Section II

*Propensity to take a risky decision-the boldness*
The boldness

Being the major component of aquatic food web, and being prayed upon by aquatic, aerial and amphibious predators, in teleost fish, decision making is a difficult task as there is no surety about whether one’s choice will augment fitness or not (Milinski, 1993). However, on several occasions fishes are compelled to take risky decisions. The propensity to take a risky decision is defined as the boldness (Wilson et al. 1994). In fishes, boldness can vary from individual to individual as well as from context to context. In the view of Coleman and Wilson (1998), individuals of a fish population can be categorized into three sub-groups based on their predisposition to take risk: bold, intermediate and shy. Bold individuals tend to be risk takers and will quickly approach novel objects and explore novel environments. By contrast, shy individuals tend to be risk averse and neophobic with behavioural responses often accompanied by fear response, such as freezing in a novel habitat (Brown and Smith, 1996; Budaev et al. 1999a; b; Templeton and Shriner, 2004). The ‘intermediates’ are placed in a position middle to the bold and shy individuals.

Though, the boldness is a flexible trait, there are standard methods for measuring the boldness of a fish (Brown et al. 2007). The major assays are focused on behaviours like tendency to inspect predators (Godin and Dugatkin, 1996), foraging under predation risk (Magnhagen, 2006), response to novel objects (Sundström et al. 2004), and open field test (Budaev, 1999a; b). According to Brown et al. (2007), measuring the latency to initiate the exploration of a novel area is now being accepted as the most recommended method of measuring the boldness of a fish.
Boldness is species specific

Gill and Andrews (2001) suggest that the boldness of a fish is correlated with species specific needs. These authors assumed that different species of fishes with difference in behaviour repertoire, adapted to different niche, exhibit variation in their inclination to take risky decisions. Recently, Yoshida et al. (2005) analysed species specific character of boldness by quantifying the latency to initiate exploration of a novel area by fishes. These authors compared the reaction of three species of fishes: bluegill sunfish (*Lepomis macrochirus*), crucian carp (*Crassius longsdorfi*) and gold fish (*Crassius auratus*), to a novel environment. The results indicate that the bluegill sunfish is boldest among the three species. The crusian carp was found to be the shyest and gold fish occupied a position in between the bluegill sunfish and crucian carp. Analysis of genetic relatedness revealed that gold fish is genetically closely related to crucian carp, but the domesticated life in artificial environment might have made them bolder than crucian carp. Therefore, Brown et al. (2007) suggested that the boldness is a species specific and genetically determined character and each species will continue to exhibit its inherited level of boldness until an external selection pressure acts on it.

Genetic correlates of boldness

Iguchi et al. (2001) have demonstrated, using two cloned strains of red spotted cherry salmon (*Onchorhynchus masou macrostomus*), that boldness has a genetic basis. To add with this result, Brown et al. (2007) have shown that the boldness of first generation individuals of *Brachiraphis episcopi* was similar to that of their parents. This result supports the contention of genetic determination of the trait, the boldness-shyness
continuum. Recently Vilhunen et al. (2008) have gone beyond just speculating genetic determination of boldness. These authors have demonstrated that genetically more variable individuals (mesaured as micro satellite heterozygosity) are more prone to take adventurous decisions under the risk of predation. Additionally, this association was significantly consistent over three populations with different backgrounds, suggesting that in this species boldness is correlated with heterozygosity. However, they failed to find any relationship between genetic variability and the degree of boldness, when threat from the predator (simulated using predator odour) was absent. These results reveal that the predation pressure can bring forward variation in genetic correlatedness of boldness in the long run of evolution (Vilhunen et al. 2008).

**Boldness and mode of locomotion**

As many studies measures boldness as the latency to initiate exploratory behaviour in a novel area, an act controlled by the motor system dedicated to the locomotor activity, Gill and Andrews (2001) analysed an interesting hypothesis: whether initiation of exploration of a novel area have any relationship with the mode of locomotion followed by the fish species. According to Westneat and Walker (1997), fishes can be divided into three groups based on their mode of locomotion: carangiform, sub-carangiform and labriform. Carangiform fishes follow stiffer, fast movements and they gain the force of propulsion by the rapid oscillation of the tail. Here, the majority of movements are concentrated in the very rear part of the body and tail (Westneat and Walker, 1997). The sub-carangiform mode of locomotion varies from carangiform in such a way that the majority of movements is concentrated on the rear half of the body,
instead of the rear end of the body. In labriform mode, the propulsive force is generated by the oscillatory movement of the pectoral fin.

Gill and Andrews (2001) measured the exploratory activity of nine species of Caribbean reef fishes in response to their first encounter with a novel environment in a large aquarium. The result demonstrated that, regardless of the mode of locomotion, all species including territorial ones initiated the exploration of novel habitat. However, their boldness varied significantly in accordance with the mode of locomotion. The carangiform fishes were more shy compared to the other two types of fishes, and initiated the exploratory activity slowly and cautiously. Though, they were slow in the beginning of the trial, their activity level slowly attained a stable value as they became familiar with the novel environment. However, there was no significant variation in the exploratory activities of the sub-carangiform and labriform fishes. These results show that genetically determined mode of locomotion has profound influence on the boldness of a species.

**Context specificity of boldness**

Though, there are reports supporting the genetic control of boldness (Wright *et al.* 2003, Stein *et al.* 2001; McCune, 1995), many fishes show context specific variation in their boldness. For example, Coleman and Wilson (1998) have demonstrated that boldness exhibited by individual pumpkinseed fish (*Lepomis gibbons*) varied significantly in presence of threatening stimulus (a red tipped meter stick) or a novel food source. Moreover, the fish that stayed still, rather approached or fled (could be considered as individuals with intermediate level of boldness), boldly foraged in presence of the predator compared, to the bold or shy individuals. According to Ward *et al.*
(2004b), reason behind this context specific variation of boldness is the options available to the fish, and each fish can initially decide whether to respond to a given stimulus and then behave in investigative or cautious manner. Thus, the fish showing an intermediate response to the threatening stimuli had simply chosen not to respond at all, in presence of a novel object (meter stick), but when confronted with an actual predator they responded boldly. This context specific alteration in the boldness is seen not only in fishes, but many other species like the big horn sheep (*Ovis canadensis*, Reale *et al.* 2000), Harris sparrow (*Zonotrichia querula*) and human beings (*Homo sapiens*, McCune, 1995) also modify their boldness depending on the context.

According to Salonen (2005), in European graylings (*Thymallus thymallus*), the boldness and its correlated factor aggression was found to be varying significantly in young ones produced from the same stock in two successive years. This author speculates that yearly changes in the atmospheric factors (which are yet to be discovered) present in different years might be considered as the suspected reason behind this unexpected result. Salonen (2005) continues her discussion by challenging the genetic control of boldness by putting forward the hypothesis that, even though, boldness has a genetic basis, it is fine tuned by the needs, niche and the quality of the habitat.

The conflict of opinion about the context specificity of boldness does not end up with the result obtained from European graylings (Salonen, 2005). Analysis of boldness exhibited by sticklebacks in four different situations challenged the context specificity of boldness itself. Ward *et al.* (2004b) showed that the boldness of a species is rather consistent and does not vary according to the context. In the light of the above
experiments, Ward et al. (2004b) opines that rigorous experimental studies are needed for ascertaining context specific expression of boldness in fishes.

**Age, size, metabolism and boldness**

According to Brown and Braithwaite (2004b) boldness of a fish is correlated with its body size and metabolic rate. Sih, (1997) suggests that the nutritional status and the rate of metabolism can affect the motivational status of the fish, which in turn reflect in the expression of boldness. The enhancing effect of hunger on activity level and boldness has been demonstrated by Weiser et al. (1992) using cyprinids fishes. Theoretically, the metabolic rate increases with the natural log of body mass, but metabolic rate per gram body weight decreases with the increase in body size (Brown et al. 2005). In teleost fishes, the resting oxygen consumption and the natural log of body mass gives a scaling exponent of 0.79 (Clark and Johnston, 1997), meanwhile, in all other vertebrates metabolic rate per gram of body weight and body size has a negative exponential allometric relation. All these observations converge to the ‘metabolic hypothesis’ which states that juvenile fishes with higher metabolic rate during the growing age are more likely to take risky decisions than larger individuals (Brown and Braithwaite, 2004b).

Many studies show that the size of a fish is correlated with its age and smaller fishes can be considered as younger than the large fishes in a population (Brown and Braithwaite, 2004b; Dowling and Godin, 2002). Small fishes have lower body weight and fat reserve, higher drag coefficient and faster metabolic rate due to the growth than large fishes (Wooton, 1994; Krause et al. 1998; Skalski and Gillaim, 2002). Due to this increased need for the metabolites, they become hungry very fast and this increased level
of hunger compels them to be bold and to emerge out from the shelter soon, as well as to try luck in front a predator. Hence, metabolic hypothesis assumes that young (small) fishes are bolder than old (large) fishes.

Though, many species obey the rules of metabolic hypothesis, it was found to be incorrect in poecilid *Brachyraphis episcopi* (Brown and Braithwaite, 2004a) and banded killifish (*Fundulus diaphanous*, Dowling and Godin, 2002). In banded killifish, the latency to emerge out from a refuge after the predator attack was found to be negatively correlated with the size and small killifishes spent more time inside the shelter, before emerging out, after the shock of a simulated predator attack (Dowling and Godin, 2002).

The metabolic hypothesis was challenged by ‘predation hypothesis’ which proposes that small fishes are more vulnerable to the predation compared to large fishes, and will exhibit reduced level of boldness by emerging slowly from the shelter than the large fishes (Sogard, 1997). Comparison of the rate of metabolism by measuring the RNA level of the bold and shy individual brown trouts with difference in their body size (and metabolism) also favoured the predation hypothesis, but not the metabolic hypothesis (Sundström *et al.* 2004). The result of this study confirmed that boldness is not correlated with metabolism (RNA level), and thus the size could not be considered as a predictor of boldness.

In order to handle this controversial context, Brown and Braithwaite (2004b) introduced two new hypotheses to support the late emergence of large fishes from the refuge provided. The first hypothesis states that, due to the increased exploratory urge and reduced level of fear towards the predator, the bold fishes will be eliminated in the earlier phases of their life by predation, and only the shy individuals might be surviving
up to the older age (Brown and Braithwaite, 2004b). This assumption can be true in an aquatic ecosystem with very high pressure of predation and limited space available for the prey species to explore and escape. The second hypothesis postulates that, the length of a fish is correlated with its age and most of the old fishes inhabiting natural habitat are well aware of the danger caused by the predator, than the small, ignorant juvenile fishes. Hence, the large fishes take more time to recover from the fear and are cautious during the emergence from the shelter. More detailed discussions dealing with the relationship between size and boldness can be expected in the future.

