KIN RECOGNITION AND SHOALING PREFERENCES IN PUNTIUS SARANA SUBNASUTUS (VALENCIENNES, 1842)

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Thesis submitted in partial fulfillment of the requirements for
the Degree of Doctor of Philosophy in Zoology
under the faculty of science of the
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Under the supervision of
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DECLARATION

I, Jilna Alex N, hereby declare that the work embodied in the thesis **Kin recognition and shoaling preferences in *Puntius sarana subnasutus* (Valenciennes, 1842)** submitted to the University of Calicut in partial fulfillment of the requirements for the award of the degree of Doctor of Philosophy in Zoology is a bonafide record of the work carried out by me under the supervision of Dr. John Thomas K., Associate Professor (Rtd.) Department of Zoology, Christ College, in Research and Post graduate Department of Zoology, Christ College, Irinjalakuda, University of calicut and no part of the thesis has formed the basis for the award of any degree, diploma or other similar titles of any university.

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This is to certify that Ms. Jilna Alex N. has completed the research work for the full period prescribed under the Ph.D. ordinance of the University of Calicut. This thesis **Kin recognition and shoaling preferences in *Puntius sarana subnasutus* (Valenciennes, 1842)** embodies the results of her investigations conducted during the period at which she worked as a research scholar. I recommend the thesis to be submitted for evaluation for the award of the degree of Doctor of Philosophy in Zoology of the University of Calicut.

Fr. JOSE THEKKAN C.M.I.
Principal

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Dedicated to………..

MY PARENTS

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ABSTRACT

Fishes are social organisms that form loose aggregations called shoals. Fish shoals have become a model system for investigating the functions and evolution of group living. Fish have shown to take a wide range of factors into account when chosing their shoal associates. Even though, many works had been already carried out in this field, still there are several unexplored areas which need more clarification. Very few studies, especially in the Indian scenario, have examined aggregating behavior of a freshwater fish species with potential for using in aquaculture. Most of the studies conducted in this field were mainly focused on a single factor that influences shoaling. In nature the fish has to make a shoaling decision by judging a number of conflicting factors. The present study considered the influence of genetic relatedness, familiarity and shoal-size in determining the shoaling preferences of *Puntius sarana subnasutus*.

Puntius sarana subnasutus (Barbodes sarana), which is a locally available fish and well known for its grouping behaviour, is the model fish in this study. As the prelude of the study, the onset of shoaling tendency of *Puntius sarana subnasutus* was confirmed by simultaneously exposing induced - bred offspring to stimulus groups of ten individuals and a single individual fish in a binary choice experiment. The ability to discriminate which shoal is larger is important for fish, since they could join the larger or the smaller shoal depending on the context. The juvenile *Puntius sarana subnasutus* successfully discriminated the sibling shoals (offspring of the same breeding pair) present in different numerical ratios and spent significantly more time with the larger shoal. In nature, the composition of fish shoals is typically nonrandom and the shoaling decisions appear to be based on the relative value of costs and benefits associated with group membership. The attractiveness of a group to potential members may also depend on a number of phenotypic characteristics such as body size, colour, species as well as genotypic characters and familiarity etc. In the present study the test fish traded off their preference to associate with a larger group when the alternate group is genetically more related, and with similar body-size.

Shoaling preferences are likely to be context-dependent. Appropriate decisions should be taken by a fish in situations where the net benefits of one could be greater than the other. For this, individuals need to be able to differentiate the various significant ‘recognition cues’. The process of recognition of these cues may be learned through experience or can be

genetically encoded. Thus in shoaling fishes, the social environment plays a substantial role in the development and expression of behaviour in individuals because of their frequent contact with other group members. When the social environment of larvae is altered from the next day of hatching onwards by rearing them in isolation or with other individuals of siblings; conspecifics; heterospecific *Puntius parrah* or a genetically more distantly related *Anabas testudineus*, the test fish preferred to associate with larger group of familiar species with which they had been reared. The results suggest that individuals learn their species identity from social environment and that this affects their social preference. *Puntius sarana subnasutus,* a fish which exhibits strong preference to associate with the larger group exhibited a severly disrupted recognition patterns when reared in total isolation. The fish was found to be more stressed with increase in the duration of isolation.

Studies are also carried out to point out the differential role of olfactory and visual senses in shoal recognition patterns of the test fish. Here, in the case of *Puntius sarana subnasutus,* it is demonstrated that the fish depend more on their visual sensory perception for discriminating the group-size. The test fish clearly recognised the group size difference between the stimulus shoals of their siblings in various numerical combinations, even when visual cues alone are available. When the test fish was exposed o unfamliar (previously not encountered) conspecifics and heterospecific stimulus shoals, the fish successfully identified their siblings and spent significantly more tme with them based only on visual cues. When the test fish was reared with heterospcifics, it exhibited a significant association with the stimulus shoal composed of siblings only when the alternate group is consisted of genetically more unrelated *Anabas testudineus*. The fish in the presence of olfactory cues alone successfully discriminated their siblings as well as the group size difference between sibling stimulus shoals but failed to differentiate the group size difference between siblings and conspecifics/heterospecifics based on odour concentration. Early exposure during the time of development to heterospecifics also did not cause any change in this behaviour. Further, the study analysed whether aquatic pollution adversely affects this sensory perception mechanisms and in turn influence the hoaling preference of the fish. Increase in turbidity, variation in water temperature and presence surfactants in the water bodies are found to be disrupting the group-size recognition abilities of *Puntius sarana subnasutus*.

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Chapter - 1

General Introduction

SHOALING

Animals often organize themselves into social groups or into territorial assemblages. How social aggregations arise and persists is central to our understanding of evolution, behaviour and animal psychology. Evolutionary divergence and speciation may occur, when social groups arise within a species. Social aggregations are particularly a common phenomenon among fishes and represent an ideal model for studying the mechanisms behind social grouping. Any collection of fish that have gathered together in some locality is called an aggregation. These aggregations may be structured or unstructured. Fish aggregations that are formed in an interactive, social way are termed shoals while a more tightly structured aggregation characterized by a high degree of polarity and synchronized swimming behavior are known as schools (Miller and Gerlai, 2012).

The ultimate mechanisms for shoaling have been extensively studied, with the primary benefits being protection from predators and enhanced foraging ability. Protection from predators can result from a number of different mechanisms, such as dilution of risk, the confusion effect (Landeau and Terborgh, 1986; Pitcher and Parrish, 1993), early detection of predators (Godin et al., 1988) and coordinated evasive manoeuvres (Magurran and Pitcher, 1987). Shoaling can also enhance foraging ability through the transfer of information if food items are patchily distributed (Pitcher et al., 1982; Ranta and Juvonen, 1993). The proximate mechanisms that govern internal shoal dynamics and maintain shoal cohesion are less well studied. Why some social systems form groups composed of kin, while others do not, has gone largely untreated in the literature. The conditions leading to group living and cooperation can be derived as a function of an intrinsic rate of growth, group carrying capacity, and cooperation parameters. One of the critical evolutionary decisions in the origin of a social system is whether or not to form groups with kin. Understanding the factors involved in this decision is key to understanding the diversity of kinship structures represented across social taxa, where at least two factors must play a role ie. their group size and degree of altruism, both are shaped by the particular ecology of a species. Conditions for evolution of altruism is then determined in terms of cost and benefit. For example, the stability of group composition through time can have consequences for the evolution of reciprocal altruism. In any given population there are stable, distinct, and co-existing cooperative strategies that predict reciprocal altruism.

It has been estimated that more than 25% of the approximately 33,112 species of teleosts form social groups throughout their lives and over 50% school as juveniles (Shaw, 1978; Eschmeyer and Fong, 2014). It is not uncommon for the young of a particular species to be social, while adults are solitary (Eg: the jewel fish, *Hemichromis bimacularis)*. Further, shoaling may be facultative or obligate; obligate shoalers, such as tunas (*Thunnus sp.*), herrings (*Clupea sp.*) and anchovy (*Engraulidae sp.*), spend all of their time in shoaling or schooling, and become agitated if separated from the group. Facultative shoalers, such as Atlantic cod (*Gadus morhua)*, Sergeant-major (*Abudefduf saxatilis*) and some carangids [jacks (*Caranx sp.*), pompanos (*Trachinotus sp.*), runners (*Caranx crysos*), Bigeye scad (*Selar crumenophthalmus*) etc.] shoal only during specific period, perhaps for reproductive purposes (Pavlov and Kasumyan, 2000). Many species of large predatory fish also school, including many highly migratory fish, such as tuna and some ocean going sharks.

Furthermore, understanding the dynamics of shoaling is important in fisheries management (Mackinson, 2000), as shoaling fish are among the most heavily exploited species in the world’s oceans and may be the key to avoiding over-exploitation and the conservation of fish stocks. Commercial fisheries have exploited the susceptibilities of shoaling fish through various fishing techniques. In many cases, this exploitation has not been sustainable and has resulted in stock collapses (Mackinson et al., 1997).

The morphological assortment of fish shoals is well documented through quantifying the composition of free ranging shoals. However, substantially less is known to what extent fish shoals represent stable entities through time. An increasing number of field investigations into the interactions of social fish species report non-random association patterns between individuals (Krause et al., 2000). Local fish populations may be considered as a network, whereby individuals are connected via associations between one other, with the strength of the association between any two individuals in the net work depending on the frequency of their co-occurrence over time. Studies on both fish and dolphin suggest that wild animal populations are characterized by a non-random social network structure. The application of the network theory provides insights into many different activities within an animal population including co-operative behaviour, mate choice and the social transmission of information and diseases (Abramson and Kuperman, 2001). In particular, networks appear to have a relatively rapid transfer of information whilst also being highly structured.

Shoaling fish can shift into a disciplined and coordinated school, then shift back to an amorphous shoal within seconds. Such shifts are triggered by changes of activity from feeding, resting, travelling or avoiding predators. When schooling fish stop to feed, they break ranks and become shoals. Shoals are more vulnerable to predator attack. The shape of a shoal or school takes depends on the type of fish and what the fish are doing. Schools that are travelling can form long thin lines, or squares or ovals or amoeboid shapes. Fast moving schools usually form a wedge shape, while shoals that are feeding tend to become circular. It is difficult to observe and describe the three dimensional structure of real world fish shoals because of the large number of fish involved. Techniques include the use of recent advances in fisheries acoustics. The researchers imaged spawning Atlantic herring (*Clupea harengus*) off Georges Bank (Makris et al., 2009). They found that the fish come together from deeper water in the evening, shoaling in a disordered way. A chain reaction triggers when the population density reaches a critical value, like an audience wave travelling around a sport stadium. A rapid transition then occurs, and the fish become highly polarized and synchronized in the manner of schooling fish. After the transition, the schools start migrating, extending up to 40 kilometres across the ocean, to shallow parts of the bank. There they spawn during the night. In the morning, the fish school goes back to deeper water again and then disbands. Small groups of leaders were also discovered that significantly influenced much larger groups.

PARAMETERS DEFINING A FISH SHOAL INCLUDE :

Shoal size - The number of fish in the shoal.

Density - The density of a fish shoal is the number of fish divided by the volume occupied by the shoal. Density is not necessarily a constant throughout the shoal where as the fish in schools typically have densities of about one fish per cube of body length.

Polarity - The shoal polarity describes the extent to which the fish are all pointing in the same direction. In order to determine this parameter, the average orientation of all animals in the group is determined. For each animal, the angular difference between its orientation and the group orientation is then found. The group polarity is the average of these differences (Viscido et al., 2004).

Nearest neighbour distance - The nearest neighbour distance (NND) describes the distance between the centroid of one fish (the focal fish) and the centroid of the fish nearest to the focal fish. This parameter can be found for each fish in an aggregation and then averaged. Care must be taken to account for the fish located at the edge of a fish aggregation, since these fish have no neighbour in one direction. The NND is also related to the packing density. For schooling fish the NND is usually between one-half and one body length.

Nearest neighbour position - In a polar coordinate system, the nearest neighbour position describes the angle and distance of the nearest neighbour to a focal fish.

Packing fraction - The packing fraction is a parameter borrowed from physics to define the organization (or state i.e. solid, liquid, or gas) of 3D fish groups. It is an alternative measure to density. In this parameter, the aggregation is idealized as an ensemble of solid spheres, with each fish at the center of a sphere. The packing fraction is defined as the ratio of the total volume occupied by all individual spheres divided by the global volume of the aggregation (Cavagna et al., 2008). Values range from zero to one, where a small packing fraction represents a dilute system like a gas.

Integrated conditional density - This parameter measures the density at various length scales and therefore describes the homogeneity of density throughout an animal group.

Pair distribution function - This parameter is usually used in physics to characterize the degree of spatial order in a system of particles. It also describes the density, but this measure describes the density at a distance away from a given point. Cavagna et al. (2008) found that flocks of starlings exhibited more structure than a gas but less than a liquid.

BENEFITS AND COSTS OF SHOALING

Shoaling confers several advantages to the members, ranging from protection from predators and foraging advantages to increased swimming efficiency, whilst there are also certain associated costs. The relative trade-off between these two determines the extent to which the shoaling behaviour is exhibited by an individual. Such a balance is potentially represented by the great degree of variations in shoal sizes in the wild (Croft et al., 2003)

BENEFITS OF SHOALING

There are four main explanations to account for why fishes form schools and shoals: protection, foraging, mating and energy saving advantages (Larsson, 2012).

Anti predator advantages:

A proximal factor that control fish shoal patterns is the antipredator advantages obtained through grouping. Behavioural patterns of fish shoals are said to be shaped by an evolutionary arms race with predators (Turesson and Bronmark, 2007). One potential method by which fish shoals avoid predator is the ‘predator confusion effect’ (Welty, 1934) whereby the hunting efficacy of predators is reduced due to disorientation and the inability of the predator to target one individual fish. Another potential antipredator effect of animal aggregations is the ‘many eyes hypothesis’ (Godin et al., 1988), which states that as the size of the group increases the task of scanning the environment for predators can be spread out over many individuals, enabling them to monitor the predator continuously. A third theory for antipredator advantages of shoaling is the ‘encounter dilution effect’ (Bertram, 1978). According to this theory an individual in a group gains advantages simply through a reduced probability of being the one attacked in an encounter with a predator. This probability is the reciprocal of the group size i.e., an elaboration of the concept of being safe in numbers (Pitcher and Parrish, 1993) and interacts with the confusion effects. Further, dense shoals can be perceived as a large active object and therefore repel predators through mimicry.

Some other common logical categories that define the ways in which fish shoals may counter attacks of predator include evasion, avoidance and abatement. Evasion can occur reducing the success of an attack by moving out of the strike range of a detected predator by flash expansion of the group as a whole. Avoidance is a strategy of evading the chances to come into attack range of predator when localization of prey is dependent on the relative mobility and visual range of predator as compared to environment and prey species. Abatement describes the combined effects of avoidance and dilution (Turner and Pitcher, 1986).

***Foraging advantages* :**

It is generally accepted that fish in large shoals find food sooner than those in small shoals (Rodgers et al. 2011). Fish foraging in shoals usually gain benefits through rapid location of food patches, more efficient feeding, increased feeding intensity, information transfer and increased opportunity for copying. As group size increases, individual fish forage for longer time consequently collecting more items per patch. Shoaling allows fish to exploit more feeding patches, possibly by allowing them to trade off the antipredator benefits of shoaling and to access patches associated with higher risk. The speed with which minnows (*Phoxinus phoxinus*) and gold fish (*Carassius auratus)* find hidden food is proportional to the shoal size (Ranta and Lindstorm, 1990). Stickleback (*Gasterosteus aculeatus*) when transferred to new environments in larger groups begin feeding sooner and with greater intensity (Ranta and Kaitala, 1991). The time spent on a food patch also increases as shoal-size increases, although these results may be confounded by the heightened fearfulness in solitary fish. Furthermore, timidity (as indicated by darting and freezing behaviours) decreases and more individuals are available for vigilance (Pitcher and Magurran, 1983).

***Hydrodynamic advantages* :**

The quantity of energy required to move in a shoal is less compared to locomotion of a solitary fish. This is brought about as less energy is exerted under certain shoal configurations due to the interactions between individuals. By maintaining a diamond formation it would appear that an overall decrease in energy expenditure to maintain a given speed is required. Jack mackerel (*Trachurus symmetricus*) and pink salmon (*Oncorhynchus gorbuscha*) swam in formations which approximated the diamond shape (Pavlov and Kasumyan, 2000). There have been many reports that provide support for a reduction in the energy required to move in a shoal demonstrating that fish in the rear of a shoal beat their tail less frequently and consume less oxygen (Herskin and Steffensen, 1998; Svendsen et al., 2003). It would seem reasonable to think that the regular spacing and size uniformity of fish in schools would result in hydrodynamic efficiencies. However, experiments in the laboratory have failed to find any gains from the hydrodynamic lift created by the neighbours of a fish within a school, though it is still thought that efficiency gains do occur in the wild.

***Mate choice* :**

There is increasing evidence that social factors play a role in mate choice in a number of fishes such as mollies (*Poecilia latipinna)*, guppies (*Poecilia reticulata*), gobies (*Pomatoschistus microps*) and the Japanese rice fish *(Oryzias latipes)* – (Witte and Ryan, 1998; Grant and Green, 1996). 'Mate choice copying' is said to have occurred when the probability of an individual selecting another as a sexual partner increases because other individuals (of the same sex) have selected the same partner. In the paradigm experiment in guppies (Dugatkin, 1992), two males are secured at the ends of an aquarium, one with a demonstrator female nearby. The observer, another female, placed centrally, watches the other female interact with one of the males. When, after the demonstrator has been removed, the observer is allowed to choose between the two males, she consistently chose the male that had the female nearby. Similar observations consistent with mate choice copying have been reported in mollies, for males (Schlupp and Ryan, 1997). One interpretation of these findings is that the observing female utilizes the presence of the female near a male as an indication of his quality and biases her male choice accordingly.

However, mate choice copying is by no means the only way that socially influenced mate choice may occur. For example, in a number of species, females prefer males with a greater number of eggs present in their nest (sticklebacks: Goldschmidt et al., 1993; sand gobies: Forsgren et al., 1996). Although the observing female may not have witnessed the laying of these eggs, conceivably their presence is a cue suggestive of mating success, or prior female choice.

Migratory advantages :

Shoaling could increase the accuracy of homing on migration, since the mean direction or route taken by several individuals is likely to be more accurate estimate of the correct destination than any one individual's choice (Larkin and Walton, 1969). The first direct evidence is that adult Coho salmon home more accurately to their natal river at higher densities (Quinn and Fresh, 1984). Secondly, studies by Helfman and Schultz (1984) proved that isolated fish when placed with new conspecifics in diurnal migration routes travelled the resident's routes to the correct location. Herrings are among the more spectacular schooling fish. They aggregate together in huge numbers. The largest schools are often formed during migrations by merging with smaller schools. "Chains" of schools one hundred kilometres long have been observed of mullet migrating in the Caspian Sea. Radakov (1973) estimated herring schools in the North Atlantic can occupy up to 4.8 cubic kilometres with fish densities between 0.5 and 1.0 fish/cubic meter. That's about three billion fish in one school. These schools move along coastlines and traverse the open oceans. Herring schools in general have very precise arrangements which allow the school to maintain relatively constant cruising speeds. Herrings have excellent hearing, and their schools react very rapidly to a predator. The herrings keep a certain distance from a moving scuba diver or a cruising predator like a killer whale, forming a vacuole which looks like a doughnut from a spotter plane (Nottestad and Axelsen, 1999).

Bluehead wrasse (*Thalassoma bifasciatum)* shows similar migratory traditions. These fish have mating locations that remain constant over many generations. Warner (1990) removed entire populations and replaced them with transplanted populations. Not only did the new fish establish new mating grounds, but these new locations remained constant over the subsequent generations. In the 12 years of studying 22 patches on the reef, not once was a new mating site established or lost, despite fluctuations in the wrasse population size. Combined with the observation that reef populations are not subjected to significant genetic differentiation, this finding provides strong evidence for social learning.

***Enhanced social learning*:**

Animals can acquire adaptive information directly, through their own asocial personal experience or through copying others. First-hand sampling allows animals to gather accurate, up to date private information about their immediate environment. Doing so can be costly, however, both in terms of the time and energy that must be invested, and also through the increased likelihood of encountering predators or exposure to other risks while gathering it. In theory, these costs can be reduced through the use of social information; by observing or interacting with others an individual can sidestep the need to sample resources directly, perhaps even gathering this information from the relative safety of cover (Hoppitt and Laland, 2008).

Many of the processes that underpin social learning lend themselves particularly well to shoals of fish. Social learning is facilitated within group-living species, allowing the opportunity for accessing and utilizing public information, such as information about places, objects and behaviour (Giraldeau, 1998). Social learning has been demonstrated in shoaling fish in a number of areas, including foraging locations, foraging routes (Laland and Williams, 1997), predator recognition and migration routes (Helfman and Schultz, 1984). Under a given set of constraining factors, the rate and outcomes of group encounters could limit information flow within a population. For example, Pyanov (1993) found that after capture, fish are more wary of fishing gear and will often exchange shoals, creating the potential for the transfer of information. It is likely that naive fish in the new shoals will learn to avoid fishing gear, as experienced fish initiate the shoal level reaction to fishing equipment (Soria et al., 1993).

Brown and Laland (2003) suggest a possible explanation of patterns of social learning taking place within a shoal. They include *local enhancement*- the behavior (or simply the presence) of one individual attracting the attention of another individual to a stimulus about which the naïve individual subsequently learn something; *social facilitation*- the behavior of one individual inducing an identical behavior in another individual and the latter learns something; *guided learning*- by following or being with a knowledgeable animal an individual is exposed to similar features of the environment and comes to learn the same behavior; *observational conditioning*- the response of a demonstrator to a stimulus elicits a mating response on the part of an observer that simultaneously perceives the original stimulus and effectively learns that response; *imitation*- learning to produce particular bodily movements through observation of others. In temporally variable environments social information has the potential to become outdated or obsolete over time, while in spatially variable environments differences in local optima mean that the relative usefulness of social information will vary between sites. The use of private versus social sources of information by animals can therefore be viewed as a trade-off between accuracy and cost. Boyd and Richerson (1985) proposed a costly information hypothesis, which suggests that when information is too costly to acquire or use personally, individuals will take advantage of the relatively cheaper information provided by others. Laland (2004), suggested this hypothesis as a 'copy-when-asocial-learning is-costly' social learning strategy.

COSTS OF SHOALING:

Regardless of the diverse benefits, shoaling also imposes certain costs on the associated individual. One of the main costs of living in a shoal is the competition for food (Krause and Ruxton, 2002). Competition increases with shoal size through interference. For instance, goldfish (*Carassius auratus*) show a reduction in the time spent catching and swallowing food objects, potentially indicating increased competition between shoal members (Street et al., 1984). Hungry fish try to minimize competition by increasing their distance from shoal mates. Generally, however, fish in shoals resume normal feeding behaviour more quickly after predator attacks or the stress of a novel environment than single ones. Fish make more feeding attempts as shoal size increases as increasing group size causes resources to become limited.

Although, the tendency of individual fish under a predation threat is to form cohesive shoals, individuals or small groups of fish are known to break off from the shoal to approach and inspect predators when these are in visual contact for the purpose of determining the threat that the predator poses. This may be accomplished by either sudden darts at the predator or in random directions followed by quick returns to the shoal, or fish may make slow approaches to the predator to a distance of 4-6 body lengths and then return. Minnows and sticklebacks have been shown to exhibit such behaviour (Magurran and Pitcher, 1987). The obvious cost involved in predator inspection is the greater risk of predation to the individual. The exhibition of predator inspection can often be seen as a form of boldness, with the maintenance of visual range to a predator considered to be a form of exploratory behaviour (Pavlov and Kasumyan, 2000). Bold individuals that inspect are more likely to forego the antipredator benefits of shoaling. Predator attacks on lone individuals are more effective, due to the fact that predator hunting success is inversely proportional to the group-size attacked whilst the inspectors in groups dilute the risk of predation. Inspectors are more likely to be predated. Fish that approach predators are unable to perform other activities, and therefore incur opportunity costs. For example, minnows, (*Phoxinus phoxinus*), that frequently approach a predator forage less than those who inspect less often (Magurran, 1986).

A further potential cost of shoaling is the spread of ectoparasites and infectious diseases (Ward et al, 2005a). A parasitized fish may be negatively selected against when choosing a shoal to join as well as to allow these individuals to join an existing shoal. In the case of banded killifish *(Fundulus diaphanous)*, fish infected with a parasite (manifested as a dark spot on the body of the host fish) are selected against in such a manner, whilst those fish actually parasitized also show marked differences in behaviour, in some case decreasing antipredator responses in general, whilst in others actually showing an increased tendency to shoal, depending on the type of parasite.

Another hypothesized cost of shoaling is that it confers competition for mates which may likely increases in groups depending on specific mating strategy and the ratio of male to female. In studies on a sexually dimorphic species such as the guppy (*Poecilia reticulata)*, it was found that males are less likely than females to join shoals, and female guppies stayed in their shoals (Griffiths and Magurran, 1998) while males spent more time moving between shoals. In studies on another sexually dimorphic species, the Siamese fighting fish (*Betta splendens*), both males and females chose to shoal with larger groups of females, but females avoided males, while males did not (Snekser et al., 2006).

Shoaling decision

The decision of with whom to associate is among the most fundamental an individual can make influencing its reproductive success, foraging rate and risk of death. A fish may actively decide to leave and join another shoal/or remain single, considering all the costs and benefits obtained from shoaling.

PHENOTYPIC AND GENOTYPIC FACTORS INFLUENCING THE SELECTION OF POTENTIAL SHOAL MATES

There exits variations in the tendency to shoal, as well as disparity in shoal composition. Fishes are known to make shoal choice on the basis of various phenotypic and genotypic characteristics of shoal mates. The phenotypic matching between fish in a shoal is necessary due to the 'oddity effect' which posits that rare and phenotypically distinct individuals within a shoal are more likely to be targeted by predators. The active choice of individuals and several other passive mechanisms may drive the shoaling decisions and the preference may be based on the following factors:

Body size / length:

Selection in terms of body length appears to be one of the most important factors, as it plays a major role in the phenotypic homogeneity of the shoal.

Several studies have shown a strong association preference for size matched individuals and free-ranging fish may frequently shoal with heterospecifics, if they are well matched in size (Krause et al., 1996a & b; Krause et al., 2000). Most teleost fishes show indeterminate growth, with size strongly correlated with age in many species so that size-structured populations in most cases also represent age-structured populations.

Body size appears to be one of the most important factors affecting the organization of fish aggregations. Numerous researchers have studied size effects and have found consistently in laboratory studies that fish prefer to group with like-sized individuals (Ranta and Lindström, 1990; Hager and Helfman 1991; Hoare et al., 2000a & b). Within multi-species shoals in the wild, shoal members tend to be of approximately the same body size, and one species usually dominates numerically (Krause et al., 1996b). Krause et al. (2000) found the composition of fish shoals to be assorted significantly by body size, particularly between shoals and among species in a given shoal. When small-sized guppies (*Poecilia reticulata*) were given the choice of shoaling with conspecifics of their own size or an equal number of larger fish, they chose the smaller fish significantly often (Lachlan et al., 1998).

Body size seems to be particularly important in forming schools and shoals because of the oddity effect. Landeau and Terborgh (1986) found that predators had more successful attacks in intermediate densities (of about eight individuals) when one or two 'odd' individuals were included in a group. Moreover, they found that the predator attacks were just as successful for 'odd' fish as they were for normal fish, which may be due to the increased ability of a predator to focus on any one member of the group, rather than only atypical individuals. This effect decreased when 'odd' individuals were more equal in number to 'normal' individuals. Peuhkuri (1997) found that large sticklebacks are more vigilant when among smaller fish than when among similar-sized individuals, but did not find the same effect with smaller fish among larger individuals. Theodorakis (1989) also found that largemouth bass (*Micropterus salmoides*) consumed odd-sized fish, in mixed shoals of small and large fish, in greater proportions than would have been predicted by their relative abundance in the shoals. Moreover, large 'odd' fish were consumed just as often as small 'odd' fish. Svensson et al. (2000) found in gobies (*Gobiusculus favescens*) that large fish preferred to shoal with large fish over smaller fish shoals, though small fish had no preference for size class. However, Lachlan et al. (1998) found in guppies that small fish prefer to shoal with small fish over large fish, but large fish had no preference. This discrepancy is unexplained. However, it appears that the oddity effect plays a role for both small and large 'odd' individuals.

Body colour:

One trait that may influence the choice of a shoal mate is the dazzling array of colours and pigment patterns found in fishes. Differences in pigment patterns may, however, be associated with other morphological or behavioural differences between fish, making it difficult to isolate the specific role of colouration in shoaling preferences. Engeszer et al. (2004), using pigment variants of the zebra fish (*Danio rerio*), have shown that fish can select shoal mates on the basis of their learned preference for particular colour patterns of their group members. The fish raised in isolation showed no shoaling preference based on colour while the fish reared in groups exhibited a very strong shoaling preference for fish with the same pigment pattern as those they were reared with, regardless of their phenotypes. With this Engeszer et al. (2004) showed that the colour preference in zebra fish is learned than being innate, since the fish did not know their own colour. Additionally, male zebrafish have been shown to be more sensitive than females to visual striping cues in shoaling assays (Engeszer et al., 2008).

Group size:

Another important factor that influences the shoal choice of an individual fish is the density and group-size of the stimulus shoal, mainly because larger groups are known to provide increased protection against predators (Krause and Ruxton, 2002). If the density is too low, individuals cannot maximize their fitness in the same manner as in larger densities. However, if the density is too high there is a greater chance for competition. For example, larger groups are more effective in detecting predators ('many eyes' effect). Furthermore, risk dilution leads to a higher survival of the individual. Banded killifish (*Fundulus* *diaphanus*) scared by the predator chose the larger of the two shoals (Krause and Godin, 1994). Barber and Wright (2001), for example, showed that European minnows (*Phoxinus phoxinus*) preferred the larger of the two groups. Many studies have been unequivocally established the preference for larger groups in a number of shoaling species (*Anabas testudineus* - Binoy and Thomas, 2004; *Betta splendens* – Blakeslee, 2009; Jordan et al., 2010; *Puntius sarana subnasutus* - Alex and Thomas, 2011). Yet the fact that whether this preference is due to larger group-size or because of an increased density of the larger group is not clearly understood. In most of the experimental studies, generally an increase in group-size came along with an increase in shoal density.

Study by Frommen et al. (2009) have shown that shoaling preferences might not always be influenced by higher number of group members but also by the density and cohesiveness of the respective groups. Indeed, there are several studies in birds, non-human primates as well as in human babies showing that there are two mechanisms to discriminate among different numerosities, one for counting small numbers (less than four) precisely and the second for quantifying large numbers approximately (Feigenson et al., 2002 & 2004). These mechanisms have recently been shown to work in fish too. Mosquitofish (*Gambusia holbrooki*) was found to discriminate small quantities, even when non-numerical indicators of quantity are unavailable, thus providing the first evidence that fish, like primates, can use numbers (Agrillo et al., 2007 & 2009). Here, the test fish either used non-numerical cues like surface area, contour length or group density or counted small numbers directly for the recognition of larger group. As in humans and non-human primates, genuine counting appears to be a ‘last resort’ strategy in fish, when no other perceptual mechanism may suggest the quantity of elements.

***Satiation*:**

Another area affecting shoaling involves the nutritional state of the fish belonging to a group and the manner in which it affects the position preferences. Krause (1993) found that fish that had been deprived of food and had a choice between differing sizes of shoals chose the larger shoals but spent significantly less time around the larger shoals as food deprivation increased. Similarly in zebrafish (*Danio rerio*) the food-deprived fish preferred to shoal with well-fed fish when presented with a choice between two shoals of food-deprived fish and well-fed fish, respectively (Krause et al., 1999). Lachlan et al. (1998) found in guppies (*Poecilia reticulata*) that food-deprived fish had no preference between shoals of well-fed and food-deprived fish.

***Parasitic Load*:**

The presence of parasites can also influence the decision of fish to join a shoal, both by altering the grouping tendency of infected individuals (Barber et al., 2000) and through avoidance of parasitized individuals by other fish (Ward et al., 2005a). Combined with passive sorting mechanisms such as reduced swimming ability in infected fish and active choice may result in wild shoals assorted by both parasite prevalence (the proportion of fish infected in a shoal) and parasite load (the number of parasites infecting each individuals) (Hoare et al., 2000a).

***Sex*:**

Association preferences with respect to sex may be influenced by mating preferences and sexual selection (Snekser et al., 2010). Preferences of large individuals are universal regardless of sex and might reflect size-assortative shoaling as well as sexually selected mating partners. Differential parental investment between sexes, common in many fishes, may also act in addition to sexual size dimorphism in generating sex differences in exploratory and learning behavior. Interestingly, sex-related shoaling differences have also been noted in species in which males and females are phenotypically similar. Female rainbow fish (*Melanotaenia eachamensis*) made shoaling choices based on relatedness of individuals within the shoal, while males did not (Arnold, 2000). Similarly, female zebrafish (*Danio rerio*) preferred to shoal with unfamiliar and unrelated males, while males showed no such preference (Gerlach and Lysiak, 2006). Zebrafish males and females also differed in their shoaling decisions when the sex of the shoal members and shoal size were varied (Ruhl et al., 2009). One aspect of shoaling behavior that has been less well represented in the literature is the effect of sex. Many studies disregard sex, either by examining only one sex or by ignoring the sex of the fish altogether. Part of this omission may be a perception that the two sexes would make similar shoaling choices, especially in species in which males and females are phenotypically similar.

Kinship:

Shoaling fish are also known to make shoal selection based on relatedness of individuals within the shoal. Many species of fishes have the ability to recognize and behave differently towards the individuals with whom they have blood relation i.e., the kin (Ward and Hart, 2003). For shoals of related individuals there is a reduction in aggressive behavior. For example, in different salmonoid species the level of aggression was lower when groups were composed of kin (Brown and Brown, 1993a). In addition to the direct benefits, kinship offers some indirect fitness to an individual through the evolution of altruistic behavior. Individuals that shoal with kin can increase their inclusive fitness (Hamilton, 1964) by increasing reproductive success of relatives through co-operative behavior during risky situations such as predator inspection. Several researchers studied relatedness between shoal members of the wild, using alloenzymes (Hauser and Ward, 1998; Peuhkuri and Seppae, 1998) or micro satellite markers (Pouyaud et al., 1999). A preference to shoal with a kin (either sibling or conspecific) would imply some kind of kin recognition mechanism that allows for the differentiation of kin from non-kin. There are two main types of kin recognition, direct familiarity and indirect familiarity (phenotype matching). In the case of direct familiarity, individuals become familiar with their siblings (members of the same litter or clutch) during early ontogeny and prefer these individuals over unfamiliar ones. In phenotype matching, an individual learns about the phenotypes of close relatives or itself (self matching) during early development and later compares unfamiliar individual with this learned phenotype to identify kin (Olsen, 1992). However, if the individuals fail to distinguish kin from non-kin, shoaling with relatives can also be disadvantageous due to the risk of inbreeding.

Investigation of kin discrimination in fish has been mainly focused on salmonids (Brown and Brown, 1996, for a review). Preference for olfactory cues from siblings rather than non-siblings has been demonstrated in Arctic charr (*Salvelinus alpinus*) (Olsen, 1989), cohosalmon (*Oncorhynchus kisutch*)

(Quinn and Busack, 1985), Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) (Brown and Brown, 1992). In the above experiments test fish were reared in kin groups, and the observed results may be due to preference for familiar odours rather than due to innate recognition mechanism. Quinn and Hara (1986) showed that cohosalmon reared with siblings and non-siblings showed no discrimination between them. Furthermore, Winberg and Olsen (1992) showed that young Arctic charr reared in isolation did not show a preference for siblings over non siblings. A study by Olsen et al. (1998) however, demonstrated that association preferences of Arctic charr are at least partly based on major histocompatibility complex (MHC) genotype, suggesting that ability for assessment of the genetic relatedness of individuals may indeed be playing a part in association decisions.

Detection of a tendency to associate with kin in the field is possible, in principle, by using genetic markers to determine relatedness among shoal members. Only Peuhkuri and Seppae (1998) and Pouyaud et al. (1999) sampled whole shoals. However, the most significant sampling problem is the need to separate shoal effects on genetic structure within a population from spatial subdivision or genetic differentiation between cohorts. For example, Hansen et al. (1997) found a non-random distribution of genotypes among samples of juvenile brown trout, which they attribute to the small number of families that contribute to the fry present in any given stretch of river. Similarly, Hauser et al., (1998) detected significant genetic structure in Lake Tanganyika sardines (*Limnothrissa miodon*) shoals.

***Familiarity*:**

The behavioural patterns of fish are the result of innate (built-in) patterns of maturation (developmental changes) and of learning process (imprinting and trial and error learning). Through learning fish can adapt to various environmental changes. A number of these studies have used fish collected from the wild and designated as 'familiar' those individuals that are associating together when caught (Brown and Smith, 1994: fathead minnows; Brown and Colgan, 1986: bluegill sunfish; Griffths, 1997: European minnows; Griffths and Magurran, 1998: Trinidadian guppies). A more robust test involves generating familiar groups at random in a laboratory environment. Preference for familiars using this method has been demonstrated in the bluegill sunfish (Dugatkin and Wilson, 1992), female Trinidadian guppies (Lachlan et al., 1998) and mixed-sex guppy fry (Warburton and Lees, 1996).

Associating with familiars may have a number of advantages. Familiarity among the members of a shoal may reduce the fitness costs of competition by reducing aggression between the contestants. Hojesjo et al. (1998) found a decrease in antagonistic behaviour with an increase in familiarity among sea trout, (*Salmo trutta*). Chivers et al., (1995) reported that shoals of fathead minnows (*Pimephales promelas*) that originated from the same shoal exhibited more effective antipredatory tactics under predator threat than groups composed of individuals taken from different shoals. It remains to be shown, however, whether this phenomenon is actually due to familiarity between individuals and whether the predation risk for the shoal is lowered as a result of social networking.

Familiarity can develop within groups of initially unfamiliar fish through social learning and previous experience with particular shoal mates (Krause et al., 2000). In selecting a familiar group, like in kin recognition, individuals evaluate the recognition cues of the group mates to a template (either their own or learned) which is an internal representation of the phenotype associated with suitable individuals (Sherman et al., 1997). Association with familiar individuals may provide shoaling preference in terms of improved co-ordination of antipredator behavior similar to shoals of related individuals (Chivers et al., 1995). Familiarity is also thought to stabilize dominance hierarchies and reduce aggressive interactions within the social groups of animals (Webster and Hart, 2006). Barber and Wright (2001) through their studies in European minnows (*Phoxinus phoxinus*) suggested that the fish perceive the value of shoaling with familiars as equivalent to the benefits gained by doubling the shoal-size. Here, the preference for familiar shoal mates was sufficient to offset the fondness to slightly larger groups.

Early Experiences in Social Environment:

Recognition of a suitable group member can be innate or learned through experiences in early social environment. Many social animals have parents, siblings or other conspecifics reliably present in the social environment, and templates can be based on the phenotype of these individuals. An individual learn a template from its own phenotype and from the individuals it has interacted with (Holmes and Sherman, 1982). Understanding the interplay of development and early experience is critical for understanding of behavior. Some behaviour is variable across ontogenic stages. The early social environment may have a disproportionate effect on recognition of siblings and conspecifics/heterospecifics later in life because of species imprinting. Manipulation of early social environment during larval stages altered the behavioural patterns of zebrafish (*Danio rerio*) (Engeszer et al., 2007a & b) while a change in social environment of fish after they had acquired a preference did not change their later preference. In three spined sticklebacks, (*Gasterosteus spp.*) rearing individuals with varying numbers of conspecifics and heterospecifics altered their shoaling preference (Kozak and Boughman, 2008). Further, rearing in isolation caused drastic behavioural and physiological changes in several of group-living species. For instance, Peuhkuri et al. (1995) demonstrated that shoaling sticklebacks grew faster than solitary ones. Zebrafish raised in isolation failed to display a colour based shoaling preference compared to the group reared ones (Engeszer et al., 2004). Fleming and Einum (1997) found that in salmon, behavioural activities are patterned according to the environmental factors in the rearing environment. The fish collected from wild was found to be more explorative and active in stream conditions while a hatchery-derived individual failed to thrive in such a condition and in the other way around.

Shoaling is a widespread behaviour, and is representative of group formation in animals in general. Even though a great deal is now known about the mechanisms and evolutionary causes governing this behaviour, further investigations are needed to unravel the genetical and neurobiological basis of this behaviour.

SCOPE OF THE STUDY

The major problem addressed in the present study is the factors that influence kin recognition and shoaling decisions of *Puntius sarana subnasutus*. As it is difficult to assess influence the genetic relationship on shoal choice of free ranging fish shoals, induced bred larvae of *Puntius sarana subnasutus* is employed throughout this study. Onset of shoaling behaviour or the time at which the newly hatched larvae of *Puntius sarana subnasutus* exhibit shoaling behaviour and whether the physical development of the fish has any importance in shoaling decisions is assessed in this work. It has been shown that shoals of fish may break up and reorganize on several occasions and individuals may leave a shoal and rejoin the same group or a different group. The ability of the test fish to discriminate the stimulus shoal size and whether the test fish trade off its affinity towards stimulus shoals composed of phenotypically and genotypically similar individuals with that of a large shoal is studied in detail. It is well documented that early rearing environment plays considerable role in determining future behavioural repertoire of several species of animals. This problem is addressed in a very comprehensive manner during the present study. Shoaling preference of the test fish to siblings, conspecifics, and heterospecifics is assessed using test fish reared along with siblings. The present study examined whether the difference in the size of stimulus shoal exert any significant influence on the shoaling decisions of the test fish in this context. Another factor tested in this section is the influence of familiarity with the conspecifics and heterospecifics developed during early rearing period influence the shoaling preference of the test fish during later stages of life. Effect of total isolation on shoaling decision and its influence of brain development and physiology of the test fish is also assessed in this study. The last section of the thesis contains the observations on the influence of aquatic pollution on shoaling decision of *Puntius sarana subnasutus* is discussed. Turbidity, drastic change in water temperature due to discharge of hot water from factories or very coldwater from ice plants and the effect of surfactants that reach the natural water bodies affect several physiological and behavioural aspects of aquatic organisms including fishes. The results of laboratory experiments carried out to examine the influence of these three pollutants are discussed in the last section of the thesis.

