for comparison between rearing treatment (F (3, 112)= 10.23, P<0.001) was found to be significant at 0.01 levels. F-value for comparison between age groups (F (1, 112)= 42.84, P<0.001) was also found to be significant (Table 1). As it was found to be significant, LSD (Least significant difference) test was carried out for pair wise comparison and the result show that number of trials taken to reach the target is lesser for fish reared in tanks with middle barrier. There was no significant difference in the number of trial to reach the target by the fish reared in tanks with upper and lower barriers. Larvae reared in tanks without any complexity (barren tanks) took highest number of trials to reach the target as compared with the other treatment groups (Figure 3 &Table 2).

Two-way Analysis of variance (ANOVA) with time taken by the fish to reach the criterion as the dependent variable and age and rearing condition as factors, revealed a significant main effect due to age (F(1, 112) = 16.78, P<0.001) and barriers in rearing condition (F(3, 112)= 49.98, P<0.001). The interaction effect of time taken to reach the criteria between age group of fish and rearing treatments was found to be non- significant (F(3, 112)= 1.78, P=0.154) (Table 3). As main effect due to age and rearing condition was found to be significant, least significant difference test (LSD) was carried out for pair-wise comparison. The results show that the mean time taken is significantly different between each rearing treatments and longer time was taken by the fish reared in the containers without any barriers and lowest time was taken by those reared in containers with middle barrier (Figure 4 &Table 4).

DISCUSSION

Juveniles of climbing perch start spatial orientation behaviour at 8th week of development (personal observation). In the first month of rearing they showed disoriented movement towards reward (food) and made more mistakes. By the end of the second month of development they were able to orient to the reward properly. The increased visits to the rewarded arm may probably be based on the information in their working memory.

The early rearing conditions and age affect the spatial learning ability of climbing perch. Fish reared with barriers in the water column of the rearing tank had better route learning ability and they were faster in locating the food reward than fish reared in the normal (barren) hatchery environment. It has been demonstrated that any environmental difference in early rearing environment feedback on brain development in teleost fishes and is likely to influence individual behavior and habitat preferences in adult life (Zaunreiter*et al.,*1991; Kotrschal and Palzenberger, 1992). Numerous studies have explored how environmental enrichment affects the rate of neuro genesis in the dentate gyrus of the hippocampus (Kempermann*et al.,*1997; van Praag*et al.,*2000).

As an obligatory air gulping species the larvae of climbing perch have to move to the surface of the water intermittently. In the present study barriers placed in the water column of the rearing tanks of climbing perch provide a complex environment for fish as compared to the water column of the normal hatchery tank. The poles fixed at the corners of the wire mesh provide additional hindrance to the free movements of the fish. Here fish may have to do more lateral twists and turns to emerge to the surface. Complex rearing environments may generate better foraging strategies, anti-predator responses and other behaviours that related to spatial learning which in turn gives the potential for better survival rate, when released into natural environment. Since hatchery reared fish larvae grow up in very different rearing environments, differential experience is likely to generate behavioural differences. Hence any deficiencies generated in early life are likely to affect later success (Salvanes and Braithwaite, 2005). Flexible learning appears to act in concert with genetic and developmental influences such that orientation behaviour is adapted for particular ecological condition and navigational demands (Odling-Smee*et al.,* 2006). Among the four rearing treatments larvae reared in tanks fitted with barriers at the middle of the water column exhibited more learning capacity than fish reared in other three conditions. They took less number of trials to reach the criteria indicating faster learning ability (Figure 5.3). As the middle barrier has poles pointing in both directions it may provide greater hindrance to the free movement and hence fish may be getting better neuronal stimulation during early development. It is reported that spatial heterogeneity in the rearing ground may be positively related to telencephalon development which in turn is related to spatial learning in fishes (Salas *et al.,*2003).

When we compare the learning ability of climbing perch larvae reared under other two experimental conditions (barriers at upper and lower level of water column), fish reared in tanks with barrier in upper water column showed more learning capacity than the fish reared in tanks fitted with barrier at the bottom of water column. In this set up the poles fitted at the corners of the wire mesh and pointing downward where the larvae have to flex the body laterally every time they emerge to surface of the water. Usually the interval between two consecutive air gulping bouts is less than two minutes (Personal observations). In other words, at every two minutes the fish has to perform body flexion to reach the water surface.