**Gender difference and boldness**

Difference in the life history priorities lead to the differences in the behaviour of sexes including boldness (Jennison and Telford, 2002; Reznick et al. 1993; 2001). In most of the species, male fishes are bolder than females (Brown et al. 2005). This elevated degree of boldness exhibited by the male fish is not easily influenced by external factors. For example, males of brown trout (Salmo trutta) never given any attention to the repeated predator attack and involved in the agonistic behaviour, even in presence of the predator (Johnsson et al. 2001). However, in comparison to the degree of boldness exhibited by males, female fishes are found to be shy and always hesitated to take risky decisions (Brown et al. 2005). In Brachyraphis episcopi males took less time to emerge out of a refuge and to engage in its normal activities (a sign of increased boldness), while females spent longer duration inside the shelter to reach a similar decision (Brown et al. 2005).

In the view of Brown et al. (2005), the proximate reason for the variation in boldness of male and female may be the variation in their hormone profile. The ultimate
is the bolder male getting more chance of insemination and hence the enhanced fitness (Evans et al. 2003). Moreover, female fishes always prefer to mate with bolder males (Godin and Dugatkin, 1996). However, the increased level of boldness makes the male fishes short lived due to the vulnerability to predation, which increases hand in hand with boldness (Brown et al. 2005). This argument was found to be true in studies using Poecilia reticulata, where females stop feeding and concentrate more on antipredator tactics in presence of a predator while males will attempt for courtship in a similar situation (Evans et al. 2003; Reznick and Endler, 1981). So, it could be concluded that the males increases their fitness by making more insemination even under the threat of predation, while females increases their fitness by increasing the longevity by avoiding predators.

**Brood and the boldness**

The tendency to take a risky decision by a fish is very high during the period of parental care. The reason for this behavioural modification during the period of nest defence and parental care is the increased cost of shyness that has to be paid by the fish. If the fish hesitate to respond boldly in this critical period of reproduction, it will lose the fitness (Huntingford, 1976a;b). As a result, the nest defending three spined sticklebacks are bolder than its non breeding conspecifics, and the former will not desert the nest, when presented with an aquatic predator, the rainbow trout (Salmo giardneri, Kynard, 1979). These territory holding males will even go to attack the intruding males even under the threat of predation from the rainbow trout (Ukegbu and Huntingford, 1988). According to Pressley (1981), the boldness shown by a breeding male is positively
correlated with the value of the brood being defended. This author established the hypothesis true, using sticklebacks and demonstrated that the boldness increases with the age of the fish and the number of eggs present in the brood. This study also revealed that the enhancing effect of brood on the boldness will decline as the breeding season reaches the climax (Ukegbu and Huntingford, 1988).

**The bold fish learns more**

The bold fishes are not only quick to make exploratory decisions, but also excel in learning various tasks (Brown *et al.* 2007). There are many studies revealing the association of boldness with different types of learning abilities of a fish. Sneddon (2003) using brown trout (*Onchorynchus mykiss*) model system demonstrated that the bold fishes learn rapidly the appetitive conditioning task than their shy counterparts (Sneddon, 2003). Additionally, Templeton and Giraldeau (1996) have shown that bold fishes are also equipped with higher level of latent learning ability, which makes their risky exploration of novel areas or objects worthwhile. This higher level of latent learning ability allows these fish to utilize the information learned during the exploration in a similar context without any reinforcement (Lieberman, 1990). The bold fishes are good social learners, and they gather more social information and achieve a dominant status in the shoal, which increases access to the biologically significant resources like food, mate etc., and make them a favourite choice of natural selection (Bumman *et al.* 1997).

Fuster (1985) binds the enhanced learning ability of bold animals with the increased activity of reticular arousal system (RAS) of the brain. RAS is a column of nerve cells extending to the cerebral cortex and thalamus from the lower parts of the
brain. This system is provided with axonal connections to spinal chord also (Carlson, 1995). When an animal starts exploration, the RAS starts to show heavy activity. This high activity of RAS in association with heightened activity of eyes, increased alertness and intensity of attention, enhances the pace of learning process by increasing the ability of an organism to discriminate between objects quickly and reliably.

**Boldness-aggression syndrom**

The probability of taking a risky decision is positively correlated with the degree of aggressiveness exhibited by that individual (Höjesjö, 2002; Sundström et al. 2004). The relationship between these two characters is evident in cichlid *Nannacara anomala*, where bold individuals are more willing to display aggression and escalate it to agonistic interaction than their shyer conspecifics, both in presence and absence of a model predator (Höjesjö, 2002).

This correlation between aggression and boldness is known as aggression-boldness syndrome as these two traits are associated even at the individual level (Salonen, 2005). According to Bell (2005), these two traits show much tight connection, so that it should be considered related to each other, both within and between populations. In the view of Tully and Huntingford (1987), the causative agent of the link between aggression and boldness is the fear induced suppression of ongoing behaviour, a common factor present in both situations. Many authors suspect that such a correlation could be the product of the genetic linkage among these two traits (Sokolowski, 2001; Van Oers et al. 2004) or owe to some underlying physiological constraints: for example an energy allocating trade-off, that is difficult to decouple or modify over evolutionary
time (Sih et al. 2003, 2004). Moreover, this correlation between boldness and aggression was found to be constant and is not at all affected by the variation in environmental quality. Although, the boldness of a species may be altered due to the early life experience, its association with aggression is maintained (Salonen, 2005). Furthermore studies of Salonen (2005) validate that the boldness changes with aggression hand in hand and the link between these two vital traits is not affected by the absence of selection force (e.g. lack of predator or cues announcing emergency) in the due course of development.

The association between boldness and aggression has ecological as well as evolutionary advantages (Sundström et al. 2004). The bold fish takes risk of the exploration of unfamiliar habitat due to their increased urge for exploration. This adventurous exploration is beneficial, as the unfamiliar habitat could provide the bold fish with more food due to lesser level of competition from conspecifics. This hypothesis is validated by the study of Wilson (1998), using pumpkin seed sunfish (*Lepomis gibbsons*). This author demonstrated that, bold individuals feed sooner in the laboratory their shy conspecifics, and they also had more food in their stomach upon capture in the wild. However, the exploration of unfamiliar area can be lethal due to the lack of knowledge about refuges, nature and tactics of predators present in the new region.

When the competition increases in a population, the shy individuals are also compelled to elevate their boldness in order to find new food sources by exploring new areas. As many fish species monitor and copy the behaviour of other individuals, the shy fishes will follow the route of bold fishes and will try to intrude into the new territory established by the bold fish (Höjesjö et al. 1998; Johnsson and Åkerman, 1998; Brown
and Laland, 2003). If the cost of taking risk of exploring a new habitat and time and energy lost in establishing a territory are to be compensated, the bold fish should defend its profitable territory. In this context, if the bold fish fail to exhibit aggression and dominance, they will be easily replaced by other fishes. Such a cost paid by the bold fish will not be favoured by natural selection, and these individuals will be eliminated from the evolutionary race. Consequently the correlation and inheritance of the boldness in combination with the aggression is a must for the survival of the bold fishes (Sundström et al. 2004; Abrahams and Dill, 1989).

According to Wilson (1998), the inheritance of behaviours in combinations is preferred by natural selection and hence it is adaptive. However, the adaptive value of this combination of behaviour will be different in different ecosystems, as each ecosystem harbours different types of predators (Price and Langen, 1992; Wilson, 1998; Sih et al. 2004; Bell, 2005). Price and Langen (1992) point out that such inheritance of behaviour could be maladaptive due to the expression of a trait only due to the compulsion to inherit and express in combination in a context where it is of little adaptive significance. For instance, the defence of territory by bold fishes will be costly in a homogenous environment as well as in areas with unpredictable food resources. Moreover, the cost of exploration will further increase if the fish tries to explore unfamiliar environment only due to its elevated boldness and curiosity, though an equal amount of resource and benefit is present in the familiar and non risky habitat (Abrahams and Dill, 1989).
Boldness in a shoal

Most of the prey fish species living under the threat of predation: practice two strategies to avoid the predator; either they invest more energy for vigilance (Krause and Ruxton, 2002) or they join a shoal by paying the costs of competition (Neil and Cullen, 1974). Though, the position of an individual in a shoal is flexible, different spatial position provides different levels of advantages like access to the food resources and rescue from the predators. As far as the success of foraging is concerned, the most appropriate position for a fish is in the front part of the shoal, at the same time, the vulnerability to predation is also at the maximum in this position (DeBlois and Rose, 1996). According to Bumman et al. (1997), leading position of a shoal is always occupied by the bold individuals enjoying a dominant rank in the group. This prominence and front position in a shoal compensate for the higher vulnerability to predation by providing greater chance for encountering and to get control over the food materials.

According to Ward et al. (2004), as the effective antipredator mechanism and active resource pursuit are mutually exclusive, bold fishes are interpreted as risking predation for increased resources (Sih, 1997; Wilson et al. 1994). For instance, sticklebacks that recovered rapidly after a shock of predator attack exhibited an increased rate of growth and always displayed a reduced tendency for shoaling. Moreover, if these fishes join a shoal, they always occupy a dominant position. In European wrasse (Symphodus ocellatus), individual fishes with the weakest shoaling tendency had the highest level of locomotion and a heightened level of spatial exploration ability (Walsh and Cummins, 1976). By contrast, the shy wrasse with higher shoaling tendency spent
more time in performing antipredator tactics like freezing, due to the elevated level of stress or fear in a novel environment (Budaev, 1997; Walsh and Cummins, 1976).

The behaviour of the fish occupying the leading position of a shoal can affect the shoal dynamics and synchronized activity of other shoal members (Bumman and Krause, 1993). This is because the followers just copy the behaviours of the leading dominant individuals in a shoal (e.g. roach *Rutilus rutilus* and stickleback, Bumman and Krause, 1993).