THE MODEL FISH:

Puntius sarana subnasutus (Barbodes sarana) was originally described as *Barbus subnasutus* by Valenciennes, 1842 from Pondicherry, India. It occurs widely in Krishna River and in all rivers south of Krishna (Menon 1963, 1999) and is endemic to the Western Ghats (Dahanukar et al. 2004). It has been recorded from Kerala (Shaji and Easa, 1995; Biju et al., 1999; Thomas et al., 2000), Karnataka, Tamil Nadu, Goa and Maharashtra. This is a common species throughout its range [Fig. 1(1)]. The species is found in rivers with medium currents, ponds and reservoirs and attains total length of 25 cm (Menon, 1999). They are tolerant to salinity and forms shoals in groups of four or five to several dozen (Pethiyagoda, 1991). It is omnivore in habit, usually feeding on aquatic insects, fish, algae and shrimps and spawns in running waters among submerged boulders and vegetation (Talwar and Jhingran, 1991). This small fish have limited demand in the aquarium trade and adults have commercial food value. Eventhough, no specific threats are currently known to the species, reclamation of wetlands, pollution from agricultural runoff and destructive fishing practices could be threatening for the species (Dahanukar, 2011).

***Short description of the species*:**

Dorsal spines (total): 3; Dorsal soft rays (total): 8; Anal spines : 2; Anal soft rays : 5. Body oblong, head, small, barbels 2 pairs - Maxillary pairs longer than orbit, rostral pairs shorter. Maximum published length: 42.0 cm TL male/ unsexed; Maximum published weight: 1,400 g (Rahman, 1989).

Puntius sarana subnasutus

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cypriniforms

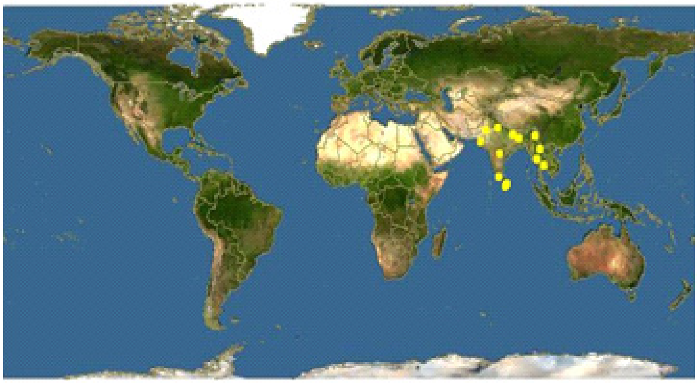
Family: Cyprinidae

Subfamily: Barbinae

Genus: *Puntius*

Species: *Puntius sarana*

Subspecies: *Puntius sarana subnasutus*



● *Puntius sarana* species distribution
@ Global Biodiversity Information Facility

Figure 1(1): *Puntius sarana subnasutus* and its distribution

A recent study by Pethiyagoda et al. (2012) based on molecular taxonomy, morphometry and osteology, suggested the genus *Puntius* which contains about 120 valid species (all together of 220 nominal species) as polyphyletic representing at least five lineages recognizable as genera. They proposed three new genera namely, Dawkinsia, Dravidia and Pethia and these new discoveries may alter the present taxonomical position of *Puntius sarana subnasutus*.

Chapter - 2

General Methodology

The study of partner-choice decisions of individual fish is a good method to glean insights into patterns of shoal structure or territorial assemblages. Most of the initial works were focused on the recognition abilities and association patterns of fish, in situations where the potential for adaptive costs and benefits is great, such as in the contexts of foraging or antipredator behavior. However, the benefits of condition independent recognition and kin discrimination may also have profound effects on patterns of movement and dispersal (Griffiths, 2003). The present study analysed the shoaling preferences and kin recognition ability of laboratory-bred offspring of *Puntius sarana subnasutus*. Available evidences proves that the fish has a tendency to associate with the larger group. Further, the experiences in the early social environment have a marked effect on patterning this shoaling preference in later life.

COLLECTION OF FISH-STOCKS :

Fishes (brood-stocks) were collected from ponds of Irinjalakuda, ($10^o25'$, $10^o18'47''$ N lat. and $76^o17'19''$, $76^o12'48''$E long), Thrissur district, India, during January - March 2009. In order to avoid relatedness among breeding pairs, we collected *Puntius sarana subnasutus* from two different ponds located in Avittathur and Konthulapuram, which are separated by more than 6km. The heterospecific species used in the study, *Puntius parrah* and *Anabas testudineus*, were collected from canals associated with paddy fields near Muriyad wetland. The fishes were acclimatized with laboratory conditions for two weeks in large cement tanks (175cm x 90cm x 90cm; length, height and width) and were fed *ad lib*. with commercially available tropical fish food (Marvel feeds, Aquarium systems, India). The tanks were filled with pond water. The temperature in the laboratory was 28 ± 2^oC, with a constant light: dark cycle of 12:12. The first part of the study assessed behavioural responses and shoaling preference among offspring of a single breeding pair of *Puntius sarana subnasutus* (Siblings of the test fish). In the next section compared the shoaling preference of *Puntius sarana subnasutus* to offspring of a different breeding pair of *Puntius sarana subnasutus* (Conspecifics of the test fish) and to *Puntius parrah* (Heterospecifics), a different species under the same genus. Then the study focused on the variations in shoaling preferences of of *Puntius sarana subnasutus* when the shoaling partner is a more genetically distantly related heterospecific species, *Anabas testudineus*. In order to ensure the genetic relatedness and uniformity of behavioural patterns, fishes used in the present study were produced by induced breeding of parental pairs collected from the wild.

TECHNIQUE OF INDUCED BREEDING :

Induced breeding is a technique whereby ripe fish breeders are stimulated by pituitary hormone or any other synthetic analogue of pituitary hormones to breed in captive condition. The stimulation promotes timely release of sperms and eggs. Selected pairs of healthy breeders from each species were induced to breed using GnRH analogue Ovaprim. Ovaprim is salmon Gonadotropin Releasing Hormone analogue (sGnRHa) in a vehicle of propylene glycol and a dopamine inhibitor, domperidon which is a potent ovulating/spermiating agent to promote and facilitate reproduction of many species of fish. Ovaprim utilizes the fish's own endocrine system to safely induce maturation and coordinate spawning time. When used during the normal spawning cycle, it can synchronize and coordinate maturation in treated fish by inducing maturation without affecting viability or fecundity. Breeders were kept separately in breeding hapas under shower for 4 to 5 hours for conditioning. At 5 pm male and female were injected peritonially with Ovaprim @ 1 ml/kg body weight (4 pairs in each set of experiment) with the help of 2 ml syringe and 26 No. needle with needle guard. After injection both the sexes were transferred to larger aquaria with hydrilla plants as the eggs are adhesive in nature [Fig. 2(1)].

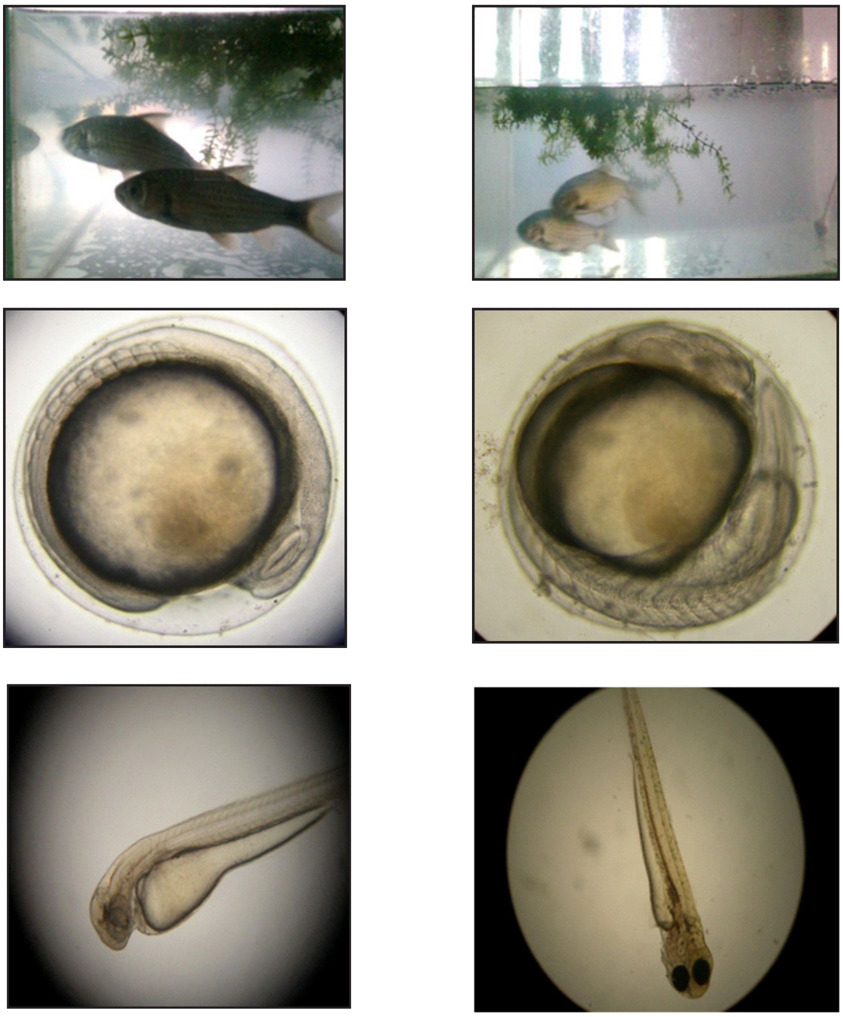


Figure 2(1): Developing egg and larvae of *Puntius sarana subnasutus*

EXPERIMENTAL DESIGN:

In all experimental protocols shoaling preferences were measured using a standard binary choice test. Experiments were conducted in a 70-litre aquarium (60 x 23 x 23 cm), which was divided into three chambers; two side chambers (15 x 23 x 23 cm each) and a central chamber (30 x 23 x 23 cm) [Fig. 2(2)]. Each end compartments were separated from the central one by perforated transparent acrylic plexiglas sheets sealed to the walls of the aquarium using fevibond glue. This enabled the test fish to have visual and olfactory contact to both stimulus shoals. In front of the each side compartment a preference zone was marked (almost equal to the length of the test fish) on the outer side of the glass walls using a permanent marker, leaving a neutral zone in the middle of the tank.

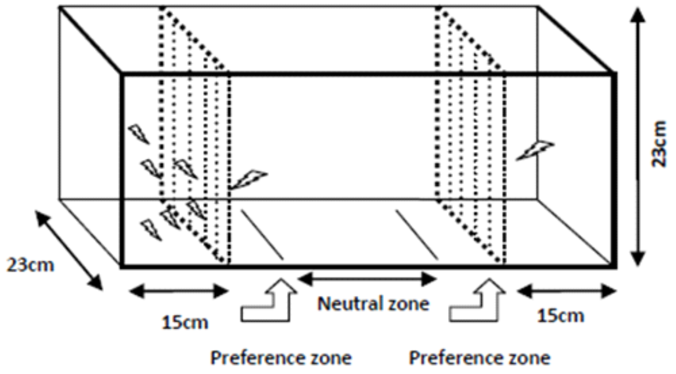


Figure 2(2): Diagramatic Representation of Experimental Set-up

An 11W fluorescent tube placed above the tank lighted the set up. The tank was filled with one-day old tap water. The water temperature at the time of testing was 24±2^{0}C. Interactions of the fish with the environment of the aquarium were prevented by covering the walls of the aquarium, with the exception of the front wall of the central compartment, with neutral grey paper. Additionally, a black curtain was tightened around the test tank. A larger tank (85 x 32 x 32 cm), in the similar set-up was used for observing sub-adult fish.

EXPERIMENTAL PROTOCOL:

For each test, we introduced stimulus shoals (number of individuals in the shoals varied according to experiments), to each of the side compartments prior to start of the choice test. The stimulus shoals were then given five minutes to acclimate. The test fish was always introduced individually into the central arena in a presentation cage made of transparent perforated acrylic sheets (15 x 10 x 27 cm) with a sliding door on the top. The bottom of the presentation cage was open so that the test fishes can be released in to the experimental arena by lifting it. This box was constructed from the same material as the barriers fixed on the inner side of the stimulus compartments allowing both visual and chemical communication between the stimulus shoals and the focal fish. The focal fish remained inside the start box for a period of five minutes before being released and allowed to assess the stimulus shoals present in the side chambers. In order to avoid interference due to the presence of the experimenter, the presentation cage was suspended using a string tied to a pulley and the test fish was released into the arena by lifting the cage. The time spent by the test fish near either of the stimulus shoals (within 15 cm from the side chamber; preference zone) or in the central area (non - preference zone) of the middle chamber was recorded using a stopwatch, by sitting behind a black screen. The time spent by the test fish in the preference zone is taken as the indication of its preference for the shoal present in the adjacent chamber. The duration of each test was six minutes (360 seconds), after which the fish was removed from the testing arena and put back into their home tank. Each experiment was repeated in twenty fish and alternated the position of stimulus shoals between tests in order to avoid any side bias. After each trial, a further 5minutes elapsed before a second focal fish was introduced and the procedure was repeated. After the experiments, the fishes were released into their native ponds. In all experiments data were analyzed using the parametric dependent sample two-tailed‘t’ test [SPSS 11.0.1 statistical package].

In this thesis, terms like shoaling preference, association preference, and shoal choice are used with same connotation. Sibling designates the larvae of the same breeding pairs (here *Puntius sarana subnasutus*) whereas conspecifics means the larvae produced from a different breeding pair of the same species (here a different pair of *Puntius sarana subnasutus*). Heterospecifics denotes the larvae produced from a congeneric (here *Puntius parrah*) or from a fish belonging to a different genus (here *Anabas testudineus*).

Chapter – 3

Onset of shoaling in *Puntius sarana subnasutus*

Groups of fishes have always attracted the attention of many scientists from ancient ages to date. As early as 4th century BC, Aristotle, in his work Historia Animalium, defined the 'shoal-fishes' as 'those caught by netting'. During the subsequent 2000 years, 'shoal-fishes' have been heavily exploited because they are found in groups and over fishing has brought about the collapse of some commercially important fish stocks (Masuda and Tsukamoto, 1999). Only in past 100 years has the interest of scientists shifted from how to catch these fishes to how to understand and protect them. Shoaling behaviour in fish has been a focus of behavioural studies for the past fifty years (Keenleyside, 1955). Apart from fishing pressures, natural stocks are known to fluctuate greatly from year to year. This fluctuation is attributed to either food availability and/or predation at some period of the early life history, the so called 'critical period' (Cushing, 1990). As shoaling is an indispensable antipredatory behavior, a proper knowledge of the development of this behaviour is crucial to understand the fluctuations in fish stocks.

Understanding the interplay of development and early experience is critical for an understanding of behaviour. Some behaviour is variable across ontogenetic stages. Knowing the onset and ontogeny of a behaviour, we can determine critical periods for behavioural development and assess the environmental and genetic factors that give rise to the behavioural phenotype. Although many fish species exhibit shoaling, they show tremendous ontogenetic variation in their timing and tendency to aggregate. Despite much work on the grouping behaviour of juvenile and adult fish (reviewed by Krause et al., 2000), there are only a few studies focusing on development of shoaling behaviour in larvae. Generally, behavioural modifications are considered to be concomitant with morphological and physiological changes (Youson, 1988). The present study analysed the influence of certain essential morphological changes with respect to days of development in patterning the shoaling behavior of larvae and juveniles of *Puntius sarana subnasutus*. At very early stages, larvae do not shoal. This lack of response is hardly surprising. On the first two days of hatching, the larvae spend their time adhering to nearby surfaces and swim only when startled. From the fourth day onwards the larvae were far more mobile and spent significantly more time on the bottom of the aquarium. The experiment was conducted from the tenth day of hatching onwards.

Materials and Method

Four gravid breeding pairs of *Puntius sarana subnasutus* were selected and induced to breed using GnRH analogue Ovaprim. Healthy larvae were reared in the laboratory in four glass tanks (45cm x 23cm x 23cm) in aerated water and fed with *artemia* nauplius larvae *ad libitum*. Five larvae of each breeding pairs were used for experimental analysis.

Experimental procedure

The shoaling behaviour of the fish was tested in 10-days-old larvae (mean body length = 8 mm) and 20-30-40-days old juveniles (when individuals are approximately 10 mm in SL, are not yet reproductively active and have complete fins, complete squamation and a nearly complete adult pigment pattern) (Mean body length 1.8, 2.6, 3.1 cm respectively). They were given an opportunity to swim near either a single sibling or a group of 10 siblings in a 30 liter a aquarium (60x23x23 cm), which was divided into two stimulus shoal compartments (measuring 15 x 23 x 23 cm each) and a central compartment (measuring 15 x 23 x 23 cm). Perforated clear Plexiglas sheets separated compartments.

An 11w compact fluorescent lamp placed above the tank lighted the set up. Three sides of the aquarium were covered with black paper to prevent interference of external stimuli. Additionally, a black curtain was tightened around the test tank. Two stimulus shoals were introduced into the side chambers. The test fish were always introduced individually into the central compartment in a presentation cage made of transparent, perforated acrylic sheets (15cm x 10cm x 27cm). The total duration of the experiment was sixteen minutes. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, the movements of the fish were recorded for six minutes using a stopwatch, sitting behind the black screen and looking through a horizontal slit on the screen. Positioning the whole body in the preference zone marked near any one of the compartments is taken as the indication of shoaling preference of the test fish towards the stimulus fish placed inside the side chambers. In half of the experiments, the positions of the stimulus shoals were interchanged to eliminate the risk of side biases.

Data were analyzed using the parametric dependent sample two-tailed‘t’ test [SPSS 11.0.1 statistical package].

Results

Ten days old larvae as well as the juveniles always preferred to shoal with a group of ten individuals rather than with a single sibling, indicating the overriding influence of group on the shoaling behavior and their ability to identify shoals. The fish clearly exhibited an increased preference to associate with a group corresponding to an increase in their age. Even though 10-days-old larvae and 20 and 30- days old juveniles explored near the single fish, they spent significant time with the larger group (t = 4.56, p = 0.001; t = 5.57, p = 0.001; t = 6.91, p = 0.001 respectively). However, 40-days old juveniles showed very little preference for the single fish (t = 12.84, p = 0.001) [Fig. 3(1)]. Morphological changes in the development of fins of the larvae with respect to increase in number of days after hatching is summarised in Table. 3(1).

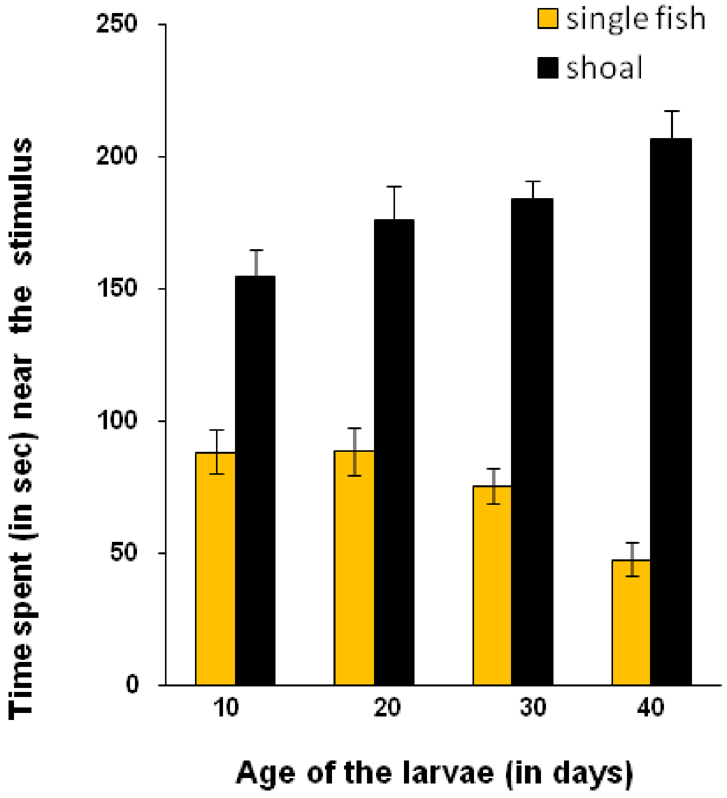


Figure 3(1): Preference of juvenile *Puntius sarana subnasutus* for Single (🟨) / ten (■) sibling stimulus shoals n = 20 Mean time (spent in sec) ± SE

No: of days	Length in cm	Characterestic features
3	0.5	Only pectoral and pelvic fins (rounded)
6	0.7	Pectoral, Pelvic, Dorsal and Caudal fins (Rounded)
10	0.9	Pectoral, Pelvic, Dorsal and Caudal fins Completed fin ray development
15	1.3	Started scale formation

Table 3(1): Morphological chances during development

Discussion

Fishes are able to discriminate shoals, based on the circumstances (Hoare et al., 2004; Wong and Rosenthal, 2005; Alex and Thomas, 2011) and always preferred to associate with a group probably due to antipredator advantages (Turesson and Bronmark, 2007), better foraging opportunity (Rodgers et al., 2011) and better transmission of information (Lachlan et al., 1998). In the present study, the larvae and juveniles of *Puntius sarana subnasutus* successfully discriminated the group of fish from a single individual and preferred to join with them. This increased preference was in turn correlated with an increase in age and subsequent developmental changes in swimming organs. Larvae started to swim regularly from tenth day of hatching (9mm in length) and from this stage onwards they exhibited mutual attraction and association preference to group. Masuda and Tsukamoto, 1999 have demonstrated the sequential development of sense organs in striped jack (*Caranx vinctus*) concomitant with increase in length. Accordingly in this fish pigmentation in retina (3.4mm length), development of vitreous humour (4.3mm length), formation of rod cells (8mm to 12mm length) and completion of formation of lateral line (23mm to 30mm length) are important in determining the schooling of fishes. In other studies on the onset of schooling behaviour in fishes, Gallego and Heath (1994) showed that herring (*Clupea harengus*) start to form schools at the length of 35mm. Silversides (*Labidesthes sicculus*) and anchovy (*Engraulis encrasicolus*) start to school at 12mm (Hunter and Coyne, 1982) and guppy (*Poecilia reticulata*) start at birth (Magurran and

Seghers, 1990). School formation by newborn guppies is considered to be consequence of viviparity, which results in the production of relatively well developed newborn.

Late appearance of the visually mediated preference is interesting given the relatively early onset of shoaling behaviour. Although juveniles and adults show a robust preference for the stimulus phenotype, with which they were reared with earlier stages do not exhibit such a preference. Zebrafish possess a functional visual system at the early stage. Some days after hatching, the larvae are able to recognize food and respond to shadows passing over them, but the full complement of photoreceptors is not expressed until approximately 12 days postfertilization. This timing roughly coincides with the transition from larva to juveniles (Engeszer et. al., 2007a). At this point in development, all receptor types are present and functioning in the retinal mosaic, which continues to grow in size as the zebrafish grow to adulthood. Ontogenetic changes in the visual system during the larval stages and through metamorphosis could, therefore, explain the late onset of the visually mediated preference. Alternatively, changes in the higher processing of visual signals in the optic pathways of the brain may account for the ontogenetic variation in the preference.

Development of behaviour is probably programmed genetically and some factors such as learning or physical and chemical environmental factors may have influence on it. Many experiments have clarified the genetic and learned basis of shoaling behaviour (Giles and Huntingford, 1984; Magurran, 1989; Magurran and Seghers, 1990). Learning is a complex ontogenic process that allows animals to acquire, store and subsequently use information about the environment. This information complements genetic prowess, allowing animals to fine tune their behavior according to circumstances. The increased preference exhibited by the juveniles to associate with a group may be aroused when their innate ability to form a shoal at the larval stage is supplemented from the learned advantages of associating with a group (Kozak and Boughman, 2008). This increased social interaction might have led to subsequent changes in the brain.

Scace et al., (2006) in a study explored the influence of social organization on the brains and behaviours of cichlid fishes using the Insel and Fernald (2004) framework for processing of social information. The social behavior of cichlid fish relied on visual stimuli –> social meaning –> social motivation –> social behavior. In fish, the telencephalon is involved in a variety of social and cognitive behaviours (Demski and Beaver, 2001) and the telencephalic size is correlated with social complexity, social learning, enhanced visual cognition and innovation (Reader and Laland, 2002). In the present study, the increased preference for a larger group with increase in age may be correlated with the increase in size of brain of the fish. In 50-52 days old sturgeon fishes the central nervous system acquires definite structure and form stabilization of conditional reflex reactions (Obukhov, 1996). Development of association preferences being the first step to active anti predatory behaviour and are essential to develop their proper size, further studies are necessary to determine the role of brain size in regulating the larval and juvenile group-size preference and the role of other factors such as surface area, contour length, density or extent of shoal movement in aiding shoaling behavior.

Chapter – 4

Shoal-Size Discrimination in Juveniles of *Puntius sarana subnasutus*

Introduction

Abilities such as recording the number of events, enumerating items in a set or comparing two different sets of objects, can be adaptive in several of ecological contexts. Two decades of comparative studies have shown that numerical abilities are widespread in the animal kingdom. Many processes affecting the survival of individuals are density-dependent, and the effects of density at both the local and population level have long been a focus of study for ecologists (Begon et al., 1996). Altering levels of predation, competition and/or aggression among individuals affect density of a population which inturn can effect their internal state, thereby potentially influencing behavioural decisions based on a cost/benefit analysis (Houston and McNamara, 1999). It is now well established that individual animal accrue significant anti-predator advantages by associating with groups composed of more number of individuals; for example in flocks of birds and schools of fishes (Queiroz and Magurran, 2005). One such trait which has been studied to varying degrees of environment is the shoaling behaviour of various fish species (Krause and Ruxton, 2002).

The local density of conspecifics can affect a great many processes including courtship (Gaskin et al., 2002), fertilisation (Powell et al., 2001), foraging effort (Davidson and Morris, 2001), and settlement of juveniles (Kent et al., 2003). Density is an increasingly important area of research for the farming industry, where the number of animals housed per unit area can greatly affect their welfare. The stocking density of commercially exploited fish, for example, can have far reaching consequences in their growth and health (Glasser and Oswald, 2001). The ease, with which small fish species can be kept in captivity, and the suitability of some species for direct field observation, make them ideal subjects for the study of density dependent behaviour. In addition to providing insights into the field as a whole, the study of the dynamics of fish shoaling behaviour allow better management and conservation of commercially exploited or endangered fish stocks (Pitcher, 1997).

Several theoretical studies show that individuals would be safer in larger groups. Most examples for this preference come from fish (e.g. minnows-

Hager and Helfman, 1991; guppies-Weetman et al., 1999; mollies-Bradner and McRobert, 2001; zebra fish-Pritchard et al., 2001; Hoare et al., 2004). Barber and Wright (2001), for example, showed that European minnows (*Phoxinus phoxinus*) preferred the larger of two groups and that this effect might outweigh preferences for familiar fish. Banded killifish (*Fundulus diaphanus*) scared by a predator chose the larger of two shoals, but only if members of both stimulus shoals were of the same size class (Krause and Godin, 1994). However, in most of the experimental studies an increase in group size came along with an increase in shoal density. Little is known whether test fish preferred the larger shoal because of the higher number of group members or because of an increase in density and thus a higher potential for predator confusion. Although the protective properties of groups have been comprehensively investigated in many fish species (Krause and Ruxton, 2002), the individual decisions on which these advantages rest are much less understood. The question of how a fish take decision on group membership by accessing the group-size of a stimulus shoals and the influence of various other factors that affect the decisions deserves particular attention, especially in the context of anti-predator advantages provided by a larger shoal. Rudimentary numerical abilities have been reported in several mammalian and avian species including elephants, dolphins, dogs, cats, robin and chicks. Nowadays, this primary quantity discrimination is also established in some fish species (Buckingham et al., 2007; Agrillo et al., 2008 & 2009). Agrillo et al. (2008) found that fish seeking safety from predators, display a rudimentary numerical ability in discriminating smaller quantities (≤ 4). However, in fishes genuine counting is a ‘last resort’ strategy when no other perceptual mechanism may suggest the quantity of elements. Most of the studies points to the mechanism of ‘relative numerosity’ (Davis and Perusse, 1988) as the basis of group-size discrimination in fishes. Relative numerosity is one of the most basic forms of number judgment and does not involve actual counting but, rather, the ability to have a general sense of ‘more versus less’. The present study analysed the ability of the juveniles of *Puntius sarana subnasutus* to use the ratio of stimulus shoals in order to determine the group-size difference between them.

Materials and Methods:

Puntius sarana subnasutus were collected from the Avittathur pond, Irinjalakuda (10°25', 10°18'47" N lat. and 76°17'19", 76°12'48"E long), Thrissur district, India, during January - March 2009. A pair of gravid fish were selected as the breeders and induced to breed using GnRH analogue Ovaprim. Fish larvae were fed with *artemia* nauplius *ad libitum*. Hundred healthy larvae were selected and reared in groups of twenty in five separate tanks (45 x 23 x 23 cm). Experiments were conducted when the larvae were three months old. Shoaling preferences were tested in 70 liter aquaria following the standard binary choice experimental procedure. The total duration of the experiment was sixteen minutes. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, using a stopwatch the time spent by the fish within the preference zone marked near the stimulus shoal compartments was recorded for six minutes, sitting behind a black screen and looking through a horizontal slit on the screen.

To test the group-size discrimination ability of *Puntius sarana subnasutus*, two sibling shoals were introduced into the stimulus compartments on either side of the central compartment in five different numerical combinations, 25 *vs*. 25, 20 *vs*. 30, 15 *vs*. 35, 10 *vs*. 40, 5 *vs*. 45, in the ratio- 1:1, 1:1.5, 1:2.3, 1:4 and 1:9 respectively). The shoaling preferences of total twenty juveniles were tested in each numerical combination. The positions of the similar shoals were interchanged (in half of the experiments) to eliminate the risk of side biases and repeated the preference experiment. The data were analysed using parametric two-tailed 't' test [SPSS 11.0.1 statistical package].

Result:

The fish always preferred to shoal with the larger sibling stimulus shoals indicating their ability to discriminate shoals of different size. In the first ratio, when the number of siblings were equal in both of the stimulus shoals, the fish did not show any preference for a particular group [Fig. 4(1): 25 *vs*. 25: $t = 1.06$, $p = 0.512$]. Nevertheless, as the number of individuals in any one of the sibling stimulus shoal increased, the test fish spent significantly more time near the larger of the two stimulus groups (Fig 4(1): 20 *vs*. 30: $t = 2.48$, $p =$

0.023, 15 vs. 35: t= 2.89, p = 0.009, 10 *vs*. 40: t= 3.24, p = 0.004 and 5 *vs*. 45: t= 6.87, p = 0.001).

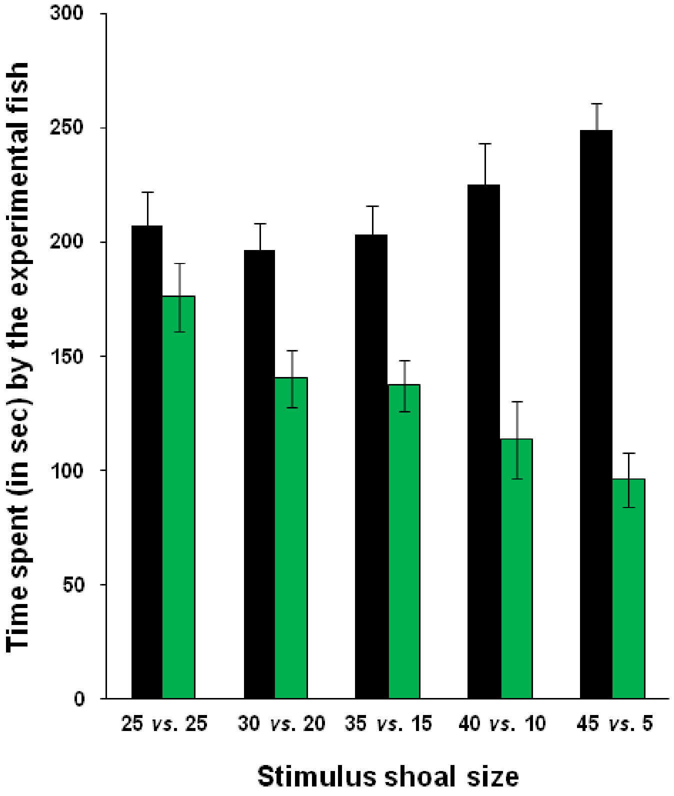


Figure 4(1): Shoal size preference of juvenile *Puntius sarana subnasutus* for sibling stimulus shoals in varying numerical combinations n = 20; Mean time (spent in sec) ± SE

Discussion:

The ability to determine quantity is one of the most useful skills an organism can possess. Numerical quantities are salient to almost every aspect of an animal's life. For example, animals may need to determine concentrations of food patches, number of potential mates or competitors, or prevalence of predators. Further it has important implications in social behaviour such as group choice. In the present study *Puntius sarana subnasutus* successfully identified the group-size difference between the stimulus shoals and associated with the larger group. Joining with a larger shoal is generally very adaptive to the fish except from some density dependent pay offs such as competition and increased risk of parasitic disease transmission (Altizer et al., 2003). It is reported that most freshwater fishes are social and live in large aggregates, gaining benefits such as hydrodynamic advantages during locomotion or increased predator confusion, collective vigilance, social information transfer and foraging benefits (Harcourt et al., 2010). Therefore, a better understanding of the group-size difference between stimulus shoals is fundamental to fish as it will directly affect their survival itself.

In lower vertebrates, especially in the case of fish, the relative comparisons between shoals are more important than absolute ones. Here, the fish may determine group size in a manner consistent with Weber's Law which states that $\Delta I/I=K$ (Sekuler and Blake, 1994) where I is the stimulus and ΔI is the change in stimulus needed to produce a noticeable sensory response K. In the context of shoaling decisions based on group size, the stimulus is the number of fish in a shoal and the response would be a shoal choice by the focal fish. The difficulty of any numerical comparison between shoals should depend on the ratio between the numbers of fish in each shoal. As the ratio (ΔI) becomes smaller, the comparison becomes more difficult i.e., discriminating between a potential group of twenty versus thirty shoaling partners (ratio of 1:1.5) may be more difficult to compare than when the choice is between five versus forty five individuals (ratio of 1:9). It is important to note here that Weber's Law is concerned with the change in stimulus need to produce some sort of response. It does not, however, tell us the discrimination abilities of the subject. For example, fish may be able to determine smaller changes in stimuli and yet not alter their response until this change is sufficiently large. The ratio sensitivity may not necessarily involve any complex cognitive mechanisms and could, instead, be based on spontaneous and rather rudimentary abilities to distinguish between 'more' or 'larger' and 'less' or 'smaller'. In an experiment on body color segregation, when mollies (*Poecilia latipinna*) were offered, a choice between shoals of three fish and six fish which corresponds to a ratio of 1:2, they preferred larger shoals. They did not, however, find a preference for larger shoals when given the choice between four versus three fish, and five versus three fish which correspond to ratios lower than 1:2 (Bradner and McRobert, 2001). In another experiment, test fish European minnows (*Phoxinus phoxinus)* was found to be exhibiting a marginal preference for the larger shoal at size ratio 1:1.2, and significant preferences in the 1:1.9

and 1:4 trials (Barber and Wright, 2001). Similarly Binoy and Thomas (2004) demonstrated that shoaling preferences of *Anabas testudineus* vary with familiarity and group-size ratios. The fish, however, preferred the larger group when two unfamiliar stimulus groups of different sizes were presented. The preference became significant when the ratio of stimulus groups was 1: 1.9 and highly significant when the ratio was 1: 4. A comparison of the influence of familiarity and group size showed that *Anabas testudineus* had a bias for the larger unfamiliar group to the smaller familiar group, when the ratio of familiar versus unfamiliar stimulus shoals was 1: 1.2 and the preference for a larger unfamiliar group was highly significant when the ratio was 1: 4. Although many studies propose a resolution limit approaching 2:1, anecdotal evidence suggests that different species may be more or less sensitive to ratio. In the present study, *Puntius sarana subnasutus* siblings are capable to discriminate the group size difference even when the ratio was 1:1.5 and this may be probably because the stimuls group is composed of their siblings. Here, the fish could enjoy the benefits offered from a sibling group apart from the advantages of joining with a larger group. Another major factor that fine tunes the group-size recognition ability of a particular fish species is the risk of predation in their locality. Krause et al. (1998) noted in their experiment on shoal choice under predation risk that creek chub *(Semotilus atromaculatus*) discriminated between shoals of five and six fish (1:1.2) while threespine sticklebacks (*Gasterosteus aculeatus*) did not. It is interesting to note that chubs are presumably at a greater predation risk than armored sticklebacks (Krause et al., 1998; Mathis and Chivers, 2003) and, therefore, finer resolutions of ratio may be advantageous to chubs.

Due to the practical limitations of rearing fish in laboratory conditions, most of the studies looked at shoals of relatively small sizes; in nature, fish shoals may reach sizes of hundreds or more (Buckingham et al., 2007). The present study have tried to analyse the group-size discrimination ability of the fish up to a difference of five versus forty five individuals. Here, the fish successfully identified the group difference and significantly spent more time with the larger group. At some point, even with very large ratios, fish may cease to discriminate between shoals once the upper limit of their ability to distinguish shoal traits is reached. This may happen, for example, in open ocean environments where shoals of species, such as herring, may be hundreds of meters long. In this situation, individuals may not be able to evaluate a shoal in its entirety. Furthermore, increases in the benefits of shoaling with a larger group may taper off as shoals reach certain critical sizes. Once this size is reached it may not be any more advantageous for a fish to join a larger group and, thus, discriminate between groups.

Group living is a flexible strategy were individuals can choose to join or leave a group based on the costs and benefits of association. Factors that favor grouping (such as protection from predators) should vie with factors that discourage grouping (such as competition). The decision to join or leave a group should reflect tradeoffs among these factors.

CHAPTER - 5

Shoal Choice : Phenotypic and Genotypic Preferences *Vs.* Group Size

Introduction

Given the ubiquity of shoaling as a behavioural strategy in fish, it is perhaps surprising how little we know about the mechanisms underlying the establishment and maintenance of the range of shoal-sizes we observe in nature (Hensor et. al., 2005). The shoal size distribution in a particular area is largely dependent on the number of individuals present and their external and internal behavioural motivations. Clearly this must integrate the social interactions among a number of individuals moving within a given area, as well as processes arising from the physical properties of the environment itself. An individual fish when deciding to join one group over another, multiple factors may influence the decision. Grouping on the basis of such attributes is purported to decrease the competition between group members and/or reduce predation risk. With respect to the anti-predator benefits, it has been suggested that it may be difficult for a predator to identify and attack any one specific individual within a group of phenotypically similar fish. This phenomenon, referred to as the confusion effect, causes predators to hesitate momentarily before attacking aggregated prey, leading to a lower capture success than when attacking solitary or dispersed prey. In a related phenomenon, the oddity effect, a phenotypically distinct individual within a shoal is more likely to be targeted by a predator. It is not surprising, therefore, that shoaling fish typically choose to associate with fish that bear a resemblance to themselves rather than fish that are phenotypically different (Ward et al., 2002).

In studies on the confusion and oddity effects, a number of phenotypic attributes have been shown to affect shoaling preferences for a variety of fish species (reviewed in Krause et al., 2000). Such attributes include body size, parasite load that causes black spots on the body (Barber et al., 1998), body shape (Hauser et al. , 1998), body coloration (McRobert and Bradner, 1998) and stripe pattern (Engeszer et al., 2004). As Rosenthal and Ryan (2005) predicted, fish are capable of discriminating between potential shoal mates on the basis of each of these phenotypic features, typically shoaling with fish that are similar in appearance. This assortative shoaling allows fish to potentially incur the benefits of the confusion effect and avoid the costs of the oddity effect.

Thus decision of a fish to join a large shoal is mainly for reducing the risk of predation. In a larger group the probability of being attacked by predator is much lower and therefore, shoal-size is considered to be the most important factor controlling the shoaling preferences in fish (Krause and Ruxton, 2002; Frommen et al., 2009). However, the attractiveness of a group to potential members may also depend on a range of other attributes such as familiarity, kinship and individual experience (Hoare and Krause, 2003). Shoaling with kin can be advantageous since closely related animals are more likely to show co-operative behaviour during risky situations such as predator inspection because helping a relative increase the indirect fitness of an individual (Hamilton, 1964). However, shoaling with relatives can also be disadvantageous through the risk of inbreeding if related individuals mate. Inbreeding can increase the genetic homozygosity and thus the possible expression of recessive deleterious mutations in offspring (Charlesworth and Charlesworth, 1987). Mechanisms to recognize kin can, therefore, are advantageous in two ways, an individual may profit from the benefits of co-operation with relatives and it can minimise inbreeding depression by avoiding mating with siblings. Several authors studied relatedness between shoal members using allozymes or microsatellite markers. The results were very ambiguos; no indication of kin structured shoals was found in european minnows *(Phoxinus phoxinus)* (Naish et al., 1993) and Tanganyikan sardine *(Limnonthrissa miodon)* (Hauser et al., 1998) while a very high significant association of kin was observed in shoals of mouthbrooding tilapia *(Sarotherodon melanopheron)* (Pouyaud et al., 1999).

To understand this diversifying role of social behavior, one must examine the internal and external influences that lead to nonrandom assortment of phenotypes. It will be very interesting to analyse what happens when preference for associating with a larger group conflicts with that of assorting on the basis of phenotypic and genotypic similarity. One aspect of shoaling behavior that has been explained explicitly in the literature is the trade-off between group-size and any other single factor such as body colour, body-size, kinship or familiarity. (Bradner and Mc Robert, 2001; Hoare et al., 2004; Binoy and Thomas, 2004; Wong and Rosenthal, 2005; Rodgers et al., 2011). However, in nature the fish is simultaneously exposed to multiple factors that influence an individual's decision to join one group over another; but we know little about how these factors interact to contribute to the complex decision of shoaling fishes.

Present study have analysed how grouping decisions of individual are affected when preferences for joining with a larger group conflicts with preference for assorting on the basis of body-size and genetic relatedness in *Puntius sarana subnasutus*. It was tested how siblings of *Puntius sarana subnasutus* (offspring from a single breeding pair) perceives group-size difference when the alternate shoal is composed of phenotypically and genotypically dissimilar individuals, ie., large body sized conspecifics (offspring from a different breeding pair of *Puntius sarana subnasutus*). If the fish considers group-size as more important than the phenotypic and genotypic characteristics of the potential shoal mates, then the fish may decide to join a larger shoal comprising dissimilar-looking fish over a smaller group of similar-looking shoaling partners. Alternatively, fish may choose randomly if the attributes in conflict are equally important. The experiments were conducted to understand the changes in group-size preferences of siblings of *Puntius sarana subnasutus*, when the size-matched sibling stimulus shoals and size-unmatched larger conspecific stimulus shoals were equal in number (25 *vs.* 25) and when the size-unmatched larger conspecifics stimulus shoals were more in number (20 *vs.* 30, 15 *vs.* 35, 10 *vs.* 40, 5 *vs.* 45).