Another interesting behaviour noted in climbing perch is that after gulping the air they very quickly return to the bottom of the water. Probably this is a behavioural strategy to avoid predation by piscivorous birds. Here again unlike the larvae reared in a normal hatchery tank, larvae reared in tanks fitted with wire mesh barriers should pay greater attention to cross the barrier accurately through the gap of the mesh without stumbling on the wire or the pole projecting from the corners of the mesh. It has been shown that increasing environmental complexity, sometimes referred to as environmental enrichment, can increase behavioural and neuronal plasticity, improve cognitive performance and increase survival rate in reintroduced species (Hunter *et al.,*2002,Kempermann *et al.,*2002,Bredy*et al.,*2003, Rabin, 2003). Similar results were observed in steelhead (*Oncorhynchusmykiss*) where complexity experienced in rearing tanks generates more flexible behaviours than those reared in conventional tanks (Lee and Berejikian, 2008). For example in a learning task, African cichlid (*Simochromispleurospilus*), that had experienced a change in food ration in early life out-performed those fish kept on constant rations, suggesting that changes in environmental quality triggered better cognitive performance in these fish (Kotrschal and Taborsky, 2010). Often the changes observed in the behaviour of the fish are ascribed to the changes in the neuronal makeup, which alter the transduction of sensory information and then behavioural responses (Browman, 1989). Thus the early rearing environment can influence the animal's behavioural phenotype, and individuals exposed to different types of environment can develop different behaviours (Marler and Peters, 1977, Wiltschko*et al.,* 1987, Braithwaite and Guilford, 1995, Caldji*et al.,*2000).

The present study also shows that orientation behaviour is also correlated with age of the fish. Our experiments indicate that applying different types of barriers in the hatchery tanks can influence the spatial learning behaviour with age. Several studies indicate that there are two different neurogenic processes in teleost fish. One system, found in the telencephalon and the hypothalamus, stops replication at an early stage of development; dividing cells in these areas are repressed or removed. The other system, found in the tectum and cerebellum, retains its replicative activity even in adult fish; the number of dividing cells in these areas seems to be maintained during development of the brain (Maeyama and Nakayasu, 2000). Neurons are added to many areas of the nervous system of fish as it grows. In this respect, the nervous systems of fish differ from the nervous systems of other vertebrates. It is possible that the comparatively greater spatial learning ability of older fish is due to the better ne urogenecity of the fish reared in complex environment. We offer the suggestion that the comparatively greater spatial learning ability of older fish may be due to ability of fish add neurons to various parts of the nervous system throughout life (Birse*et al.,*1980). As well as provide for plasticity in how fish brains develop and respond to environmental variability.

When comparing the spatial learning ability of juveniles with that of adult fish, fish reared in complex environment show greater learning capacity in adult stage of its life (Figures 3 and 4). Among the four rearing treatments, fish reared in tanks fitted with barriers at the middle of the water column exhibited more learning capacity in adult phase than fish reared in other three conditions. This indicates that the experience of the fish larvae during early phase of life confers better learning ability and even transfers that to later period of life. Adult climbing perch learns better than the juveniles, due to increased exposure to complex environment and experience in their life.

It appears that shift in the behaviour with age is geared towards maximizing fitness potential as selection regimes, life history priorities and individual experience change during ontogeny (Brown *et al.,*2007). The effect of spatially enriched rearing environments on rodent behaviour has been shown to affect behavioural flexibility, neuroanatomy and stress responses (Harris *et al.,*2009). Thus, early rearing condition promotes better spatial learning ability in later life that may help the fish to develop problem-solving strategies, features that will be important for tackling variable natural ecosystems. It can be suggested that behavioural studies should be part of applied research for developing aquaculture, as well as for research concerning restocking and conservation of freshwater fish resources. This study also suggests a mechanism of how early rearing conditions during ontogeny enhance learning ability persistently in adult allowing the fish to acquire better learning abilities to cope with changing complexity of natural environment.