**Boldness and habitat quality**

In nature, different areas of a large aquatic habitat show much variation in physico-chemical as well as biological properties that it can be divided into microhabitats with different levels of selection pressures. Hence, only the fishes equipped with flexibility in behaviours and high level of adaptability can live and reproduce in a wide variety of environments. However, the variation in behaviour with reference to the ecological quality of habitat reflects in the boldness-shyness continuum of populations of a species originated from different environmental conditions (Brown and Braithwaite, 2004a).

The major ecological parameters affecting the boldness of a fishes are food availability, foraging competition, distance to be travelled to obtain the food (Dowling and Godin, 2002; Godin, 1997; Sih, 1997; Lima and Dill, 1990). According to Brown and Braithwaite (2004a) the predation pressure can modify many types of cognitive abilities of the fish. This assumption was found to be true in the case of boldness also (Brown *et al*. 2007). Many studies support the idea that fishes from an area of lower risk of predation show high level of boldness and they emerge from the shelter soon after a frightening experience (Pitcher and Parrish, 1993). These findings support the theory,
that predator allopatric population of fish is less likely to respond fearfully to predators than the predator sympatric population (Pitcher and Parrish, 1993).

However, the relevance of this theory has been questioned by the experiments in Brachyraphis episcope collected from habitats with different levels of threat from predators (Brown et al. 2005). This study demonstrated that fishes from the areas of increased pressure of predation exhibit lesser level of shyness and they emerge out from the shelter sooner than the fishes collected from areas with lower risk of predation. The fishes from the areas with increased risk of predation cannot wait for a long time in a refuge, because these fishes expect predator at any moment in their home range. Moreover, for an individual inhabiting such a habitat, encounter with predator is not a rare event, and the fishes have to search for food and mate in the shadows of unpredictable predator attack. Hence, the prey species living under the risk of predation are well equipped with the tactics to escape from predators.(Brown and Braithwaite, 2004a).

A second proposition based on metabolic hypothesis is also available to explain the increased level of boldness exhibited by the fishes collected from habitat with high level of predation pressure (Brown et al. 2005). It is stated that fishes leading sympatric life with predators get only reduced access to food, that they seldom reach satiation. It is the hunger that compels them to emerge out of the shelter soon to compensate the metabolic needs.
Application of boldness for the welfare of the fish

Usually the fishes born and brought up in a homogenous environment of the hatchery exhibit converged and increased level of boldness due to the absence of spatial complexity and lack of predator stimuli (Salonen, 2005). Usually, induced bred larvae reared in artificial hatcheries, a habitat with extreme levels of homogeneity, are used for restocking and reintroduction of endangered fish species. As these fishes have no experience with predators, they are bolder than fingerlings developed in the natural environment. Thus these bolder individuals can become a threat to local population when released in to the natural water bodies (McMichael et al.1999). The bold newcomers with increased aggression and dominant status will get hold of the biologically significant resources like the food and mates soon after their entry in to the new area. This problem becomes further complicated as female fishes prefer bolder males to mate with (Godin and Daugatkin, 1996). As a result less aggressive native fishes will loose their territories and ultimately their fitness (McMichael et al.1999; Einum and Fleming, 2001).

The inflow of bold fishes will, not only affect the equilibrium in an existing population but also alter the genetic composition of existing population, since boldness is inherited. Ultimately the immigration of genes for elevated boldness will lethally affect the resident fish population (Koskinen et al. 2002; Susnik et al. 2004; Madeira et al. 2005). Hence, the knowledge about the boldness of the individual fish species living together in a habitat is an important factor to be considered, while developing strategies to restock and reintroduce endangered species (Brown and Day, 2002; Brown and Laland, 2001).
However, the increased fitness of bold individual is a short lived phenomenon as the risk of predation increases hand in hand with boldness (Salonen, 2005). According to Brown and Day (2002), the bold individuals will even go to explore predators and unfamiliar areas where danger is unpredictable. As a result, most of the bold fishes will be eliminated from their habitat due to the increased curiosity, exploratory urge and aggression; the behaviours that helped them to get control over the resident conspecifics. This reduction in the survival due to the increased boldness has been highlighted by the study of Olla et al. (1998). Their study shows that, hardly less than five percent of reintroduced young fishes reached adolescence. Hence, the fluctuation in the boldness of individuals inhabiting an ecosystem can make the population unstable (Brown and Laland, 2001).

Study on the development of boldness using European graylings (Thymallus thymallus), that are reared in a homogenous environment in hatchery, gave a contradictory result. In this species, the fishes reared in a homogenous condition exhibited a reduced level of boldness compared to their counterparts collected from the natural water bodies (Salonen, 2005). However, the reduction of boldness is as lethal as elevation of it during the restocking and reintroduction, as the shy naïve fishes cannot withstand the pressure of competition from the resident individuals. Hence, the elimination fishes before reaching the phase of reproduction will badly affect the success of re-introduction programme itself (Miller et al. 2004).

Applications of data analyzing the boldness of a species are used in sea-ranching (Sundstorm et al. 2004). In sea-ranching, fish larvae are collected from the field and reared up to a certain size in artificial conditions, and then released into the wild with the
hope of significantly reducing larval mortality. For example, youngones of brown trout are collected and kept for the first two years in artificial conditions, before placed back in to sea (Sundström et al. 2004). The alteration in the boldness due to this early life experience in a homogenous environment can affect the success of sea ranching programme (Sundström et al. 2004).

A recent study by Salonen (2005) shows that the degree of flexibility in boldness required to survive successfully varies in accordance with the characteristics of the ecosystem. For instance, European graylings (*Thymalus thymalus*) inhabiting the lotic water of rivers are bold and aggressive compared to the pond dwelling conspecifics. This variation is evident in the boldness of young ones produced from the parents collected from lentic and lotic ecosystems (Peuhkuri and Salonen, 2004). Additionally, the boldness of the young ones produced by the cross breeding between parents collected from different ecosystems is unpredictable. Hence, Salonen (2005) suggests the use of progenies produced from parents living in ecosystems with properties similar to that of the water body proposed for the restocking.

Even though the variation in boldness is not a desired condition during the reintroduction programs, these young fishes could be trained to attain a desirable level of boldness, before introducing them in to the natural water bodies (Sneddon, 2003). Recent works demonstrate that, if kept in an environment with spatial complexity or maintained with variation in the availability of food materials, the hatchery reared shy cod (*Godus morhua*) larvae will become bolder and grow fast. Such larvae are better learners compared to their conspecifics reared in a homogenous habitat and will quickly locate shelters and take refuge very fast in response to a predator attack. This enhanced learning
ability helps them to adapt quickly to the spatial complexity of the natural ecosystem in which they are introduced (Braithwaite and Salvaness, 2005). Moreover, fishes with experiences in a complex habitat will easily shift to the new menu available in the natural habitat, ability absent in shy fishes born and brought up in the homogenous habitat with constant food availability.

Braithwaite and Salvaness, (2005) postulates that the increment in the boldness and correlated negative impact on the antipredator behaviour and survival of the fishes resulting from long term stocking in the captivity can be reduced by making variation in the spatial properties and food availability. Hence they expect a new system of bio-manipulation for the management boldness and allied behaviours, to increase the success rate of reintroduction programmes and the sustainable maintenance of the existing fish stocks.
Chapter I

Evaluation of the effect of repeated exposure on boldness

INTRODUCTION

The urge to explore a novel area or novel object is a part of behavioural profile of all most all animal species (Crusio, 2001). However, exploration of a novel area is dangerous as the explorer is unaware of pitfalls present in such an environment, while the same behaviour helps to find new food sources and escape routes (Sundström et al. 2004). The bold individual will exhibit a quick initiation of exploration and active search of a novel area, while the shy or emotional ones show less interest for exploration and take more time to start locomotory activity and exploration (Crusio, 2001). The ‘intermediates’ are put in position middle to the bold and shy individuals (Coleman and Wilson, 1998)

The exploratory activity and boldness of animals always fascinated the scientists. The earlier attempts for the scientific quantification of these behaviour patterns comes from the studies of Hall (1901 quoted in Silverman, 1978) using rats. The trendiest version of apparatus used for analyzing different aspects of boldness and other behaviour patterns connected with exploratory behaviours is known as ‘open field’. This apparatus is a well illuminated homogenous open area without any complexity. Here, the focal animal is introduced in to the well-lighted central arena of the open field, and activity of the animal is recorded and quantified, either manually or using electronic devices
The bold (less emotional) animals visit the bright lit area soon after the entry into the apparatus. By contrast, the shy (emotional) animals show latency to move around and prefer the areas with lower luminescence to focus the searching activity. The duration of the behavioural measurement in open field also varies (Crusio, 2001): some authors study the activity only for a few minutes (Flint et al. 1995), while others observe the animal for twenty minutes or more (Foshee et al. 1965). Survey of literature reveals that, circular open field with bright inner sides and floor marked in segments to quantify the locomotor activity is the most popular version (Silverman, 1978). Nowadays, open fields of different shape and size are used according to the species and hypothesis tested (Crusio, 2001).

Even though, the open field apparatus is widely used even now to measure the exploratory activity and boldness, many authors question the validity and usefulness of the data obtained from this apparatus (Archer, 1973; Walsh and Cummins, 1976). The ambiguity in interpreting the behavioral response in an open field arises partly from the open field situation itself, where animals are forced to explore the novel environment. In such a situation, the effect of compulsion cannot be separated from the boldness (Yoshida et al. 2005). Hence, many researchers studying exploratory behaviour and boldness of animals preferred another apparatus named run-way apparatus.

The run-way apparatus consists of a shaded start chamber and an illuminated straight runway (Fujita, 1984). The runway is separated from the start chamber by a guillotine door. The shaded start chamber serves as a shelter, where the emotional or shy animal can stay away from all the external stimuli. Additionally, the shaded ceiling of the start chamber increases the refuge value of the start chamber (Yoshida et al. 2005). The
data obtained from using run-way apparatus for measuring boldness is considered to be more precise, as the decision to explore or hide is left to the animal (Fujita, 1984; Fujita et al. 1994), a condition which is not possible in open field apparatus. Hence, the shy animals are seen staying longer inside the start chamber of run-way apparatus than the bold animals (Brown et al. 2007).

The trend shift from the open field apparatus to the run-way apparatus is also reflected in studies using fish model systems (Yoshida et al. 2005). The exploratory behaviour and boldness of the fishes are being quantified using aquatic version of open field apparatus. Here, the well lighted aquarium (with all the specification of open field apparatus used for testing terrestrial animals) filled with water up to the level required for the free movement of the focal fish, called swim-way apparatus is used (Warren and Callaghan, 1976; Gerlai, et al. 1990; Gerlai and Crusio, 1994).