Materials Methods :

The siblings used in the present study were, induced bred larvae of gravid pairs of *Puntius sarana subnasutus*. Experiments were conducted when they were three months old (standard length 5.7 ± 2.15 cm and body weight 11 ± 2 mg). Conspecifics less than one year old (standard length 11.3 ± 2 cm and body weight 18.75 ± 4.12 mg) were collected from ponds of Irinjalakuda. Shoaling preferences were tested following the procedures of standard binary choice experiments. The total duration of the experiment was sixteen minutes. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, the time spent by the focal fish within the preference zone was recorded for six minutes using a stopwatch, sitting behind the black screen and looking through a horizontal slit on the screen. Data were analyzed using the parametric dependent sample two-tailed‘t’ test [SPSS 11.0.1 statistical package].

Results:

The present study unequivocally demonstrate the focal fish’s preference to associate with more genetically related group in spite of the anti-predator benefits gained from joining with a larger group of phenotypically dissimilar and genetically less related conspecifics. Here, the preference was found to be conflicting between genetic relatedness and oddity effect. The focal fish spent more time near smaller sibling shoal, till the number of individuals in the conspecific shoal became forty and forty five. The experimental fish failed to exhibit any preference to size-unmatched conspecific shoal (25siblings *vs.* 25conspecifics: t=8.040, p=0.001), even though a shoal composed of 25 large conicspecifics may appear larger than a shoal composed of 25 smaller siblings. Increase in the number of individuals in the size-unmatched conspecific shoal to thirty and thirty-five, keeping the total number of individuals in both stimulus shoals to fifty (20siblings *vs.* 30conspecifics: t=3.536, p=0.002; 15siblings *vs.* 35conspecifics: t=3.470, p=.003) did not change the preference of the focal fish. However, a further increase in the number of size-unmatched conspecifics into forty and forty-five and subsequent decrease in the number of size-matched siblings into ten and five respectively, decreased the test fish’s preference towards the smaller size-matched siblings shoal and the fish moved randomly between the compartments (10siblings *vs.* 40conspecifics: t=1.901, p=0.073; 5siblings *vs.* 45conspecifics: t=2.896, p=0.009) [Fig. 5(1)].

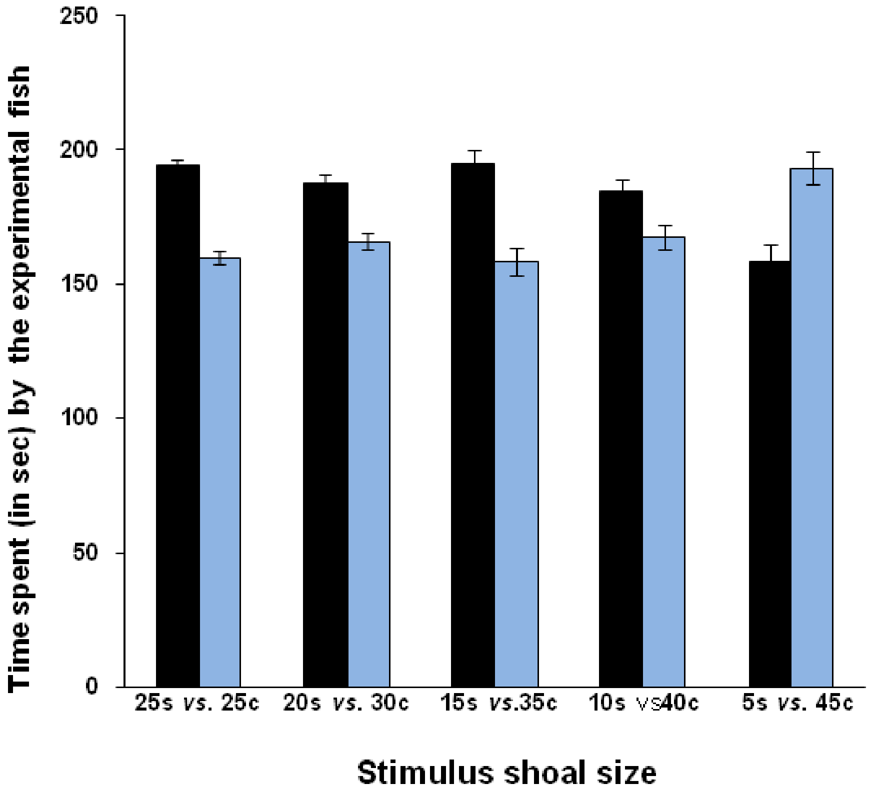


Figure 5(1): Preference of individual *Puntius sarana subnasutus* for size-matched sibling stimulus shoals (■) and size-unmatched larger conspecifics (🟦)stimulus shoals. Mean time (spent in sec) ± SE n = 20

Discussion:

Collective behaviour of large aggregations of animals is a truly amazing natural phenomenon. Particularly interesting is the case when aggregations self-organize into complex patterns with no need of an external stimulus. Simple rules of interaction among the individuals are sufficient to produce collective behaviour. Unfortunately, we have very scarce empirical information about the precise nature of such rules. Alex and Thomas (2011) reported that *Puntius sarana subnasutus* preferred to associate with larger shoal when a choice was given to associate with size-matched smaller sibling shoal and a larger sibling shoal. The present study indicates that the fish prioritized their preferences according to the context, taking into consideration the group-size as well as genetic relatedness in guiding the shoaling preferences. It is said that in size discriminations the fish spontaneously use the relative magnitude of continuous variables such as the total area of the stimulus or the sum of their contour to estimate which group is larger or smaller (Agrillo et al., 2009). However, present study shows that an increase in the total area of the stimuli alone cannot always attract the fish towards the larger shoal. This is indicated by the fact that the test fish failed to associate with the size-unmatched stimulus shoal with larger conspecifics. However, when the number of individuals in the size-matched sibling stimulus shoal was very less compared to the alternate stimulus shoal of size-unmatched larger conspecifics, the test fish actively judged the differences and associated with the size-unmatched larger conspecifics group. Here, the fish traded-off their phenotypic similarity and genotypic relatedness with the sibling shoal and associated with the larger group as an indispensible strategy for predator avoidance.

The main goal of the interaction among individuals is to maintain cohesion of the aggregation. This is a very strong biological requirement, shaped by the evolutionary pressure for survival (Turesson and Bronmark, 2007) Flocks and shoals, however, live in an open space, and yet undergo enormous density changes without losing cohesion, in a very flexible way. Membership in a larger group is always beneficial to the fish through earlier detection of predators and/or to dilute the individual risk of being attacked. However, in this context the homogeneity of group members within a shoal is very important in order to avoid the so called ‘oddity effect’ (Rodgers et al., 2011) as individuals that differ in appearance from other members of a group are more likely to be preyed upon selectively. In stickleback *(Gasterosteus aculeatus)* foraging activity was found to be lower when a single fish in a shoal of 3, 6 or 12 was larger than others compared to the foraging activity of large individuals in a shoal dominated by large fish or those in a size-assorted shoal (Peuhkuri, 1997).

Teleost fishes show indeterminate growth, many populations exhibit a broad range of individual body lengths. An individual’s fitness varies as a function of shoal size and shoal composition, and this relationship is potentially body length dependent. In natural circumstances, the characteristics of groups, such as size and composition, fluctuate along side changing phenotypic and genotypic variables. In the present study *Puntius sarana subnasutus* traded-off their preference for phenotypically similar and genetically related sibling stimulus shoal only when the group-size ratio of stimulus shoals was 1:9 (5 size-matched siblings *vs.* 45 size unmatched larger conspecifics). Here the test fish may enjoy the advantage of 'dilution effect' in spite of the 'oddity effect' because of the larger difference in number and size. The prediction that fish should trade off the relative costs and benefits of associating with genetically related, phenotypically similar shoals, depending on group-size, is supported by the present results. Critically, however, It was found that body size has an overriding influence on shoaling decisions of an individual, suggesting that the anti-predator benefits to grouping, such as a reduction in individual risk through dilution, oddity, and confusion effects are dependent upon individual characteristics.

A preference to shoal with kin would imply some kind of kin recognition mechanism that allows the differentiation of related conspecifics from non kin. There are two main types of kin recognition, direct familiarity and indirect familiarity (phenotype matching). In the case of direct familiarity, individuals become familiar with members of the same litter or clutch during early ontogeny and prefer these individuals over unfamiliar ones. In the case of phenotype matching, an individual learns about the phenotype of close relatives or itself (self-matching) during early development and later compares unfamiliar conspecifics with this learned phenotype to identify kin. Phenotype matching implies a correlation of the phenotypic traits used for kin recognition and the genotype, because only heritable phenotypic traits can be true indicators for relatedness (Tang-Martinez, 2001). Phenotype matching requires a correlation between phenotypic similarity and genotypic similarity, so that individuals with traits that most closely match an animal's template are its closest kin. Self-referent phenotype matching, the ability of animals to learn and use their own phenotypes as referents for recognition of relatives (dubbed the 'armpit effect' by Dawkins (1982), enables the most accurate assessment of the degree of relatedness between two individuals because one's own cues will generally reflect one's own genotype more accurately than the cues of close kin. Self matching should be favoured in species with multiple paternity or maternity or when individuals commonly encounter older (or younger) siblings after dispersal. This mechanism may mediate nepotistic behaviours (dispensing benefits only to kin; Sherman et al., 1997), mate-choice decisions (optimizing the costs and benefits of inbreeding and outbreeding) or both.

In the present study individuals in conspecific stimulus shoal were phenotypically very much similar to the test fish in their body colouration but differed in their body-size. Here the number of individuals in each stimulus shoal was found to be more important in prioritizing the preference to other phenotypic characters. When the number of individuals in the sibling stimulus shoal was sufficiently larger the focal fish gave more importance for homogeneity of the shoal and when the number was very less the fish preferred stimulus shoal having dissimilar body-sized conspecifics with matching body colouration.

Nevertheless, in the present study, the alternate shoal being the conspecifics of the test fish; the influence of genetic relatedness could not be completely ruled out. Apart from this, The study found that the test fish, *Puntius sarana subnasutus* is not merely associating with a larger shoal which is perceived to be bigger but actively judges the characteristics of the group before exhibiting its associational preference to a particular group. This capacity to judge, in turn, may dependent on the fish's ability for social cognition and social information processing. The question how social environment could influence the shoal choice of a fish is biologically relevant in this context and is necessary to understand the factors that favour the social learning ability of fishes.

Chapter - 6

Influence of rearing environment on shoal choice

Introduction

In fishes shoaling preferences are likely to be context-dependent and appropriate decisions are taken by a fish in situations where the net benefit of one is greater than the others. Several shoaling fishes evaluate various cues available in the surroundings and are able to take appropriate decisions. The process of recognition of cues may be learned through experience or can be genetically encoded. Many social animals have parents, siblings or other conspecifics reliably present in the social environment and differential behaviour patterns to a related/familiar individual and to another unrelated individual take place based on the social information transfer from these individuals. Social learning, or learning from others, provides a means for animals to acquire adaptive information about their environment rapidly and efficiently.

In order to select group members, individuals need to be able to recognize those that possess suitable phenotypes or genotypes. Recognition involves the production of a cue or a signal by one individual and the evaluation of that cue or signal by another individual (Sherman et al., 1997). Recognition can be based on cues, such as body size, colour patterns, chemical signals and evolved signals, such as vocalizations (Smith and Harper, 2003). Individuals evaluate cues by comparing the cue to a template, which is an internal representation of the phenotype associated with suitable individuals (Beecher, 1982). Templates may consist of visual, auditory or chemical cues (Gerlach and Lysiak, 2006). Whereas some species recognize group members using genetically determined templates (Queller et al., 2003), others use learned templates (Mateo, 2004). However, for many species, it is not sure that if templates are genetically encoded or learned. An individual can learn a template from its own phenotype or from the phenotypes of individuals it has interacted with (Holmes and Sherman, 1982). The template is likely to be based on a phenotype that is reliably present and easy to learn (Sherman et al., 1997).

The social environment provides individuals with an opportunity to gather and use information resulting from the behaviour of others. Help directed toward kin (altruism) is an important example of social behaviour. Such helping behaviour requires a mechanism to distinguish kin from non-kin. The prevailing kin recognition hypothesis is that when familiarity is a reliable cue of relatedness, other mechanisms of recognition will not evolve. However, when familiarity is an unreliable cue of relatedness, kin recognition by phenotype matching is predicted to evolve (Hain and Neff, 2007). When kin recognition by familiarity is used, individuals learn phenotypic cues of conspecifics encountered during early development and 'remember' these specific individuals as kin. When kin recognition by phenotype matching is used, individuals, instead learn the phenotypic cues of their rearing associates (or its own cues) and use these cues to form a 'kin template.' Individuals later compare phenotypic cues of putative kin to the template and, based on the similarity of the cue to the template, determine the degree of relatedness of the individual. So, in phenotype matching, specific individuals are not remembered as kin. Self-referencing is the special case of phenotype matching where individuals use their own cues to form their kin template.

Evidence is accumulating that learning plays a role in individual, group member, and kin recognition in several social species. The ability to recognize kin can be learned from the early social environment in mammals (Heth et al., 1998), birds (Hatchwell et al., 2001), amphibians (Waldman, 1981), fish (Winberg and Olsen, 1992; Griffths and Magurran, 1999; Griffths, 2003; Hain and Neff, 2006; Frommen et al., 2007a), and some insects (Schausberger, 2007). Social insects learn to recognize the distinct odour of their colony from nest mates and reject individuals that do not possess the correct cuticular hydrocarbon blend (Lorenzi et al., 1999). Social insects can also learn to recognize familiar non-nest mate individuals (Nowbahari, 2007). Preferences for familiar group members (not necessarily kin) might be based on learning individually distinctive phenotypes in many species (Griffths and Magurran 1997a & b; Dale et al., 2001; Tibbetts, 2002; Ward and Hart, 2003; D'Ettorre and Heinze, 2005). Alternatively, these preferences may be based on learning familiar phenotypes, such as familiar habitat odor cues (Ward et al., 2005b & 2007), familiar color cues (McCann and Carlson, 1982; Engeszer et al., 2004; Spence and Smith, 2007), or familiar species cues (Warburton and Lees, 1996). Whether recognition of kin, group members, and familiar individuals is based on shared or distinct mechanisms is a matter of debate (Tang-Martinez, 2001).

Moreover, still it is not clear how often social recognition processes are learned rather than genetically determined. Similarly, it is also difficult to predict when learning is likely to be involved in recognition. Social recognition may change over ontogeny as predation risk, diet, and habitat change (Butler et al., 1999; Despland and Hamzeh, 2004; Gramapurohit et al., 2006). The role of learning in social recognition could also change across life stages. The early social environment may have a disproportionate effect on the recognition of kin and conspecifics later in life because some species imprint on parental (Immelmann, 1972; Kruijt et al., 1983) or sibling phenotypes (Schutz, 1965). In ants, nest mate recognition in based on learning early in life: during the larval stage (Isingrini et al., 1985; Carlin et al., 1987) or during the early days as an adult (Feneron and Jaisson, 1995). Social recognition may also differ between species that differ in levels of social behavior. Selective pressures for increased sociality might change the strength of preferences for group members. It may also alter the phenotypes that suitable group members should possess. However, we do not know if changes in social ambience would alter the role, learning plays in social recognition.

Evidences of kin recognition in fishes by phenotypic matching, come from a number of teleost species e.g. in coho salmon, *Oncorhynchus kisutch,*(Quinn and Busack, 1985), Arctic charr, *Salvelinus alpinus* L., (Olsen, 1989; Winberg and Olsen, 1992); rainbow trout, *Oncorhynchus mykiss*, (Brown et al., 1993) and African cichlid , *Neolamprologus pulcher,* (Le vin et al., 2010) or by familiarity, e.g. in guppies, *Poecilia reticulata,* (Griffiths and Magurran, 1999); bluegill sunfish, *Lepomis macrochirus*, (Hain and Neff, 2006) and sticklebacks, *Gasterosteus aculeatus,* (Frommen et al., 2007b). Because all these mechanisms of recognition may be used by the same individual, the genetic and sensory basis of recognition is difficult to disentangle.

Evidences are accumulating that learning plays a crucial role in kin/ familiar recognition and discrimination of kin from a non-kin in a number of animal species (Olsen and Winberg, 1996). Early social environment may have a disproportionate effect on the recognition of kin and conspecifics later in life because some species imprint on parental (Kruijt et al., 1983) or sibling phenotypes (Schutz, 1965). Imprinting is a kind of rapid learning process occurring at a crucial period of life by which a new born or very young animal acquires the skill for recognition and attraction to another animal.

A) Kinship and shoal choice

Kinship is a kind of relationship between any entities that share a, genealogical origin, through biological descent. In group-living animals, theory predicts special benefits in co-operating with relatives mainly via the evolution of kin-directed altruism (Hamilton, 1964). More recently, fishes have also proved to be worthwhile model organisms in research works regarding kin-recognition and kin-biased association patterns. They clearly have cognitive skills necessary for distinguishing kin from non-kin (Griffiths, 2003; Ward and Hart, 2003) and for the evolution of sociality and cooperative net works (Croft et al., 2006).

The advantages of kin discriminating behaviour are often cited to explain a number of direct and indirect fitness advantages. Direct benefits are conferred by reduced levels of aggression among kin groups (Olsen et al., 1996) which lessens the risk of injury and reduces the risk of being preyed on. Shoaling in a kin-group also provides improved shoal cohesion and decreases the conspicuousness (Evans and Kelly, 2008). Furthermore, when aggression is low, animals have more time to forage and attend to other activities (Krause and Godin, 1996). Indeed siblings housed together in aquaria or small enclosure, exhibited faster growth and reduced variation in size probably because of less stressful environment (Gerlach et al., 2007) which can lead to higher direct fitness based on increased survival and early reproduction. The indirect benefits of shoaling in kin-groups are acquired through inclusive fitness. In nature resources may be limited and fish compete with one another for food and suitable habitats. Under these conditions fishes may share a portion of available resources with their relatives. The improved survival thus conferred on altruistic fish has important implications not only for the fishes, but also for the fisheries' managers and biologists responsible for handling the fish stocks (Freon and Misund, 1999).

However, living with kin can have several disadvantages, for example, an increased risk of mating with a close relative. Inbreeding is deleterious as it increases the overall genetic homozygosity and expression of recessive deleterious mutations in offspring. A number of studies have suggested that fishes solve this problem by changing their preferences for kin on attaining sexual maturity. When the olfactory preferences for kin was tested in laboratory-bred juveniles and reproductively active adult Zebrafish (*Danio rerio*), Gerlach and Lysiak (2006) found that adult females preferred the odour of unfamiliar, unrelated males to familiar brothers, indicating inbreeding avoidance. Adult male zebrafish showed no preference for the odour of related or unrelated females. Juveniles of mixed-sex groups spent more time on the side of unfamiliar kin than unfamiliar non-kin, and preferred familiar kin to unfamiliar kin.

To date, evidence for assortment based on genotype in the natural environment is equivocal. A few studies have reported evidence for kin associations in free-ranging shoals of Eurasian perch, (*Perca fluviatilis*) (Gerlach et al., 2001) and common shiner (*Luxilus cornutus*) (Ferguson and Noakes, 1981). The only study so far that provide clear evidence for kin associations in free-ranging shoals is that of Pouyaud et al., (1999) in mouth-brooding tilapia (*Sarotherodon melanotheron*). At present, the technique for genetic sampling to resolve relationships is largely dependent on the polymorphism of the chosen markers. Microsatellite loci tend to have high levels of heterozygosity, offering perhaps the most powerful approach available at present (Marshall et al., 1998). The Trinidadian guppy (*Poecilia reticulata*) has life history traits that might promote kin structure of shoals such as internal fertilization and small brood size in contrast to many other fish species. A genetic analysis on 40 shoals from four populations using ten microsatellite markers showed that the frequency of sib dyads among juveniles within shoals was significantly larger (Piyapong et al., 2011). Work on free-ranging shoals of sticklebacks suggests that, although shoals tend to continually break up and reform, small closely related subsets of full-sibs or half-sibs remained together always (Ward et al., 2002). The opposite of kin selection, heterogenous advantage, predicts a proportional relationship between the levels of relatedness and competition in a group, as a result of variability in genotype being expressed as differences in resource utilization. Thus different explanations seem plausible in individual recognition depending on the context and life history traits of fishes.

B) Familiarity and shoal choice

Shoaling fish, like many other social animals often demonstrate an association preference for unrelated individual or individuals, outside of a mate choice context. "Familiarity" as this phenomenon is known, also acts to enhance the benefits of shoaling. Fish shoals result from the grouping decisions of individuals which tradeoff the costs and benefits of shoal membership. Many of the benefits of shoaling are maximized when members are phenotypically similar, leading to the selection for homogeneity within shoals. However, in nature mixed-species groups occur frequently (Coolen et al., 2003) and apparently it has been shown that the use of social information is not restricted to interactions between conspecifics. Indeed, social information use may be the primary benefit gained by some constituents of mixed-species groups, allowing them to exploit the greater ability of their heterospecific group mates to locate food resources or detect approaching predators. For social animals, other group members are a source of information about the environment, both via active signaling and communication and through their reactions to external stimuli. The behaviour of one fish may provide another fish with information concerning aspects of environment of which they are unaware, and learned behaviour may be influenced by interactions with others through social learning. Fish may form mixed-species groups both passively, when different species come together to exploit resources within a given area and actively, when individuals are drawn to and preferentially associate with heterospecifics.

The ability to associate with individuals on the basis of familiarity (previous experience with them) has been reported in a range of fish species. Such preferences may lead to a reduction in predation risk, perhaps facilitated by the faster and more effective flow of information between familiar fish (Swaney et al., 2001). Fathead minnows (*Pimephales promelas*) which associate with familiars are also known to reduce investment in epithelial alarm substance cells, possibly in response to a perceived reduction in predation risk (Wisenden and Smith, 1998). Further, familiarity among group members are thought to stabilize dominance hierarchies in trout (*Salmo trutta*) and provides both antipredator and foraging benefits, due to direct consequence of reduced aggression (Griffiths et al., 2004). Similarly, familiarity reduces the intensity of competitive interactions in three-spined stickleback, (*Gasterosteus aculeatus*) (Utne-Palm and Hart, 2000) and shoals of familiar sticklebacks forage more effectively than shoals of unfamiliar fish (Ward and Hart, 2005). In addition, familiarity has been shown to enhance social learning in guppies (*Poecilia reticulata*) (Swaney et al., 2001).

The role of familiarity in mediating shoal choice is constrained by the ability of individuals to recognize familiars. Preferences for familiar group members might be based on learning individually distinctive phenotypes (Ward and Hart, 2003; D' Ettorre and Heinz, 2005). Alternatively, this preference may be based on learning familiar phenotype, such as familiar habitat odour cues (Ward et al.,2005b & 2007), familiar colour cues (Engeszer et al., 2004; Spence and Smith 2007) or familiar species cues (Warburton and Lees, 1996). However, the degree to which social behaviour is phenotypically plastic is mainly influenced by conditions that an individual experience during early development (Chapman et al., 2008). Another important factor that controls the expression of familiarity is the group-size. The tendency of female guppies to shoal with familiar fish declined as the group-size in which they naturally live increases (Griffiths and Magurran, 1997a).

Thus association with familiars is known to convey significant benefits similar to the advantages gained from grouping with siblings. In this context it is very interesting to estimate the strength of preference for shoaling with familiar individuals when the choice is between familiar siblings *vs*. familiar conspecifics/heterospecifics. A closer examination of the literature, however, suggests that the role of familiarity in shoal choice is less well demonstrated than is often claimed. A number of studies have used fish collected from the wild and distinguished as 'familiar' those individuals that are associating together when caught. An observed preference for these 'familiars' does not rule out the possibility that some factors other than familiarity may be mediating shoal choice both in the wild and the test environment. A more robust test involves generating familiar groups at random in a laboratory environment and testing their preferences.

C) Isolation and shoal choice

Animals acquire skills and knowledge from other animals, and fish are no exception. Socially transmited learning is described as the process whereby animal 'B' learns "aspects of behavioral similarity" from conspecific 'A' (Whiten and Ham, 1992). It is generally assumed that social learning is beneficial because naïve individuals can quickly and efficiently acquire locally adaptive behaviour from more knowledgeable individuals, for instance, without having to incur the costs of exploration or the risks of learning about predators. The clearest way to demonstrate the importance of certain social stimuli for the normal development of an organism is to deprive them of such stimuli and observe the resulting deficits. Social isolation beginning early in development has marked effects on adult behaviour. Rats, isolated as early as 14 days after birth show normal sexual behavior, although neonatal isolation disrupts adult mating behavior (Gruendel and Arnold, 1969). Isolated male guinea pigs, cats, and dogs exhibit poorly organized sexual behavior patterns and rhesus monkeys reared in isolation show profound disruption of adult social behavior (Davis and Kassel, 1975). In some birds the development of a juvenile social attachment influences adult sexual behavior. Male ducks, geese, turkeys, and zebra finches selectively court females of the imprinted species. Sex differences in avian sexual imprinting could be related to external sexual dimorphism (Schein, 1963).

Throughout the scientific history, for a long period social learning was assumed to be seen in or even restricted to 'intelligent' or 'large brained' taxa. There have been only a few investigations on early social experience and the ontogeny of species-typical behavior in fishes. The available results indicate that specific social interactions are not necessary for reproductive development. Lifelong social isolation does not block the development of sexual behavior in male platyfish (*Xiphophorus maculatus*) (Shaw, 1962). Briefer reports on experiments in threespined stickleback (*Gasterosteus aculeatus*), African jewelfish (*Hemichromis imaculatus*) and Siamese fighting fish (*Betta splendens*) and paradise fish (*Macropodus opercularis*) (Tinbergen, 1953; Kuo, 1960) also indicate that social behavior develops in individuals which are isolated from the time of hatching. However, there are now strong experimental evidences that many species of fish exhibit social learning in much traditional behaviour such as antipredator behaviour, migration and orientation, foraging, mate choice etc (Brown and Laland, 2003). The way in which a population's antipredator behaviour is modified, when animals are isolated from predators depends on its underlying developmental mechanisms. Experience dependent behavior may be lost after the isolation. In contrast, more 'hard-wired' antipredator behavior may persist for years following isolation (Berger et al., 2001; Blumstein, 2002). Similarly, foraging decisions involve the interplay between several distinct systems of learning and memory, including those that relate to habitat, food patches, prey types, conspecifics and predators (Warburton, 2003). A study by Ryer and Olla (1999) demonstrated that isolated juvenile chum salmon, *(Oncorhynchus keta*) when visually exposed to conspecifics feeding group gained more information about foraging opportunities and ate more.

Social environment is also implicated as an important factor in shaping the ontogeny and evolution of fish brain architecture. Social isolation was found to decrease the number of new neurons in the dentate gyrus of prairie voles (*Microtus ochrogaster)* (Fowler et al*.,* 2002), while greater social complexity increased neuronal recruitment in birds (Adar et al., 2008). In comparison to mammals, fish have a much higher prevalence and rate of adult brain cell proliferation (Grandel et al., 2006; Kuroyanagi et al., 2010). As in mammals, the rate of adult brain cell proliferation in fish can be regulated by external factors including environmental complexity and interaction with conspecifics. For instance, a study by Sorensen et al. (2011) have recently revealed that the stress experienced by subordinate rainbow trout in social hierarchies leads to a marked suppression of brain cell proliferation in the telencephalon and that is accompanied by an increase in plasma levels of cortisol. Similarly, when nine-spined sticklebacks (*Pungitius pungitius*) from two marine and two pond populations were reared in the laboratory from eggs to adulthood either individually or in groups, group reared fish from both pond and marine populations developed larger tecta optica and smaller bulbi olfactorii than individually reared fish (Gonda et al., 2009).

Even though some works have been already conducted to analyse the recognition strategies of isolated fish to their kin and conspecifics, very few studies had been conducted to analyse the group size recognition ability of an isolated fish. Utne-Palm (2001) has shown that brown trout, which acquired familiarity with conspecifics, gradually lost their acquaintance within 4 weeks of isolation. However, European minnows (*Phoxinus phoxinus*) remembered their familiar conspecifics even living in isolation for six months (Bhat and Magurran, 2006). Additionally, studies using Atlantic salmon (*Salmo salar*) have shown that that isolation from the egg stage makes the fish unable to recognize their conspecifics due to the underdevelopment of templates resulting from the lack of social stimuli (Olsen and Winberg, 1996). Arctic charr (*Salvelinus alpinus*) reared in isolation from egg stage do not discriminate kin from non-kin (Olsén and Winberg, 1996), suggesting that the recognition template by which fish discriminate siblings from unrelated individuals is probably learned.

Materials and methods

Fish used in the present study were laboratory bred offspring of adult fish collected from from the fields of Irinjalakuda (10^{o}25’, 10^{o}18’47" N lat. and 76^{o}17’19", 76^{o}12’48"E long.), Thrissur district, India, during January – March 2009. The focal fish were offspring of *Puntius sarana subnasutus* from a single breeding pair collected from Avittathur pond, Irinjalakuda. In order to avoid relatedness and familiarity among breeding pairs, the stimulus group conspecific (*Puntius sarana subnasutus*) and heterospecifics (*Puntius parrah* and *Anabas testudineus*) parental pairs were collected from another pond (Konthulapuram) in the same locality but separated by more than 10km from Avittathur pond. The fishes were acclimatized with laboratory conditions for two weeks in large cement tanks (175cm x 90cm x 90cm). The tanks were provided with sand substratum and water level was maintained at 60 cm. The temperature in the laboratory was constant at 26^{0}C, with a constant light : dark cycle of 12 : 12. The larvae were produced by the technique of induced breeding using GnRH analogue ovaprim. Under laboratory conditions, they were fed *ad libitum* with commercially available tropical fish food (Marvel feeds, Aquarium systems, India).

Shoaling preferences were tested following standard binary-choice procedure (Refer Chapter 2 of the thesis). Twenty sibling fish from each of the four rearing groups were used for each experimental analysis. As the previous experiments have established a strict preference of *P.sarana subnasutus* for a larger group of their siblings, the present study analysed the trade-off of this behaviour with respect to changes in rearing environment, genetic relatedness and group-size of stimulus shoals. It tested the preference of focal fish, for a sibling stimulus group when exposed simultaneously to their sibling and conspecifics (*P.sarana subnasutus* offspring of a different parental pair) or heterospecific (*P. parrah* from same genus) or for a more distantly related *A.testudineus* stimulus groups in five different ratios.

Rearing of Larvae and Experimental Set-up

A: Larvae of a single breeding pair of *P. sarana subnasutus*

On the second day of hatching, larvae (siblings) of *P.sarana subnasutus* (Avittathur breeding pair), were divided into four groups of 10, 20, 30 and 40 individuals and reared in four separate aquaria (20cm x 23cm x 23cm), (40cm x 23cm x 23cm), (60cm x 23cm x 23cm), (80cm x 23cm x 23cm) respectively. This experimental set-up was replicated thrice. Apart from this, another set of 150 larvae of the same breeding pair were reared separately in three large glass tanks (85cm x 32cm x 32cm) (each containing fifty individuals) without any visual or olfactory interventions from the experimental group. They served as stimulus groups of siblings in the experiments. Similarly, the larvae of *P. sarana subnasutus*, *P. parrah* and *A.testudineus* (from breeding pairs collected from

Konthulapuram pond) were also reared separately and they served as stimulus shoal of conspecfic/heterospecifc in the present study. Special care was taken to avoid the development of any kind of familiarity between the experimental group and stimulus group during the rearing period. Fish larvae were fed with *Artemia* nauplius *ad libitum*. The first set of experiments was conducted when the larvae were three months old. After three months the experimental groups were transferred to larger aquaria based on their size and the whole set of experiments were repeated when they were 8 months old (Table 6 A(1).

The first set of experiments have analysed the kin recognition ability of *Puntius sarana subnasutus* by providing them an opportunity to select between siblings and conspecifics/heterospecifics. The study assessed the following three aspects of shoal preference:

- Whether genetic relatedness among individuals has an overriding influence on group-size
- Whether the number of individuals in the rearing environment (immediate surrounding) and subsequent social information transfer affect association preference and group-size recognition ability of the test fish
- Whether sexual maturity influence shoaling preference of the fish

Experi-ment	Stimulus shoal	Subset Experiments	Stimulus shoal group-size			Stimulus shoal Ratio
I: Group of 10 siblings	Sibling *vs.* Conspecific	TEST FISH (n=20)	SIBLING (*P.sarana*)		CONSPECIFIC (*P.sarana*)	
		i	25	*vs.*	25	1:1
		ii	20	*vs.*	30	1:1.5
		iii	15	*vs.*	25	1: 2.3
		iv	10	*vs.*	35	1:4
		v	5	*vs.*	45	1:9
	Sibling *vs.* Hetrospecific	TEST FISH (n=20)	SIBLING (*P.sarana)*		HETEROSPECIFIC (*P.parrah)*	
		i	25	*vs*	25	1:1
		ii	20	*vs*	30	1:1.5
		iii	15	*vs*	25	1: 2.3
		iv	10	*vs*	35	1:4
		v	5	*vs*	45	1:9
	Sibling *vs.* Hetrospecific	TEST FISH (n=20)	SIBLING (*P.sarana)*		HETEROSPECIFIC (*A. testudineus)*	
		i	25	*vs*	25	1:1
		ii	20	*vs*	30	1:1.5
		iii	15	*vs*	25	1: 2.3
		iv	10	*vs*	35	1:4
		v	5	*vs*	45	1:9
II: Group of 20 siblings	___ do ___					
III: Group of 30 siblings	___ do ___					
IV: Group of 40 siblings	___ do ___					

Experimental set-up: Table: 6(A)-1

In experiment i), the focal fish is taken from a rearing group composed of 10 individuals and exposed to their sibling and conspecific stimulus shoals in equal number (25 v*s.* 25). Then in subsequent experiment ii to v, we reduced the number of individuals in the sibling stimulus group alternatively increasing the number of individuals in conspecific and heterospecifc stimulus group [20 *vs.* 30; 15 *vs* 35; 10 *vs.* 35; 40 *vs.*10; 5 *vs.* 45 (Ratio – 1:1, 1:1.5, 1:2.3, 1:4 and 1:9)]. In experiment II, III & IV, we followed the same procedure but the test fish is taken from different rearing groups composed of varying number of individuals (20, 30 and 40). Here, the study tested whether an increase in the number of individuals in a genetically less related group (with subsequent decrease in alternate sibling group) can alter the shoaling preference of the test fish. However, in all combinations the total number of individuals in both stimulus shoals remained fifty. All the subset experiments are repeated in 20 individuals from each of the four (10, 20, 30, 40) rearing groups. It was hypothesised that if genetic relatedness has an overriding influence on the group-size preference, the test fish will trade off their preference for a larger group and exhibit associational preference for siblings, when the alternate larger stimulus shoals is composed of genetically more unrelated individuals (conspecfic and heterospecifc).

B: Larvae of *P. sarana subnasutus* reared with either conspecifics or heterospecifics

The larvae were reared from the second day of hatching onwards in four different groups of 10, 20, 30 and 40 individuals along with equal number of conspecifics/heterospecifics in tanks divided into two chambers using transparent, perforated acrylic sheets allowing exchange of visual and olfactory cues but without direct contact [Table: 6B (1)].

Experimental Group	Divisions of experimental group
Group I	10 siblings of P.sarana + 10 Conspecific P.sarana 10 siblings of P.sarana + 10 Heterospecific P.parrah 10 siblings of P.sarana + 10 Heterospecific A. testudineus
Group II	20 siblings of *P.sarana* + 20 Conspecific *P.sarana* 20 siblings of *P.sarana* + 20 Heterospecific *P.parrah* 20 siblings of *P.sarana* + 20 Heterospecific *A. testudineus*
Group III	30 siblings of *P.sarana* + 30 Conspecific *P.sarana* 30 siblings of *P.sarana* + 30 Heterospecific *P.parrah* 30 siblings of *P.sarana* + 30 Heterospecific *A. testudineus*
Group IV	40 siblings of *P.sarana* + 40 Conspecific *P.sarana* 40 siblings of *P.sarana* + 40 Heterospecific *P.parrah* 40 siblings of *P.sarana* + 40 Heterospecific *A. testudineus*

Experimental set-up: Table: 6(B)-1

Experiment	Stimulus shoal(familiar)	Subset Experiments	Stimulus shoal group-size			Stimulus shoal Ratio
Group I: 10siblings reared along with a)10 conspecifics *P. sarana*	Sibling *vs.* Conspecific	TEST FISH (n=20)	SIBLING (*P.sarana*)		CONSPECIFIC (*P.sarana*)	
		i	25	*vs.*	25	1:1
		ii	20	*vs.*	30	1:1.5
		iii	15	*vs.*	25	1: 2.3
		iv	10	*vs.*	35	1:4
		v	5	*vs.*	45	1:9
b) 10 heterospecifics *P.parrah*	Sibling *vs.* Hetrospecific	TEST FISH (n=20)	SIBLING (*P.sarana*)		HETEROSPECIFIC (*P.parrah*)	
		i	25	*vs.*	25	1:1
		ii	20	*vs.*	30	1:1.5
		iii	15	*vs.*	25	1: 2.3
		iv	10	*vs.*	35	1:4
		v	5	*vs.*	45	1:9
b) 10 heterospecifics	Sibling *vs.* Hetrospecific	TEST FISH (n=20)	SIBLING (*P.sarana*)		HETEROSPECIFIC (*A. testudineus*)	
		i	25	*vs.*	25	1:1
		ii	20	*vs.*	30	1:1.5
		iii	15	*vs.*	25	1: 2.3
		iv	10	*vs.*	35	1:4
		v	5	*vs.*	45	1:9
Group II:	20siblings reared along with 20 conpecifics/heterospecific ___ do ___					
Group III:	30siblings reared along with 30 conspecifics/heterospecific ___ do ___					
Group IV:	40siblings reared along with 40 conspecifics/heterospecific ___ do ___					

Experimental set-up: Table: 6(B)- 2

Apart from this another set of 150 larvae of all the four breeding pairs were reared separately in three large glass tanks (each containing fifty individuals) without any visual or olfactory interventions from the experimental group which served as stimulus group. Fish larvae were fed with *Artemia* nauplius *ad libitum*. The experiments are conducted when the larvae were 3 and 8 months old.

Shoaling preferences is tested in a 70 liter aquarium (60cm x 23cm x 23cm) following the standard binary-choice procedure. Larvae of *Puntius sarana subnasutus* is simultaneously exposed to familiar siblings and familiar conspecifics/ heterospecific stimulus groups [Conspecific: *Puntius sarana subnasutus* off spring of a different parental pair, Heterospecific: *Puntius parrah,* from same genus or *Anabas testudineus,* from a more distantly related group] in five different ratios. Twenty sibling fish from each of the twelve rearing groups were used for each subset of experiments. The experiments were conducted when they were 3 months and 8 months old [Table 6 B(2)].

The present study analysed the trade-off between genetic relatedness and group-size when the stimulus shoal is familiar to the test fish. It assessed the following three aspects of shoaling preference:

- The tradeoff between sibling preference and familiarity with the preference for a larger group in *Puntius sarana subnasutus*
- Whether the number of individuals in the rearing environment (immediate surrounding) and subsequent social information transfer affect association preference and group-size recognition ability of the test fish
- Whether sexual maturity influence shoaling preference of the fish.

C: Larvae of *P. sarana subnasutus* reared in isolation

In order to study the effects of isolation on sibling recognition and group-size discrimination ability of *Puntius sarana subnasutus*, The larvae were reared in complete isolation from the second day of hatching onwards. The study assessed the following aspects of the effect of isolation on shoaling preference of *P.sarana subnasutus*:

- Whether the isolated individuals are able to recognize their siblings from conspecifics/heterospecifics

- Whether the individuals reared in total isolation are capable of distinguishing the group-size (number) difference between two sibling stimulus shoals
- Effects of isolation and changes on brain architecture and physiology

Experimental procedure:

I) BEHAVIOURAL ASSAY

Siblings of *Puntius sarana subnasutus* were reared in an entirely isolated environment from the second day of hatching onwards. More than hundred healthy siblings were selected and allocated them to hundred separate glass containers of 500 ml capacity. All sides of the containers are then properly covered using black paper in order to avoid any visual contact with the neighbours. The entire behavioural assays were conducted as binary choice experiments. The first set of experiments, compared the sibling recognition ability of the test fish isolated for one month, isolated for three months with that of a control group (individuals reared with ten other siblings). Here, the stimulus shoals consisted of equal number of individuals. Sibling shoal is placed in one of the stimulus chamber and conspecifics (*Puntius sarana subnasutus,* of another breeding pair) or heterospecifics (*Puntius parrah/ Anabas testudineus*) is placed in the other stimulus chamber.

The second set of experiments compaired the ability of isolated individuals (both isolated for one month and three months) to assess shoal size of siblings presented in various numerical combinations (25 *vs. 25;* 30 *vs*. 20; 35 *vs*. 15; 40 *vs.* 10 and 45 *vs*. 5) with that of a control group (individuals reared with ten other siblings). The third set of experiments tested whether isolation at critical period of development (here, an average 45 days ie, three-fifth of the maximum days of larval life: Chambers et al., 2001) influence behavioural repertoire of the test fish during later stages of life. Here, the group-size discrimination abilities of control fish (fish reared in groups of 10 siblings) and permanently isolated fish (for one month) was compared with those i) isolated for first 45days after hatching and then reared in group for 1month and with those ii) reared in group for first 45days after hatching and then isolated for 1 month.

II) COMPARISON OF FOREBRAIN USING SCANNING ELECTRON MICROSCOPY

The fore brain of the fish, both the isolated test fish as well as the control fish, were examined using scanning electron microscopy (SEM centre, NIT, Calicut) and photographed 10 kV 32.4mm X 500SE.

III) ANALYSIS OF THE PHYSIOLOGICAL CHANGES

Glucose: Plasma glucose is measured using commercial GOD/POD test kit (based on the hexose kinase method), Span Diagnostics Ltd., New Delhi. The concentration of glucose was measured colourimetrically (in mg/dl) in a Systronic Spectrophotometer 2202 (Systronics, New Delhi)

T4: Plasma T4 concentrations were measured by Electro Chemi Luminescence Immuno Assay (ECLIA) using Elecsys T4 reagent kit, based on a competitive antibody binding test principle on Elecsys 1010/2010 and ‘cobas e’ 411/601 immunoassay analyzers. Results were determined via a calibration curve instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

Cortisol: Plasma cortisol concentrations were also measured similarly by Electro Chemi Luminescence Immuno Assay (ECLIA) using Elecsys T4 reagent, based on a competitive antibody binding test principle on Elecsys 1010/2010 and ‘cobas e’ 411/601 immunoassay analyzers. Results were determined via a calibration curve which instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

In all behavioural assays data were analyzed using parametric dependent sample two-tailed ‘t’ test [SPSS 11.0.1 statistical package].