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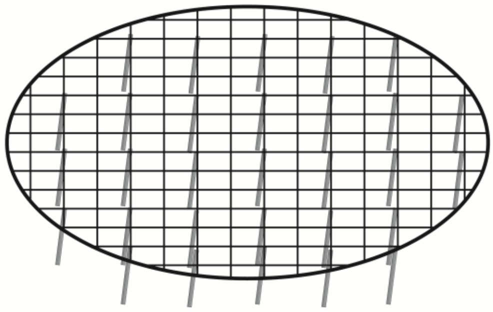
FIGURESAND TABLE

Figure 1.A



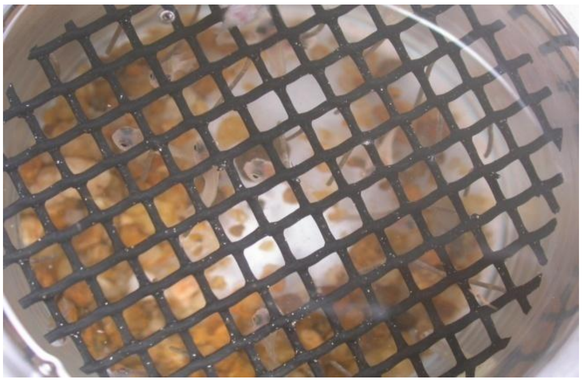


Figure 1.C

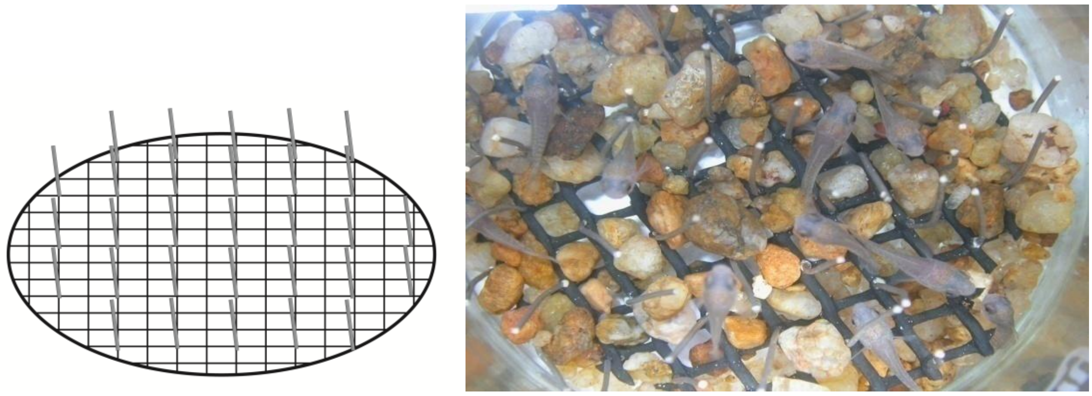


Figure 1.D

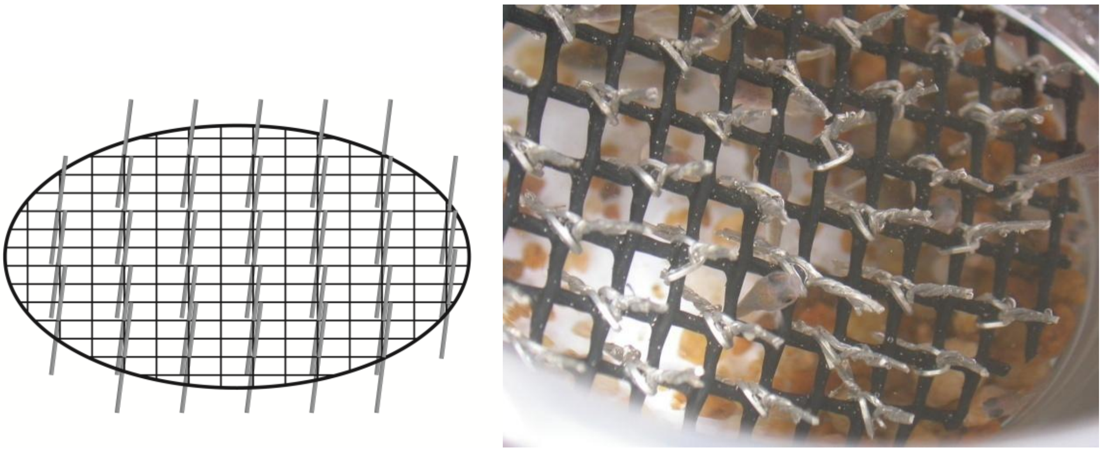


Figure 1 Hatchery rearing environment