The fish exhibit behaviours like freezing, reduced locomotion, startle response or hyperactivity in a novel environment (Russel, 1967a; Yoshida et al. 2005). For example, the guppy (Poecilia reticulata) showed startle responses and rapid swimming around the tank when introduced into the unfamiliar experimental arena inside the open field apparatus (Russel 1967a, b). By contrast, the crucian carp has taken a longer latency (of $17 \pm 3.63$ minutes) to initiate the exploratory activity (Yoshida et al. 2005). The basis for the hyper reactivity in a novel environment may be the fear evoked by the novelty itself. This fear initiated hyperactivity can affect the quantification of boldness as the separation of initial startle response from the boldness-dependent initiation of exploration is very difficult (Yoshida et al. 2005). The situation becomes further complicated in the light of the study using paradise fish (Macropodus opercularis) model system, which revealed
that the fishes will show high level of startle reactivity and movements when handled by
the human beings (Davies et al. 1974).

The influence of neophobia and hyperactivity can be controlled effectively by
habituating the focal fish with the experimental arena by giving repeated exposure
(Yoshida et al. 2005). The attenuation of hyperactivity through familiarisation with
novel experimental arena was unequivocally demonstrated by Gomez-Loplaza and
Morgan (1991). These authors have demonstrated that the isolated angelfish,
(Pterophyllum scalare) decreased its hyper activity as the time spent in the novel area
increased.

The present study is designed to investigate the response of climbing perch in a
novel environment and to quantify its boldness by estimating the latency to initiate the
exploration of a novel area in an open field situation. The scope of this study also
includes the quantification of variation in boldness in response to the experience
(repeated exposure) with the unfamiliar environment.

**Apparatus**

Boldness of the climbing perch was analysed using an apparatus developed by
combining the characteristics of open-field and the swim-way apparatus (Binoy and
Thomas, 2003). Apparatus consists of a rectangular aquarium with a start box (Fig. 1).
An aquarium (60 x 32 x 32 cm) was divided into two chambers, A (20 x 32 x 32 cm) and
B (40 x 32 x 32 cm) with transparent acrylic sheets. An enterance (8 x 4 cm) was
provided in one corner of the partition wall. Three sides of the aquarium were covered
using black paper to reduce the external interference. Differing from the swim-way
apparatus, here the partition between the start box and the swim-way was made using a transparent acrylic sheet with an entrance. In this apparatus no guillotine door was used and the cover on the ceiling of the start chamber was also avoided to reduce the refuge value of the start chamber.

**Figure 1.** Diagramatic representation of the apparatus used to study the boldness of climbing perch.
**Testing the boldness**

The focal fish was introduced individually into the chamber A, and the latency to initiate the exploration of chamber B, was recorded. The initiation of exploration is defined as the time taken by the fish to come out of the Chamber A, through the entrance provided. After coming out of the chamber A, the focal climbing perch was given five minutes for the exploration of chamber B. If any fish failed to come out from chamber A after 6 minutes, the trial was terminated and the fish was allocated a ceiling value of 360 seconds. Focal fishes were returned to its home tank, and tested using the same procedure on the next day. This procedure was continued for the five consecutive days. 28 individual climbing perch were tested as mentioned in the procedure. Fish once tested in an experiment was never used again. A compact fluorescent lamp (20w) on the top of the assembly lighted the apparatus, and all observations were made sitting behind a black screen with slits. All fishes were released back into the site of collection after the experiment.

**RESULTS**

Analysis of the latency to initiate exploration of a novel area for five consecutive days using Kruskal-Wallis test has revealed that, in climbing perch boldness enhanced concomitantly with the experience in a novel environment ($\chi^2 = 73.873; N= 31; P<0.001$, Fig. 2).
Figure 2. Variation in the boldness of climbing perch in response to the repeated exposure in a novel environment for five consecutive days.
DISCUSSION

On the first day of exposure to the novel environment inside the apparatus, the majority fish spent a short period inside the start chamber, afterwards they slowly initiated the exploration of chamber B. However, a minority of individuals (less than 5% of total number of individuals tested), did not show any interest to explore chamber B and spent whole test period inside the chamber A. Usually the fishes introduced into a novel habitat exhibit startle response and enhanced level of locomotor activity due to the fear evoked by the novelty (Gomez-Laplaza and Morgan, 1991). The other responses seen in the novel area are staying motionless or initiate the exploration after a long period of latency (Crusio, 2001). Interestingly, the initial startle response and enhanced locomotor activity reported in other fishes (Fujita, 1994; Yoshida et al. 2005) were not found in climbing perch. Instead, this fish exhibited a gradual increase in the locomotor activity as the days of exposure proceeded. Hence, it could be assumed that, such a reduced level of initial startle activity may either be a species specific characteristic or might have resulted from the presence of the start chamber (Chamber A) near the open swim way. Utilization of start chamber of the apparatus as a refuge in presence of any fear evoking stimulus by climbing perch is already reported by Binoy and Thomas (2003).

Brown and Braithwaite (2004a) states that predator sympatric population of *Brachyraphis episcope* were bolder and reached a quick decision to explore a novel habitat compared to their conspecifics collected from an area with reduced level of predation pressure. Brown et al. (2007) states that such a variation in the boldness of predator allopatric and predator sympatric population is due to the variation in the degree of exposure to the predator during the course of life. Young sticklebacks chased
repeatedly by their father were bolder than their siblings without such experience (Tully and Huntingford, 1987), thus, the exposure to a situation simulating predator attack during the early phase of life is an unavoidable factor, as far as the development of boldness of a fish is concerned. Hence, it could be suggested that having a sympatric life with various types of aquatic predators during different phases of life in its tropical aquatic habitat might have resulted in the enhancement of boldness in climbing. In such habitats with high pressure of predation, the hyperactivity may be lethal to the fish as the agile fishes will attract attention of the predators. Hence, climbing perch starts exploration cautiously and avoids the startling response and hyperactivity.

Reduction in the degree of responsiveness towards a stimulus after getting familiarity with it (often referred as habituation), has been reported in almost all animal species. Brown (2001) has reported such an influence of repeated exposure on the behaviour of rainbow fish. The rainbow fish performed antipredator activities more effectively and enhanced the success rate of escape response, as they became familiar with the environment. The boldness of the climbing perch was also found to be sensitive to repeated exposure and the latency to reach a risky decision of exploring a novel area reduced gradually as the familiarity with the environment increased. The latency to initiate the exploration of chamber B reduced dramatically from 360 to 42 seconds on the fifth day of exposure. Therefore this species can be used for tracing out the neural mechanism behind the positively correlated elevation of boldness with the familiarity, acquired as a result of repeated exposure to a novel habitat by a fish species. Hence, climbing perch offers an excellent model system to study biological, behavioural and neurobiological basis of boldness.
Chapter II

Taking risky decisions in presence of a predator or predator cues

INTRODUCTION

The fishes are considered to the excellent cost-benefit analyzers, taking only the decisions beneficial to enhance their fitness (Hart, 1993). However, none of the fish is able to take only beneficial decisions, as the web of life is too complex in natural habitats due to the multilevel inter and intra specific interactions and competition. In the due course of life, most of the fishes will have to face challenging situations frequently and only those fishes who dare to face threat by taking risky decisions can enhance the fitness in natural habitats (Godin and Davis, 1995).

The risky situations are always associated with fear. Johnes (1997) defined fear as a psycho-physiological response and phenomena, which act as a powerful motivation to evade threat. In fishes, fear has been characterized through freezing response (Brown and Smith, 1996; Budaev et al. 1999 a, b; Templeton and Shriner, 2004), increased branchial activity (Chandroo et al. 2004) etc. In a frightened situation, the total physiological condition of a fish will be altered due to the excessive production of hormones involved in the management of emergency. As far as fishes are concerned, predator is one of the most important sources of fear (Vilhunen et al. 2008). Consequently, Brown et al. (2005) states that, in presence of a predator, making a decision after assessing the cost and benefit by a prey fish species may show significant variation in the latency and its
translation in to action. Additionally, Brown et al. (2007) validated this hypothesis by demonstrating that fishes born and grown in habitats with different levels of predation risk, shows variation in their ability to take a decision in a challenging situation. Their study shows Brachyraphis episcopi collected from an area of higher risk of predation exhibited higher disposition to take challenging decisions, compared to their conspecifics living in areas with lower risk of predation.

Different fish species sense the threat of predator by perceiving different cues emitted by the predator. Some species utilizes visual characteristics of the predator for the recognition (Hartman and Abraham, 2000), whereas, others depend upon the odour of the predator or the chemical cues present in the diet of the predator (Brown et al. 2000a). On sensing such cues the fish will shift to antipredator behaviours, which may vary from the refuge seeking and total avoidance of the area where threat is present (Dowling and Godin, 2002), to intense inspection of the areas where alarm cues are present (Brown, 2003). Some other fishes may take extremely audacious decision of inspecting the predator to understand its motivational status (Kelley and Magurran, 2003). For example, the banded killifish (Fundulus diaphanous) and the three spined stickleback (Gastrosteyus aculeatus) took more time to take a bold decision of leaving the refuge on the detection of the presence of an aquatic or aerial predator (Dowling and Godin, 2002, Krause et al. 1998). On the other hand, species like Poecilia reticulata or Cichlasoma nigrofasciatum, exhibited increased predator inspection on sensing the presence of the predator (Brown, 2003).

In addition to the predator odour, and the chemicals present in the faecal matter of the predator, a third type of chemical cue which can initiate fear response in a fish is
hypoxanthine-3N-Oxide (H$_3$NO), the putative ostariophysan alarm pheromone (Brown et al. 2000b; 2001; Smith, 1999). This chemical is present inside the skin cells of the fish and is released into the water from wounds as an announcement of emergency to others (Brown, 2003). According to Brown (2003) these chemicals possess the power to escape digestion, and being present in the fecal matter of predator, can communicate the threat not only to conspecifics, but also to the heterospecifics. However, Korpi and Wisenden (2001) have demonstrated that, in many cases the exposure to alarm pheromones alone cannot initiate the fear response, but a combination of alarm pheromone with other characteristics of predator can induce fear in fishes. For example, zebra fish, which never exhibited any sign of fear on exposure to the conspecific skin extract, displayed antipredator behaviour, when presented with a combination alarm chemicals and live aquatic predator (Korpi and Wisenden, 2001).