RESULT:

6.A: SHOALING PREFERENCE OF *PUNTIUS SARANA SUBNASUTUS* REARED WITH SIBLINGS (10, 20, 30 AND 40 INDIVIDUALS) TO STIMULUS SHOAL

6. A: I a) Shoaling preference of larvae of *P. sarana subnasutus* to stimulus shoal of their siblings and conspecific *P. sarana subnasutus* at 3 months

The results indicate that early social ambience and age have considerable influence on shoaling preference of the larvae of *Puntius sarana subnasutus*. It should be noted that all test fishes are reared with their siblings till the time of experiments (3 months). At three months of age, the test fish larvae reared at population density of 10, 20, 30 and 40 preferred sibling shoal when the size of the shoal is equal (**25 conspecifics *vs.* 25 sibling and 30 conspecifics *vs.* 20 siblings**; at the ratio of 1:1 and 1:1.5). The behaviour of the larvae of *Puntius sarana subnasutus* changed, when the number of the conpecifics increased and the number of siblings decreased in the stimulus shoals **(35 conspecifics *vs.* 15 siblings; 40 conspecifics *vs.* 10 siblings and 45 conspecifics *vs.* 5 siblings; at ratio 1:2.3, 1:4 and 1:9)**. The larvae reared in group of 10 and 20 siblings exhibited slight preference for larger conspecifics shoal in a binary choice test between siblings and conspecifics at shoal size **35 conspecifics *vs.* 15 siblings**. But test fish reared with 30 and 40 other siblings preferred their siblings, when the stimulus shoal size is **35 conspecifics *vs.*15 siblings**. It is interesting to note that test fish reared along with 10, 20 and 40 other siblings during their initial days of life, exhibited drastic change in their preference and spent significantly more time near the larger shoal composed of conspecifics when the stimulus shoals size is **40 conspecifics *vs.* 10 siblings and 45 conspecifics *vs.* 5 siblings**. Whereas, test fish reared along with 30 siblings during their initial days of life exhibited almost equal preference to stimulus shoals composed of conspecifics and siblings, when the stimulus shoals size consisted of **40 conspecifics *vs.* 10 siblings and 45 conspecifics *vs.* 5 siblings**. These results clearly indicate that early rearing conditions and social ambience profoundly influence the shoaling preference of *Puntius sarana subnasutus* [Fig. 6A (1-4); Table 6A (2)]

6A (1): Test fish - Larvae of *Puntius sarana subnasutus* reared with 10 siblings for 3 months

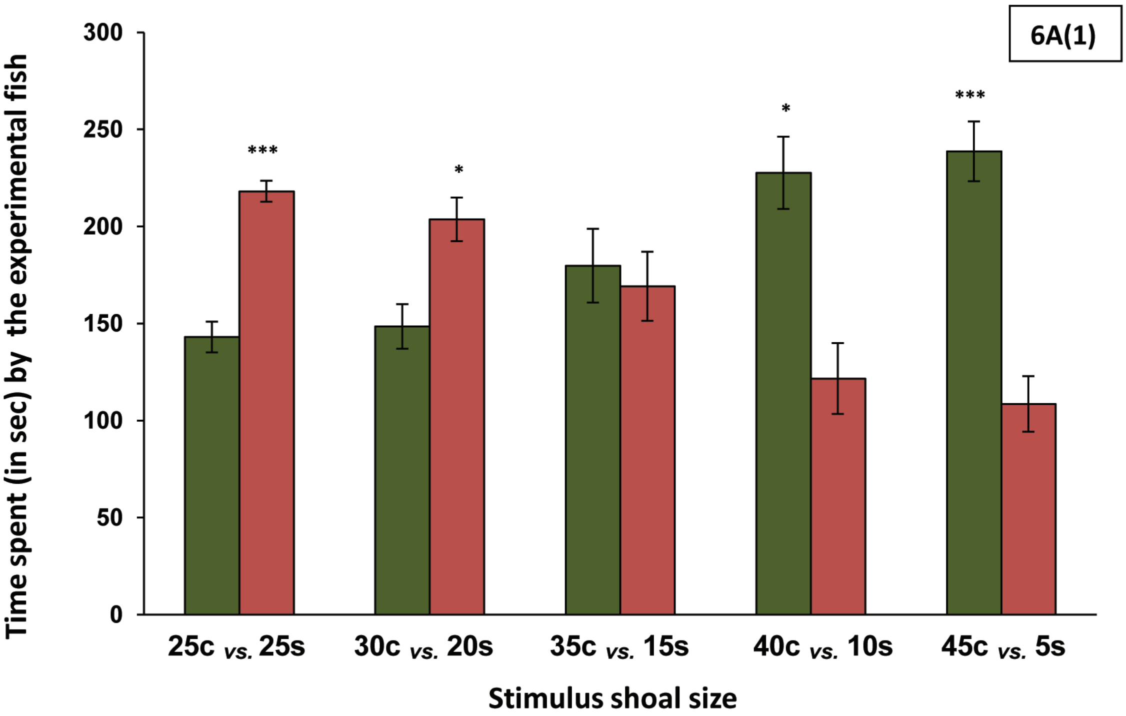


Figure 6A (1): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6A (2): Test fish - Larvae of *Puntius sarana subnasutus* reared with 20 siblings for 3 months

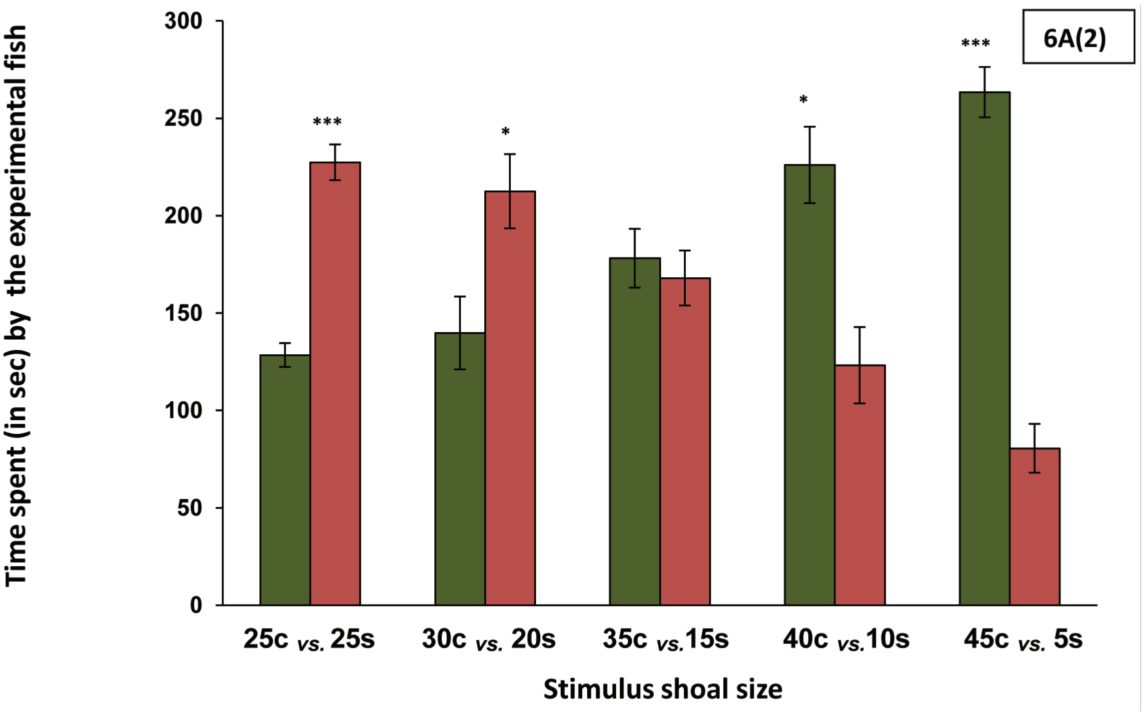


Figure 6A (2): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6A (3): Test fish - Larvae of *Puntius sarana subnasutus* reared with 30 siblings for 3 months

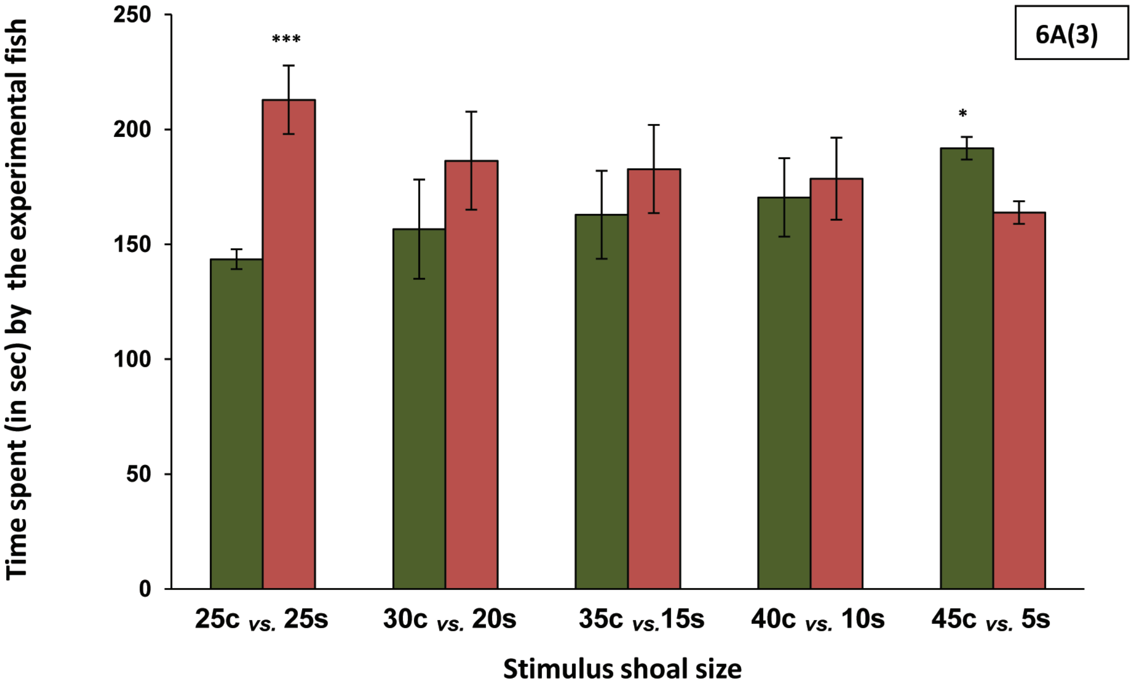


Figure 6A (3): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6A (4): Test fish - Larvae of *Puntius sarana subnasutus* reared with 40 siblings for 3 months

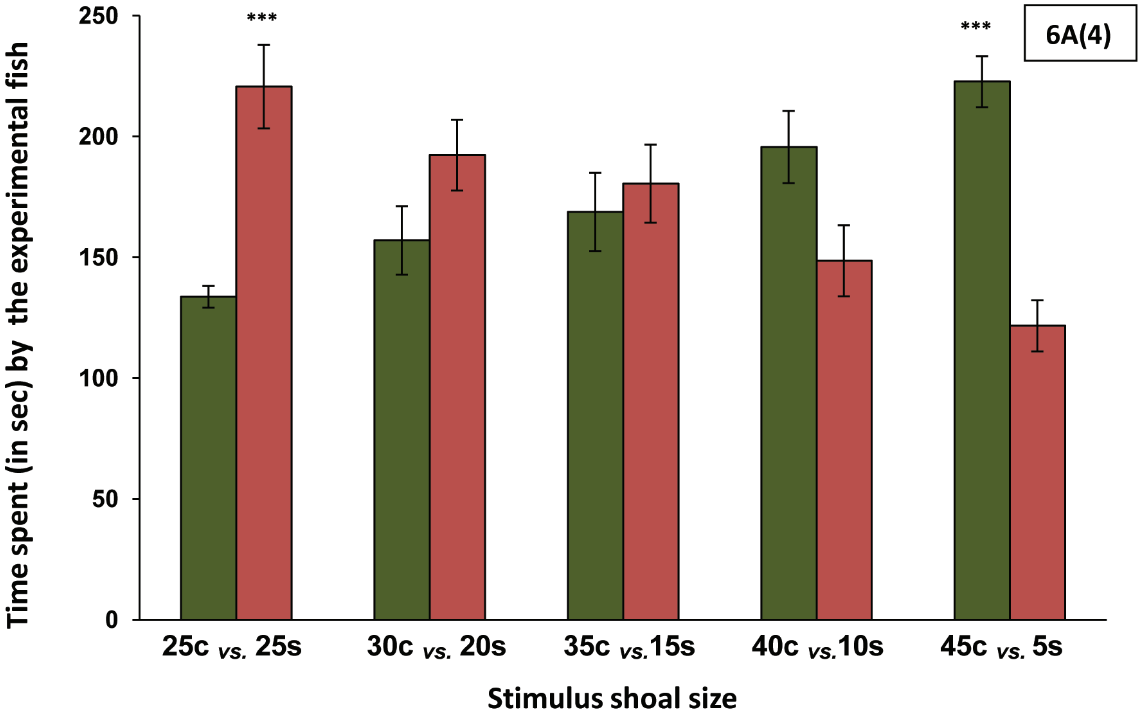


Figure 6A (4): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6. A: I b) Shoaling preference of larvae of *P. sarana subnasutus* to stimulus shoal of their siblings and conspecific *P. sarana subnasutus* at 8 months

Larvae of *Puntius sarana subnasutus* reared in **group of 10 siblings** for 8 months, preferred to stay near sibling shoals, when the stimulus shoal size is **25 conspecifics *vs.* 25 siblings; 30 conspecifics *vs.* 20 siblings; 35 conspecifics *vs.* 15 siblings**. When the shoal size of the conspecifics was increased to **40 conspecifics *vs.* 10 siblings and 45 conspecifics *vs.* 5 siblings**, the test fish significantly preferred the larger shoal.

The test fish reared in **group of 20 siblings** for 8 months preferred to stay near sibling shoals when the stimulus shoal size is **25 conspecifics *vs.* 25 siblings**. They exhibited almost equal preference for stimulus shoals consisted of **40 conspecifics *vs.* 10 siblings**. However, when the stimulus shoal size is **30 conspecifics *vs.* 20 siblings; 35 conspecifics *vs.* 15 siblings and 45 conspecifics *vs.* 5 siblings**, larvae preferred larger shoal of conspecifics to that of sibling shoal. The preference exhibited by the test fish to the larger shoal of conspecifics is very significantly different from that the fish exhibited to siblings.

Larvae of *Puntius sarana subnasutus* reared in **group of 30 siblings** for 8 months exhibited preference for siblings, but significant preference for siblings to conspecifics is seen only when the stimulus shoal size is **25 conspecifics *vs.* 25 siblings; 30 conspecifics *vs.* 20 siblings.** Preference to stimulus shoals is almost equal when the stimulus shoal size is **35 conspecifics *vs.* 15 siblings**. However, the test fish showed significant preference to larger conspecific stimulus shoal to smaller sibling shoal **40 conspecifics *vs.* 10 siblings and 45 conspecifics *vs.* 5 siblings.**

Larvae of *Puntius sarana subnasutus* reared in **group of 40 siblings** for 8 months exhibited preference for siblings, but significant preference for siblings to conspecifics is seen only when the stimulus shoal size is **25 conspecifics *vs.* 25 siblings; 30 conspecifics *vs.* 20 siblings**. Preference to stimulus shoals is not significant when the stimulus shoal size is **35 conspecifics *vs.* 15 siblings and 40 conspecifics *vs.* 10 siblings**. However, the test fish showed significant preference to larger conspecific stimulus shoal to smaller sibling shoal (**45 conspecifics *vs.* 5 siblings)** [Fig. 6A (5-8); Table 6A(2)]

6A (5): Test fish - Larvae of *Puntius sarana subnasutus* reared with 10 siblings for 8 months

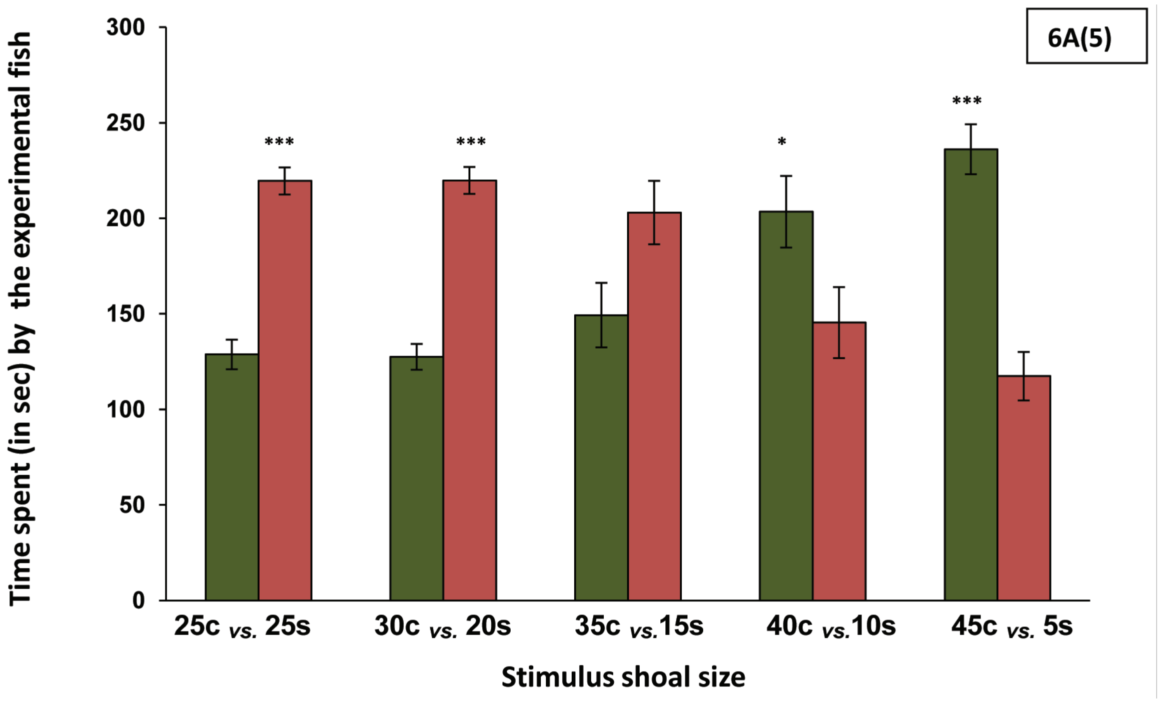


Figure 6A (5) : Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6A (6): Test fish - Larvae of *Puntius sarana subnasutus* reared with 20 siblings for 8 months

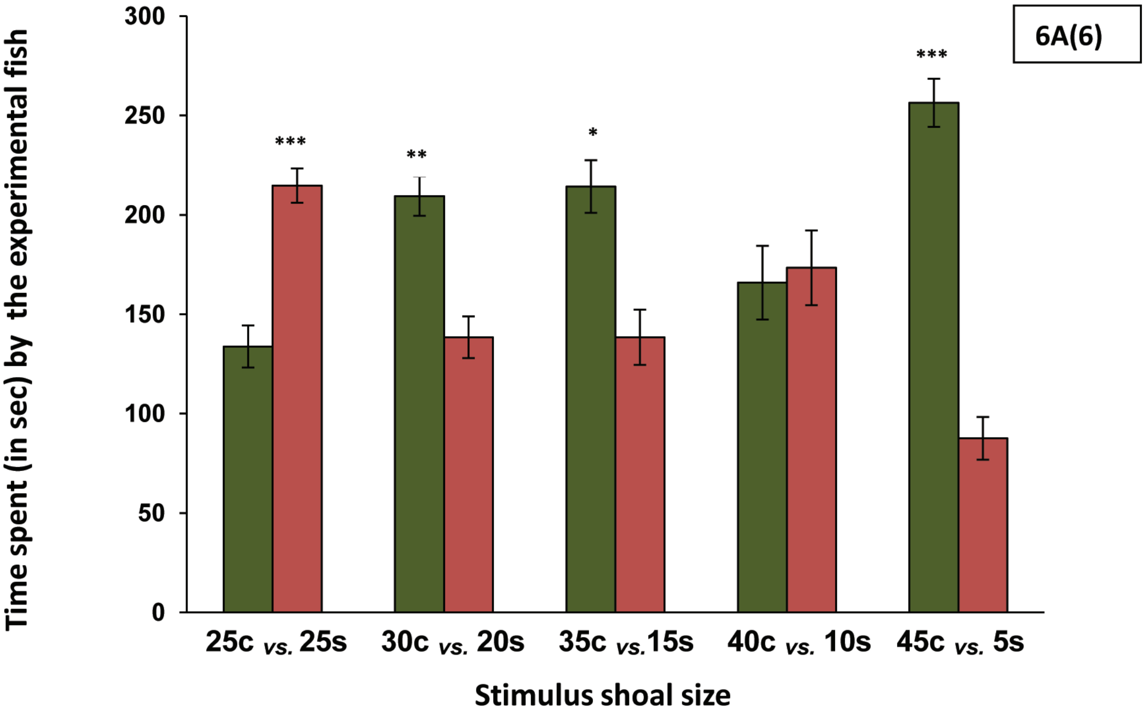


Figure 6A (6) : Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6A (7): Test fish - Larvae of *Puntius sarana subnasutus* reared with 30 siblings for 8 months

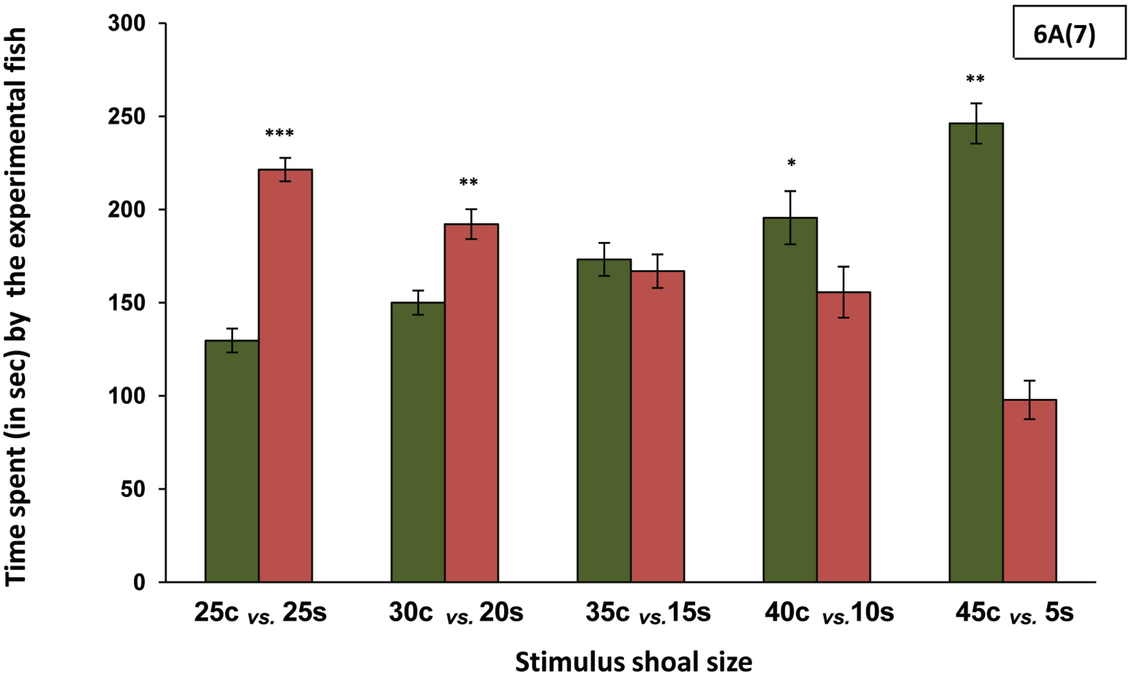


Figure 6 A (7) : Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6A (8): Test fish - Larvae of *Puntius sarana subnasutus* reared with 40 siblings for 8 months

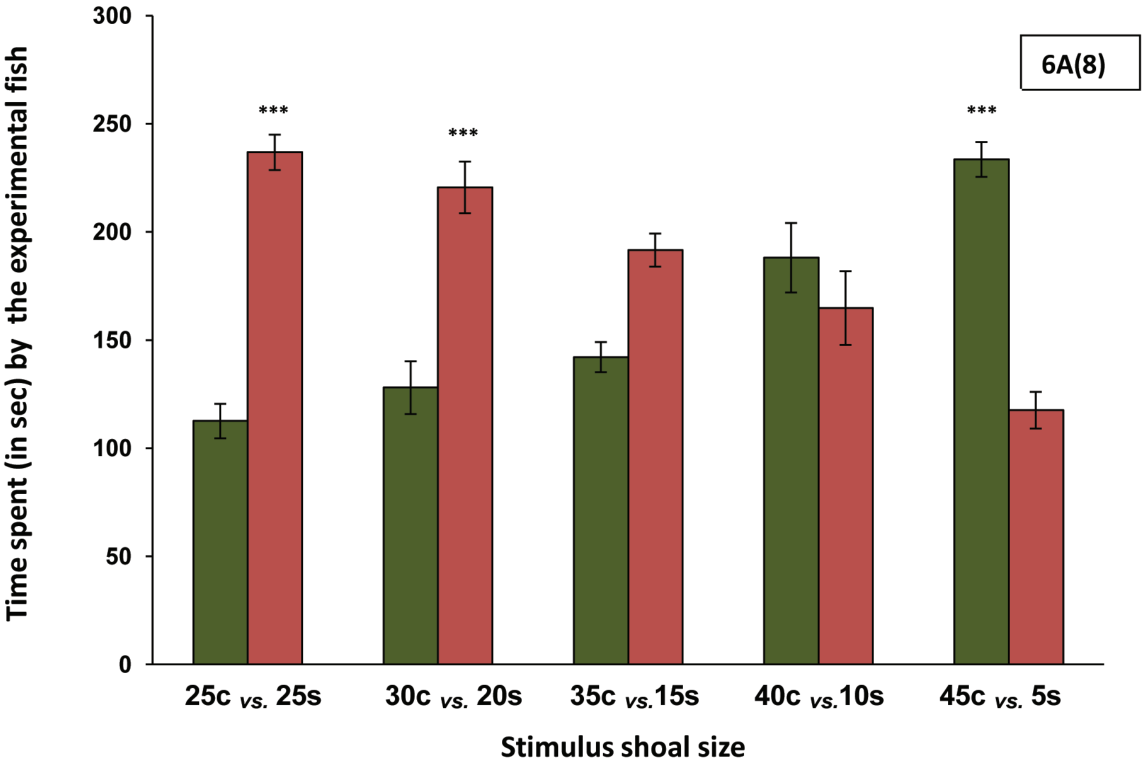


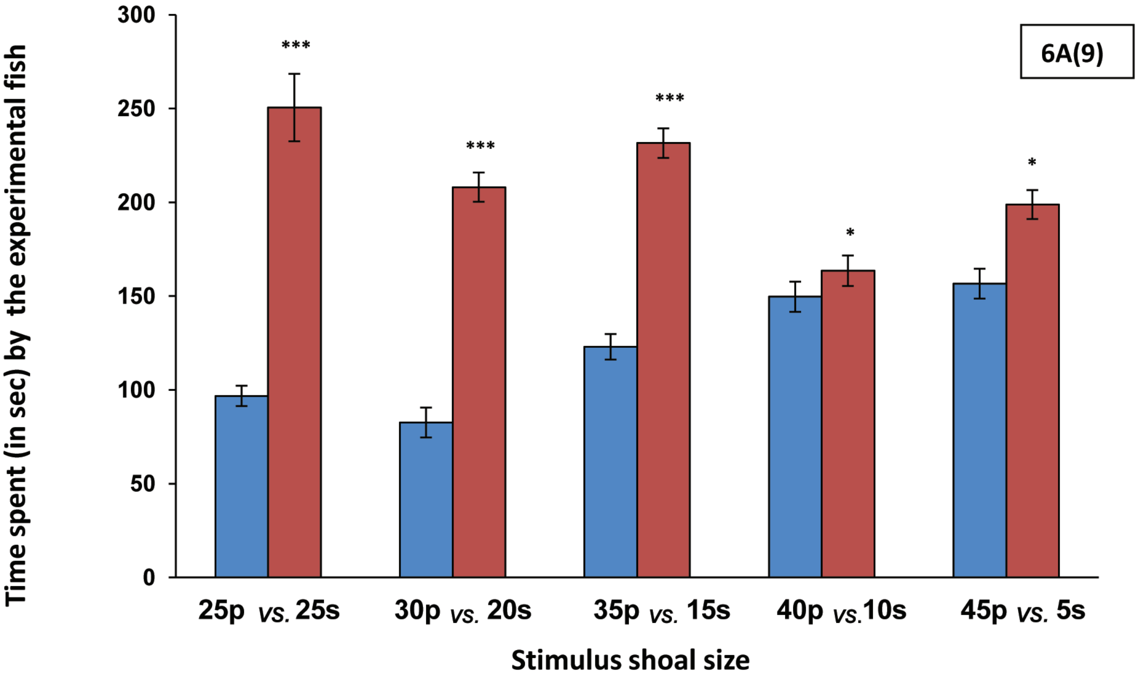
Figure 6A (8): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

Test fish	**Rearing group**	**Age of the larvae**	**Stimulus shoal-size Conspecific group (c) *vs.* Sibling group (s)**				
			25c vs. 25s	***30c vs. 20s***	***35c vs. 15s***	***40c vs. 10s***	***45c vs. 5s***
Larvae reared with individuals of the same breeding pair	**10**	3 Months	t= 6.248 p=0.001***	t= 2.436 p=0.025*	t=0 .289 p=0.776	t= 2.877 p=0.010*	t= 4.423 p=0.001**
		8 Months	t= 6.230 p=0.001***	t= 6.872 p=0.001***	t= 1.441 p=0.166	t= 1.737 p=0.099*	t= 4.711 p=0.001***
	20	3 Months	t= 8.013 p=0.001***	t= 1.931 p=0.069*	t=0.351 p=0.730	t= 2.638 p=0.016*	t= 7.206 p=0.001***
		8 Months	t= 4.318 p=0.001***	t= 3.503 p=0.002**	t= 2.802 p=0.011*	t= 0.200 p=0.843	t= 7.461 p=0.001***
	30	3 Months	t= 6.991 p=0.001***	t= 0.697 p=0.494	t= 0.520 p=0.609	t= 0.234 p=0.817	t= 2.806 p=0.011*
		8 Months	t= 7.322 p=0.001***	t= 3.063 p=0.006**	t=0 .227 p=0.823	t= 2.255 p=0.036*	t= 7.078 p=0.001***
	40	3 Months	t= 9.388 p=0.001***	t= 1.232 p=0.233	t= 0.363 p=0.721	t= 1.592 p=0.128	t= 4.828 p=0.001***
		8 Months	t= 7.695 p=0.001***	t= 3.856 p=0.001***	t= 1.627 p=0.120	t= 1.517 p=0.146	t= 7.140 p=0.001***

Table: 6A (2) Preference of *Puntius sarana subnasutus* to associate with the larger group when the choice is between siblings and conspecifics at 3months and 8 months old stage

6. A: II a) Shoaling preference of larvae of *P. sarana subnasutus* to stimulus shoal of their siblings and heterospecific *P. parrah* at 3 months

Larvae of *Puntius sarana subnasutus* reared for 3 months along with 10, 20, 30 and 40 siblings failed to exhibit any preference to a shoal of heterospecific fish, *Puntius parrah*, even when the number of heterospecific shoal to sibling shoal increased to **40 *vs.* 10 and 45 *vs.* 5**, except the larvae reared with 30 siblings which exhibited significant preference to heterospecific *Puntius parrah* at simulus shoal size **45 *Puntius parrah vs.* 5 *Puntius sarana subnasutus*** (at ratio 1:9) [Fig. 6A (9-12); Table 6A (3)].



6A (9): Test fish - Larvae of *Puntius sarana subnasutus* reared with 10 siblings for 3 months

Figure 6A (9): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (10): Test fish - Larvae of *Puntius sarana subnasutus* reared with 20 siblings for 3 months

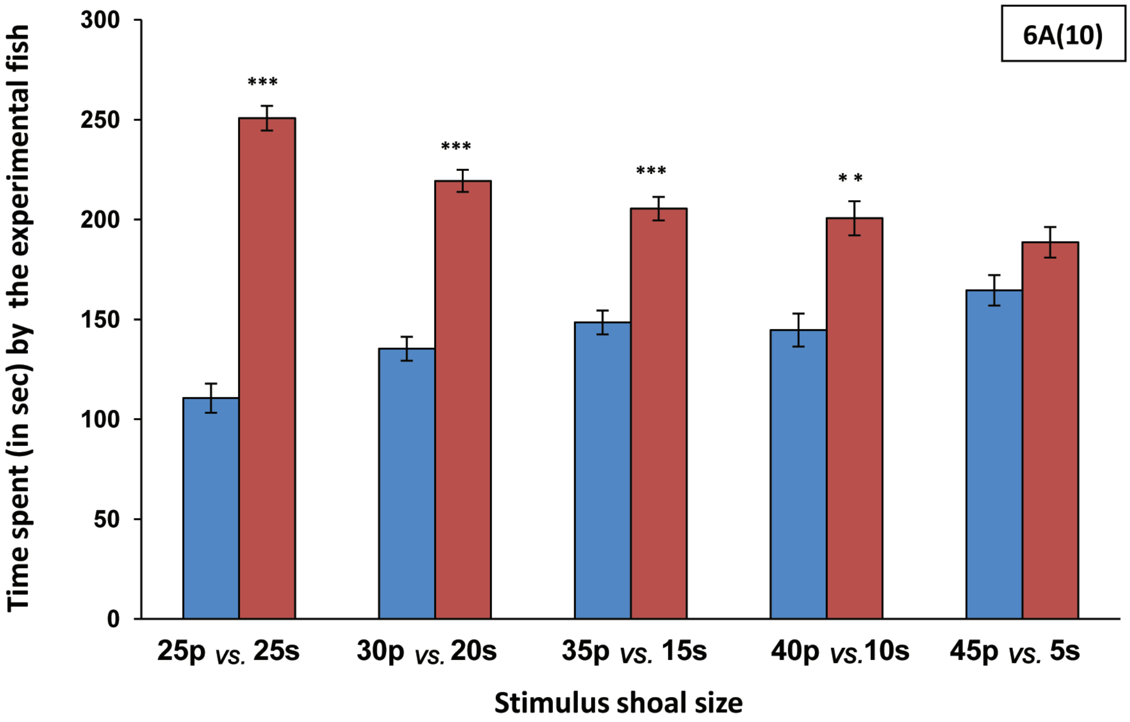


Figure 6A (10): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations.

6A (11): Test fish - Larvae of *Puntius sarana subnasutus* reared with 30 siblings for 3 months

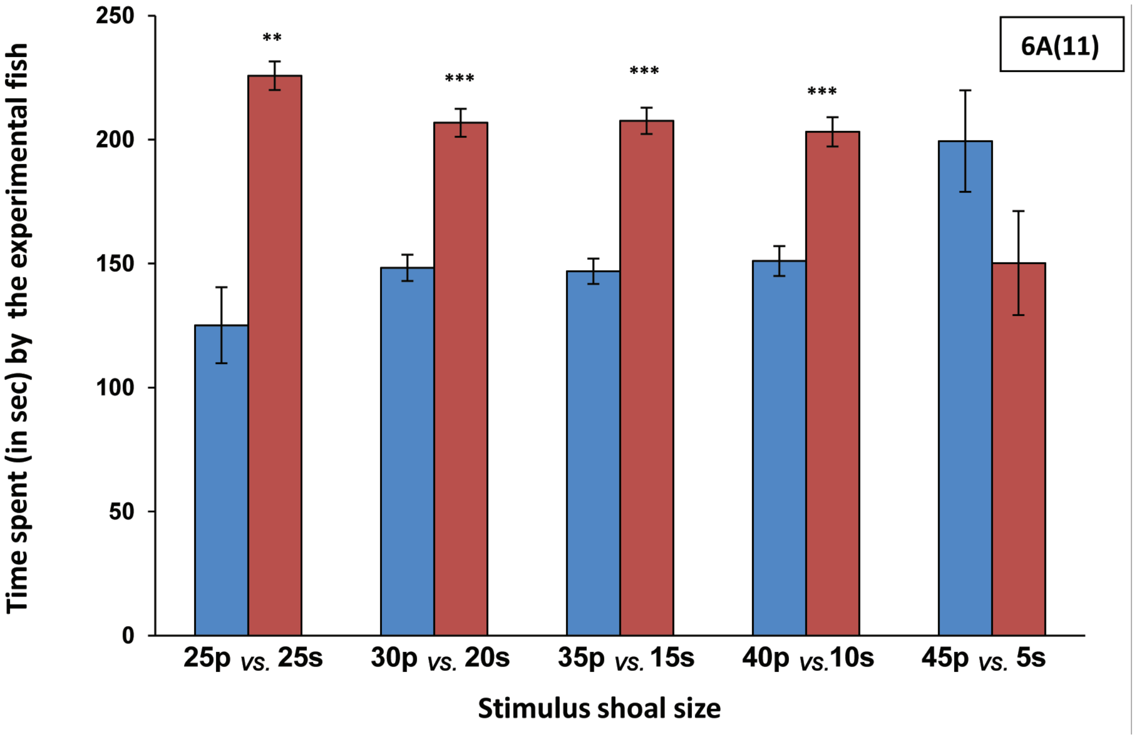


Figure 6(A) 11: Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations.

6A (12): Test fish - Larvae of *Puntius sarana subnasutus* reared with 40 siblings for 3 months

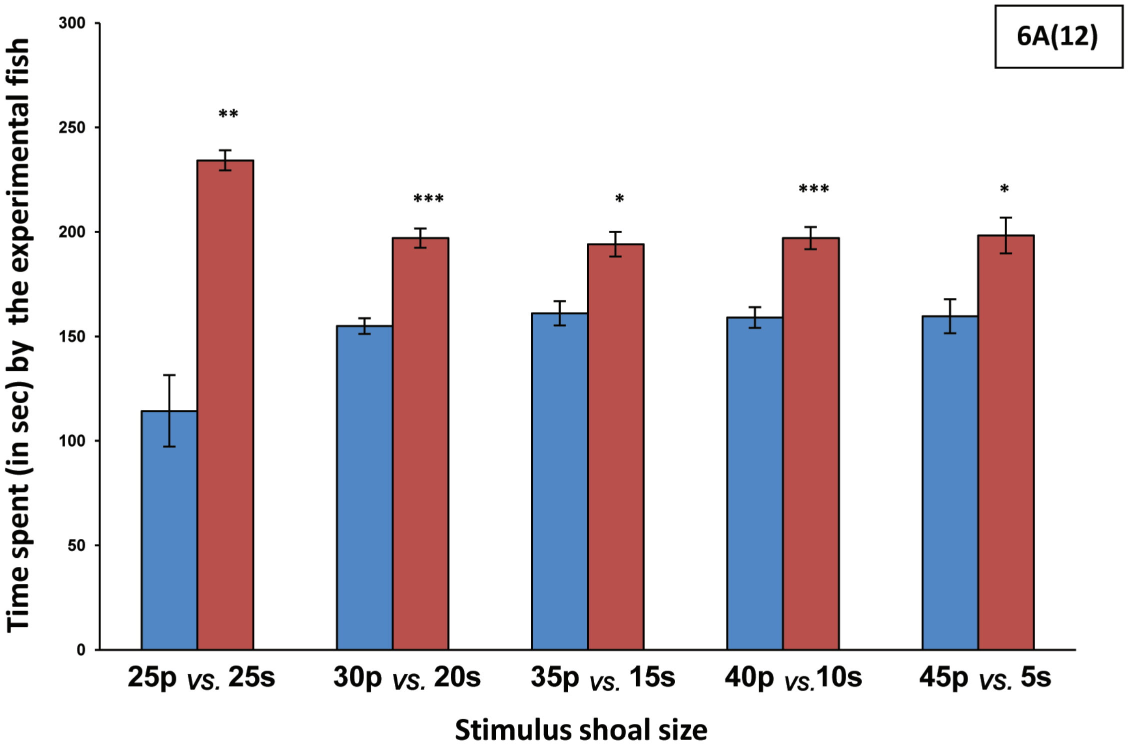


Figure 6A (12): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6. A: II b) Shoaling preference of larvae of *P. sarana subnasutus* to stimulus shoals of their siblings and heterospecific *P. parrah* at 8 months

However larvae of *P. sarana subnasutus* reared for 8 months along with 10, 20, 30 and 40 siblings for 8 months of age showed associational preference to siblings when given a binary choice between siblings and heterspecific, *Puntius parrah* when the shoal size is **25 *vs.* 25; 30 vs. 20; 35 *vs.* 15**. But at shoal size **40 *vs.* 10 and 45 *vs.* 5** the test fish exhibited a tendency to prefer both shoals irrespective of whether the shoal is composed of heterospecifics or siblings [Fig. 6A 13-16; Table 6A (3)].