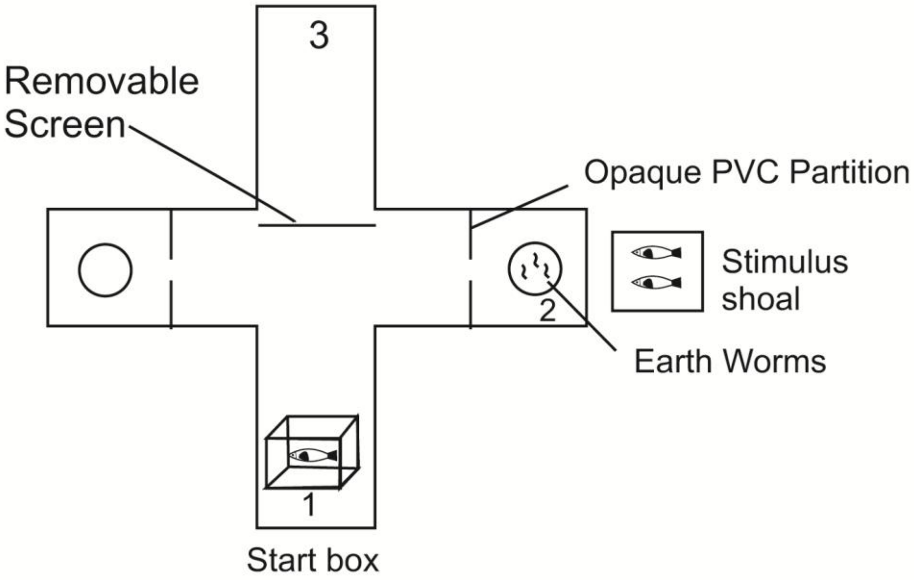


Figure 2 Plus maze used for studying the spatial learning in *Anaba*

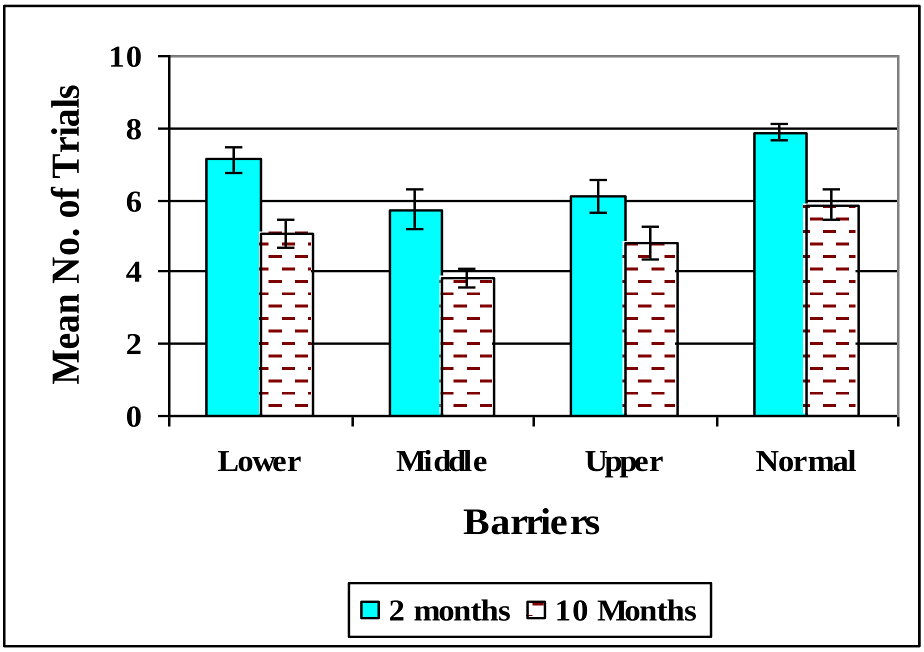


Figure 3: Mean number of trial taken to reach the reward by juvenile and adult climbing perch exposed to different spatial barrier in hatchery (± S.E)