In many cases, exaggeration of certain morphological characteristics of predator can induce or enhance the fear response in prey species. The features like presence of large eyes, position of the mouth, and the movement of predator model can induce fear and initiate anti-predator behaviours in many prey fishes (Guthrie and Muntz, 1993; Karplus et al. 1982). For example, in paradise fish (*Macropodus opercularis*), degree of the expression of the anti-predator behaviour is positively correlated with the eye size of the predator model (Altbäcker and Csányi, 1990).

Many authors point out that, not only the presence of predator but also the presence of novel objects in the habitat can also influence risk taking tendency of the fish (Fraser et al. 2001). Coleman and Wilson (1998) have shown that the presence of a novel object like a red coloured meter stick in the habitat can influence the boldness of the
pumpkinseed sun fish. Chapter II of this section analyses the ability of climbing perch to take a risky decision in various contexts shadowed with fear of predation.

**MATERIALS AND METHODS**

*Apparatus*

All experiments were conducted in an aquarium (60 x 32 x 32 cm) partitioned with transparent acrylic sheet into two chambers: ‘A’ (20 x 32 x 32 cm) and ‘B’ (40 x 32 x 32 cm; Fig.1). A guillotine door (8 x 4 cm) was provided in one corner of the partition wall. Washed fine river sand spread on the bottom of the tank acted as the substratum, and water level was kept at 28 cm. A compact florescent lamp (11 W) on the top of the assembly lighted the room and observations were made from behind a black screen with slits.

*General procedure*

Normally, when a fish is introduced into the chamber A of the apparatus, it will start moving inside the chamber within a short duration and will extend the exploratory activity to the chamber B, passing through the gate provided. The time taken by the test fish for the initiation of exploration of chamber B was taken as the measure of trading off neophobia with the decision to explore (Yoshida *et al.* 2005) and shorter period of latency to enter the chamber B, is assumed to indicate greater level of boldness and vice versa. The latency of initiation of exploration is defined as the time taken by the test fish to come out of chamber ‘A’ through the gate provided. If the fish failed to emerge from the chamber A after 6 minutes, the trial was terminated and it was allocated a ceiling
value of 360 seconds. Each experimental fish was given seven minutes daily for four consecutive days to make it familiar with the apparatus and to avoid the error resulting from handling and the hyper reactivity in novelty (Russel, 1967a;b; Gomez-Laplaza and Morgan, 1991). The data obtained in these days were not taken for analysis.

On the fifth day of exposure all most all fishes reached chamber B within 42.5 seconds (median). This value is taken as control (I). 28 individual fishes were used in each experiment and none of the fish was used more than once in any experiment.

*Experiment I: Aerial predator*

In order to study the influence of the presence of an aerial predator on the boldness of the climbing perch, a stuffed crow (*Corvus splendense*) was used as described by Milinski (1993) and Krause *et al.* (1998). The stuffed bird was fixed on the partition wall of the apparatus in such a way that, the shadow of the bird fell near the gate. The test fish introduced in the chamber A had to cross the ‘predator above’, in order to reach the chamber B.
Figure 1. Diagrammatic representation of the apparatus used for studying the influence of aerial predator on the boldness of climbing perch.

**Experiment II: Odour of aquatic predator**

The mucilaginous skin secretions of the most common predator of climbing perch, striped murrel (*Channa striatus*) was used for testing the effect of predator odour on the boldness. To extract the odour producing skin secretion, murrels (of size 20±2 cm S.L. ± S.E.) were kept individually in 5 litre of water in a bucket for 12 hours. Mucilaginous skin secretion was extracted from 7 individual fishes and the secretion from each fish was filtered and stocked in separate air tight bottles inside the refrigerator until it is used.
One litre of water containing skin secretion of the aquatic predator was mixed with the water in chamber B, after closing the door. The door was opened after 2 minutes, and a focal climbing perch was introduced in to the chamber A and the latency to emerge out to chamber B was recorded.

**Experiment III: Aquatic predator**

To test the influence of the aquatic predator on the boldness of climbing perch, chamber B of the apparatus was further divided in to two chambers (B and B₁; Fig. 2), using perforated transparent acrylic sheet. A striped murrel (*Channa striatus*; standard length S.L. 20 ± 2 cm, mean ± S.E.) was introduced into chamber B₁. This set-up allows the focal fish in chamber A to see the aquatic predator in chamber B₁, while entering into chamber B. After thirty minutes, the test fish was introduced in chamber A and the latency to visit the chamber B was recorded. A control (II) was also carried out using the same experimental set-up, but in the absence of any predator to check whether alteration in the apparatus had any influence on the boldness of the fish.
Figure 2. Diagrammatic representation of the apparatus used for testing the influence of aquatic predator on the boldness of climbing perch.

**Experiment IV: Alarm pheromone**

In order to test the effect of the presence of the conspecific alarm pheromones on the propensity to take a risky decision by climbing perch, the skin extract was prepared by sacrificing 10 donor climbing perch by decapitation with a sharp knife without giving much suffering to the animal (Nordell, 1998). Anaesthetics were not used before sacrificing the fish in order to avoid the possible confounding effect of anaesthetic odour with pheromone (if present) in the subsequent experiments. The skin (which is considered to be a potent source of alarm substances) was removed from each side of the fish and placed in 50 ml of chilled distilled water. Then, it was ground with fine sand and filtered to remove larger particles. The extract was kept in freezer until it is used (Mirza et al. 2000; Brown, 2003).
10ml of skin extract was introduced into the water of chamber B and mixed thoroughly using a glass rod. The door on the partition wall was closed while stirring the water, in order to avoid immediate mixing of the alarm chemical with the water in the chamber A. The test fish was introduced into the chamber A, after keeping the door open and the latency to initiate the exploration of chamber B was recorded.

**Experiment V: Alarm pheromone and aquatic predator**

The potency of alarm pheromones to affect the boldness of the climbing perch in presence of an aquatic predator was also analyzed. Experiment was carried out in the apparatus described in experiment II. After mixing the skin extract (10 ml.) with water in chamber B (as mentioned in the experiment IV) an aquatic predator [striped murrel, 20 ± 2 cm. (SL ± SE)] was introduced into the chamber B. The boldness of the focal fish was measured by introducing it in chamber A. The apparatus was cleaned thoroughly and the stimulus predator was changed after each trial.

**Experiment VI: Eye spot**

To analyze the influence of the presence of an enlarged eye spot on the boldness of the climbing perch, a yellowish brown coloured screen (32 X 32 cm, Fig. 3) with a shining black spot (diameter 3 cm) in the centre was placed in the chamber B. The test fish was introduced into the chamber A and the latency to initiate the exploration of chamber B was recorded. The boldness of climbing perch in presence of a yellowish brown screen without the black spot (control III) and a black acrylic sheet (control IV) was also tested.
Yellowish brown screen with black spot (3 cm in diameter) in the centre

Figure 3. Diagrammatic representation of the apparatus used for testing the influence of presence of a novel object (eye spot) on the boldness of the climbing perch (Top view).
RESULTS

No significant difference was found in the latency to initiate the exploration of chamber B by control fishes (I, II, III and IV), (Kruskal-Wallis, Test, $\chi^2 = 0.710$, N=28; P> 0.05), and most of them entered the chamber B with in 42 seconds (median). This result shows that slight alterations in the apparatus did not cause any marked influence on the boldness of the climbing perch. The present study indicates that the climbing perch exhibited variation in its boldness, when tested in presence of different fear evoking stimuli (Kruskal-Wallis Test, $\chi^2 = 612890$, N=28: P<0.001; Figure 4). Post-hoc analysis of the data using ‘Steel test’ (statistical programme KyPlot) has shown that the presence of the predator (both aquatic and aerial), and odour of the aquatic predator failed to affect the boldness of the climbing perch. Additionally, the skin extract of the conspecific alone or in presence of a potent aquatic predator, were found to have no influence on the boldness. However, the presence of a black shining spot on the centre of a yellowish brown screen (‘eye spot’) significantly reduced the boldness in the fish (Table 1).
<table>
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<th>Experiment</th>
<th>t1j</th>
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<tr>
<td>1 Control X aerial predator</td>
<td>0.1516</td>
<td></td>
<td>&gt;0.05</td>
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<tr>
<td>2 Control X aquatic predator</td>
<td>−1.0329</td>
<td></td>
<td>&gt;0.05</td>
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<tr>
<td>3 Control X predator alarm + Aquatic predator</td>
<td>0.29513</td>
<td>28</td>
<td>&gt;0.05</td>
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<td>4 Control X yellowish brown screen without black spot</td>
<td>0.6480</td>
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<tr>
<td>5 Control X black acrylic sheet</td>
<td>0.6221</td>
<td></td>
<td>&gt;0.05</td>
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<tr>
<td>5 Control X yellowish brown screen with a black spot</td>
<td>6.1464</td>
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Table 1. Post–hoc (Steel test) analysis of the influence of the presence of different fear evoking stimuli on boldness of climbing perch.
Figure 4. Influence of the presence of different fear evoking stimuli on the boldness of climbing perch (data is represented as median quartile); N=28 for each treatment.
DISCUSSION

It is well established that the presence of predator has a profound influence on the performance of normal behavioural repertoire of the prey fish species (Kelly and Margurran, 2003). In presence of a potent predator, most of the prey species will adopt antipredator strategies like taking refuge or moving away from the place of danger (Dowling and Goding, 2002, Krause et al. 1998). Sometimes, the cues announcing the presence of the predator, like the odour, can do the job even in the absence of a live predator (Kelly and Magurran, 2003). For instance, when tested to measure boldness in presence of predator odour, two spotted gobies (Gobisculus flavescence) avoided the areas with the odour of the predator cod (Godus morhua, Utne-Palm, 2001). However, neither the predator odour nor the presence of predator (no matter whether the predator is aerial or aquatic) was able to influence the climbing perch, while taking a bold decision. The focal climbing perch emerged out of the start chamber in presence as well as in absence of predator/predator odour taking almost the same latency.

The climbing perch did not show any variation in boldness in presence of the skin extract of the conspecifics, which may contain probable alarm pheromones. Even, simultaneous presentation of skin extract with an aquatic predator also failed to initiate fear response in the fish. In contrary to the expected variation in the latency to take a risky decision in the shadow of predator attack, climbing perch never paid any attention to the predator even in presence of conspecifics’ skin extract.