6A (13): Test fish - Larvae of *Puntius sarana subnasutus* reared with 10 siblings for 8 months

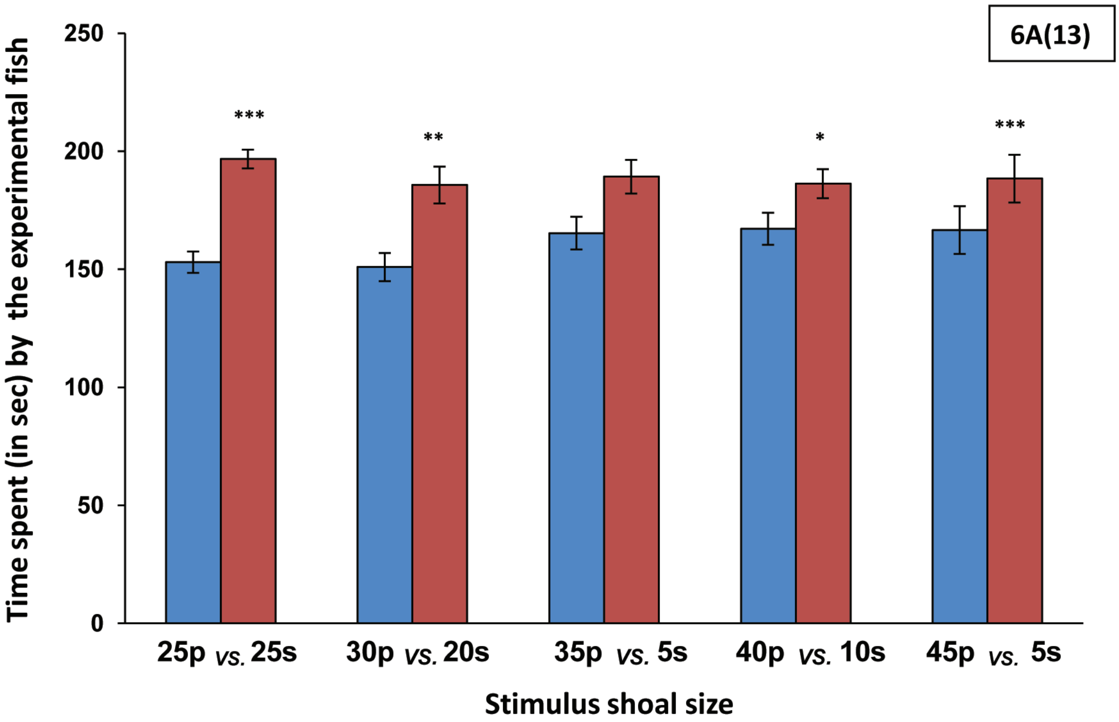


Figure 6A (13): Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P. sarana subnasutus (S)*** in varying numerical combinations

6A (14): Larvae of *Puntius sarana subnasutus* reared with 20 siblings for 8 months

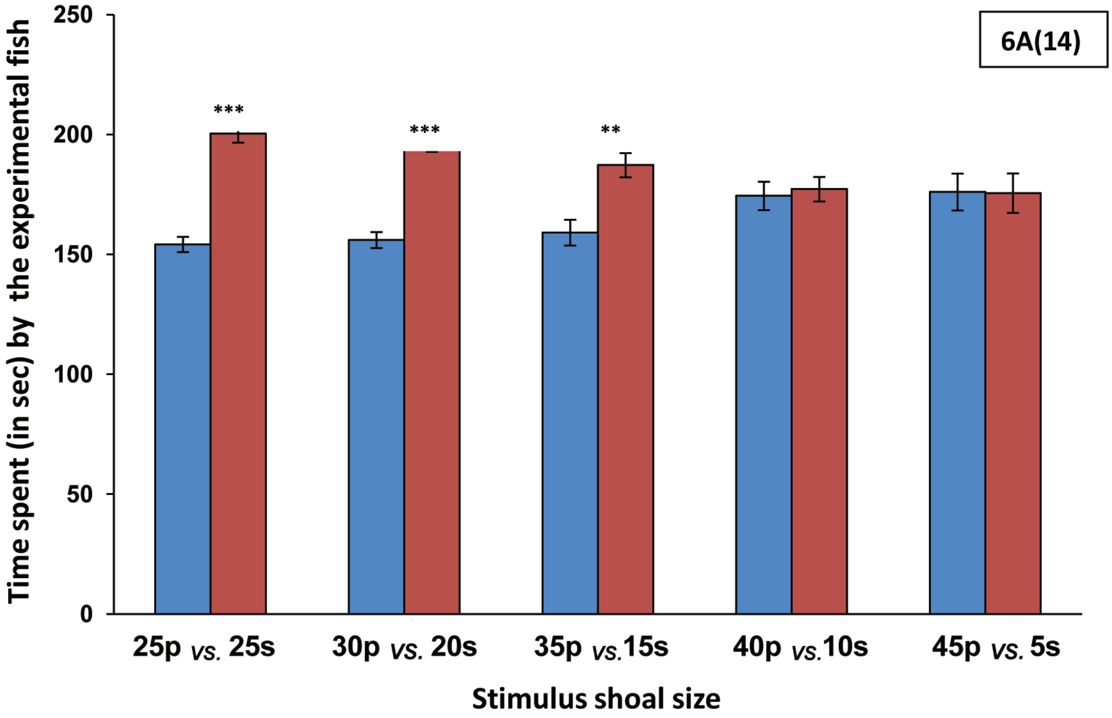


Figure 6A (14): Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (15): Test fish - Larvae of *Puntius sarana subnasutus* reared with 30 siblings for 8 months

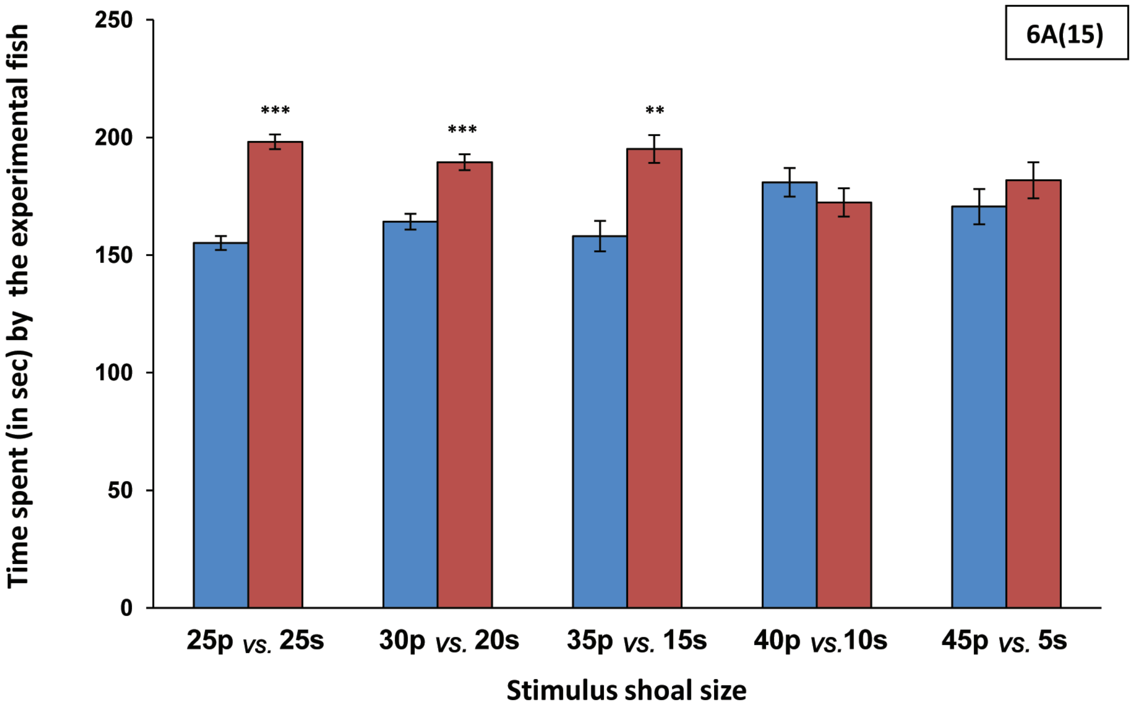


Figure 6A (15): Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (16): Test fish - Larvae of *Puntius sarana subnasutus* reared with 40 siblings for 8 months

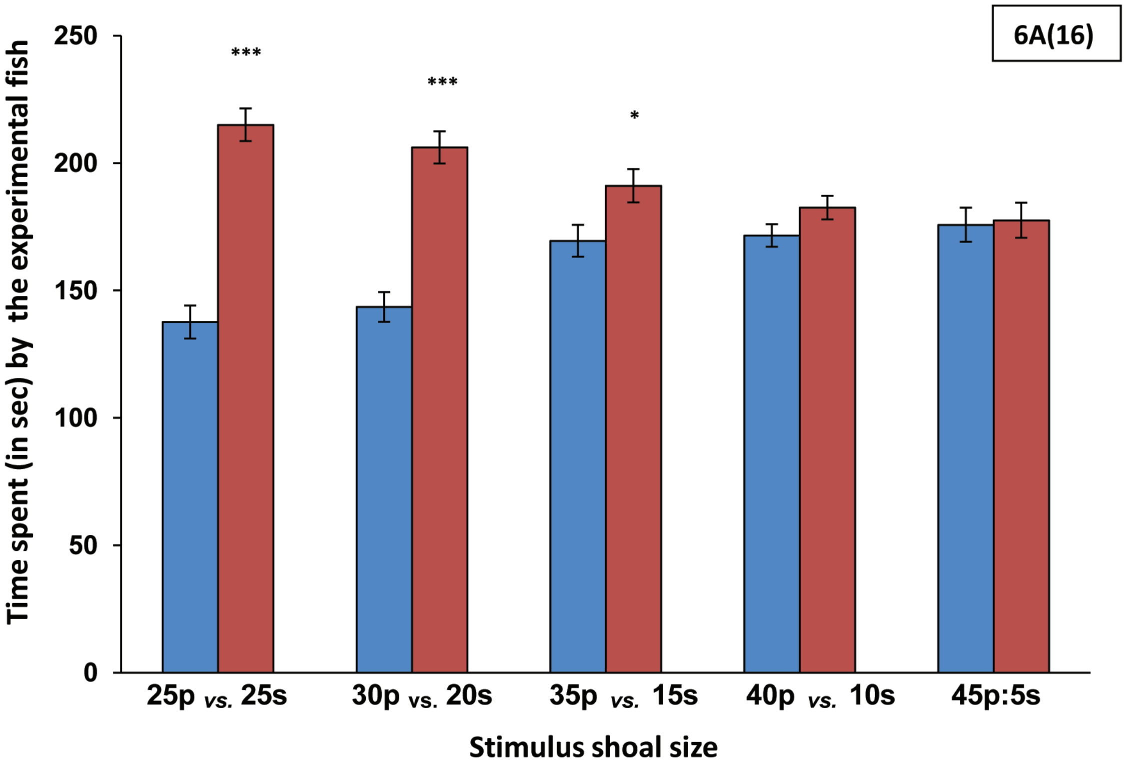


Figure 6A (16): Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***heterosprcific P. parrah (p)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

Test fish	**Rearing group**	**Age of the larvae**	**Stimulus shoal-size Heterospecific *Puntius parrah* (p) *vs.* Sibling group (s)**				
			25p vs. 25s	***30p vs. 20s***	***35p vs. 15s***	***40p vs. 10s***	***45p vs. 5s***
Larvae reared with individuals of the same breeding pair	**10**	3 Months	t= 4.174 p=0.001***	t= 4.338 p=0.001***	t= 6.741 p=0.001***	t= 2.142 p=0.045*	t= 2.692 p=0.014*
		8 Months	t= 6.089 p=0.001***	t= 3.252 p=0.004**	t= 1.715 p=0.103	t= 1.487 p=0.153	t= 1.079 p=0.294
	20	3 Months	t= 9.356 p=0.001***	t= 7.322 p=0.001***	t= 4.827 p=0.001***	t= 3.565 p=0.002**	t= 1.579 p=0.131
		8 Months	t= 6.854 p=0.001***	t= 5.918 p=0.001***	t= 3.546 p=0.002**	t= 0.257 p=0.800	t= 0.032 p=0.975
	30	3 Months	t= 3.367 p=0.003**	t= 5.408 p=0.001***	t= 5.807 p=0.001***	t= 4.370 p=0.001***	t= 1.187 p=0.250
		8 Months	t= 7.107 p=0.001***	t= 3.771 p=0.001***	t= 3.014 p=0.007**	t= 0.707 p=0.488	t= 0.738 p=0.470
	40	3 Months	t= 3.497 p=.002**	t= 5.297 p=0.001***	t= 2.833 p=0.011*	t= 3.790 p=0.001***	t= 2.343 p=0.030*
		8 Months	t= 6.063 p=0.001***	t= 5.768 p=0.001***	t= 1.775 p=0.092*	t= 1.208 p=0.242	t=0 .132 p=0.896

Table: 6A (3) Preference of *Puntius sarana subnasutus* to associate with the larger group when the choice is between siblings and heterospecific *Puntius parrah* at 3months and 8 months old stage

6. A: III Shoaling preference of larvae of *P. sarana subnasutus* to their siblings and heterospecific *Anabas testudineus* at a) 3 months and b) 8 months

It is interesting to note that given a choice between sibling shoal and a shoal of a fish belonging to a different genus, *Anabas testudineus*, *P.sarana subnasutus* reared for 3 months and 8 months along with 10, 20, 30 and 40 siblings exhibited very clear preference for sibling shoal, [**25 heterospecifics *vs.* 25 siblings; 30 heterospecifics *vs.* 20 siblings, 35 heterospecifics *vs.* 15 siblings, 40 heterospecifics *vs.* 10 sibling and 45 heterospecifics *vs.* 5 siblings**] at the ratio of 1:1, 1:1.5, 1:2.3, 1:4 and 1:9. This indicates the ability of the fish to identify the siblings and the overriding influence of genetic relatedness in the context of shoal choice in this fish [Fig. 6A (17-24); Table 6A (4)]

a) Shoaling preference of larvae reared in shoaling group for 3 months

6A (17): Test fish - Larvae of *Puntius sarana subnasutus* reared with 10 siblings for 3 months

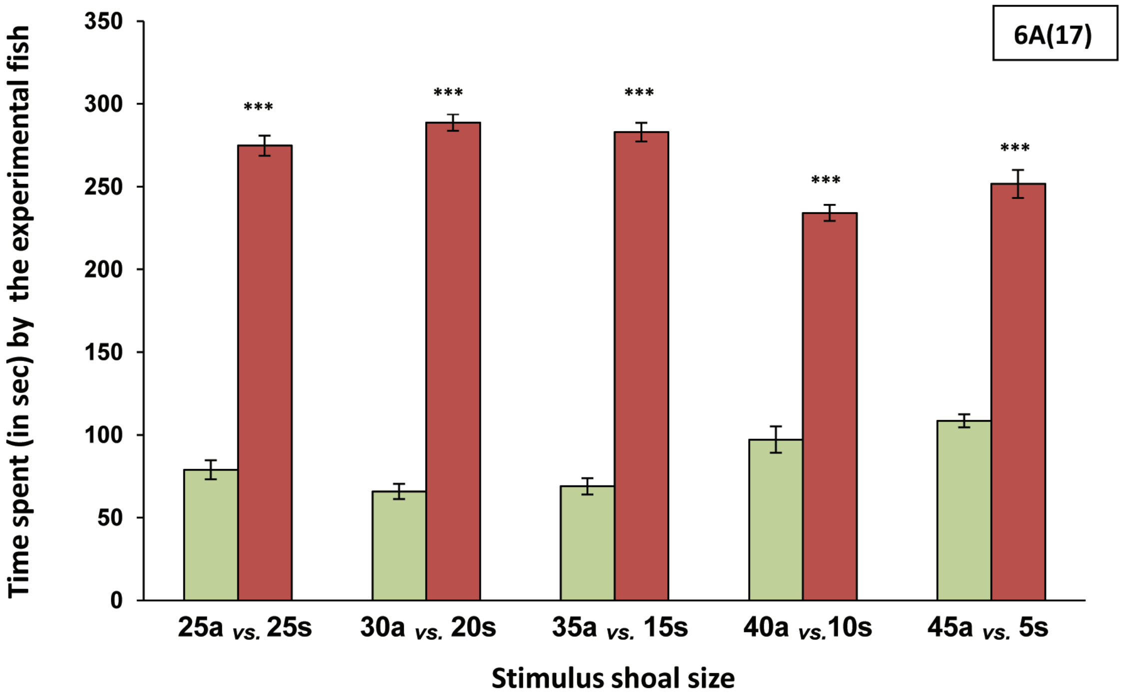


Figure 6A (17): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***Heterospecifics A.testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (18): Test fish - Larvae of *Puntius sarana subnasutus* reared with 20 siblings for 3 months

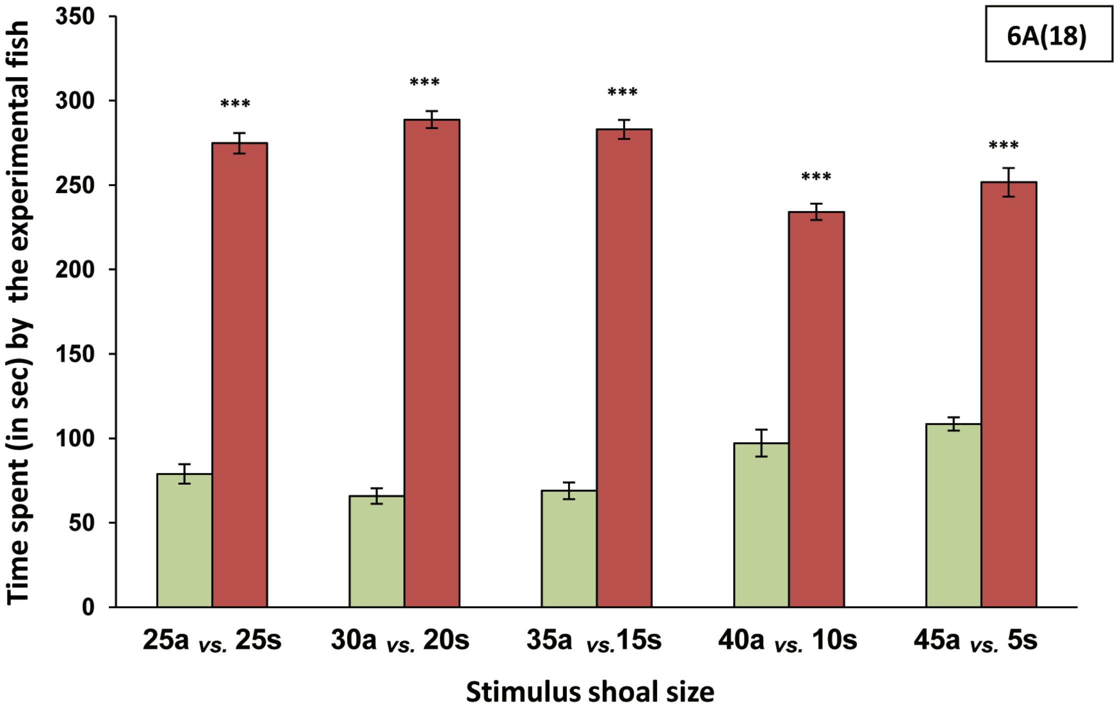


Figure 6A (18): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***Heterospecifics A.testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (19): Test fish - Larvae of *Puntius sarana subnasutus* reared with 30 siblings for 3 months

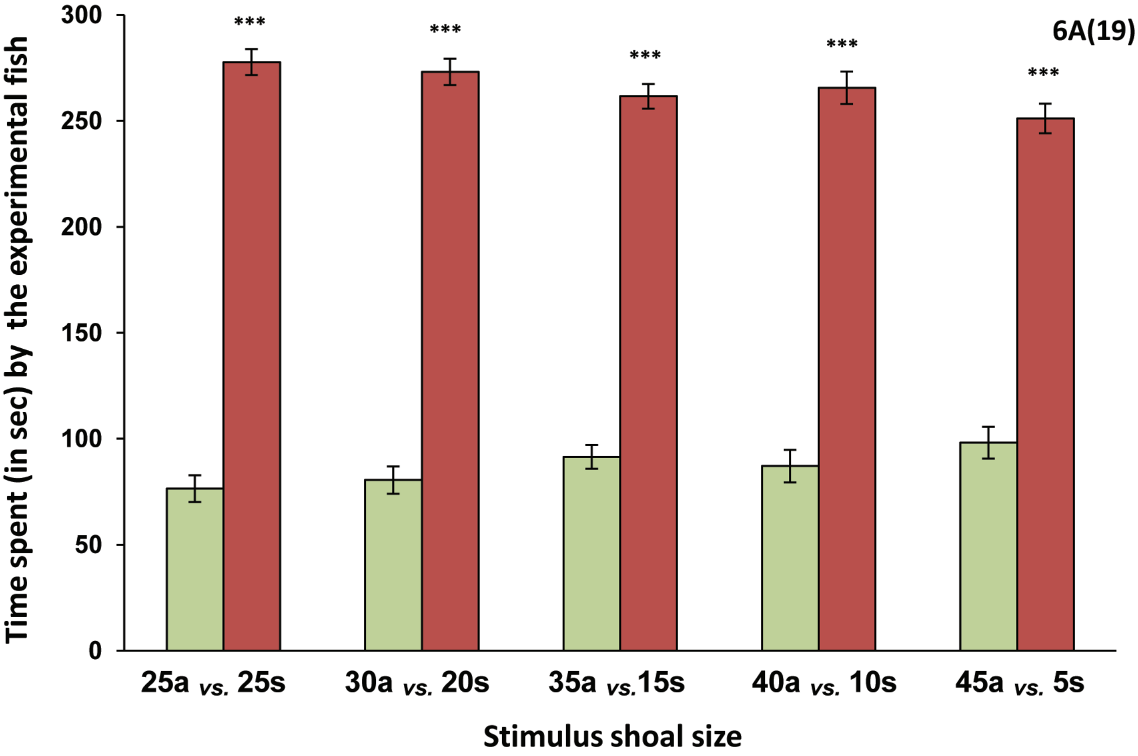


Figure 6A (19): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***Heterospecifics A.testudineus (a)*** and ***siblings of P.sarana subnasutus (S)***in varying numerical combinations

6A (20): Test fish - Larvae of *Puntius sarana subnasutus* reared with 40 siblings for 3 months

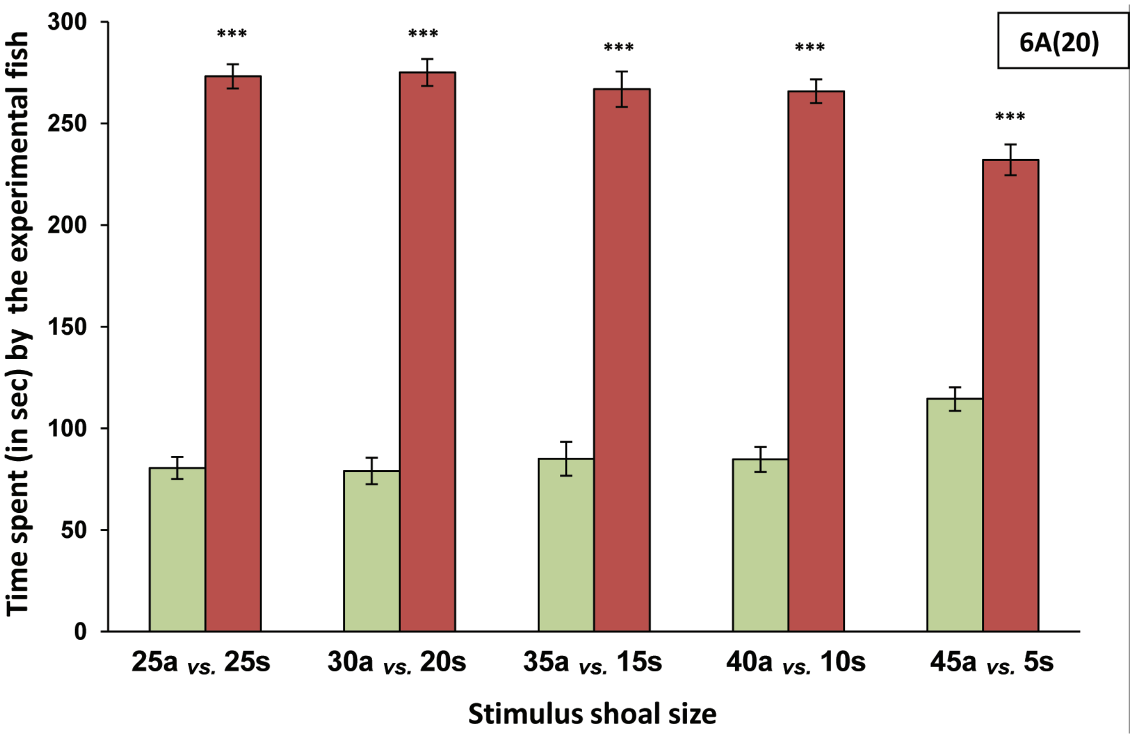


Figure 6A (20): Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***Heterospecifics A.testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

b) Shoaling preference of larvae reared in shoaling group for 8 months

6A (21): Test fish - Larvae of *Puntius sarana subnasutus* reared with 10 siblings for 8 months

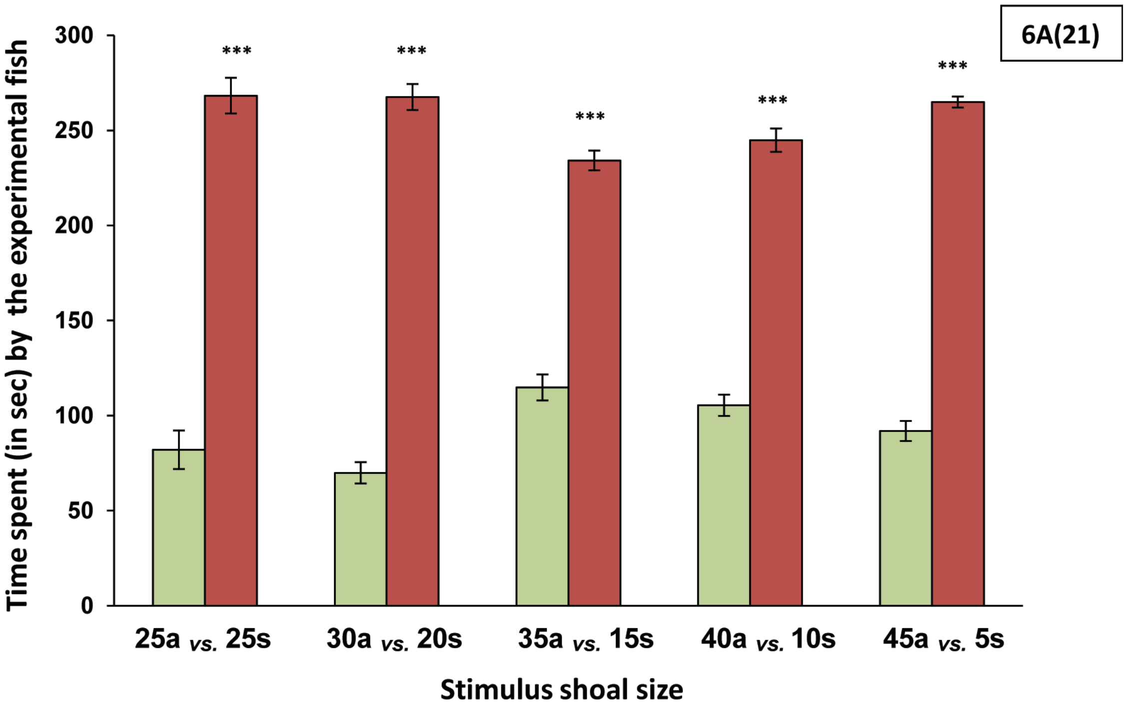


Figure 6A (21): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***Heterospecifics A. testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (22): Test fish - Larvae of *Puntius sarana subnasutus* reared with 20 siblings for 8 months

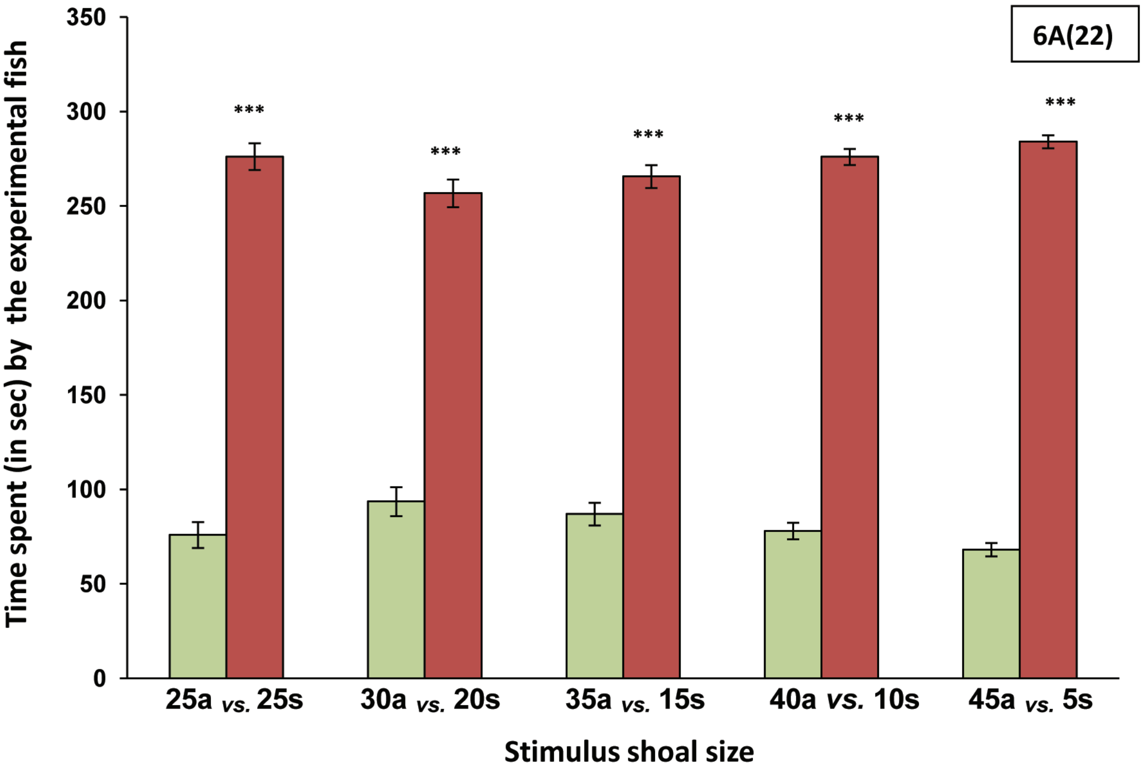


Figure 6A (22): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of ***Heterospecifics A. testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (23): Test fish - Larvae of *Puntius sarana subnasutus* reared with 30 siblings for 8 months

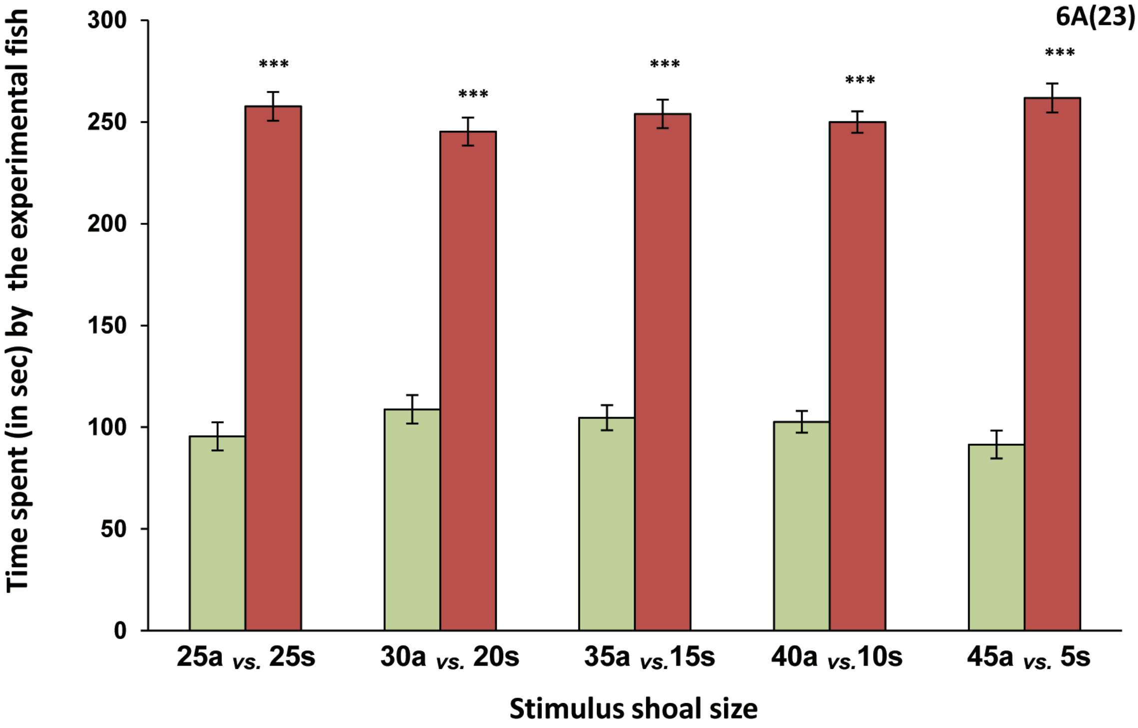


Figure 6A (23): Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***Heterospecifics A.testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

6A (24): Test fish - Larvae of *Puntius sarana subnasutus* reared with 40 siblings for 8 months

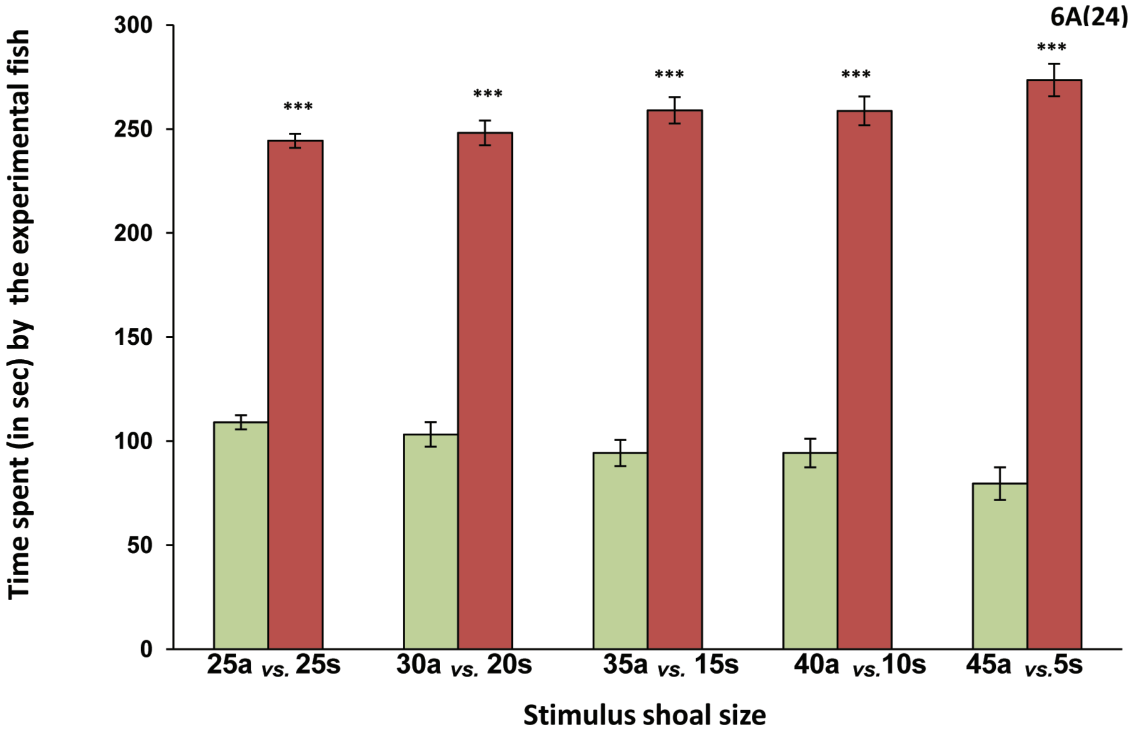


Figure 6A (24) : Time spent in seconds (Mean ± SE) by the test fish(n=20) near the stimulus shoals composed of ***Heterospecifics A.testudineus (a)*** and ***siblings of P.sarana subnasutus (S)*** in varying numerical combinations

Test fish	**Rearing group**	**Age of the larvae**	**Stimulus shoal-size Heterospecific *Anabas testudineus* (A) *vs.* Sibling group (s)**				
			25a vs. 25s	***30a vs. 20s***	***35a vs. 15s***	***40a vs. 10s***	***45a vs. 5s***
Larvae reared with individuals of same breeding pair	**10**	3 Months	t= 16.667 p=0.000***	t= 23.262 p=0.001***	t= 20.433 p=0.001***	t= 9.633 p=0.001***	t= 15.311 p=0.001***
		8 Months	t= 9.819 p=0.001***	t= 16.503 p=0.001***	t= 10.383 p=0.001***	t= 12.254 p=0.001***	t= 24.555 p=0.001***
	20	3 Months	t= 13.116 p=0.000***	t= 12.006 p=0.001***	t= 11.876 p=0.001***	t= 11.782 p=0.001***	t= 10.467 p=0.001***
		8 Months	t= 14.439 p=0.001***	t= 10.917 p=0.001***	t= 14.821 p=0.001***	t= 23.063 p=0.001***	t= 31.147 p=0.001***
	30	3 Months	t= 16.353 p=0.000***	t= 15.249 p=0.001***	t= 14.930 p=0.001***	t= 11.683 p=0.001***	t= 10.684 p=0.001***
		8 Months	t= 11.625 p=0.001***	t= 9.838 p=0.001***	t= 12.539 p=0.001***	t= 13.886 p=0.001***	t= 12.249 p=0.001***
	40	3 Months	t= 16.874 p=0.000***	t= 15.280 p=.001***	t= 10.704 p=0.001***	t= 15.246 p=0.001***	t= 9.596 p=0.001***
		8 Months	t= 20.077 p=0.001***	t= 12.261 p=0.001***	t= 13.056 p=0.001***	t= 11.954 p=0.001***	t= 12.403 p=0.001***

Table: 6A (4) Preference of *Puntius sarana subnasutus* to associate with the larger group when the choice is between siblings and heterospecific *Anabas testudineus* at 3months and 8 months old stage

6. B: SHOALING PREFERENCE OF *PUNTIUS SARANA SUBNASUTUS* (REARED IN GROUPS OF 10, 20, 30 AND 40 WITH EQUAL NUMBER OF CONSPECIFICS, HETEROSPECIFIC *PUNTIUS PARRAH* AND HETEROSPECIFIC *ANABAS TESTUDINEUS)*

6. B: I a) Shoaling preference of larvae of *P. sarana subnasutus* reared with conspecifics to stimulus shoal of their siblings and conspecifics *P. sarana subnasutus* at 3 months

The present study indicates that early rearing environment has profound influence on the shoal choice of *Puntius sarana subnasutus*. Group of larvae of *Puntius sarana subnasutus* reared along with equal number of conspecific fish (larvae of *Puntius sarana subnasutus* of a different breeding pair) for 3 months preferred conspecifc shoals to that of sibling shoal irrespective of the rearing population density [group of larvae (10, 20, 30,and 40) reared along with equal number of conspecifics]. Significant preference to conspecifics were observed in all numerical combinations of the stimulus groups [**30 conspecifics *vs.* 20 siblings, 35 conspecifics *vs.* 15 siblings, 40 conspecifics *vs.* 10 sibling and 45 conspecifics *vs.* 5 siblings**] at the ratio of 1:1.5, 1:2.3, 1:4 and 1:9 respectively, except in when the stimulus shoals of familiar conspecifics and siblings were equal 1:1 (**25 conspecifics *vs.* 25 siblings**) [Fig. 6B (1-4); Table 6B (3)]

6B (1): Test fish – 10 larvae of *Puntius sarana subnasutus* (siblings) reared with 10 larvae of conspecifics *Puntius sarana subnasutus* for 3 months

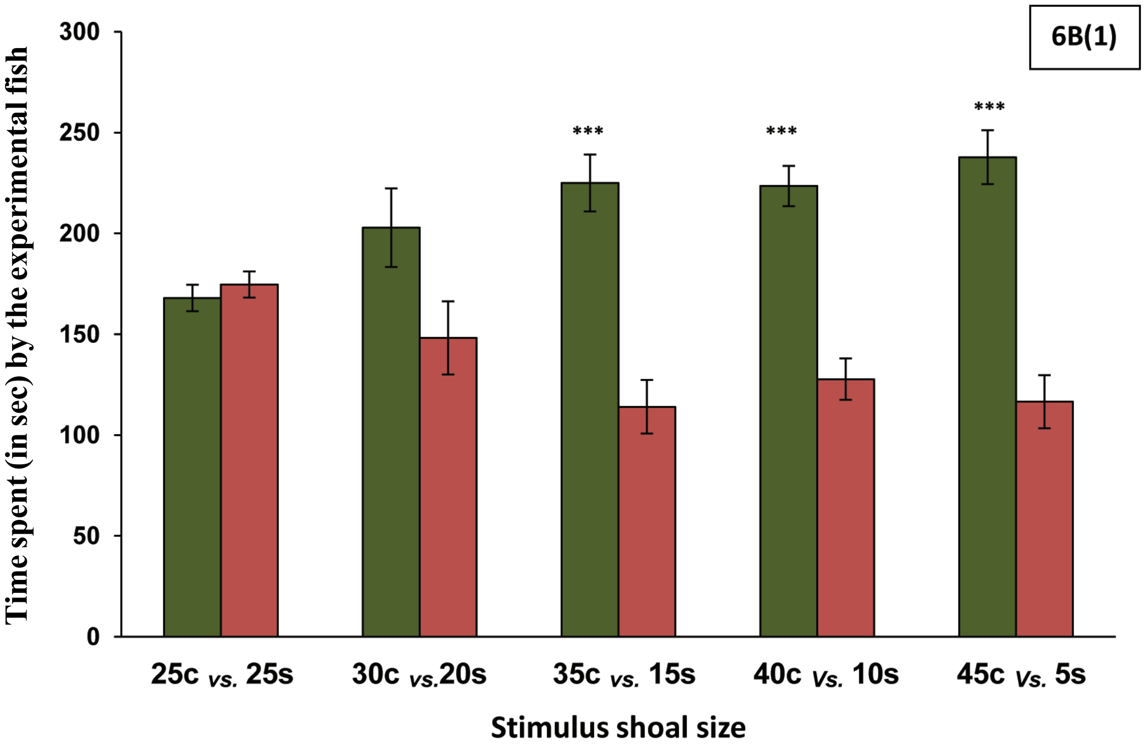


Figure 6B (1): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (2): Test fish - 20 larvae of *Puntius sarana subnasutus* (siblings) reared with 20 larvae of conspecifics *Puntius sarana subnasutus* for 3 months

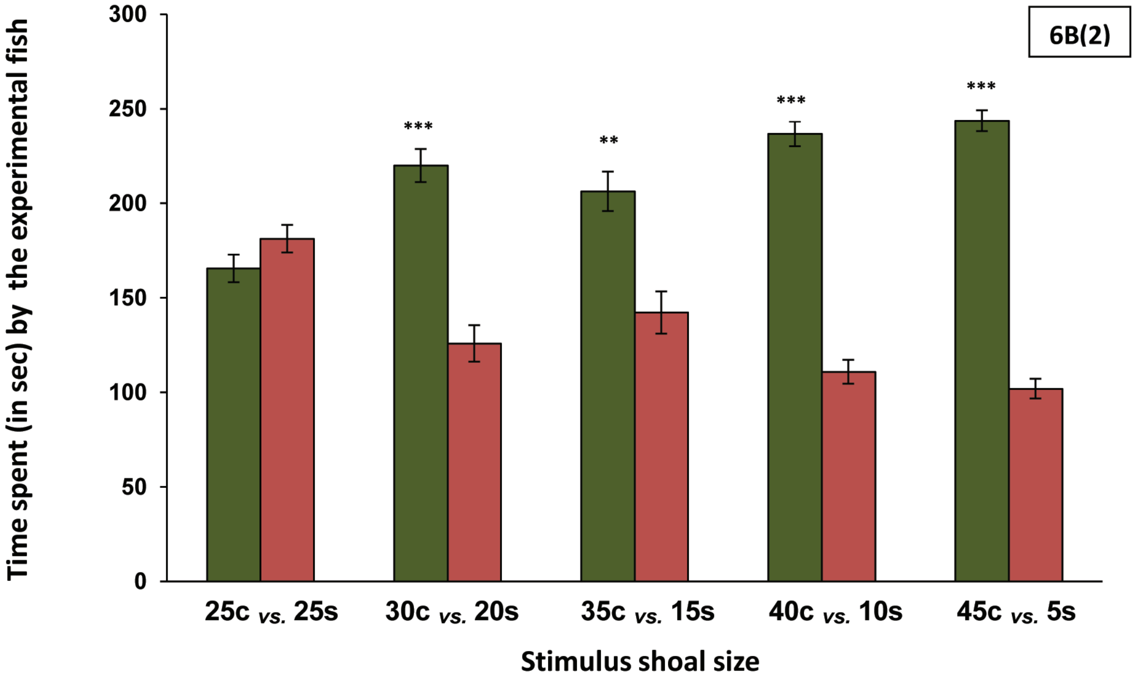


Figure 6B (2): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations when tested