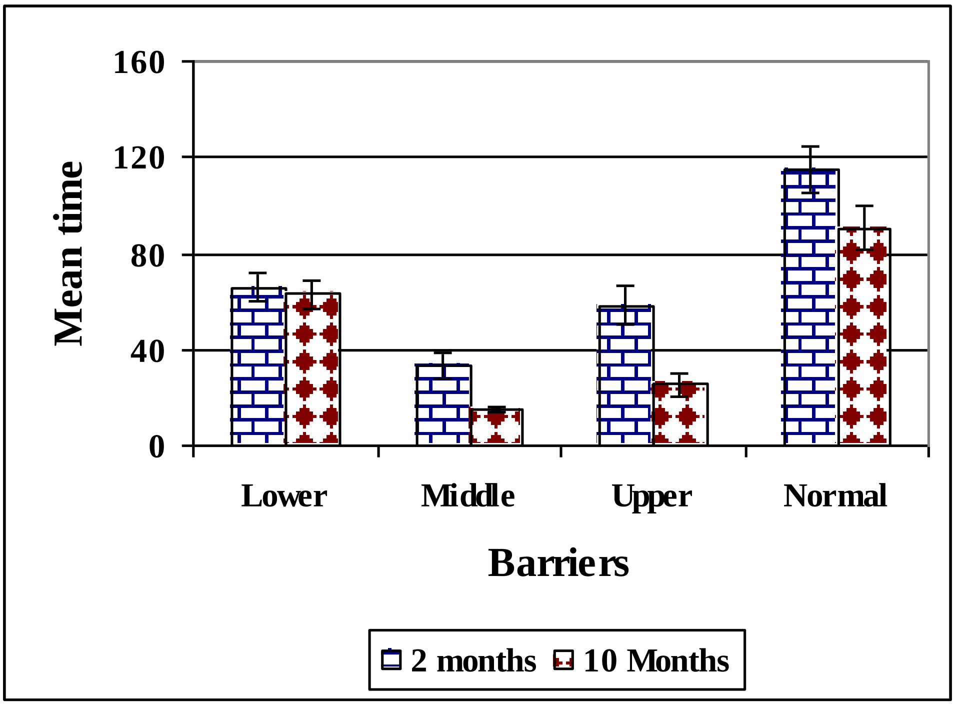


Figure 4 Mean time taken to reach the reward by juvenile and adult climbing perch exposed to different spatial barrier in hatchery (± SE)

DESCRIPTIVE STATISTICS TABLE

TABLE 1 Results of two- way ANOVA (comparing number of trial to reach the reward)

Source	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Age	1	100.83	100.83	42.84**	<0.001
Barriers	3	72.20	24.07	10.23**	<0.001
Age x Barriers	3	2.57	0.86	0.36^{ns}	0.779
Error	112	263.60	2.35		
Corrected Total	119	439.20			

TABLE 2 Average numbers of trials to reach the reward

Rearing treatments(Barriers)	Age		Overall
	2 months	10 months	
Lower	7.13	5.07	6.10^{ab}
Middle	5.73	3.80	4.77^{c}
Upper	6.13	4.80	5.47^{bc}
Normal	7.87	5.87	6.87^{a}
Overall	6.72	4.88	

TABLE 2 Average numbers of trials to reach the reward

Means with same letter as superscript are homogeneous within a column

TABLE 3 Results of two- way ANOVA (comparing time taken to reach the reward)

Source	Degrees of freedom	Sum of squares	Mean square	F-value	p-value
Age	1	11505.21	11505.21	16.78**	<0.001
Barriers	3	102803.49	34267.83	49.98**	<0.001
Age x Barriers	3	3669.89	1223.30	1.78^{ns}	0.154
Error	112	76798.40	685.70		
Corrected Total	119	194776.99			

TABLE 4 Average time taken to reach the reward

Rearing treatments (Barriers)	Age		Overall
	2 months	10 months	
Lower	65.87	63.07	64.47^{b}
Middle	33.47	15.33	24.40^{d}
Upper	58.40	25.27	41.83^{c}
Normal	114.80	90.53	102.67^{a}
Overall	68.13	48.55	

Means with same letter as superscript are homogeneous within a column