All these results converge to the point that climbing perch is a bold fish and the presence of the predator or predator cues have no influence on the tendency of this fish to take risky decisions. The consistency of boldness shown by the climbing perch in
situations announcing emergency may be due to the genetically determined and species specific decision making ability. This argument is supported by the findings of Yoshida et al. (2005) which demonstrated that the boldness is a species specific characteristic and certain species are bolder compared to other species.

The climbing perch never stopped its exploration just after the entry in to the chamber B; instead, it extended its searching even to the area near the partition wall separating the aquatic predator. Hence, it could be assumed that the climbing perch may have a natural tendency to inspect predators, due to which it neglects the predator or predator cues during the initiation of exploration of chamber B. Kelly and Magurran (2003) reported that on sensing the presence of the predator, many prey fish species increase their search activity to locate it. At the same time, some individuals will even try for the close assessment of the predator to get an overview of its motivational status. Though, a highly adventurous job, the predator inspection behaviour provides the benefits like visual alarm signalling (Murphy and Pitcher, 1987), predator deterrence (Dugatkin and Godin, 1992 a; Godin and Davis, 1995) and bolder males enjoy the preference of females during mate choice situations (Godin and Dugatkin, 1996). Hence, it is possible that climbing perch is a fish showing predator inspection than predator avoidance, as it exhibits exploration of a novel area even in presence of predator or cues associated with the predator.

The introduction of alarm pheromones in presence or absence of the predator is said to have an enhancing effect on the exploratory behaviour of some fish species (Brown, 2003; Kelly and Magurran, 2003). The reason behind this increased interest of the fish to take a risky decision in presence of the predator alarm cues is the reduction of
uncertainty of local risk gained, when the position of the predator is located (Kelly and Magurran, 2003). However, in climbing perch there was no variation in the latency to take a bold decision of exploring a novel area, either in presence of conspecifics’ skin extract or when the skin extract was presented in combination with the aquatic predator. Hence, these results could be interpreted as, this species possesses an enhanced level of boldness, that it neglects alarm pheromone even in the presence of a predator or the skin cells of the climbing perch does not harbour any alarm substance to initiate fear response.

The consistency of climbing perch in taking a risky decision in presence of biologically significant fear evoking predator stimuli vanished totally, when an eyespot was presented in the novel area. None of the climbing perch tested were bold enough to explore the chamber B, in presence of the eyespot. Therefore, it is assumed that the black spot may resemble the eye of some large organism or it may be acting as a supernormal stimulus for the eye of a predator. This argument is supported the study of Karplus et al. (1982), which demonstrated that the shape and size of the eye of aquatic predator have a functional role in the recognition of the predator, when recognition is dependent only upon the frontal view of the approaching predator fish.

According to Rowland (1995), the fishes recognize and respond to two-dimensional and three-dimensional images. However, Bovet and Vauclair (2000) argue that in animals, reaction to pictures is more likely to express confusion between the objects and the picture than discrimination and active correspondence between the two. As far as the exploratory behaviour is concerned, uncertainty of an object present in a novel environment can significantly reduce the exploratory urge of an organism (Berlyne,
1960; Keller, 1987). Thus the climbing perch might have avoided taking a risky decision in presence of the black spot, due to the confusion in the fish evoked by the novel object (Bovet and Vauclair, 2000). The analysis of the motivational basis of boldness using climbing perch as a model system may provide more insight to the risk management strategies of fishes.
Chapter III

Environmental correlates of risky decisions in climbing perch

INTRODUCTION

All animals, including fishes, take adaptive decisions to maximize net energy intake, while simultaneously minimizing the risk of predation (Dill, 1987). However, the nature of the environment (both physical and biological) in which the fish lives, can influence the outcome of any decision (Brown and Braithwaite, 2004a; Brown et al. 2007). According Urban (2007), the criterion satisfied while taking a risky decision in one habitat will not provide the same benefit in another habitat with different types of selection pressures. For example, in ecosystems where prey fishes lead an allopatric life with predators, the individuals may hesitate to take risky decisions. While, predator sympatric fishes are more prone to take the risk of exploration of a novel area, because the encounter with a predator is not a rare event in their habitat (Brown and Braithwaite, 2004a). In predator sympatric population the fish can reduce the cost of predator vigilance, if it grows beyond a size, even by taking risky decision to feed in front of a predator (Urban, 2007).

According to Silverman (1978) animals love to explore a novel area because the exploration itself is rewarding. Moreover, the information primacy theory (IPT; Inglis et al. 2001) postulates that the animals always patrol their habitat and nearby areas to gather information continuously to deal with the environmental variability. The extension of
activities to a new area or the areas with significant difference in properties is correlated with the latent learning, where learning occurs on non-reinforced trials, but remains unused until the reintroduction of a reinforcer provides an incentive for using it (Liberman, 1990). When competition in a safe habitat increases or the benefits from safe habitat decrease, the fish can utilize the knowledge obtained during adventurous exploration and can shift to new areas with more benefit. For example, gold fish is seen to switch patches, when profitability changes (Warburton, 1990).

Like almost all other animals, fishes also extend their activities to the novel areas surrounding their home range (Gerlai and Crusio, 1994). Sometimes, the explorer may perish in predator attack with in a short duration after the entry in to the novel habitat. Hence, Abraham and Dill (1989) predicted that the fish will explore unknown and risky habitat only if it is obligatory. This hypothesis is supported by the observation of Brown et al. (2005) which shows that Brachyraphis episcopi prefers to spend more time in areas providing more refuge value, even in its familiar habitat. Additionally, Chapman and Mackay (1984) have demonstrated that the small pikes were not at all interested to leave areas with vegetation cover and spend very little time in open water area of their home range to avoid the risk of aggression from other individuals.

In social fish species, the propensity to take an adventurous decision is not only influenced by the microhabitat conditions but also by the presence of conspecifics (Milinski, 1990a; b). For example, many fish species perform risky jobs like inspecting the motivational status of the predator in pairs to reduce the risk. Sundström et al. (2004) point out that the bold individuals are followed by some shy ones to get the benefits of the risk taken by the bold individuals, which may escalate fight and affect the decision to
extent the exploration to a new area. Additionally, Jain and Sahai (1989) have demonstrated that several cognitive abilities of the social fish species are affected, when isolated from the group. This chapter describes the influence of the habitat quality and presence of a conspecific on the boldness of climbing perch.

MATERIALS AND METHODS

All fishes were tested in apparatus mentioned in the Chapter 1, after making necessary modification, and boldness was quantified following the methodology described in Chapter II. The effect of variation in the habitat quality on the boldness was studied by altering physical or biological properties of the start chamber (chamber A). In all experiments the focal fish was introduced in the chamber A, and the time taken for the initiation of exploration of chamber B was recorded.

Control

In this experiment boldness of the focal fish was measured by introducing it into the chamber A, without making any modification.

Experiment I: Sandy substratum

The bottom of chamber A was covered with washed fine river sand (thickness: 2 cm).
**Experiment II: Cobble substratum**

The floor of chamber A was covered with a single layer of rock pieces (Cobbles; size 90 ± 20 mm., Friedman and Sanders, 1978.)

**Experiment III: Vegetation cover**

The water surface of chamber A was covered with the aquatic plant hydrilla (water thyme; Hydrilla verticillata). Here, the bottom of the aquarium (without sand) served as the substratum.

**Experiment IV: Vegetation cover and sandy substratum**

The substratum of chamber A was covered with sand as described in experiment I and a vegetation cover was provided by spreading Hydrilla on the water surface.

**Experiment V: Vegetation cover and cobble substratum**

Here the rock pieces were spread over the bottom of chamber A and Hydrilla cover was provided on the water surface.

**Experiment VI: Presence of a conspecific**

In order to assess the influence of the presence of a conspecific on the boldness of climbing perch, chamber A of the apparatus was divided into two sub chambers using a transparent Plexiglas (A and A*; Fig. 1). The partition between A and B was replaced with opaque acrylic sheet. In this arrangement the test fish can see the conspecific only
when it remains inside chamber A. A conspecific from the home tank of the test fish was introduced in the Chamber A*, ten minutes before the experiment. The test fish was introduced in the chamber A and the boldness was recorded. A control (II) was also carried out using another set of fish in the absence of conspecific, to rule out the influence of the alteration in the spatial properties of chamber A of the apparatus.

**Figure** 1. Diagrammatic representation of the apparatus used for testing the influence of the presence conspecific on the boldness of climbing perch.
RESULTS

The results show that alteration in the quality of habitat or the presence of conspecific can influence the boldness of climbing perch (Kruskal- Wallis test, $\chi^2 =27.462; N=28; P<0.001$, Fig. 2). The control fishes reached chamber B with in 42 seconds (median). No significant difference was found in the latency of the initiation of exploration by two control groups (CI and CII; Mann Whitney ‘U’ test, $U=362, N=28; P>0.05$). This result indicates that the reduction in the space of chamber A, due to the partition does not influence the ability of this fish to take risky decisions. Post-hoc analysis using Steel test (statistical programme KyPlot) shows that sandy substratum, vegetation cover (Steel test, $t_{ij}=0.492, N=28; P>0.05$), and the combination of sandy substratum with vegetation cover failed to influence the boldness of the climbing perch. Meanwhile, a substratum composed of rock pieces (Steel test, $t_{ij}=-3.485, N=28; P<0.01$) or the presence of a conspecific (Steel test, $t_{ij}=-3.195, N=28, P<0.01$) considerably reduced the boldness of climbing perch. When vegetation cover was given in combination with rocky substratum, the fish retained its normal boldness (Steel test, $t_{ij}=-2.006, N=28; P>0.05$).
Figure 2. The influence of different environmental conditions and presence of a conspecific on the exploratory behaviour of the climbing perch. Data is represented as median and quartile.
DISCUSSION

The results of the present study demonstrate that the chance of taking a risky decision by a fish is significantly influenced by the properties of the habitat in which the decision is taken. The climbing perch took more time to take a decision of leaving a microhabitat with a substratum composed of rock pieces and to explore a homogenous area without any complexity. The climbing perch restricted most of its activities in the space between the rock pieces and came out of the crevices only for the periodic gulping of the atmospheric air. By contrast, there was no variation in the latency to emerge out from the chamber A from control condition, when chamber A was provided with sandy substratum.