6B (3): Test fish - 30 larvae of *Puntius sarana subnasutus* (siblings) reared with 30 larvae of conspecifics *Puntius sarana subnasutus* for 3 months

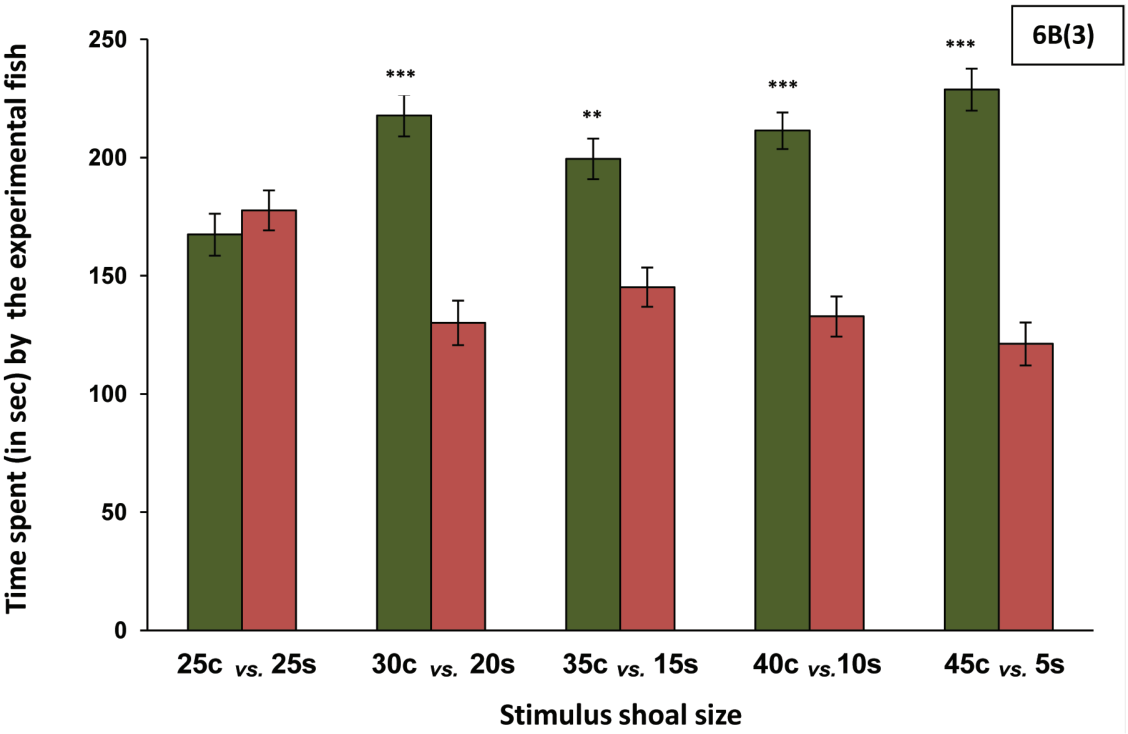


Figure 6B (3): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (4) : Test fish - 40 larvae of *Puntius sarana subnasutus* (siblings) reared with 40 larvae of conspecifics *Puntius sarana subnasutus* for 3 months

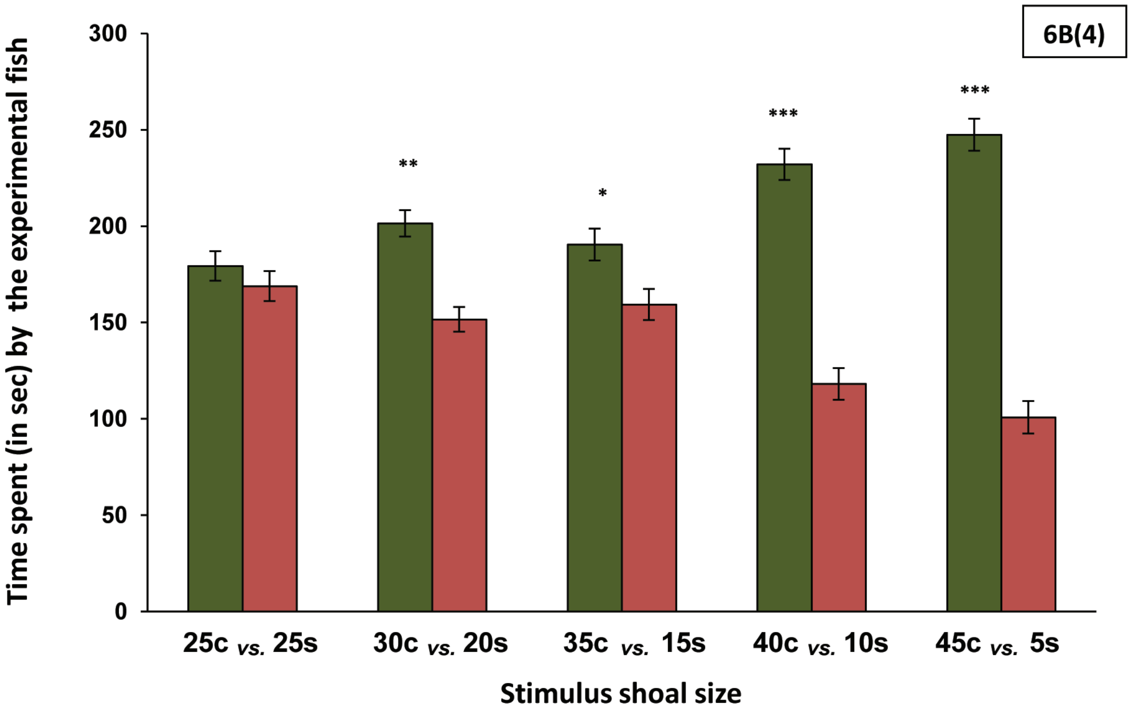


Figure 6B (4): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6. B: I b) Shoaling preference of larvae of *P. sarana subnasutus* reared with conspecifics *Puntius sarana subnasutus* to stimulus shoal of their siblings and conspecifics *Puntius sarana subnasutus* at 8 months

Group of larvae of *Puntius sarana subnasutus* reared in group of 10, 20, 30 and 40 along with equal number of conspecific fish (larvae of *Puntius sarana subnasutus* of a different breeding pair) for 8 months preferred sibling shoal to that of familiar conspecific when the binary choice between stimulus shoal of **25 conspecifics *vs.* 25 sibilings**. In all other numerical combinations [**30 conspecifics vs. 20 siblings, 35 conspecifics vs. 15 siblings, 40 conspecifics vs. 10 sibling and 45 conspecifics vs. 5 siblings;** at the ratio of 1:1.5, 1:2.3, 1:4 and 1:9 respectively] irrespective of the rearing density [group of larvae (10, 20, 30 and 40) reared along with equal number of conspecifics] irrespective of the rearing density [group of larvae (10, 20, 30 and 40) reared along with equal number of conspecifics, significant preference to conspecifics were observed [Fig. 6B (5-8); Table 6B (3)].

6B (5): Test fish: 10 larvae of *Puntius sarana subnasutus* (siblings) reared with 10 larvae of conspecifics *Puntius sarana subnasutus* for 8 months

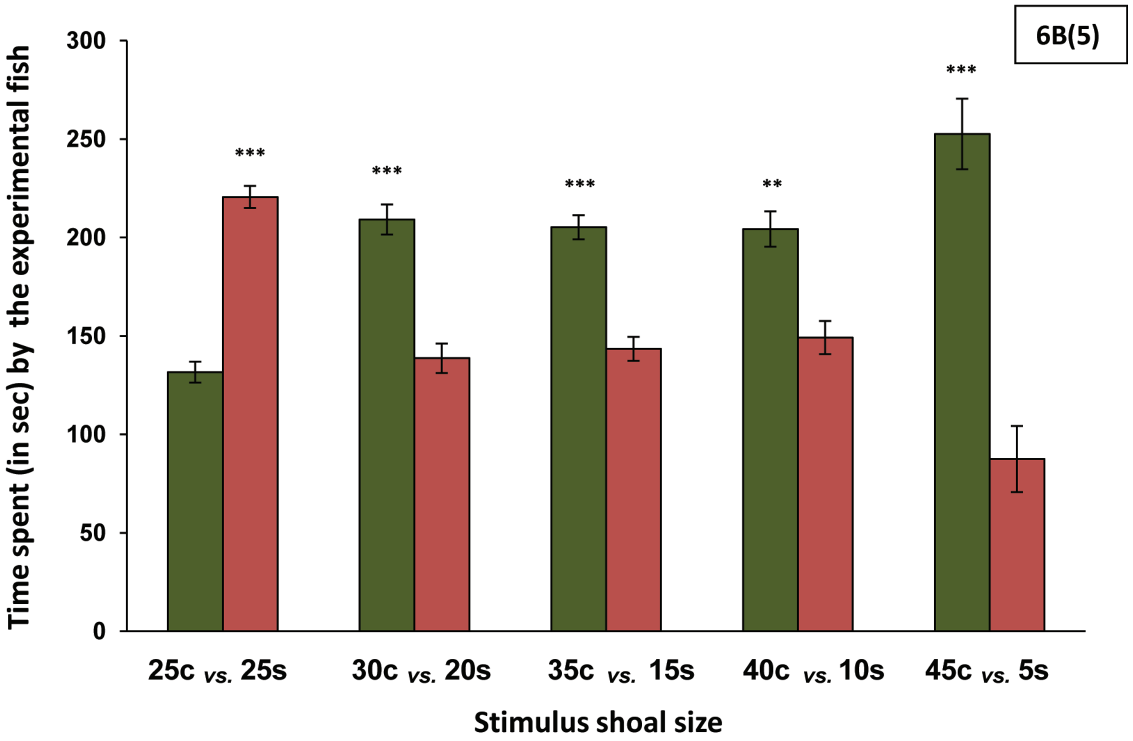


Figure 6B (5): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (6): Test fish: 20 larvae of *Puntius sarana subnasutus* (siblings) reared with 20 larvae of conspecifics *Puntius sarana subnasutus* for 8 months

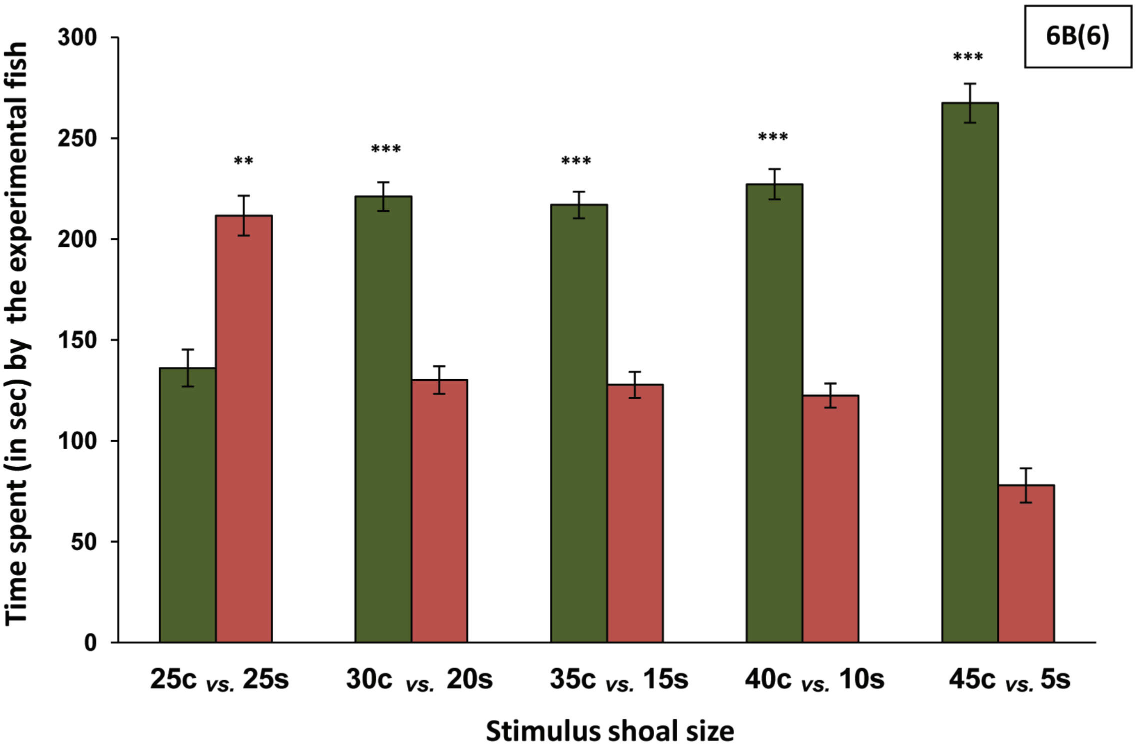


Figure 6B (6): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (7): Test fish: 30 larvae of *Puntius sarana subnasutus* (siblings) reared with 30 larvae of conspecifics *Puntius sarana subnasutus* for 8 months

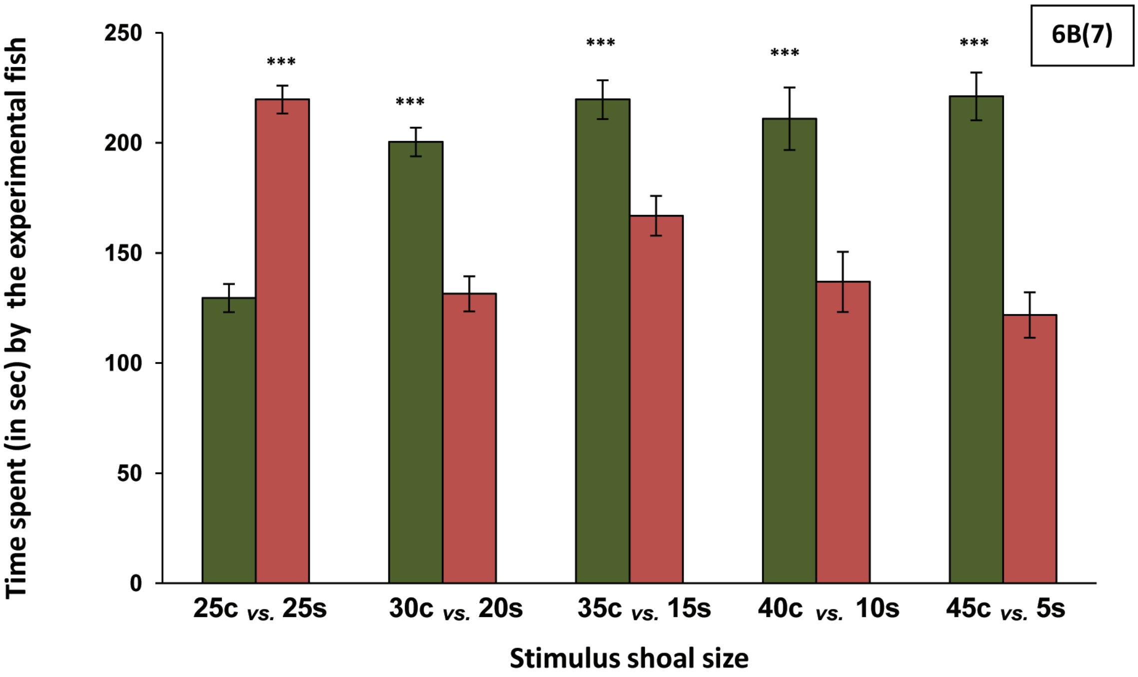


Figure 6B (7): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (8): Test fish: 40 larvae of *Puntius sarana subnasutus* (siblings) reared with 40 larvae of conspecifics *Puntius sarana subnasutus* for 8 months

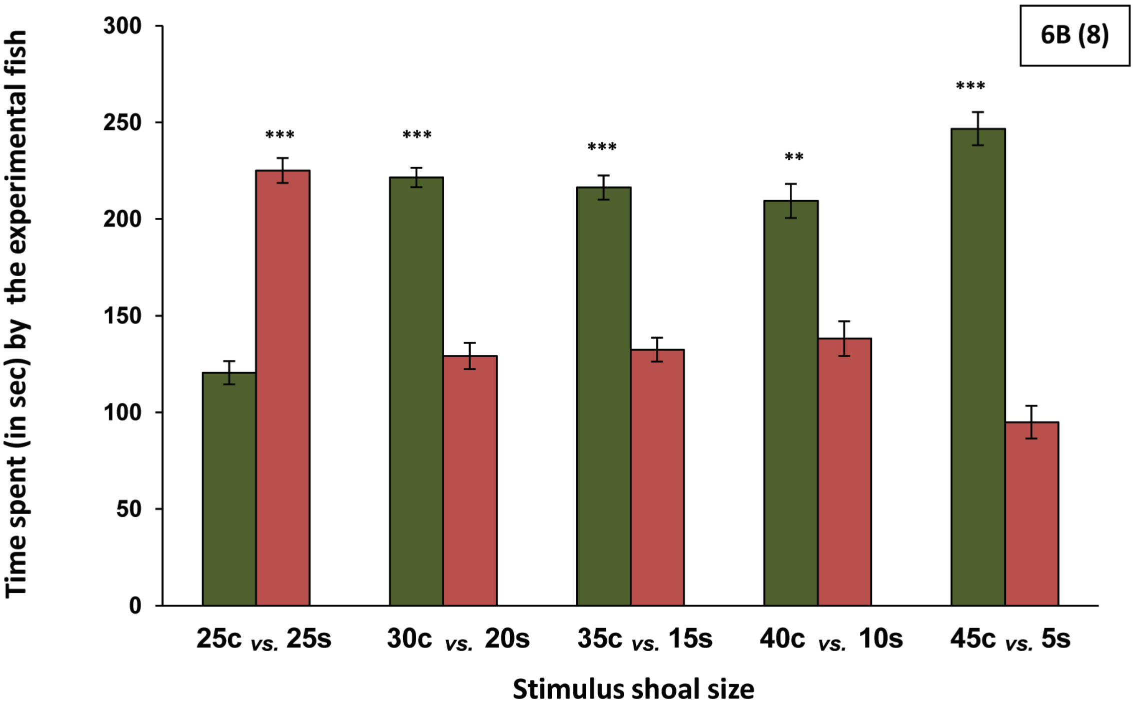


Figure 6B (8): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***conspecifics (C)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

Test fish	**Rearing group**	**Age of the larvae**	**Stimulus shoal-size Sibling group (s) *vs.* Conspecific group (c)**				
			25c* Vs *25s	***30c* Vs *20s***	***35c* Vs 1*5s***	***40c* Vs *10s***	***45c* Vs *5s***
Larvae reared with individuals of conspecific *P. sarana subnasutus*	**10**	3 Months	t= 0.522 p=0 .608	t= 1.461 p=0.160	t= 4.078 p=0.001**	t=4.728 p=0.000***	t= 4.582 p=0.000***
		8 Months	t= 8.167 p=0.000***	t= 4.714 p=0.000***	t= 5.126 p=0.000***	t= 3.189 p=0.005**	t= 4.825 p=0.000***
	20	3 Months	t= 1.090 p=0.289	t= 5.227 p=0.000***	t=2.996 p=0.007**	t=9.885 p=0.000***	t= 13.460 p=0.000***
		8 Months	t= 3.980 p=0.001**	t= 6.521 p=0.000***	t= 6.936 p=0.000***	t= 7.959 p=0.000***	t= 10.795 p=0.000***
	30	3 Months	t= 0.596 p= 0.558	t= 4.850 p=0.000***	t= 3.249 p=0.004**	t= 4.861 p=0.000***	t= 6.016 p=0.000***
		8 Months	t= 5.889 p=0.000***	t= 5.554 p=0.000***	t= 9.140 p=0.000***	t=4.607 p=0.000***	t= 6.694 p=0.000***
	40	3 Months	t=0 .679 p=0.505	t= 3.935 p=0.001**	t= 1.910 p=0.071*	t= 6.996 p=0.000***	t= 8.912 p=0.000***
		8 Months	t= 8.554 p=0.000***	t= 6.757 p=0.000***	t= 6.868 p=0.000***	t= 4.012 p=0.001**	t=9.022 p=0.000***

Table: 6B (3) Preference of *P.sarana subnasutus* to associate with the larger group when the choice is between siblings and familiar conspecifics at 3months and 8 months old stage

6. B: II a) Shoaling preference of larvae of *P. sarana subnasutus* reared with heterospecifics *P. parrah* to stimulus shoal of their siblings and heterospecifics *P. parrah* at 3 months

Larvae of *Puntius sarana subnasutus* reared in group of 10, and 20, reared along with equal number of a heterospecific fish, (*Puntius parrah*) larvae for 3 months preferred sibling shoal when the size of the shoal **25 heterospecific *vs.* 25 sibling; 30 heterospecific *vs.* 20 siblings and 35 heterospecific *vs.* 15 siblings**. Larvae of *P.sarana subnasutus* reared in group of 30, and 40, reared along with equal number of a heterospecific fish, (*Puntius parrah*) larvae for 3 months exhibited preference for familiar heterospecifc shoal when stmulus shoal size is **30 heterospecific *vs.* 20 siblings; 35 heterospecific *vs.* 15 siblings; 40 heterospecific *vs.* 10 siblings and 45 heterospecific *vs.* 5 siblings.** But when the stimulus shoal size is equal (**25 heterospecific *vs.* 25 sibling**) the test fish preferred sibling stimulus shoal [Fig. 6B (9-12); Table 6B (4)]

6B (9): Test fish: 10 larvae of *Puntius sarana subnasutus* (siblings) reared with 10 larvae of heterospecifics *Puntius parrah* for 3 months

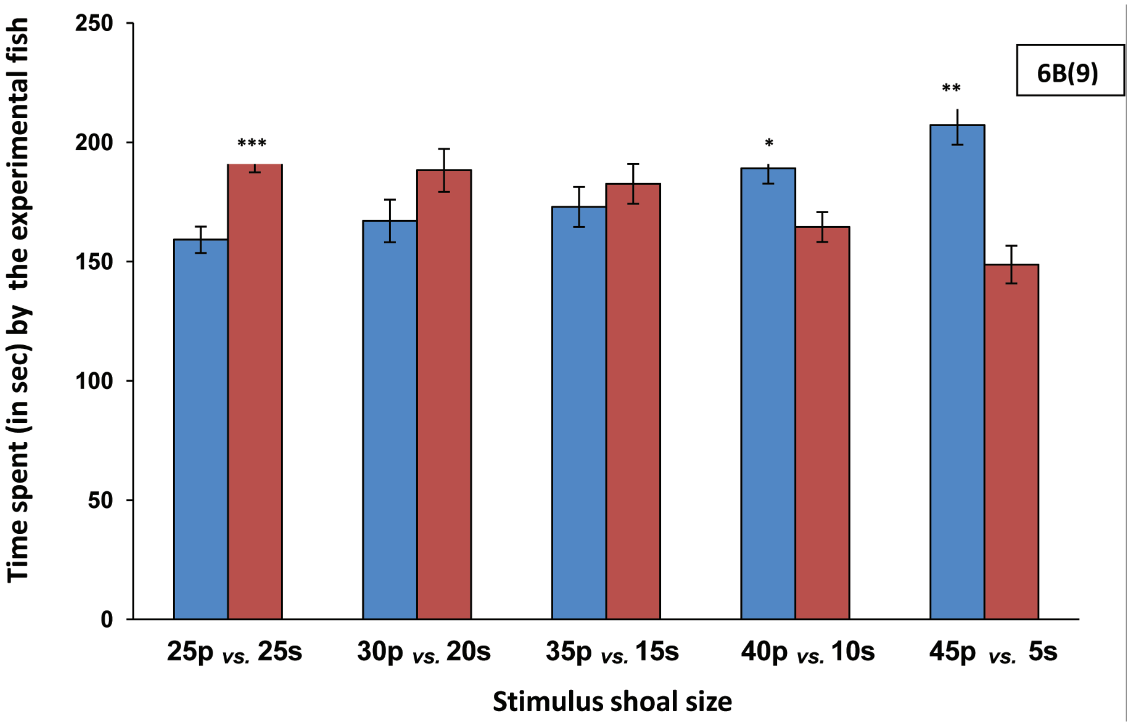


Figure 6B (9): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (10): Test fish: 20 larvae of *Puntius sarana subnasutus* (siblings) reared with 20 larvae of heterospecifics *Puntius parrah* for 3 months

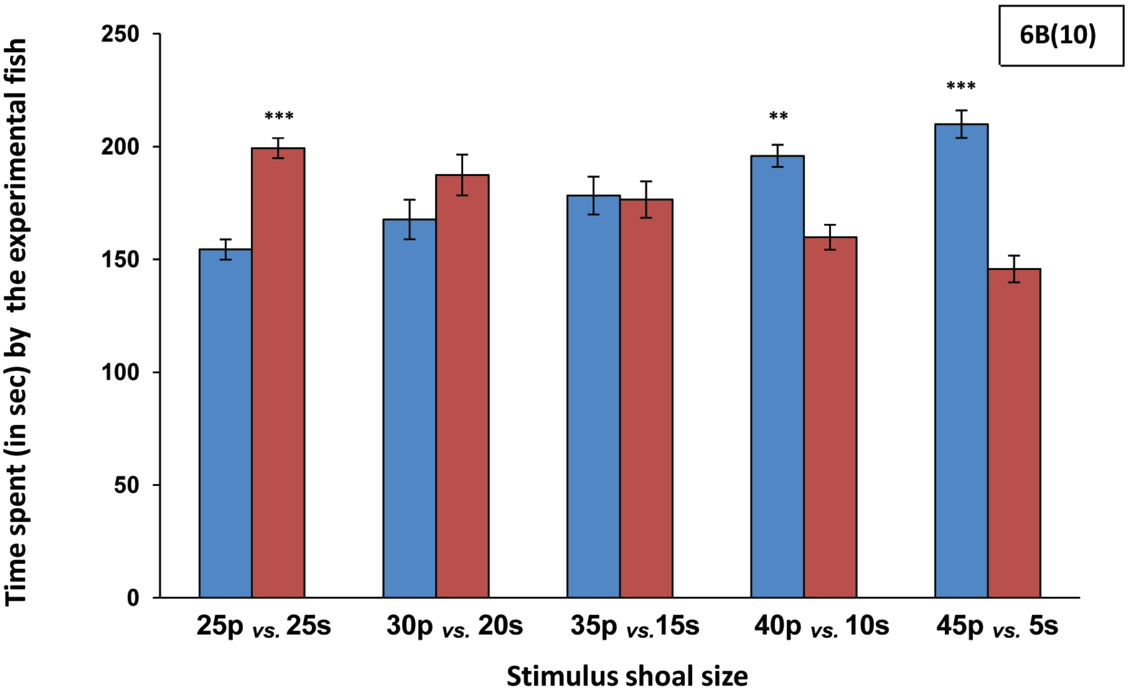


Figure 6B (10): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (11): Test fish: 30 larvae of *Puntius sarana subnasutus* (siblings) reared with 30 larvae of heterospecifics *Puntius parrah* for 3 months

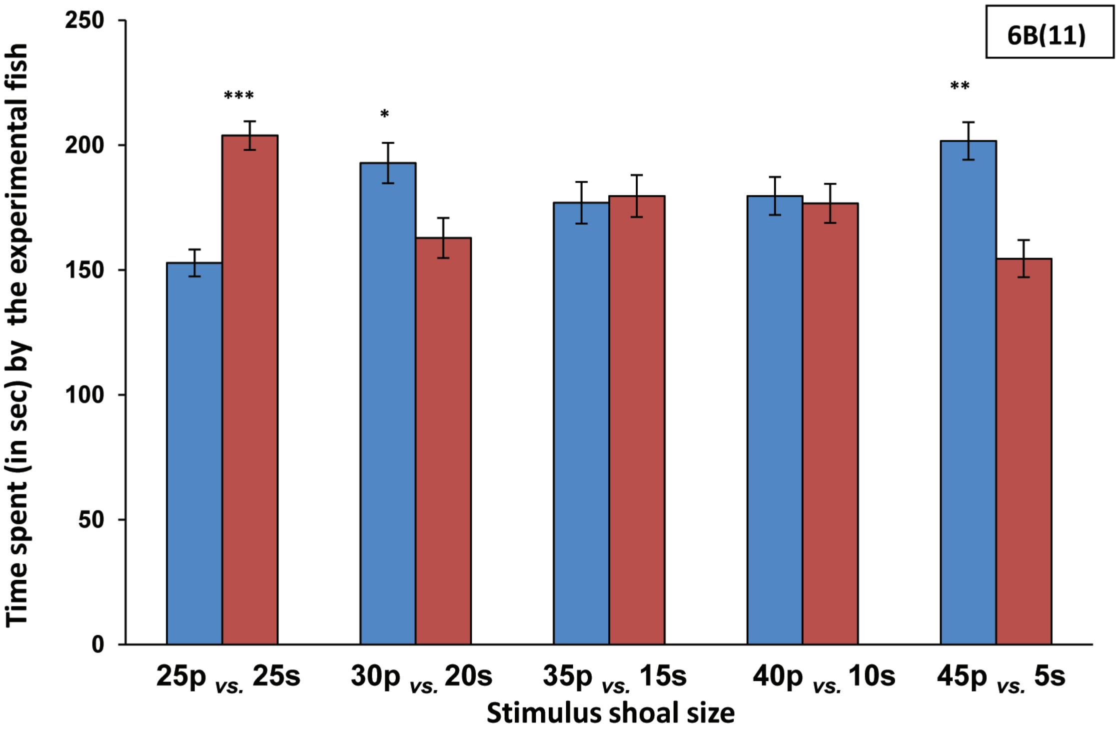


Figure 6B (11): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (12): Test fish: 40 larvae of *Puntius sarana subnasutus* (siblings) reared with 40 larvae of heterospecifics *Puntius parrah* for 3 months

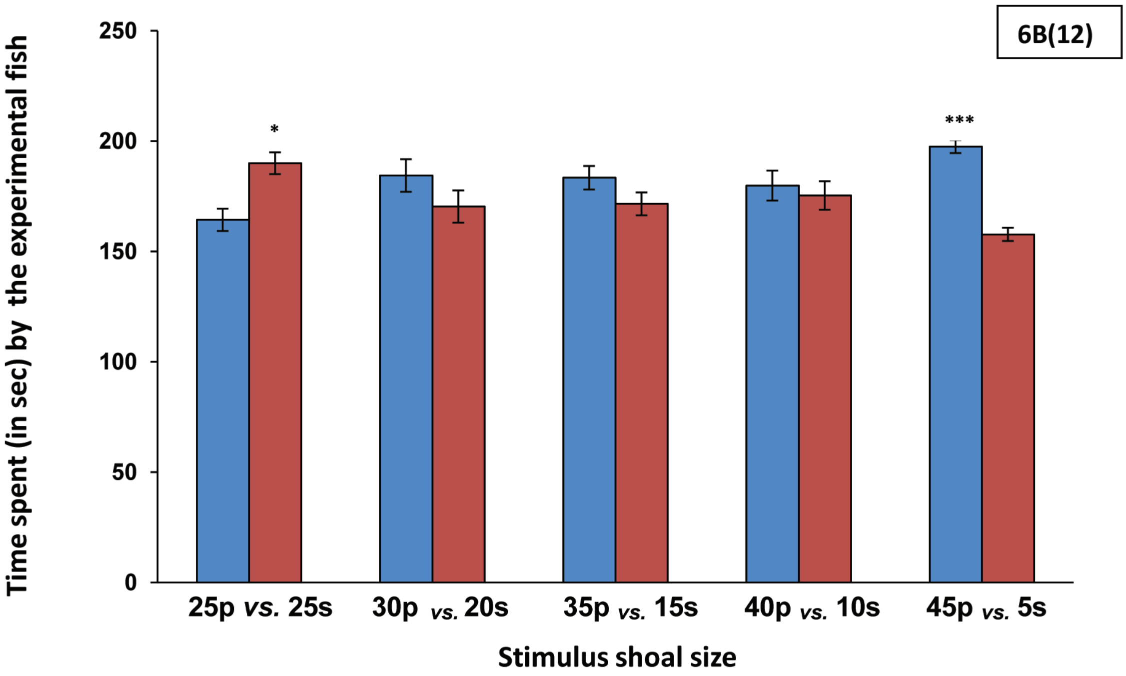


Figure 6B (12): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6. B: II b) Shoaling preference of larvae of *P. sarana subnasutus* reared with heterospecifics *P. parrah* to stimulus shoal of their siblings and heterospecifics *P. parrah* at 8 months

Larvae of *Puntius sarana subnasutus* reared in group of 10, 20, 30 and 40 reared along with equal number of a heterospecific fish, (*Puntius parrah*) larvae for 8 months preferred familiar heterospecific shoal when the stimulus shoal size is **30 heterospecific *vs.* 20 siblings and 35 heterospecific *vs.* 15 siblings; 40 heterospecific *vs.* 10 siblings; 45 heterospecific *vs.* 5 siblings**. However when the shoal size is equal (**25 heterospecific *vs.* 25 sibling**) the test fish of all rearing groups exhibited a tendency to prefer stimulus shoal of siblings [Fig. 6B (13-16); Table 6B (4)].

6B (13): Test fish: 10 larvae of *Puntius sarana subnasutus* (siblings) reared with 10 larvae of heterospecifics *Puntius parrah* for 8 months.

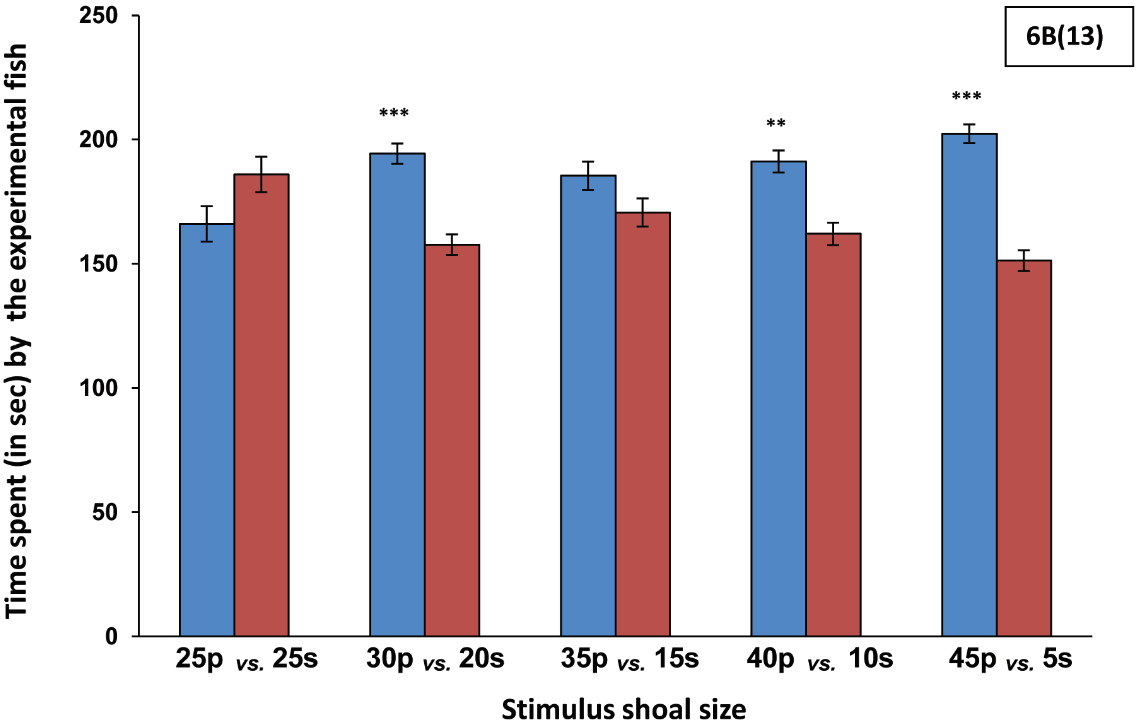


Figure 6B (13) : Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (14): Test fish: 20 larvae of *Puntius sarana subnasutus* (siblings) reared with 20 larvae of heterospecifics *Puntius parrah* for 8 months

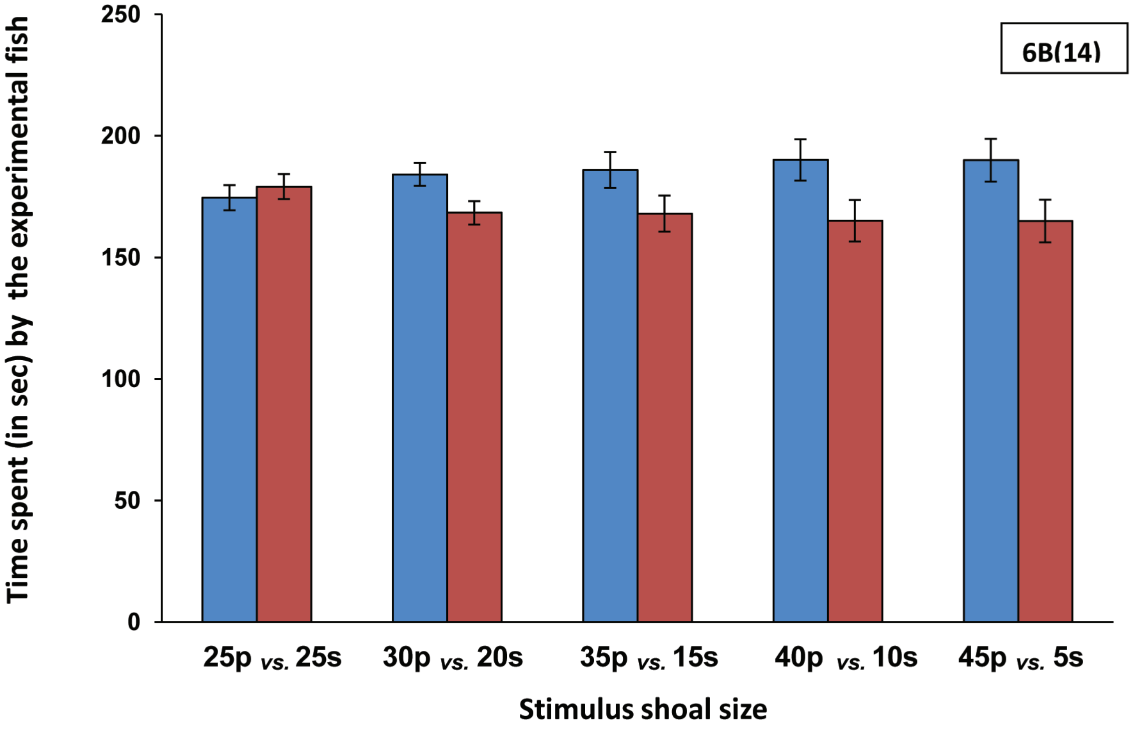


Figure 6B (14): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (15): Test fish: 30 larvae of *Puntius sarana subnasutus* (siblings) reared with 30 larvae of heterospecifics *Puntius parrah* for 8 months

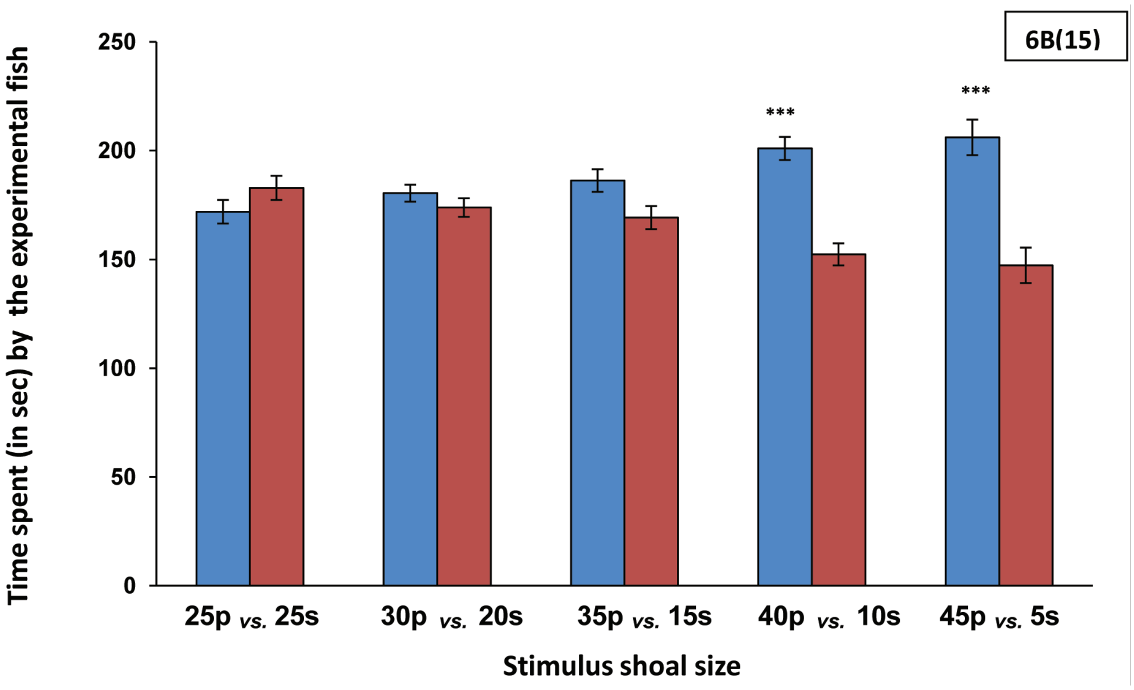


Figure 6B (15) : Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (16): Test fish: 40 larvae of *Puntius sarana subnasutus* (siblings) reared with 40 larvae of heterospecifics *Puntius parrah* for 8 months

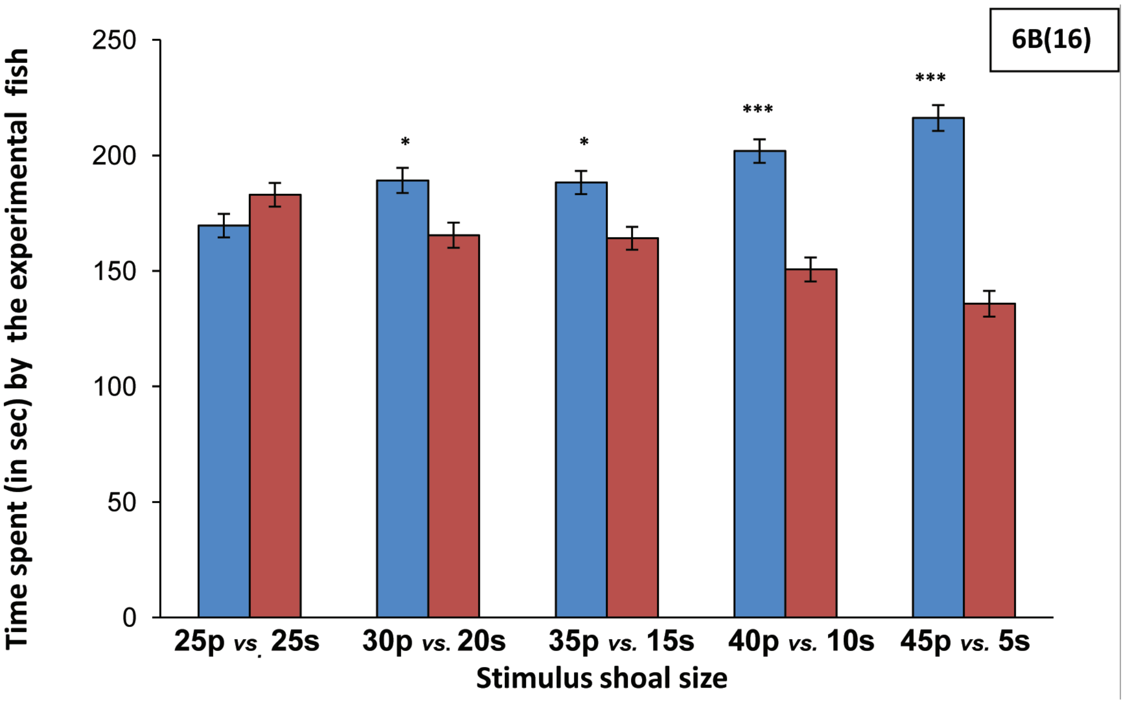


Figure 6B (16): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific P. parrah (P)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

Test fish	**Rearing group**	**Age of the larvae**	**Stimulus shoal-size Sibling group (s) *vs.* Heterospecific *Puntius parrah* (p)**				
			25p vs. 25s	***30p vs. 20s***	***35p vs. 15s***	***40p vs. 10s***	***45p vs. 5s***
Larvae reared with individuals of hetero-specific *Puntius-parrah*	**10**	3 Months	t= 3.023 p=0 .007**	t=1.182 p=0.252	t= 0.574 p=0.573	t= 1.949 p=0.066*	t= 3.640 p=0.002**
		8 Months	t= 1.399 p=0.178	t= 4.444 p=0.001***	t= 1.302 p=0.209	t= 3.252 p=0.004**	t= 6.485 p=0.001***
	20	3 Months	t= 5.027 p=0.001***	t= 1.109 p=0.281	t= 0.106 p=0.916	t= 3.467 p=0.003**	t= 5.337 p=0.001***
		8 Months	t= 0.446 p=0.661	t= 1.657 p=0.114	t= 1.207 p=0.242	t= 1.466 p=0.159	t= 1.423 p=0.171
	30	3 Months	t= 4.621 p=0.001***	t= 1.863 p=0.078*	t=0 .161 p=0.873	t= 0.194 p=0.848	t= 3.152 p=0.005**
		8 Months	t= 1.000 p=0.330	t= 0.807 p=0.430	t= 1.620 p=0.122	t= 4.669 p=0.001***	t= 3.605 p=0.001***
	40	3 Months	t= 2.572 p=0.019*	t= 0.955 p=0.351	t= 1.123 p=0.276	t=0 .337 p=0.740	t= 6.694 p=0.001***
		8 Months	t= 1.309 p=0.206	t= 2.189 p=0.041*	t= 2.441 p=0.025*	t= 4.977 p=0.001***	t= 7.230 p=0.001***

Table: 6B (4) Preference of *Puntius sarana subnasutus* to associate with the larger group when the choice is between siblings and familiar heterospecific *Puntius parrah* at 3months and 8 months old stage

6. B: III Shoaling preference of larvae of *P. sarana subnasutus* reared with heterospecifics *Anabas testudineus* to stimulus shoal of their siblings and heterospecifics *Anabas testudineus* at *a) 3 months and b) 8 months*

Larvae of *Puntius sarana subnasutus* reared in group of 10, 20, and 30 reared along with equal number of a heterospecific fish *Anabas testudineus*, larvae for 3 months and 8 months preferred sibling shoal when the size of the stimulus shoal is **25 heterospecific *vs.* 25 sibling; 30 heterospecific *vs.* 20 siblings; 35 heterospecific *vs.* 15 siblings; 40 heterospecific *vs.* 10 siblings and 45 heterospecific *vs.* 5 siblings**. But larvae reared in group of 40 along with equal number of a heterospecific fish *Anabas testudineus* larvae, exhibited equal preference to stimulus shoal of familiar heterospecifc to sibiling shoal, except when the stimulus shoal consisted of **25 heterospecifics *vs.* 25 siblings**. This results unequivocally shows the overriding influence of genetic relatedness in shoaling preference of *Puntius sarana subnasutus* [Fig. 6B (17-24); Table 6B (5)].

a) Shoaling preference of larvae Puntius sarana subnasutus of reared with heterospecifics Anabas testudineus for 3 months

6B (17): Test fish: 10 larvae of *Puntius sarana subnasutus* (siblings) reared with 10 larvae of heterospecifics *Anabas testudineus* for 3 months

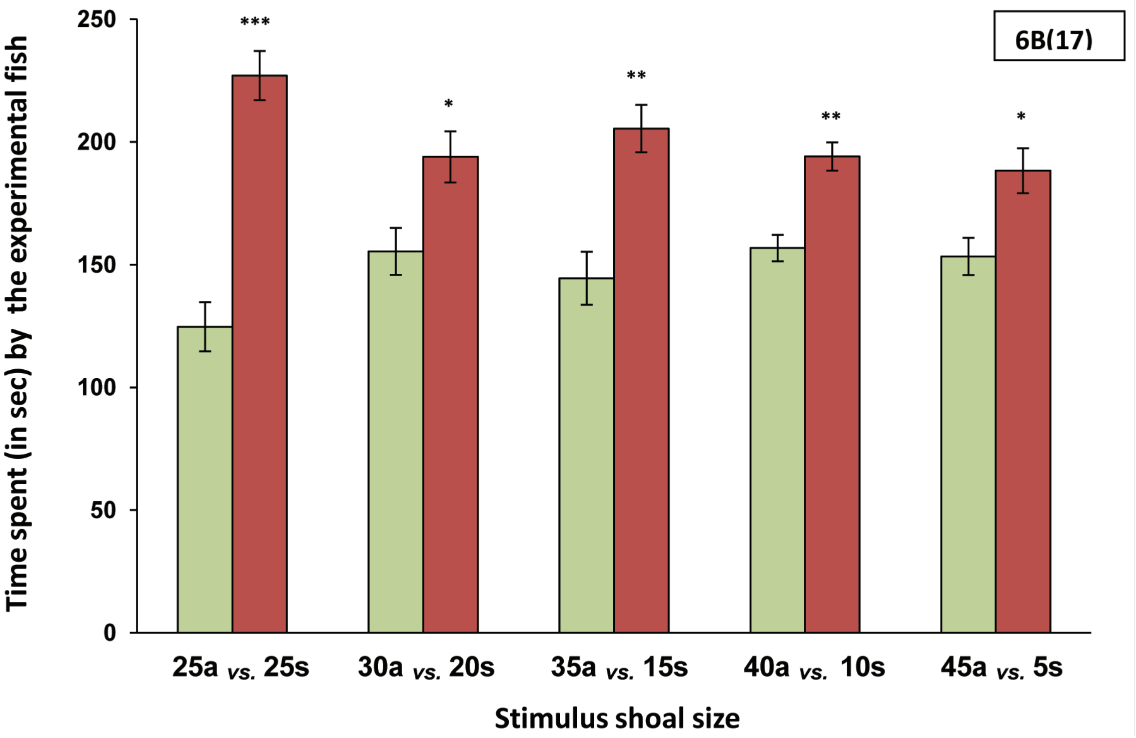


Figure 6B (17): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (18): Test fish: 20 larvae of *Puntius sarana subnasutus* (siblings) reared with 20 larvae of heterospecifics *Anabas testudineus* for 3 months

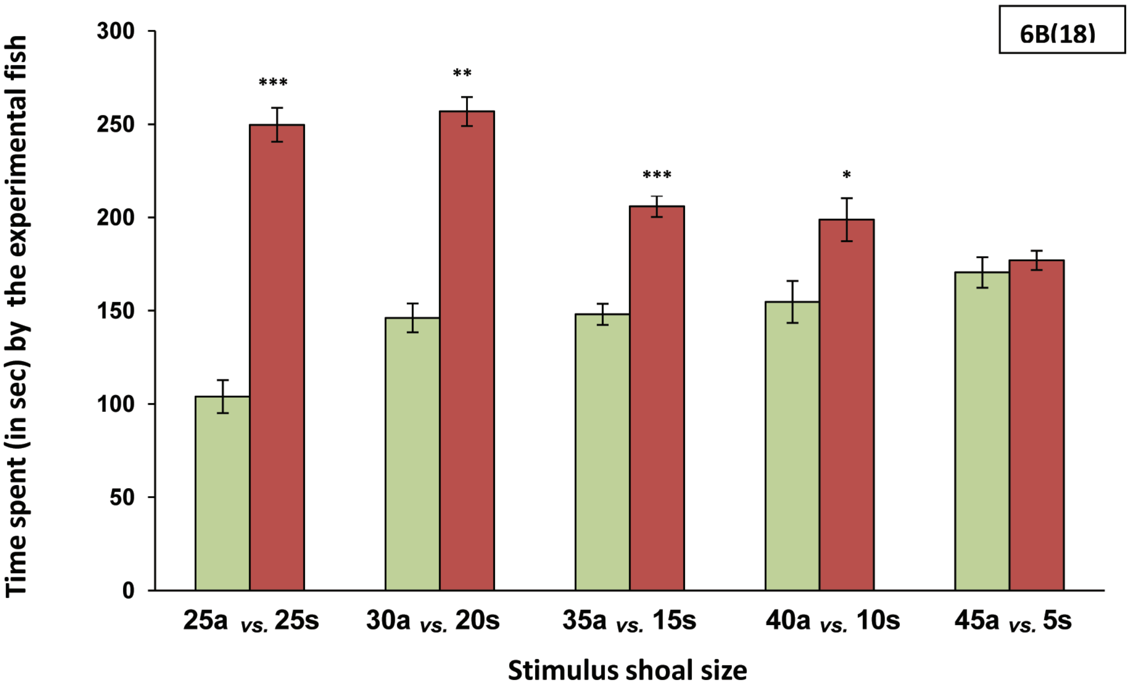


Figure 6B (18): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (19): Test fish: 30 larvae of *Puntius sarana subnasutus* (siblings) reared with 30 larvae of heterospecifics *Anabas testudineus* for 3 months

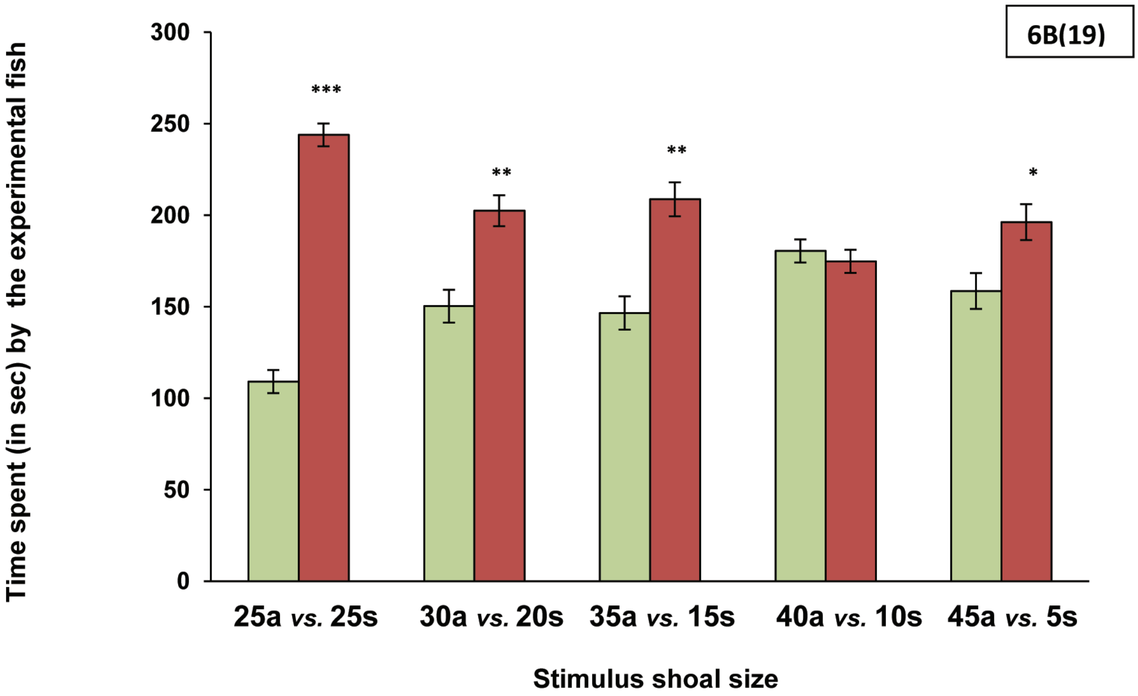


Figure 6B (19): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (20): Test fish: 40 larvae of *Puntius sarana subnasutus* (siblings) reared with 40 larvae of heterospecifics *Anabas testudineus* for 3 months

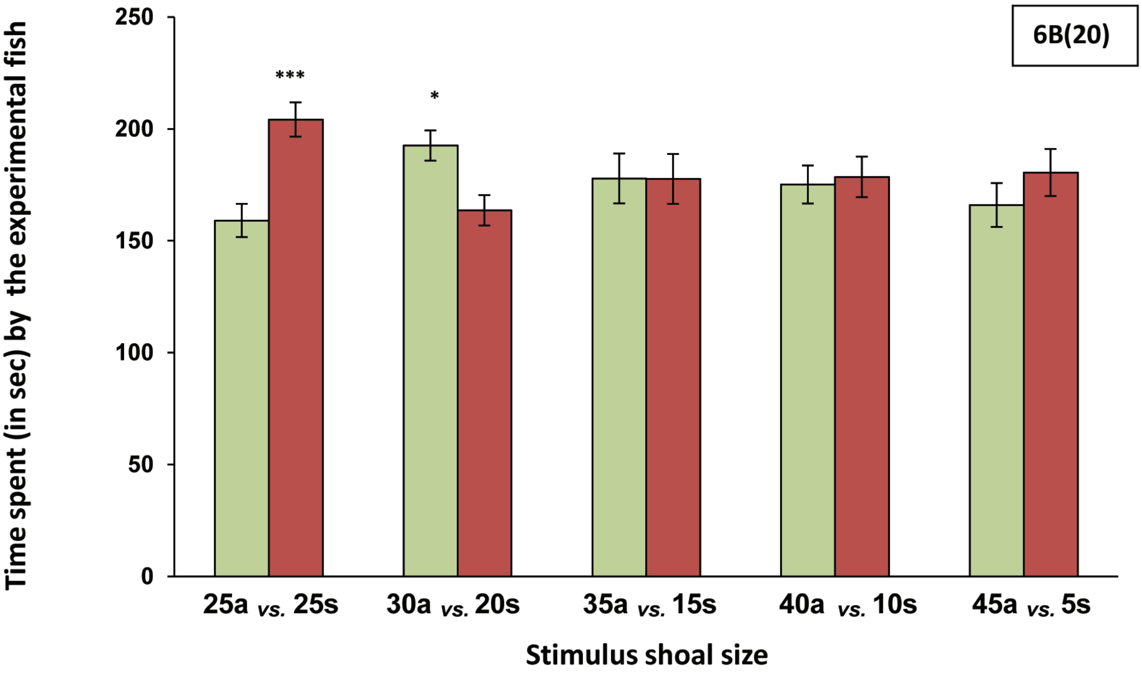


Figure 6B (20): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

b) Shoaling preference of larvae of Puntius sarana subnasutus reared with larvae of *heterospecific Anabas testudineus for 8 months*

6B (21): Test fish - 10 larvae of *Puntius sarana subnasutus* (siblings) reared with 10 larvae of heterospecifics *Anabas testudineus* for 8 months

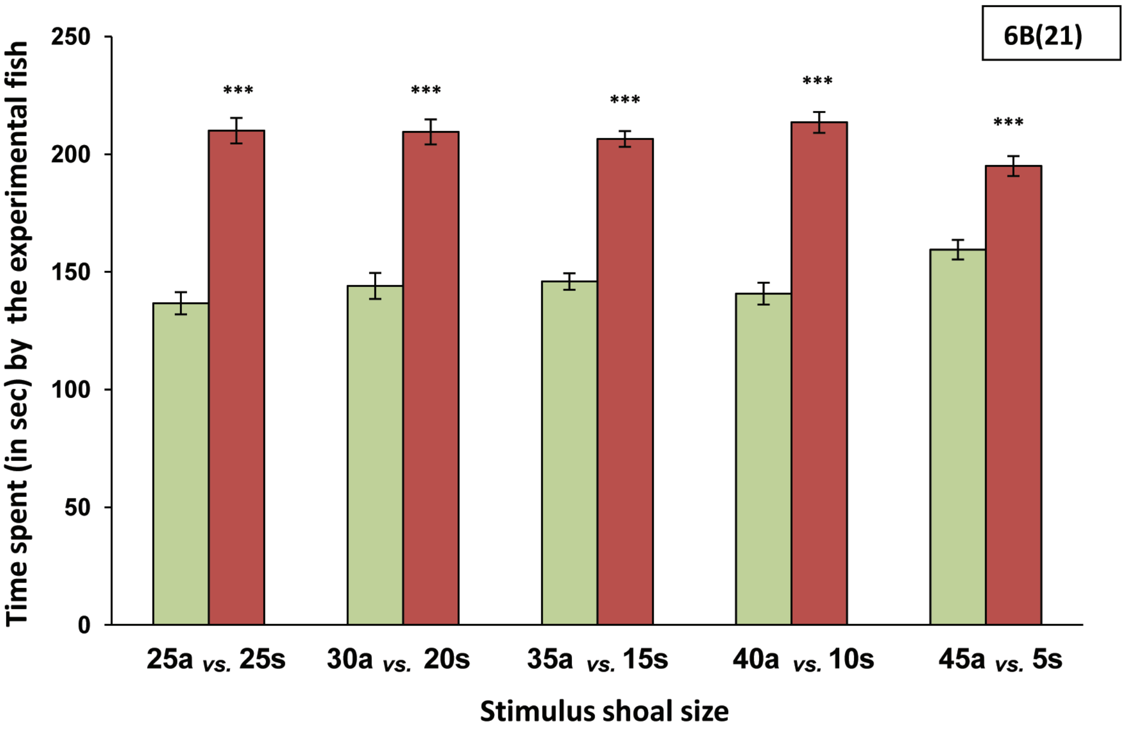


Figure 6B (21): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations when

6B (22): Test fish - 20 larvae of *Puntius sarana subnasutus* (siblings) reared with 20 larvae of heterospecifics *Anabas testudineus* for 8 months

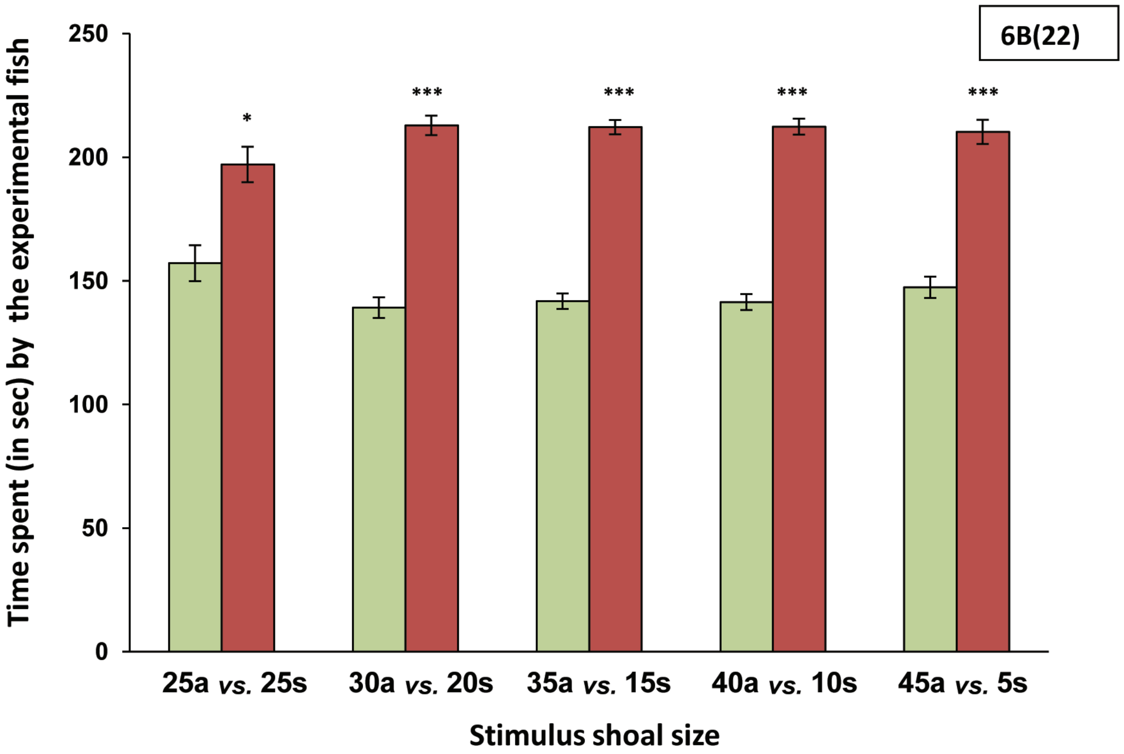


Figure 6B (22): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B(23): Test fish -30 larvae of *Puntius sarana subnasutus* (siblings) reared with 30 larvae of heterospecifics *Anabas testudineus* for 8 months

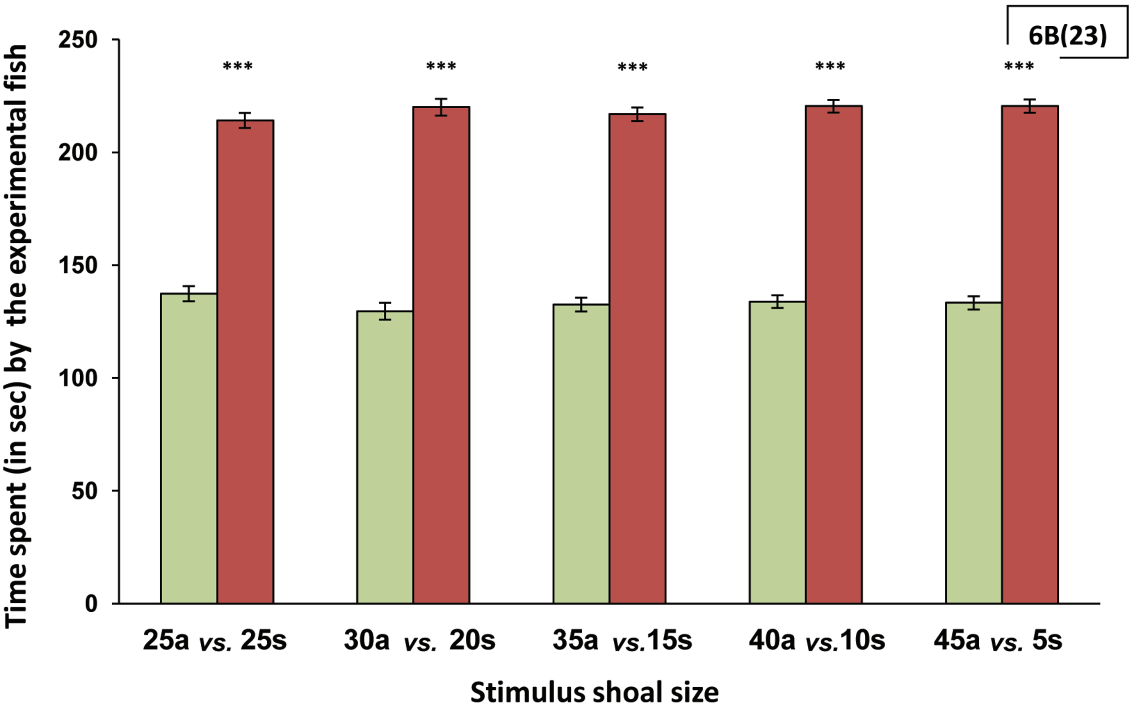


Figure 6B (23): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

6B (24): Test fish: 40 larvae of *Puntius sarana subnasutus* (siblings) reared with 40 larvae of heterospecifics *Anabas testudineus* for 8 months

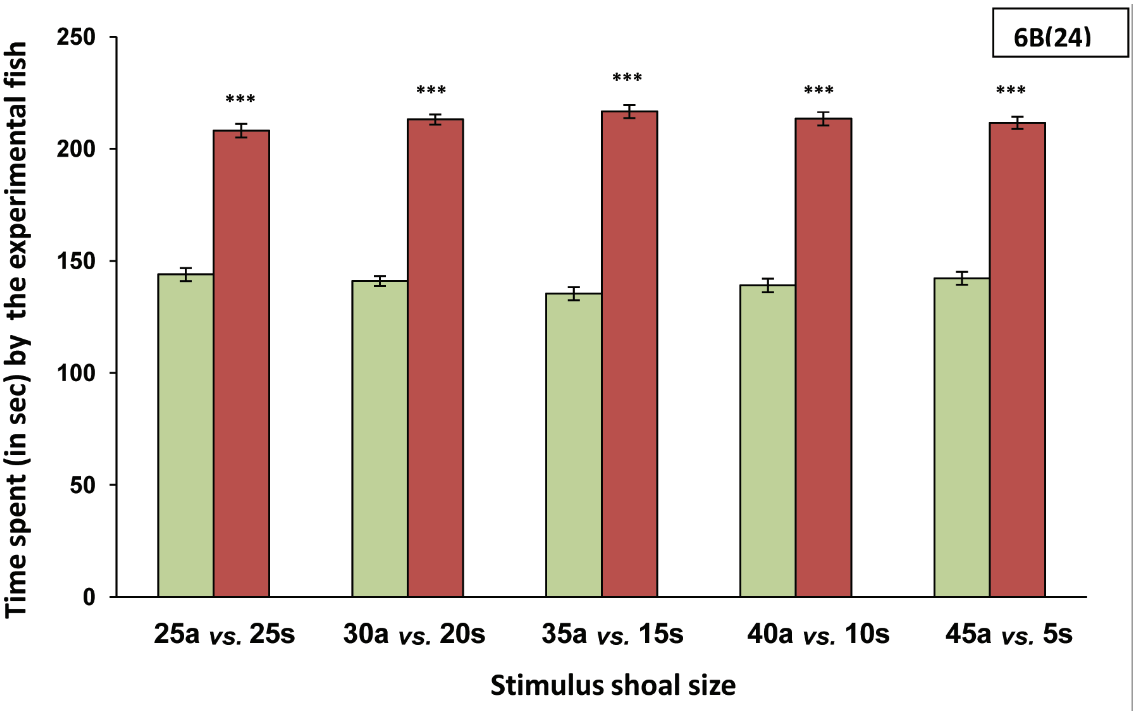


Figure 6B (24): Time spent in seconds (Mean ± SE) by the test fish (n=20) near the stimulus shoals composed of familiar ***heterospecific A.testudineus (a)*** and ***siblings (S)*** of ***P. sarana subnasutus*** in varying numerical combinations

Test fish	**Rearing group**	**Age of the larvae**	**Stimulus shoal-size** **Sibling group (s) *vs.* Heterospecific *Anabas testudineus* (a)**				
			25a vs. 25s	***30a vs. 20s***	***35a vs. 15s***	***40a vs. 10s***	***45a vs. 5s***
Larvae reared with individuals of hetero specific *Anabas testudineus*	**10**	3 Months	t= 5.116 p=0.001***	t= 1.944 p=0.067*	t= 3.081 p=0.006**	t= 3.387 p=0.003**	t= 2.291 p=0.034*
		8 Months	t= 8.584 p=0.001***	t= 6.035 p=0.001***	t= 8.887 p=0.001***	t= 8.014 p=0.001***	t= 4.247 p=0.001***
	20	3 Months	t= 8.148 p=0.001***	t= 3.833 p=0.001**	t= 5.094 p=0.001***	t= 1.934 p=0.068*	t=0 .517 p=0.611
		8 Months	t= 2.757 p=0.013*	t= 9.273 p=0.001***	t= 11.748 p=0.001***	t= 11.035 p=0.001***	t= 7.083 p=0.001***
	30	3 Months	t= 10.830 p=0.001***	t= 3.004 p=0.007**	t= 3.379 p=0.003**	t= 0.448 p=0.659	t= 1.954 p=0.066*
		8 Months	t= 11.613 p=0.001***	t= 13.034 p=0.001***	t= 14.286 p=0.001***	t=15.225 p=0.001***	t= 14.343 p=0.001***
	40	3 Months	t= 8.961 p=0.001***	t= 2.142 p=0.045*	t=0 .009 p=0.993	t= 0.194 p=0.848	t= 0.735 p=0.471
		8 Months	t= 10.873 p=0.001***	t= 16.158 p=0.001***	t= 14.339 p=0.001***	t= 12.469 p=0.001***	t= 12.437 p=0.001***

Table : 6B (5): Preference of *Puntius sarana subnasutus* to associate with the larger group when the choice is between siblings and heterospecific *Anabas testudineus* at 3 months and 8 months old stage

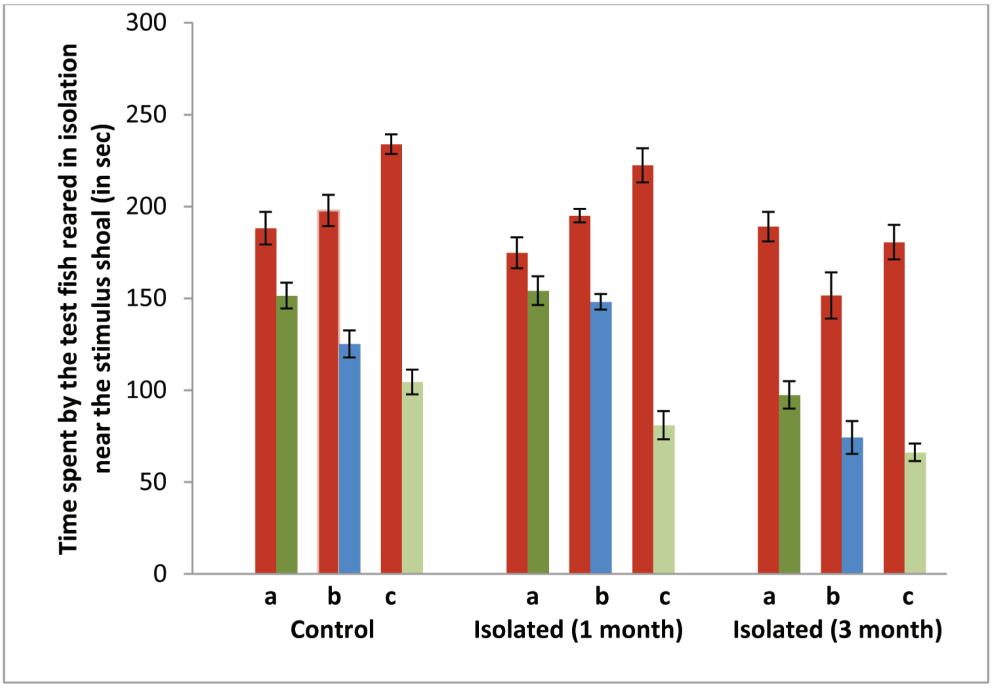
6.C: Larvae of *P. sarana subnasutus* reared in isolation

I) BEHAVIOURAL ASSAY

a) Sibling preference in isolated individuals

Isolation from the next day of hatching onwards did not cause any marked effect on the sibling recognition abilities of *Puntius sarana subnasutus* compared to the control individuals those were reared in groups of ten individuals. Isolated fish (both isolated for a period of one month and three months) similar to the control fish effectively identified their siblings from conspecific when the number of individual in the stimulus shoal were same. However a slight decrease in sibling preference was observed when the larvae reared in isolation for one months (t = 2.599, p= 0.057). When the alternate stimulis shoal is composed of heterospecifc *Puntius parrah* and *Anabas testudineus*, larvae from all treatment group associated more significantly with their siblings. Further, prolonging the period of isolation from one month to three months also did not cause any difference in sibling recognition [Fig. 6C (1), Table 6C (1)].

Figure 6C (1): Preference of isolated individuals to associate with the sibling stimulus shoal when the alternate stimulus shoal is composed of conspecific *P. sarana subnasutus* and heterospecifics *Puntius parrah and Anabas testudineus* in equal number (25 *vs.* 25)



a) Stimulus shoal 1: 🟥 25 *P. sarana subnasutus* (Siblings of the test fish)
Stimulus shoal 2: 🟩 25 *P. sarana subnasutus*(Conspecifics of the test fish)

b) Stimulus shoal 1: 🟥 25 *P. sarana subnasutus* (Siblings of the test fish)
Stimulus shoal 2 : 🟦 25 *P. parrah*(Heterospecific of the test fish)

c) Stimulus shoal 1: 🟥 25 *P. sarana subnasutus* (Siblings of the test fish)
Stimulus shoal 2: 🟩 25 *Anabas testudineus* (Heterospecific of the test fish)

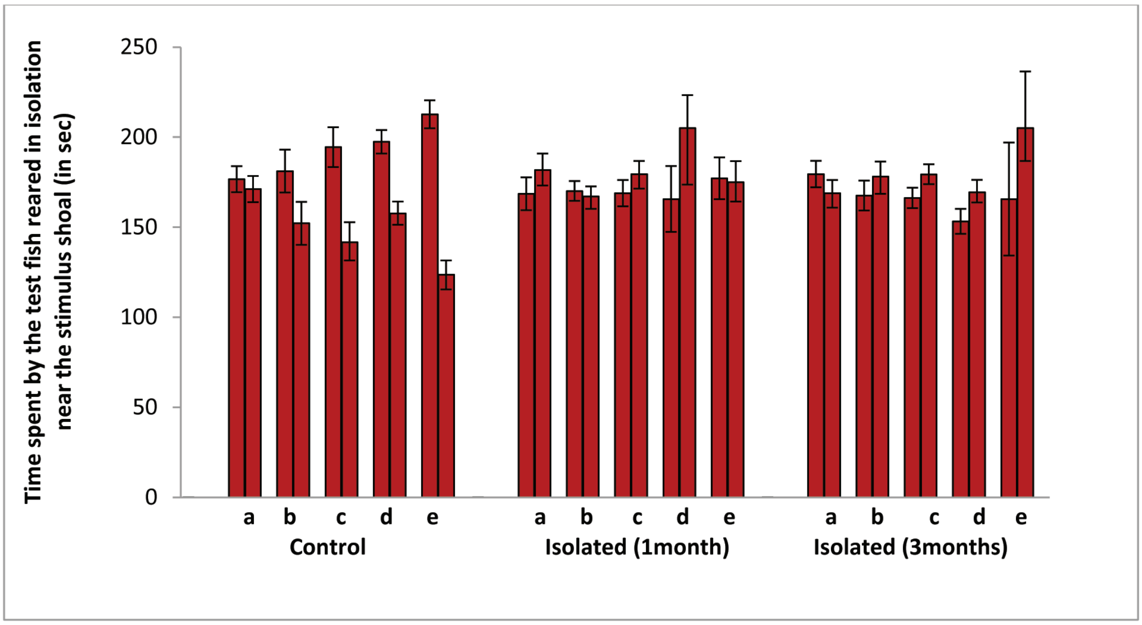
Expt.	**Test fish** (*Puntius sarana subnasutus*)	**Stimulus Shoals** (25siblings *vs.* 25conspecifics/hetero specifics)		
		P. sarana subnasutus *vs.* *P. sarana subnasutus* (Conspecific)	*P. sarana subnasutus* *vs.* *Puntius parrah* (Heterospecific)	*P. sarana subnasutus* *vs.* *Anabas testudineus* (Heterospecific)
I	**Control** (Larvae reared in group of 10siblings)	t = 3.361 p = 0.007**	t = 5.087 p = 0.001***	t = 14.224 p = 0.001***
II	**Permanently isolated** (1 months)	t = 2.599 p = 0.057*	t = 4.135 p = 0.001***	t = 10.120 p = 0.001***
III	**Permanently isolated** (3 months)	t = 3.178 p = 0.004**	t = 6.178 p = 0.001***	t = 9.574 p = 0.001***

Table 6C (1): Preference of isolatd individuals to associate with genetically more related stimulus shoal when the shoal choice is between siblings and conspecific *P. sarana subnasutus* and heterospecifics *Puntius parrah* and *Anabas testudineus*

b) Sibling group size discrimination in isolated individuals

Eventhough isolation did not cause any marked changes on sibling recognition abilities of *Puntius sarana subnasutus*, it entirely disrupted the fish's shoal size discrimination/ numerical ability. The fish (both isolated for a period of one month and three months) failed to properly identify the larger group and never spent significantly more time with the larger sibling stimulus shoal at any of the numerical combination [25 siblings *vs.* 25 siblings, 30 siblings *vs.* 20 siblings, 35 siblings *vs.* 15 siblings, 40 siblings *vs.* 10 sibling and 45 siblings *vs.* 5 siblings at the ratio of 1:1, 1:1.5, 1:2.3, 1:4 and 1:9 respectively]. The lack of social interactions during the period of development prevented them from gaining the benefits of shoaling behaviour [Fig. 6C (2), Table 6(2)].