The latency to take a decision of extending activities from a complex microhabitat to a novel homogenous habitat may be an outcome of the variation in the antipredatory strategies utilised by this fish. Hoar and Krause (2003) states that fishes enhance their safety by either joining a shoal or by investing more energy for vigilance. Being isolated from the shoal and provided with a refuge to hide from predator the climbing perch may be utilizing latter strategy, taking refuge than searching for a conspecific or a preferred habitat.

Another hypothesis put forwarded to explain the delay in taking a bold decision in a habitat with substratum made up of rock pieces, is that climbing perch could have a natural preference for a substratum composed of cobbles. Such a natural preference for the substratum of cobbles have been demonstrated in Atlantic salmon, and Kennedy
advocate practical strategies for the stock enhancement of juvenile Atlantic salmon using stones to satisfy various microhabitat needs.

In the view of Hartzler (1983), substratum with spatial complexity can improve the growth and biomass of the fish by reducing the predation risk, competition and aggression. This hypothesis has been validated using brown trout model system and trout acquired more bio-mass in habitat provided with half-log covers (Hatzler, 1983). So the lack of complexity in the substratum is the suspected reason behind the quick decision to leave the habitat with sandy substratum. Hence, spending more time to take a risky decision to visit an open area, leaving a safe and more beneficial microhabitat with substratum composed of rock pieces, is quite tenable.

According to Killgore et al. (1989), the weed cover and bank side vegetation impart determinant effect on the lives of many fish species. The weed cover provides refuge for the prey species and many small sized species restrict their activities under the cover vegetation and always avoid the open areas of the water body to reduce the predation, inter and intra-specific aggression (Durocher, et al. 1984; Chapman and Mackay, 1984; Grimm, 1981 a;b). Though, the substratum composed of cobbles increased latency of taking a risky decision, the vegetation cover was unable to impart any influence on the boldness of the climbing perch. Moreover, the reduction of boldness induced by the substratum composed of cobbles was abolished and the climbing perch left that area, when a vegetation cover was given in combination.

The result can be explained by the following hypothesis: the presence of a vegetation cover increases the refuge value of chamber A (Brown et al. 2005) and climbing perch can boldly extend its activities to an open area, because it can quickly
retreat to the safety of vegetation cover, in case an aerial attack. Hence, it could be assumed that presence of a vegetation cover near the open area might be improving the boldness of climbing perch that it moves from beneficial microhabitat with substratum composed of rock pieces to risky open area.

In presence of a conspecific, the climbing perch reduced its boldness and spent more time in the shelter chamber. Here, instead of exploring a novel area, the fish always tried to join the conspecific present in the nearby chamber. The reduction in the tendency to explore a novel area in presence of conspecific may be the result of shoal living nature of this species. Many studies show that shoaling fish give more importance to the conspecific (Hoare and Krause, 2003). Climbing perch may also be giving more importance to stay with conspecifics than obtaining benefits from exploring a novel area.

It could be concluded that in climbing perch, the nature of microhabitat in which the decision is taken as well as the presence of a conspecific can influence the boldness. This result is supported by the finding of Coleman and Wilson (1994), which states that the propensity to take a risky decision is a context specific behaviour and the fish may exhibit variation in the boldness according to the characteristics of microhabitat in which the risk is taken. In addition to the habitat quality, the sociobiologically important factor conspecific is also found to be influencing the boldness of the climbing perch. Hence, the studies dealing with the genetical and environmental correlates of propensity to take a risky decision can throw more light on the biological basis of the boldness and its influence on decision making ability of fishes.
Chapter IV

Development of boldness

INTRODUCTION

Environmental conditions experienced during the early phases of life are crucial for the normal development of vital behaviour patterns of an animal (Futyama, 1998; Salonen, 2005). This is also true in the case of the trait boldness-shyness continuum. The determinant effect of early experiences on boldness of a fish is demonstrated by Tully and Huntingford (1987) using sticklebacks. These authors demonstrated that the boldness of this fish is affected by the level of chasing by fathers during early life. A recent study using the species *Brachyraphis episcopi* also supports this finding (Brown et al. 2007). Here, the first generation larvae reared in a homogenous laboratory condition, but experienced repeated chasing using a net, exhibited increased level of boldness. Additionally, Salonen (2005) points that the fish larvae developed in the hatchery conditions, with reduced environmental complexity, exhibited variation in boldness compared to their conspecifics living in the natural water bodies.

According Braithwaite and Salvaness (2005) safety and homogeneity provided by the hatchery conditions are the basic causative factor for the abnormal development of boldness in hatchery reared fish. Thus, the lack of variation in the stimuli to elicit diversity in the behavioural profile and the reduced need of behavioural flexibility in the due course of early life in a homogenous condition could create an adult fish with reduced cognitive ability and malformed behavioural and neural plasticity (Hunter et al.}
The major ecological parameters that have most often suggested to affect the development of risk taking ability of a fish are high rearing density (Cabellero and Castro-Hdez, 2003; Sundström et al. 2004), feeding in excess at a predictable place (Grand and Guha, 1993), and the lack of presence of predators (Braithwaite and Salvaness, 2005).

There are studies showing that simple exposure to varying spatial and foraging cues during the early days of life can help the fish larvae and check the malformed development of boldness. For example, cod (Godus morhua) larvae developed in a plain hatchery tank were with very poor boldness, while larvae experienced spatial heterogeneity either on its own or in combination with varying food availability in the hatchery tanks were quick in taking risky decisions like leaving the enclosed start box to explore a novel area (Braithwaite and Salvanes, 2005).

In natural water bodies, juvenile fishes will have to face many situations where bold decisions are to be taken (Brown et al. 2007). They have to learn new routes to the feeding grounds and shelters for escaping from predators (Sundström et al. 2004). Young inexperienced fishes develop a menu from the food materials available in its home water by exploring novel objects. Many of these explorations are carried out under the threat of predation (Magnhagen, 2006). According to Brown (2003) many antipredator tactics are learned either from a real situation or from conspecifics through social learning.

If the larvae develop in a homogenous condition, the chances to get experience with different environmental factors are scanty and the conspecifics are also ignorant of predators and novel food items, the chance of any sort of social learning is denied. For
example, when the food is assured and provided *ad lib.* at a particular place during younger days of development, the fishes will not be able to develop a menu (due to the poorly developed exploratory behaviour) and will consume even non palatable materials resembling food materials present in its vicinity (Brown and Day, 2002). Additionally, these inexperienced fishes will exhibit poorly developed antipredatory tactics and will take lethal decisions like exploring a hungry predator due to the underdeveloped predator inspection ability. Moreover, these fishes will not be able withstand a real predator attack, because, to escape from a predator requires more than just an ability to recognize the threat (Braithwaite and Salvanes, 2005). Hence, fish bred in such undiversified habitat for several generations will become bolder due to the relaxed selection pressure on antipredator behaviour (Fleming and Einum, 1997).

According to Salonen and Peuhkuri (2004), the alteration of boldness does not require the selection for many generations in a less complex habitat. In a study using European graylings (*Thymallus thymallus*) these authors have demonstrated that only one generation in a homogenous habitat can increase the time required for taking a risky decision by a species. However, European greylings originated from the same hatchery stock, in two different years and undergone the same course of experience in a habitat without any complexity, showed variation in boldness (Salonen, 2005). The present chapter gives an account of the development of boldness in two groups of climbing perch kept in homogenous condition for two different durations. Additionally, the boldness of these two groups of fishes was compared with the conspecifics of similar age collected from the natural water bodies.
MATERIALS AND METHODS

Collection and maintenance of breeders

Breeders were collected from ponds of Irinjalakuda, Thrissur District, Kerala State, India and maintained in circular cement tanks, outside the laboratory. Each tank was with a dimension of 3 m diameter and a depth of 2 m. Jumping out of fishes was prevented by covering the mouth of the tanks with iron grids. A shade was provided using cotton cloth to reduce the excessive sunlight and temperature fluctuation in the water column. Water level was maintained at the height of 1.7 m. Fifty fishes were stocked in each tank without segregating the sex. Artificial food pellets (Higashimaru, Japan) was given ad lib. twice daily (morning and evening). The water was changed once in a month. All fishes were stocked during the period of April–May.

Induced breeding

In order to acclimatize the fishes with the laboratory conditions, healthy females (gravid) and males were selected and transferred to glass aquarium (80 x 40 x 60 cm) seven days before subjecting them for induced breeding. In this species, males are smaller in size compared to the females and the former had a slender body form in comparison to the latter. The gravid females of size 7 ± 2 (standard length S.L. ± S.E.) and the males having the size 4 ± 0.5 cm (S.L ± S.E.) were sorted for breeding. The sexual maturity of the males was confirmed by stripping and only the males with the presence of milt were used. A pair of males and a female was kept in an aquarium (50 x 40 x 30 cm) covered with a steel grid, one day before the administration of artificial hormone. The water level was maintained at a level of 40 cm. The fishes were induced
to breed only after the onset of monsoon (after June 15), a season when most of the fish species inhabiting the freshwaters of Kerala breed.

The males and females were administered with artificial Gonadotropine releasing hormone (GnRH; OVAPRIM; Syndal Lab, Vancouer, Canada) on the caudal peduncle. The dosage of this intramuscular injection was 0.5 ml/kg body weight of the fish. The injected breeders were transferred back to their home aquaria and simulated rain was given using a sprinkler. The aquarium was covered using the steel grid and fishes were kept in an ambience without much disturbance. The hormone injection was given in the evening (after 5.00 p.m).

**Collection of eggs**

The spawned male and female were removed from the breeding tank on the next morning of hormone injection. Eggs were collected from breeding aquarium by siphoning water to a net using a rubber hose. The unfertilized eggs (milky white in colour) were removed. The healthy eggs (transparent in nature) were separated from the debris and transferred 15 eggs each to 500 ml beakers filled with filtered pond water. The filtering of the pond water was carried out to remove the zooplankton which may destroy the eggs or hatched out larvae. All beakers were covered with fine mosquito net to avoid the consumption of the larvae by aquatic insects. Water level was maintained at a height of 8cm. Up to 48 hours after the fertilization, 98 ± 3% of eggs were hatched out in to the larvae. The dead eggs, chorion, and the larvae with morphological deformities were removed.


**Maintenance of larvae**

The number of larvae was maintained to 15 in all beakers by adding larvae (in case of death) from other beakers. However, no larvae were added or removed after fifth day of hatching to avoid any confounding affect on the normal development of their social cognitive abilities. The sides and bottom of the larval rearing beakers were covered using white paper to avoid the interference of any external cues and to simulate a homogenous atmosphere present in the hatchery conditions.

The larvae were fed with artemia nuplii for 14 days, after the complete absorption of yolk (4th day after hatching). From 14th day onwards artificial larval food Piscimix was given in combination with artemia nuplii. As the larvae grown in size, the menu was shifted to food pellets (Higashimaru, Japan).

One month old juveniles were transferred from the beaker to white fibre tubs (45 x 30 x 30 cm) covered with steel grids. The fibre tubs provided the larvae more space for locomtion and a homogenous environment without any spatial or structural complexity, a condition similar to the hatchery. Only the beakers containing 14 to 15 juveniles were emptied to the tubs. Food pellets were given *ad lib.* twice daily morning and evening. Water was changed with in an interval of seven days. These juvenile fishes were allowed to grow in the same tub up to the date of experiment. Only the fishes from the tubs with at least 13 members were used for the experiment.
Controls

Climbing perches were collected from nearby ponds and fishes of age 0+ and 1+ were segregated. The age of the fish was determined by counting the growth rings on the scale (Lagler et al. 1962; Tandon and Johal, 1996). Only fishes, with a size similar to that of six month old fishes grown in artificial cement tanks maintained like natural ponds, were sorted to use as control I. These fishes were with a size of 5.2 ± 1.3 cm (S.L. ± S.E.) and were olive green in colour with dark stripes on the body. They were with a prominent dark spot on the caudal peduncle.

Fishes of the age 1+ (size 7.6 ± 1cm; S.L. ± S.E.) were used as control II. These fishes were also dark olive green in colour, but the black stripes on the body and the spot on caudal peduncle were faded in nature.

Focal fishes (reared in the laboratory)

1. six month old fish

These fishes were pinkish white in colour and were very small in size (3.4 ± 0.5cm; S.L. ± S.E.; N=50) compared to the fishes of the same age, collected from the natural ponds. The stripes on the body of these fishes were not dark and clear.

2. 14 month old fish

These fishes were small in size (4.5 ± 0.5cm S.L. ± S.E; N=50) and whitish in colour compared to their conspecifics collected from the natural habitats.
Testing of boldness

Swim-way apparatus

An aquarium of size 60 x 32 x 32 cm was converted into the swim-way apparatus by dividing it into two chambers [Chamber A (20 x 32 x 32 cm) and Chamber B (40 x 32 x 32 cm)]. The partition was an opaque Plexiglas sheet with a gate (8 x 4 cm) on a corner. The chamber A was made opaque using black acrylic sheets. Three sides of the aquarium were covered using black paper to avoid any sort of external interference. Here, the small chamber (chamber A) functioned as the start chamber and chamber B was the swim-way. The ceiling of the start chamber was covered using an opaque acrylic sheet to provide the necessary shade for the fish staying in chamber A. This apparatus was illuminated by a compact fluorescent lamp (20 W) suspended on the top of the assembly. Differing from the apparatus used in other experiments, the swim-way apparatus was used in all experiments described in this chapter only, because the ambience available in the home tanks of the fishes born and brought up in the laboratory had many similarities with the apparatus used in the earlier experiment. Hence, the confounding effect of the difference in experience with the habitat on the boldness can be avoided, if swim-way apparatus is used for the quantification of boldness, as the fish collected from the field and fish reared in the laboratory were unfamiliar with such an environment.
Figure 1. Diagrammatic representation of the swim way apparatus used for testing the boldness of climbing perch.

All fishes used in this experiment were given experience of five minutes, with the experimental arena for four days consecutively. After coming out of the chamber A, the focal climbing perch was given five minutes for the exploration of chamber B. On the fifth day, individual focal fish was introduced into the chamber A and the latency to initiate the exploration of chamber B was recorded. The initiation of exploration is defined as the time taken by the fish to come out of the chamber A through the gate provided. If any fish fail to come out from chamber A after 6 minutes the trial was terminated and the fish was allocated a ceiling value of 360 seconds. 28 individual
climbing perch were tested from each group. After the experiment, all fish collected from the natural water bodies were released back into the site of collection and the lab reared fishes joined the induced the breeding programme conducted by Animal Behaviour and Wetland Research Laboratory, Christ College.

**RESULTS AND DISCUSSION**

According to Brown and Day (2002), development of fish larvae in undiversified atmosphere can lead to the malformation of decision making ability. For example, the brown trout developed in a homogenous condition were bolder in nature (Sundström *et al.* 2004), while European graylings experienced a similar condition exhibited a reduced level of boldness (Salonen, 2005), compared to their conspecifics living in a heterogeneous environment. Moreover Álvarez and Nicieza (2003) using brown trout model system have demonstrated that one generation in a homogenous habitat can generate divergence in the the ability to make risky decisions. In contrary, the first generation climbing perch born and brought up in a consistent condition for a duration of 14 months failed to exhibit any significant variation in their propensity to take a risky decision compared to their counter parts collected from the natural habitat (Kruskal Wallis test; N=20; P> 0.05; Fig. 2) The laboratory reared fishes came out of the opaque start chamber quickly and started the exploration as done by the fishes collected from the wild habitat.

This, result leads to a hypothesis that, in climbing perch, boldness have a genetical background and this inherited boldness may not vary up to one generation even in a homogenous condition. A similar result was obtained by Brown *et al.* (2007) in a
poecilid fish *Brachyraphis episcopi*, where, the first generation fishes developed in a homogenous atmosphere took a risky decision of entering a novel habitat from a shelter chamber with a latency equivalent to the wild fish. The genetical background of boldness is also proven by Iguchi *et al.* (2001) using two strains of cloned red-spotted cherry salmon, *Oncorhynchus masou macrostomus*, and by Vilhunen *et al.* (2008) using brown trout model system.

Though, there are evidence supporting genetic control of boldness, Braithwaite and Salvanes (2005) argue that living for a long time in a homogenous condition can affect the development of boldness in a fish. Their study using cod larvae shows that exposure for 14 weeks in a homogenous condition increased the tendency of the larvae to initiate exploration of a novel area. The elevated levels of boldness reduced subsequently as the time spend in the homogenous condition increased. However, living for six months in a homogenous condition in the laboratory did not affect the boldness of climbing perch. This fish exhibited a similar propensity to take a risky decision as the six month old juveniles from the wild habitat. Additionally, the comparison of the latency to take a risky decision by the climbing perch of age six months and fourteen months also failed to show any significant difference. This result suggests that genetic factors have a dominant role over environmental factor on the development of boldness of the first generation young ones of climbing perch.

The climbing perch born and brought up in the hatchery conditions were very small in size compared to the individuals of the same age inhabiting natural water bodies (ANOVA $F_{3,199} = 23.510; P<0.001$, Fig. 2). According to Ryer and Olla (1997), the fishes developed in heterogeneous condition spend much of their energy in tasks other than
feeding, with the cost of lower growth rate. This result is supported by the study of Braithwaite and Salvanes (2005) using cod (*Godus morhua*). However, climbing perches developed in homogenous condition with food *ad lib.* exhibited a reduced growth rate. The reason behind the reduced growth rate of climbing perch developed in artificial homogenous habitat with food *ad lib.* offers a new area for future research.

The variation in the size of climbing perch developed in the lab and field conditions become significant, when compared with studies of Brown *et al.* (2005; 2007) in *Brachyraphis episcopi*. These authors have demonstrated that larger fish always spend more time in side the shelter chamber before taking a risky decision compared to the smaller fish, indicating a correlation of boldness with the body size. By contrast, in climbing perch the trait boldness was found to be independent of the body size. Here, six month old fishes reared in the laboratory condition as well as 1+ climbing perch from the wild habitat exhibited almost similar boldness, though there was a pronounced variation in their body size. This results also indicate that the application of metabolic hypothesis of boldness (stating that the higher metabolic rates of young fishes will compel them to take risky decisions quickly in order to compensate the high energy demand) is not possible in the case of climbing perch. The results of the present support the observations of Sundström *et al.* (2004) which deny any correlation between metabolic rate and boldness.

Brown *et al.* (2004) suggest that experience with predator can reduce shyness, and the individual fishes leading a predator sympatric life are more prone to take risky decisions. Contrary to the above hypothesis, Brown and Laland (2001) state that predator naive fishes from homogenous atmosphere will be more interested in taking risky
decisions due to the unawareness about the danger. However, comparison of the boldness of climbing perch reared in the laboratory (in absence of any predator cues) with members of natural population living under high levels of selection pressures, like spatial complexity and unpredictable food availability, and predators failed to show any significant variation. Hence, the scientific basis of the consistent boldness in climbing perch is to be analyzed in detail. Moreover, future studies analyzing the development of boldness in climbing perch will help us to understand the scientific basis of development of boldness in fishes.
Figure 2. Comparison of development of boldness of climbing perch reared in a homogenous habitat and collected from natural habitat.
Figure 3. Influence of the rearing conditions on the growth of climbing perch
Section summary

**Boldness: the propensity to take a risky decision**

The climbing perch was found to be a bold fish; dare to take risky decisions in most of the experimental situations. Their shyness: the latency to initiate the exploration of a novel habitat, reduced with the increase in the familiarity with the environment after repeated exposure. Moreover, these fishes will attain steady level of boldness by the fourth day of repeated exposure. Furthermore this level of boldness was almost consistent in various occasions. For example, the presence of fear evoking cues like predator (both aquatic and aerial), skin extract of the conspecific (probable source of alarm pheromone), and the combination of predator with predator alarm cues failed to affect the boldness of this fish. In contrast, the presence of a novel object simulating an eyespot was found to be potent to inhibit the fish from taking a risky decision of exploring a novel area.

The nature of the habitat in which the fish makes a decision also can affect its boldness. The climbing perch retained their natural boldness in habitats with covering vegetation and sandy substratum. The combination of sandy substratum with vegetation cover also failed to affect the boldness of the fish. However, in a habitat with substratum made of cobbles, this fish preferred to stay inside the crevices present between the cobbles and avoided taking any risky decisions. This reduced boldness in a habitat with a substratum composed of cobbles retained its normal value, when a vegetation cover was given in combination with it.

The climbing perch born and brought up in the homogenous habitats for one year exhibited similar degree of boldness shown by the fishes of same age collected from the
wild habitats. These results point to the assumption that, in this species boldness is a genetically determined trait.