Figure 6C (2): Ability for group-size discrimination in isolated individuals when the alternate stimulus shoal is composed of *Puntius sarana subnasutus* (siblings of the test fish) in varying numbers



a) Stimulus shoal: 🟥 25 *P. sarana subnasutus* (Siblings of the test fish)
🟥 25 *P. sarana subnasutus*(Siblings of the test fish)

b) Stimulus shoal: 🟥 30 *P. sarana subnasutus* (Siblings of the test fish)
🟥 20 *P. sarana subnasutus* (Siblings of the test fish)

c) Stimulus shoal: 🟥 35 *P. sarana subnasutus* (Siblings of the test fish)
🟥 15 *P. sarana subnasutus*(Siblings of the test fish)

d) Stimulus shoal: 🟥 40 *P. sarana subnasutus* (Siblings of the test fish)
🟥 10 *P. sarana subnasutus* (Siblings of the test fish)

e) Stimulus shoal: 🟥 45 *P. sarana subnasutus* (Siblings of the test fish)
🟥 5 *P. sarana subnasutus*(Siblings of the test fish)

Expt	Test fish *(Puntius sarana subnasutus)*	**Stimulus Shoals of *Puntius sarana – subnasutus* varying Group-size (number)**				
		25 vs. 35	***20 vs. 30***	***15 vs. 35***	***10 vs. 40***	***5 vs. 45***
I	**Control** (Larvae reared in group of 10)	t = 0. 486 p = 0.623	t = 1.010 p = 0.325	t = 1.908 p = 0.072*	t = 4.214 p=0.001***	t = 6.085 p=0.001***
II	**Permanently isolated (1month)**	t = 0.954 p =0.380	t = 0.677 p = 0.507	t = 0.139 p = 0.891	t = 0.325 p =0.742	t = 0.056 p = 0.956
III	**Permanently isolated (3 months)**	t = 1.210 p = 0.247	t = 0.754 p = 0.460	t = 0.234 p = 0.817	t = 0.539 p = 0.596	t = 0.084 p = 0.934

Table 6C (2): Significance of time spent by the isolated individuals near stimulus shoals composed of *Puntius sarana subnasutus* (siblings of the test fish) in various numbers

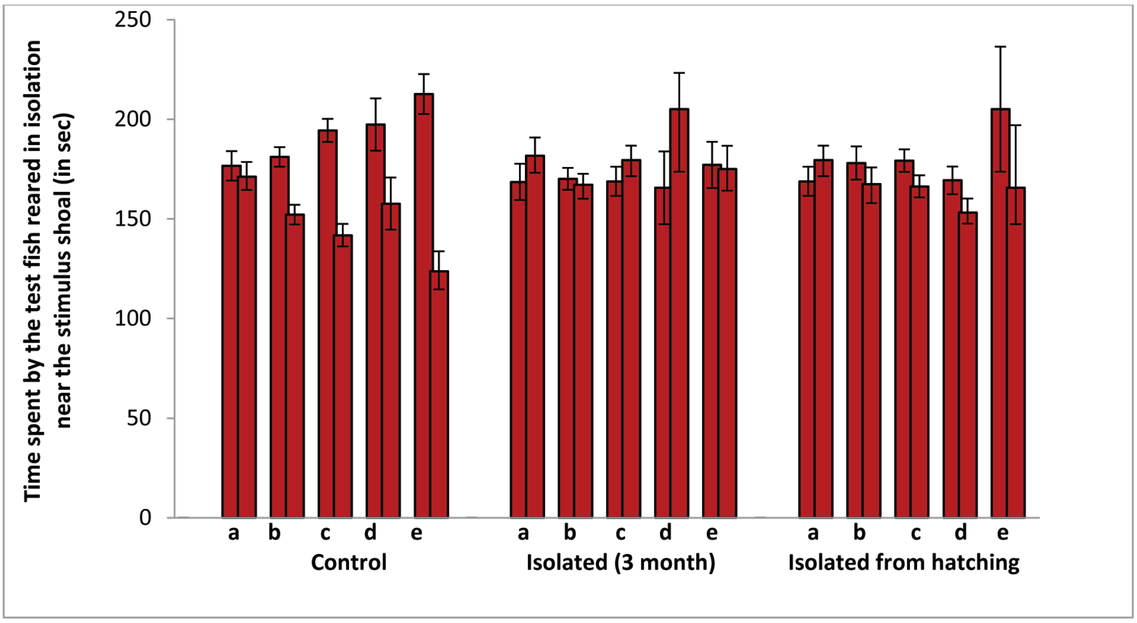
c) Effect of isolation during the early periods of development

The findings of the present study strongly indicate that group-size discrimination ability of *Puntius sarana subnasutus* is determined by the social interactions of the fish during the early developmental stages. The control fish gradually shifted their preference towards larger siblings with respect to an increase in the number of individuals in the sibling stimulus shoal [25 siblings *vs.* 25 siblings, 30 siblings *vs.* 20 siblings, 35 siblings *vs.* 15 siblings, 40 siblings *vs.* 10 sibling and 45 siblings *vs.* 5 siblings at the ratio of 1:1, 1:1.5, 1:2.3, 1:4 and 1:9 respectively]. Those individuals separated for a period of 45 days from the next day of hatching and then reared in group for one month behaved similar to the individuals those reared in total isolation for three months. They failed to discriminate the larger sibling group from the smaller sibling shoal in all numerical combinations. On the other hand, the individuals reared in group for a period of 45 days from the day of hatching and then reared in isolation for a period of one month behaved as that of controls identifying the group size differences between the sibling stimulus shoals. The fish spent significantly more time with the larger sibling stimulus group. The preference was very high at the ratios1:4 and 1:9 (40 siblings vs. 10 sibling and 45 siblings vs. 5 siblings) [Fig. 6C (3) & Fig. 6C (4); Table: 6C(3)].

i) EFFECT OF ISOLATION FROM THE DAY OF HATCHING ONWARDS

Figure 6C (3): Ability for group-size discrimination in isolated individuals

(*Permanently isolated larvae and larvae isolated for 45 days from the next day of hatching onwards and then reared in groups for 1 month*)



a) Stimulus shoal: 🟥 25 *P. sarana subnasutus* (Siblings of the test fish)
🟥 25 *P. sarana subnasutus*(Siblings of the test fish)

b) Stimulus shoal: 🟥 30 *P. sarana subnasutus* (Siblings of the test fish)
🟥 20 *P. sarana subnasutus* (Siblings of the test fish)

c) Stimulus shoal: 🟥 35 *P. sarana subnasutus* (Siblings of the test fish)
🟥 15 *P. sarana subnasutus*(Siblings of the test fish)

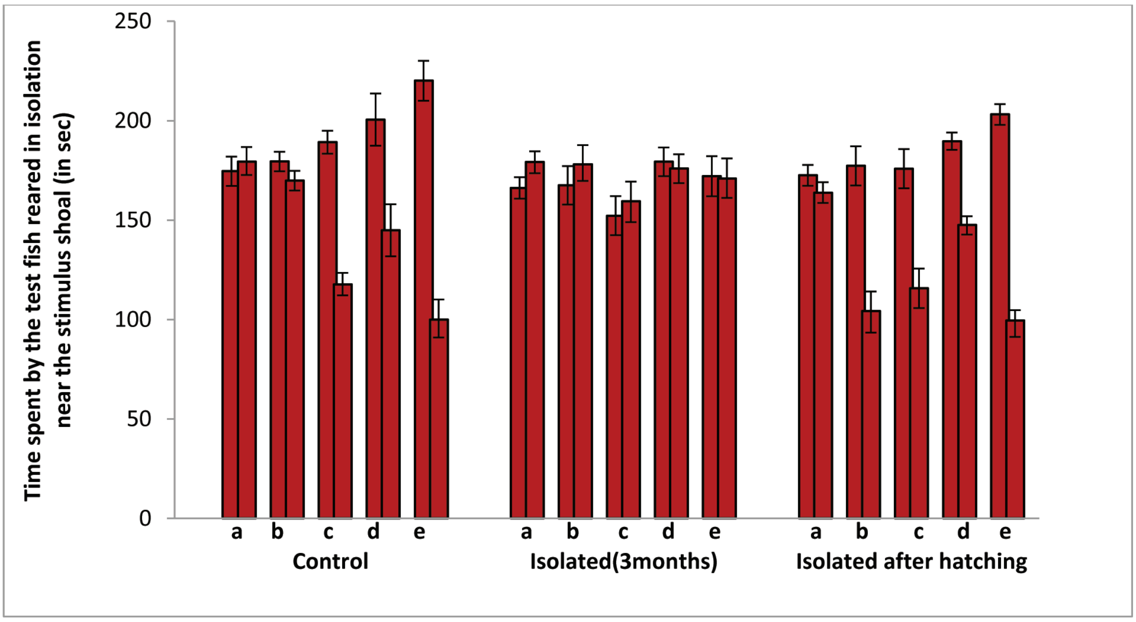
d) Stimulus shoal: 🟥 40 *P. sarana subnasutus* (Siblings of the test fish)
🟥 10 *P. sarana subnasutus* (Siblings of the test fish)

e) Stimulus shoal: 🟥 45 *P. sarana subnasutus* (Siblings of the test fish)
🟥 5 *P. sarana subnasutus*(Siblings of the test fish)

ii) EFFECT OF ISOLATION AFTER GROUP REARING IN EARLY STAGES

Figure 6C (4): Ability for group-size discrimination in isolated individuals

(*Permanently isolated larvae and larvae isolated after 45 days hatching and then reared in isolation for 1 month*)



a) Stimulus shoal: 🟥 25 *P. sarana subnasutus* (Siblings of the test fish)
🟥 25 *P. sarana subnasutus*(Siblings of the test fish)

b) Stimulus shoal: 🟥 30 *P. sarana subnasutus* (Siblings of the test fish)
🟥 20 *P. sarana subnasutus* (Siblings of the test fish)

c) Stimulus shoal: 🟥 35 *P. sarana subnasutus* (Siblings of the test fish)
🟥 15 *P. sarana subnasutus*(Siblings of the test fish)

d) Stimulus shoal: 🟥 40 *P. sarana subnasutus* (Siblings of the test fish)
🟥 10 *P. sarana subnasutus* (Siblings of the test fish)

e) Stimulus shoal: 🟥 45 *P. sarana subnasutus* (Siblings of the test fish)
🟥 5 *P. sarana subnasutus*(Siblings of the test fish)

Expt	Test fish (*Puntius sarana subnasutus*)	Stimulus Shoals of varying Group-size				
		25 vs 25	20 vs 30	15 vs 35	10 vs 40	5 vs 45
I	Control	t = 0.385 p = 0.704	t = 1.840 p=0.081*	t = 1.908 p=0.072*	t = 3.100 p =0.006**	t = 6.085 p=0.001***
	Permanently isolated (3 months)	t = 0.734 p = 0.490	t = 0.264 p = 0.795	t = 0.691 p = 0.498	t = 0.918 p = 0.370	t = 0.092 p = 0.928
	45 days in isolation & grouped for 1 month	t = 0.691 p = 0.498	t = 0.593 p = 0.560	t = 1.195 p = 0.247	t = 1.350 p = 0.193	t = 0.918 p = 0.370
II	Control	t = 0.343 p =0.735	t = 1.013 p =0.352	t = 2.200 p=0.040*	t = 3.722 p=0.001**	t = 7.116 p=0.000***
	Permanently isolated (3 months)	t = 1.195 p = 0.247	t = 0.593 p = 0.560	t = 0.539 p = 0.596	t = 0.234 p = 0.817	t = 0.056 p= 0.956
	45 days in group & isolated for 1 month	t = 0.128 p =0.900	t = 2.114 p=0.048*	t = 4.214 p=0.001**	t = 4.692 p=0.001***	t = 9.380 p=0.000***

Table 6C (3): Significance of time spent by the isolated individuals (under three different experimental set-up) along with stimulus shoals composed of *Puntius sarana subnasutus* (siblings of the test fish) in varying numerical combination

II) COMPARISON OF FOREBRAIN USING SCANNING ELECTRON MICROSCOPY

The images obtained after scanning electron microscopy of fore brain of *Puntius sarana subnasutus* exhibited marked variation according to the rearing environment. The fore brain regions of fish reared in isolation were more constricted which may be an indication of lesser neuronal development compared to the control group reared in groups [Figure 6C (5) & 6C (6)]

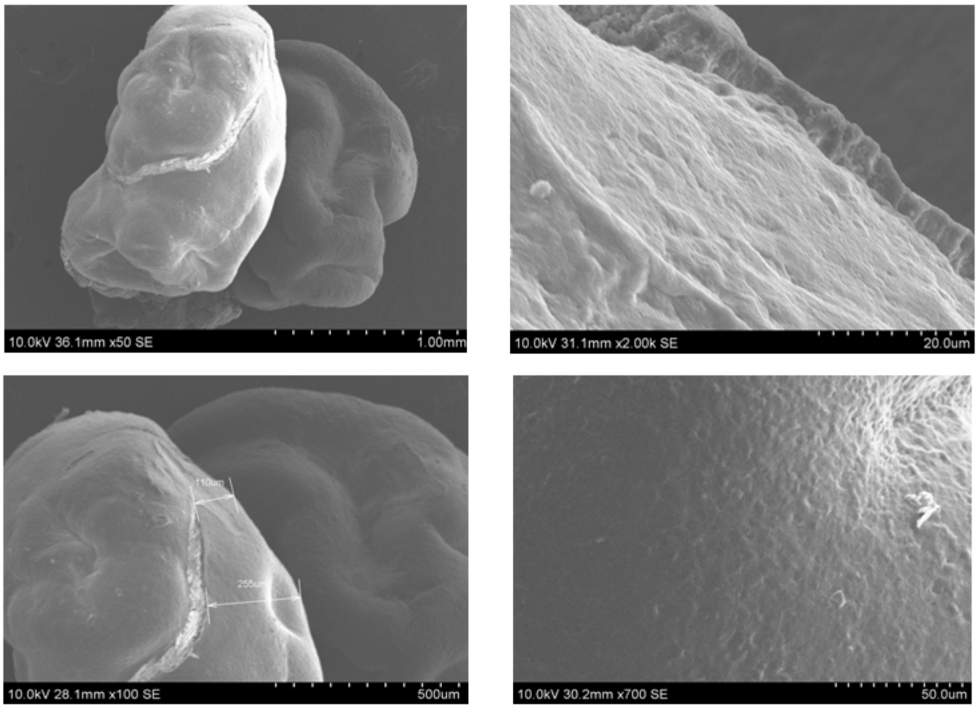


Figure 6C (5): SEM images of forebrain of *Puntius sarana subnasutus* reared in isolation

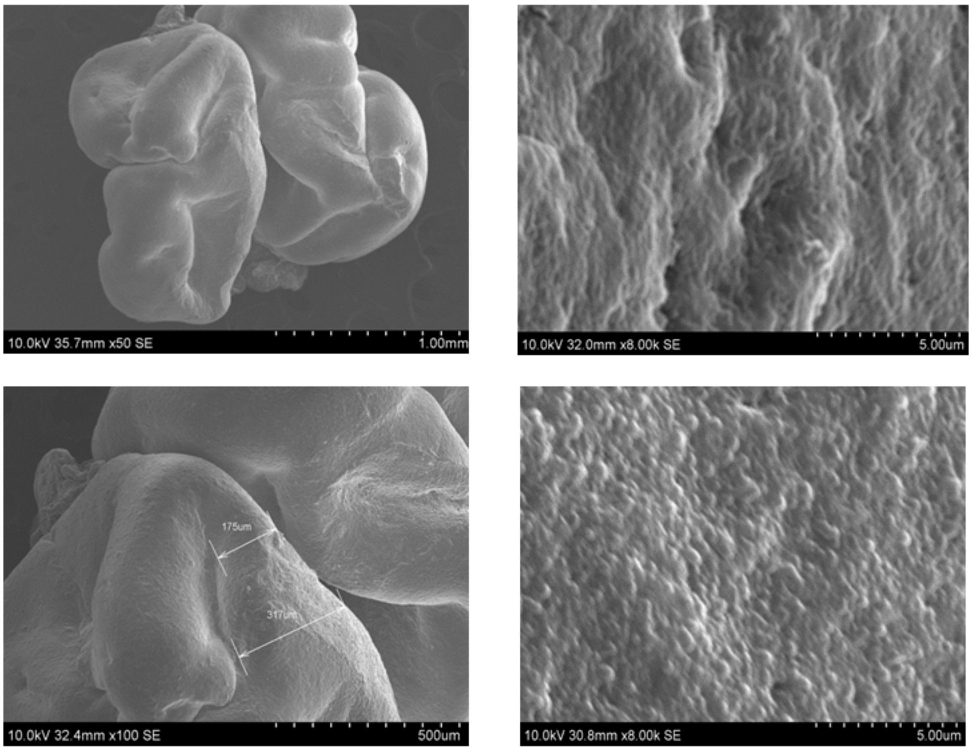


Figure 6C (6): SEM images of forebrain of *Puntius sarana subnasutus* reared in group of ten (control)

III) ANALYSIS OF THE PHYSIOLOGICAL CHANGES

Glucose:

Control (n=7)	32mg/dl
Experiment (n=7)	17mg/dl

Cortisol:

Control (n=7)	4.22 µg/dl
Experiment (n=7)	9.30µg/dl

T4:

Control (n=7)	5.28 µg/dl
Experiment (n=7)	8.04 µg/dl

DISCUSSION

A) Kinship and shoal choice

Group living is a common strategy across animal taxa and involves trade-off between the costs and benefits of joining a group or remaining solitary. When studying the adaptive benefits of sociality, the fitness consequences of living in groups of different sizes and compositions should be considered effectively (Jordan et al., 2010). It is well established that as shoal-size increases, per capita predation risk for shoal members is expected to decrease as a result of 'group vigilance' and 'confusion effect' and a shoal may also be able to find and access food resources more readily. By joining and thus increasing the size of the shoal, especially those of relatives, a fish can certainly maximize both its direct fitness as well as inclusive fitness. Additionally, if genetic homogeneity within a shoal translates into phenotypic homogeneity this may further enhance the confusion effect. Such phenotypic homogeneity is also expected to increase competition for food within a shoal although this may be reduced by individuals exercising 'competitive restraint' when competing with kin (Waldman, 1988).

The present results show that *Puntius sarana subnasutus* clearly recognized their siblings and preferred to spent more time with them when the group-size were equal. Preference for siblings rather than non-siblingss has been demonstrated in some other species like Coho salmon, Arctic charr, Atlantic salmon, rainbow trout and sticklebacks (Olsen, 1989; Quinn and Busack, 1985; Brown and Brown, 1992; Fitzgerald and Morrissette, 1992). In further analysis we found that this preference for siblings can be readily reversed by increasing the group-size of stimulus shoal of conspecifics *vs.* sibling shoal. Obviously, this raised the question whether genetic relatedness or increase in group-size matters more in the association preferences of the test fish. Even though, many studies have analysed the shoal-choice preferences between siblings and half sibs / conspecifics in terms of number, no studies have ever investigated the trade-off between preferences for siblings with that of larger shoal of heterospecifics.

Mixed-species groups, however, are still common across a range of environments, including shallow fresh water habitats (Krause et al., 1996b; Peuhkuri, 1997), tropical and sub-tropical reefs (Overholtzer and Motta, 2000) and temperate pelagic communities (Freon and Misund, 1999). Multispecies groups may result when the antipredator benefits of membership of a large shoal outweigh the costs of phenotypic oddity (Landeau and Terborgh, 1986) and resource partitioning between species could reduce food competition in heterospecifics shoals (Ehrlich and Ehrlich, 1973). However, in the present study when we compared the shoaling preferences of *Puntius sarana subnasutus* with their conspecifics and heterospecifics, the preference towards heterospecifics were very less. When the heterospecifics group was composed of *Puntius parrah* of the same family cyprinidae, the focal fish exhibited a fairly increased preference towards them that too only when the group-size was much larger compared to the alternate sibling stimulus shoal. Further when the heterospecifics stimulus shoal was composed of genetically more distant *Anabas testudineus,* even an increase in group size failed to attract the fish towards them. This lack of preference may be because of the peculiarities in the rearing environment. Experiences in the early social environment play an important role in shaping various behavioural patterns in later life. In the present study we reared the experimental fish in pure sibling groups from the next day of hatching onwards without any exposure to the cues from conspecifics and heterospecifics. This alteration in rearing environment might impose certain changes in the individual recognition mechanisms employed by the fish.

A fish encountering another individual may recognize its specific individual identity, or it may simply distinguish some other, more general cue, enabling it to differentiate between subsets of the population. These alternative mechanisms are not mutually exclusive, although the extent to which each is used may vary between populations and species according to their ecology. However a distinction between these two recognition systems is very important. Specific individual recognition is gradually learned over a period of time while more general form of recognition does not require individuals to have any prior experience of each other (Ward et al., 2007; Webster et al., 2007). In the present study, since the focal fish did not get any prior experience/encounters with the other species the fish failed to implement properly the specific recognition mechanism of identification. The results clearly specify the importance of early social interactions with other species, especially during it's critical learning period, which may otherwise affect the survival of the species itself.

The general recognition mechanism is more genetically hard-wired and the fish could successfully identify their conspecifics. Recen research works have established the role of major histocompatibility complex (MHC) genotypes in recognition among related individuals. Studies by Ward et al. (2009) in Stickle backs identified that recognition of general odour traits allows individuals to access potential group mates without the need for actually having previously encountered them. MHC genes are highly polymorphic, and because of this, each individual often has a highly characteristic genotype. In addition to this, MHC affects the odour of its carrier allowing detection and recognition between individuals (Penn, 2002). High level of relatedness increases the likelihood of a comparably high degree of similarity between MHC genotypes. Olsen et al., (2002) reported that in juvenile Salmonids, even when the choice given was between two sibling odours, the focal fish demonstrated a preference for siblings with matching MHC genotypes. However, in some other species studied, kin recognition ability was only fully expressed when both visual and olfactory signals were communicated, for example Sticklebacks (Steck et al., 1999), Rainbow fish (Arnold, 2000).

Whatever may be the mechanism of recognition (whether general or specific) learning is an important component. Phenotypic cues, which may be visual or olfactory, an individual should learn to acquire such a signal and compare this to a template (Waldman, 1999). This template may be genetically hard-wired (in general recognition) or alternatively it may be gained during a sensitive developmental period (in specific recognition). Where the signal matches the template, recognition is said to have occurred. If effective learning does not take place appropriately in nascent learning stage during which a template is formed, the individual may lose its ability for recognition without further reinforcement. When Arctic charr were reared in isolation, they become subsequently unable to discriminate kin from non-kin (Winberg and Olsen, 1992). Similarly, individuals raised with both kin and non-kin were subsequently unable to differentiate between them. Warburton and Lees (1996) by raising juvenile guppies (*Poecilia reticulata*) with sword tails (*Xiphophorus helleri*) found that guppies raised along with other guppies subsequently displayed typical pattern of conspecifics preference; however, those raised with sword tails preferred to associate with heterospecific sword tails rather than conspecifics. Thus the template used for recognition of individual may be flexible according to the social environment.

B) Familiarity and shoal choice

For group living animals, individual fitness varies as a function of group size, so that group choice of an individual reflects a dynamic trade-off between the costs and benefits of membership. Typically, competition for resources increases, where as predation risk and certain foraging costs decrease with increasing group size. One best strategy to reduce competition for resources without compromising predation risk and foraging costs is to form heterogenous groups with other species that exploit various food resources in a same locality (Ward and Hart, 2005). A number of recent studies have reported preferences for familiar individuals in a range of taxa. Familiarity develops over a period of time and when once developed, it may persist for several weeks, even without reinforcement. Associating with familiars can potentially provide individuals with important benefits, including enhanced group antipredator behaviour and foraging success (Barber and Wright, 2001). In fishes, shoals composed of familiar individuals are more cohesive, potentially increasing the shoal's antipredatory function. Shoals of familiars outperform random shoals in foraging tasks (Ward and Hart, 2004). The 'dear enemy' phenomenon of reduced aggression between familiar individuals acts to stabilize dominant hierarchies and mediate competitive interactions in many species (Utne-Palm and Hart, 2000). In addition, familiarity has been shown to promote social learning (Swaney et al., 2001).

In the present study, the test fish were allowed to interact with the unfamiliar (either conspecifics or heterospecifics) from the next day of hatching onwards. Exposure during this critical period of development leads to tremendous changes in the social recognition and shoal choice of *Puntius sarana subnasutus*. Here the individuals traded off their gains from associating with their siblings when presented with a larger group of familiar conspecifics or heterospecifics. Here also the influence of genetic relatedness is evident. The test fish preferred familiar conspecific stimulus shoal to unfamiliar sibling shoal. When presented with stimulus shoals of familiar heterspecific *Puntius parrah* and shoals of siblings the test fish exhibited significant preference to familiar heterospecifics. However, when the alternate stimulus shoal is composed of genetically distantly related *Anabas testudineus*, the test fish never exhibited preference to the shoal of familiar *Anabas testudineus* irrespective of the size of the shoal.

Familiarity, as it is generally understood develops gradually. In the guppy, frequent interactions over a 12day period are required before the fish begin to demonstrate association preferences for familiar individuals (Griffiths and Magurran, 1997a). In addition, investigations in this species has shown that there is an upper limit of (around 40) the number of individuals that fish can become familiar with (Griffiths and Magurran, 1997b). Metcalfe and Thompson (1995) examined the schooling preferences of European minnows kept in groups of seven for 12 and 20 days. Some previous investigations of the role of familiarity in decision making have examined groups of fish that were kept together for longer periods. Magurran et al. (1994) looked at grouping preferences for familiar fish in groups of up to 15 guppies that have been together for 2 months. Dugatkin and Wilson (1992) found that bluegill sunfish, *Lepomis machrochirus*, that had been in groups of six for more than 3 months preferred to associate with familiar individuals.

In the present model, group-size is an emergent property of the system in which individuals used context dependent decision rules based on the costs and benefits between genetic relatedness and familiarity. Familiarity is, by definition, reliant upon social recognition, which may be achieved by learning the individual identities of frequently encountered individuals over time or by a more general recognition of some characteristics common to members of the local environment, such as habitat specific chemical cues (Ward et al., 2004). The study shows that *Puntius sarana subnasutus* is capable of using temporarily flexible, local habitat – specific cues for social recognition. The association preference observed in the current study suggests that the fish use learned individual recognition based on previous social experience to mediate their shoaling decisions. Apart from this, fish can also use self referent matching of chemical cues. The current work suggests that rather than being based solely on simple fixed templates, social recognition in fish may be at least partially dependent on spatially and temporally flexible cues relating to their recent habitat, diet and social experience. Such cues acquired through interaction may allow individuals to discriminate between subsets of their local application in the context of foraging, predator avoidance etc.

Taken as a whole, the findings suggest that social recognition mechanisms and association preferences of *Puntius sarana subnasutus* are based primarly on individual recognition by previous social experience. This explains the current paradigm of the mechanism underlying familiarity. When the fish was previously not exposed to the heterospecifics especially to a genetically distantly related *Anabas testudineus,* the test fish never exhibited preference towards them irrespective of the shoal-size. These cues gained through early interaction with conspecifics and heterospecifics stabilize the local social interactions of fishes and provide a context in which more specific individual recognition could develop. The importance of various recognition cues to the behaviour of aquatic animals has been demonstrated in contexts as diverse as mate choice and reproduction (Sorensen and Hobson, 2005), predatory-pray relationship (Mirza and Chivers, 2001) and shoaling preferences (Behrmann-Godel et al., 2006). Swaney et al. (2001) found that guppies learned a foraging task faster from familiar than unfamiliar conspecifics, suggesting that socially learned information may diffuse more rapidly through familiar sub-groups.

Time is one factor that mediates shoaling preferences for certain individuals. Prolonged interactions between familiar individuals can provide fish with important information about individual differences in their shoal mate's behaviour and allow shoaling fish to co-ordinate behaviour or exploit actions of others. In the present study preference towards conspecifics and heterospecifics *Puntius parrah* was found to be increasing with an increae in the period of exposure (3 months and 8 months). As familiarity among individuals may be based on past encounters and behavioural experiences with each other over some period, such social familiarity is more likely to develop under conditions where individuals live in small and stable groups, encounter each other and share socially transmitted informations often and for prolonged periods, and when the benefits of social association based on familiarity equal or exceed the associated costs. During this time the preference for genetically related individuals may be much stronger in order to facilitate mate selection. Further research works on these aspects is necessary ascertain this phenomenon undoubtedly. In the current study preference with familiar individuals exhibited by *Puntius sarana subnasutus* is consistent with the expectations based on the previous studies (Griffiths, 2003; Ward and Hart, 2003). These results also show that the advantages offered by kinship can be overridden by the composition of a familiar group.

C) Isolation and shoal choice

Social isolation refers to a lack of contact with members of a species. Isolated individuals report fewer interactions with others, physiological / emotional imbalances and lower levels of activity. Social isolation is more closely related to the quality than quantity of social life (Cacioppo and Hawkley, 2009). A fish may respond differentially to the same stimulus on different occasions because of motivational changes. Imprinting and social learning profoundly affects the motivational state of the animal. Imprinting refers to the development of social and habitat attachments as the result of experience during a brief sensitive period in early life. In covict cichlids *(Archocentrus nigrofasciatus)*, fear increases with social isolation (Gallagher et al., 1972). Social isolation also has been found to decrease aggressiveness in a number of species (Heiligenberg and Kramer, 1972).

The present results suggest that social isolation did not severely affect the sibling recognition ability of *Puntius sarana subnasutus,* even though, it disrupted the group-size discrimination abilityof the individuals. It is likely that recognition of a suitable group member can be innate or it can be learned through experience. Waldman (1991) proposed that individual recognition might develop following a simple sequence of events. Phenotypic signals, which may be visual or chemical in nature, are exchanged between individuals. When an individual acquires such a signal, it compares the signal to a template. Where the signal matches to the template recognition may be said to have occurred. In the present study, sibling recognition might have taken place with the help of the "genetically hard-wired" templates. Further, relatedness increases the likelihood of a comparably high degree of similarity between MHC genotyoes. Olsen et al., (2002) reported that when given a choice between two sibling odours, focal fish demonstrated a preference for sibling odour with matching MHC genotypes.

Neverthless, the present results demonstrate that social isolation adversely affects the numerical ability of an isolated fish compared to a fish reared in groups. Currently, few studies shoals numerical competence in lower vertebrates. For example, fish seeking safety from a predator display a rudimentary numerical ability in selecting a larger shoal (Agrillo et al., 2009; Agrillo and Dadda, 2007; Wong and Rosenthal, 2005). There are indeed many real-life situations in which using quantitative abilities can be useful, and there is no reason to believe that selective pressures in favour of the ability to quantify different magnitudes should have acted only on hominids. Quantitative abilities can permit animals to optimize foraging, enabling them to rapidly select the larger of two available sources of food; this ability also represents a powerful tool for anti-predator defense, letting animals join the largest available group of conspecifics, reducing the probability of being spotted by predators. In animals quantitative abilities are shown in social interactions; chimpanzees, lionesses and hyenas are more likely to attack other groups of conspecifics when they perceive themselves as being part of a larger group (Piffer et al., 2013). For instance, teleost fish have been shown to recognize up to forty familiar individuals, cooperate to achieve a common goal, learn new habits from experienced conspecifics, use tools, have cultural traditions, and use numerical information (Bisazza, 2010).

Even though, this ability for numerical discrimination among group members does not necessarily requires any high cognitive abilities but demands some sort of information processing abilities such as the ability to use relative magnitude of continuous variables like total area of the stimuli or the sum of their contours etc. In most cases these occur through learning and social interaction. Therefore, the size discrimination ability of an isolated fish may be very less because of the lack of a socio-sexual context as well as sets of abstract elements. Further, little is known about social behavior in early stages of development in fish. In some species, newborns appear to be solitary and their social behavior tends to increase across development. In Atlantic silver sides (*Menidia menidia*), schooling behavior starts when they are about twenty days old and develop gradually (Shaw, 1960). In anchovy larvae (*Engraulis mordax*), schooling becomes clearly established when they are about thirty days old. Recently, Buske and Gerlai (2011) conducted a laboratory study on the development of shoaling behavior in zebrafish (*Danio rerio*) from day seven post-fertilization to over five months of age, reporting that a few days after hatching fish do not form cohesive shoals, but shoaling tendency increases during development. Isolation during this critical period of development may make the individual deprived of a social behaviour like shoaling.

Further, the present results indicate the importance of social interaction during the early period of development. The larvae isolated from the next day of hatching for a period of 45 days and reared in groups for next one month was unable to discriminate the group-size difference between stimulus shoals while those larvae reared in group for first 45 days and then isolated for 1 month, successfully discriminated the group-size difference between stimulus shoals. In fish, the telencephalon is involved in a variety of social and cognitive behaviours, including courtship, grouping, paternity and learning (Demski and Beaver, 2001). Pollen et al. (2007) showed that the telencephalon of monogamous species is 15-20% larger than that of polygamous species, suggesting enhanced cognitive needs in monogamous fish. It has also been shown that changes in demand alter the number and size of component elements, making the relative size of different brain parts; a reliable predictor of their importance for the organism in question (Kotrschal et al., 2012). In the present experiment individually reared fish by no means get any information from other individuals, while visual, chemical and tactile cues were all available for the group-reared fish. Hence, one could expect that brain structures have more modifications in a group reared fish.

Dunbar (1995) has suggested that neural capacity limits the extent of social networks in higher vertebrates. A comparative study of 38 genera of primates revealed a significant link between relative neocortex volume and size of the social group in which the animals typically live. Social insects such as ants also demonstrate a positive trend between group-size and complexity of their neural apparatus (Jaffe and Chacon, 1995). The most social (those which form the largest colonies) of 13 species of Formicidae ants were found to have both the most complex chemical communication system and the most highly developed corpora pedunculata and olfactory lobes. It seems plausible that brain size in fish too is constrained by social relationships and ineractions based on individual recognition. In the present study it is observed that the size of the fore-brain regions of the isolated fish is smaller than that of the control fish reared in group. It is plausible that this may acocunt for the disruption in group-size discrimination of the ability of the isolated fish. It would be informative to use laboratory manipulations to further explore the relationship between brain architecture and social recognition.

Further, as suggested in a recent study (Sorensen et al., 2011) environmental stress may directly inhibit the brain cell proliferation in the telencephalon and is accompanied by an increase in plasma level of cortisol. Cortisol is the main corticosteroid hormone in fish. In addition to its homeostatic function in osmoregulation and energy metabolism cortisol is typically elevated during acute and chronic stress (Bonga, 1997). An increased level of glucose and T4 in isolated fish also indicates the high stress level (Abbas and Authman, 2009; Martínez-porchas et al., 2009). The results of the present study indicates that isolation of a group-living animal like fish, is a dreadful social stressor and has a marked effect on the development of social cognition of *Puntius sarana subnasutus*.

In conclusion, it is become clear that individual members of groups can enhance their fitness further by associating with kin or familiar individuals rather than interacting with unfamiliar individuals. Such groups are more cohesive and thus can increase their cooperative behaviours such foraging and predator avoidance compared with a shoal composed of unrelated or unfamiliar individuals. Shoal size is another important determining factor in the shoal choice of *Puntius sarana subnasutus*. The social environment at which the fish is exposed during the early periods of development plays a very important role in this context.

Chapter – 7

Role of visual cues in shoal choice and shoal-size discrimination

Communication between individuals is one of the key factors behind any sort of social aggregation. 'Communication systems' mean the combined expression, transmission and reception of signals in the visual, chemical, acoustic or electric modalities. Despite the generally poor quality of underwater images, fish depend a great deal on vision as a source of sensory information. Many studies on fish communication emphasize the importance of visual signals in the fish recognition mechanisms (Albert et al., 2007; Kemp et al., 2008; Wisenden and Dye, 2009). The adult fish brain, with relatively large optic lobes, a small forebrain and small cerebellum indicate that vision is the dominant sense. The eyes are so large with their median curvature adjacent to each other have displaced the small forebrain dorso-caudally (Schwassmann and Kruger, 1965). In threespine sticklebacks *(Gasterosteus aculeatus*) for example, the eyes are conspicuously large in proportion to the rest of the body with the retina compriing as much as 3.5% of the total body surface and the olfactory epithelium about 0.5% (Beukema, 1968). The lobes of the optic tectum of the stickleback brain are also relatively large, in keeping with the importance of vision in this family.

In freshwater fishes, social recognition is achieved using a combination of visual and chemical cues (Brown and Smith, 1994). Vision is of primary importance to fishes in short-range detection (Douglas and Hawryshyn, 1990). Kin recognition and differential treatment of kin and non-kin including familiar individuals can be a fundamental process in the evolution of social behaviour. Several mechanisms of kin recognition have been identified (reviewed in Tang-Martinez, 2001; Mateo, 2004) including phenotype matching. It has been shown that recognition of even unfamiliar kin is possible because individuals establish an olfactory, visual or acoustic template for their kin during early development and compare this template to cues from unfamiliar individuals later in life. In the aquatic environment, phenotype matching is demonstrated in several species, for instance African cichlid *(Neolamprologus pulcher)* (Le Vin et al., 2010), monogamous cichlid *(Pelvicachromis taeniatus)* (Hesse, et al., 2012), guppies, *(Poecilia reticulata)* (Hain and Neff, 2007) and bluegill sunfish, *(Lepomis macrochirus)* (Hain and Neff, 2006) etc.

Evidence for the neurobiological basis of the remarkable olfactory and visual imprinting has been recognized in zebrafish. Terminal nerve (TN) fibres containing neuropeptide FMRF (Phe-Met-Arg-Phe) amide project to telencephalon and tectal targets and, importantly to the contralateral retina (Maaswinkel and Li, 2003). Apparently, in the TN input to the retina upon olfactory stimulation has an effect on behavioural visual sensitivity (Maaswinkel and Li, 2003; Whitlock et al., 2003). In another teleost, the dwarf gourami, *(Trichogaster lalius)*, TN cell bodies receive input from brain areas involved in sensory functions such as olfaction and vision (Yamamoto and Ito, 2000) and may integrate this information. Thus, rather than being a sensory nerve, the TN is in a position to initiate olfacto-retinal modulation.

The significance of vision in shoaling is affected by environmental conditions governing the ability of fish to obtain visual information. There are six general activity patterns in fish shoals at dusk that were repeated in reverse order at dawn: an increased movement of diurnal fish, group break up, cessation of feeding, reduced swimming speed, initiation of activity by nocturnal fishes and cessation of activity by diurnal fish (Pitcher and Parrish, 1993). The dispersal of shoals in the evening may be due to visual constraints and once illumination has fallen below a critical level the fish may no longer be able to maintain shoal formation, at which point they settle at the bottom of the water column, significantly reducing their activity. Critical levels vary greatly between different species (Pavlov and Kasumyan, 2000).

Learning that involves the use of social information is termed as 'social learning'. The present study analysed the influence of visual cues in the social recognition mechanisms of *Puntius sarana subnasutus* in the absence of other cues. The major objectives of the present study were:

i) Do the fish estimate the group-size difference between two stimulus sibling shoals based on visual cues alone

ii) Assess the unique role of visual cues in the sibling recognition ability of *Puntius sarana subnasutus*

iii) Whether the familiarity (previous experience) with the shoal members influence the recognition and shoal-size discrimination ability of *Puntius sarana subnasutus* based on visual cues alone

Materials and method

A pair of healthy breeders of (*Puntius sarana subnasutus, Puntius parrah* and *Anabas testudineus*) were collected from the ponds of Irinjalakuda, (10^o25’, 10^o18’47" N lat. and 76^o17’19", 76^o12’48"E long) Thrissur district, India, during January - March 2011. In order to avoid relatedness among breeding pairs, we collected *P. sarana subnasutus* from two different ponds located in Avittathur and Konthulapuram, which were separated by more than 6km. The heterospecific species used in the study, *Puntius parrah* and *Anabas testudiensis*, were collected from canals associated with paddy fields near Muriyad wetland. Fish were induced to breed using ovaprim. On the next day of hatching, the larvae of *Puntius sarana subnasutus* were divided into two groups. The first group (80 larvae) is further divided into four subgroups of 20 siblings and allowed to grow in four different aquaria (60 cm x 23 cm x 23 cm). The second group of 240 larvae is further divided into sub-groups of 20 and four subgroups were reared along with equal number of conspecifics (*Puntius sarana subnasutus*), four other subgroups with equal number of heterospecific *Puntius parrah,* and other four subgroups with equal number of *Anabas testudineus* allowing contact with both olfactory and visual cues. Further, 100 larvae of all breeding pairs were reared separately for using as the stimulus shoals. Experiments are conducted when the larvae were eight months old.

Experimental set up and protocol

The experimental set-up consists of a central large (60 x 30 x 30cm) aquarium and two smaller (15 x 30x 30 cm) aquaria placed on both sides [Figure 7 (1)]. The test fish is introduced into the central aquarium (experimental arena) and the smaller aquaria on the sides acted as the stimulus chambers. To removable opaque barriers were placed on eitherside of the central aquariam in between the side chambers and the central chamber. The experimental fish is introduced into the central chamber and allowed to acclimatize for ten minutes and after that the opaque screen is removed and observed for further ten minutes. First four minutes were given to the test fish assessing the stimulus shoals. The time spent by the test fish in the preference zone marked near each stimulus shoal within six minutes of observation period is recorded. All experiments were repeated using twenty different individual fish.

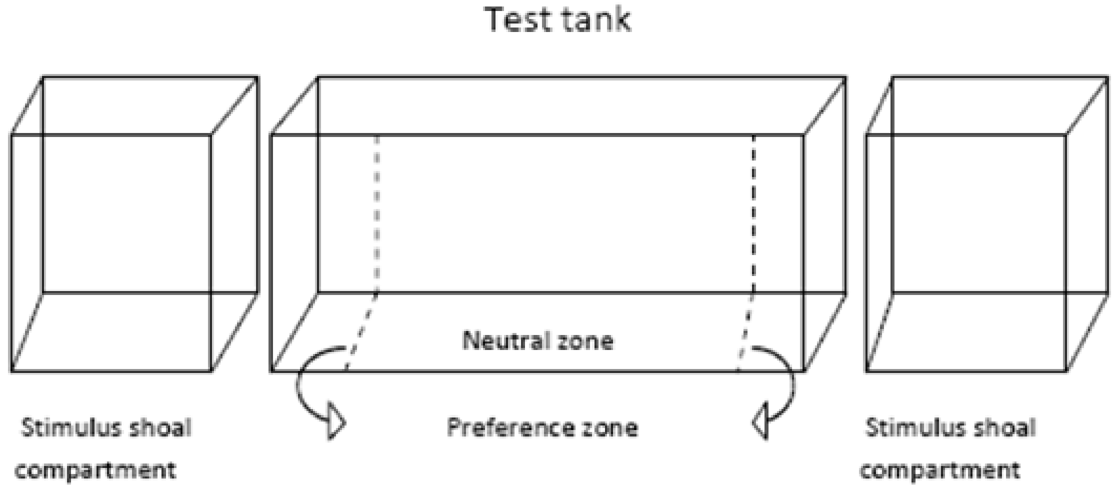


Fig. 7(1). Experimental set-up for testing the shoaling preferences of the test fish using visual cues alone.

The present study tested the group-size discrimination ability of *Puntius sarana subnasutus* based only on visual cues from the stimulus shoals. In all experiments the stimulus shoals were housed in side chambers and hence the test fish has accessed only the visual cues of the stimulus shoal. In the first set of experiment the test fish was simultaneously exposed to two stimulus shoals composed of their siblings in five different ratios [1:1, 1:1.5, 1:2.3, 1:4 and 1: 9]. In the second set of experiments, 25 siblings are introduced into one of the side chambers and 25 heterospecifics (*Puntius parrah*) in the other chamber and tested the shoaling preference of *Puntius sarana subnas*utus. Similarly their shoaling preference was tested using conspecifics, heterospecifics and *Anabas testudineus* in the ratio of 1:1. In order to test the influence of familiarity on shoal choice of *Puntius sarana subnas*utus, the test is repeated using the focal fish that were reared with heterospecifcs (*Puntius parrah* and *Anabas testudineus*) for eight months from the next day of hatching onwards.

Further, in order to understand the role of visual cues in group-size recognition, The stimulus shoals were provided in varying ratios (1:1.5, 1:2.3, 1:4 and 1:9) where the larger stimulus shoal is composed of heterospecifics and a smaller group with that of siblings of the test fish. Here, tested the influence of familiarity in identifying siblings from un-familiar conspecifics and heterospecifics and determined how group-size influenced their shoal choice with a conspecifics and hetrospecific based only on visual cues. The experiment was repeated using focal fish reared in sibling groups of fifty as well as those reared with equal number of conspecifics (*Puntius sarana subnasutus*) or heterospecifics (*Puntius parrah* and *Anabas testudineus*). Data were then normalized using arcsine transformations. Since the correlation between the samples were significant, the data were analyzed using parametric dependent sample two-tailed‘t’ test.

Result

The present study shows that *Puntius sarana subnasutus* is capable of assessing group size and exhibit shoaling preferences towards the larger group based on visual cues alone. In the first set of experiment the test fish clearly recognized the group-size differences among the stimulus shoals composed of their siblings in different numerical combinations (1:1, 1:1.5, 1:2.3, 1:4 and 1: 9) and was able to choose the larger shoal [Table 7(1); Fig. 7(2)].

In the second set of experiments, when the test fish was exposed to stimulus shoals composed of equal number of (25 *vs.* 25) siblings and unfamiliar (previously not encountered) conspecifics/heterospecifics like, *Puntius parrah* and genetically distantly related shoal of *Anabas testudineus*, the fish successfully identified the shoal composed of siblings and spent significantly more time near them, when alternate shoal is composed of *Puntius parrah* and *Anabas testudineus* [Table 7(2), Fig. 7(3) i]. However, when the test fish was previously exposed to conspecifics and heterospecifics, they exhibited a significant preference towards siblings only when the alternate shoal is composed of more genetically unrelated *Anabas testudineus* [Fig. 7(3) ii].

The results of the next set of experiment reveal that *Puntius sarana subnasutus* is able to discriminate shoal size differences between unfamiliar and familiar shoals of conspecifics and heterospecifics based only on visual cues. Given a choice between a shoal composed of sibling, and unfamiliar conspecific or unfamiliar *Puntius parrah* or genetically distantly related shoal of *Anabas testudineus* (20 *vs.* 30 & 15 *vs.* 35) the test fish exhibited higher preference for stimulus shoal composed of siblings when the alternate shoal is composed of genetically unrelated and dissimilarly coloured *Anabas testudineus* [Fig. 7(4) i & ii, Table 7(2)] However, when the size of the sibling shoal decreased further to that of the unfamiliar conspecific, or a heterospecific *Puntius parrah* or genetically distantly related shoal of *Anabas testudineus* (10 *vs.* 40 and 5 *vs.* 45), the test fish exhibited significant preference to larger shoal of unfamiliar conspecific.

But, *Puntius sarana subnasutus* randomly switched over the preference zone near a large shoal composed of heterospecific shoal of *Puntius parrah* and a smaller shoal composed of siblings. Similar behaviour is exhibited by the test fish when a binary choice of a large shoal of genetically distantly related *Anabas testudineus* (10siblings *vs* 40 *A. testudineus;* 5 siblings *vs* 45 *A. testudineus*) is given indicating that they have no significant preference to any one of the stimulus shoals. It is evident that the test fish traded off its preference for a smaller shoal of genetically related individuals to that of a larger shoal of unfamiliar unrelated fish [Fig. 7(4) iii & iv, Table 7(2)].

Interestingly familiarity with conspecifcs; heterospecific *Puntius parrah and Anabas testudineus* acquired through coexistence during early rearing period altered the associational preference of *Puntius sarana subnasutus* during later stage of life. The test fish exhibited significant preference to associate with larger familiar shoals of conspecifcs and heterospecific *Puntius parrah* to that of smaller sibling shoals (20 *vs.* 30; 15 *vs.* 35; 10 *vs* 40 and 5 *vs.* 45) irrespective of the genetic relationhip of the test fish with the stimulus shoal. Eventhough, the test fish never exhibited a significant preference towards the familiar larger shoal of heterospecific *Anabas testudineus*, it traded off the preference towards smaller sibling shoal [Fig. 7(5) i, ii, iii & iv; Table 7(3)]. This indicates that familiarity and shoal size play significant role in the shoal choice of *Puntius sarana subnasutus*. The results clearly established the unique role of visual cues in the sibling recognition, group-size discrimination and development of familiarity among individuals.

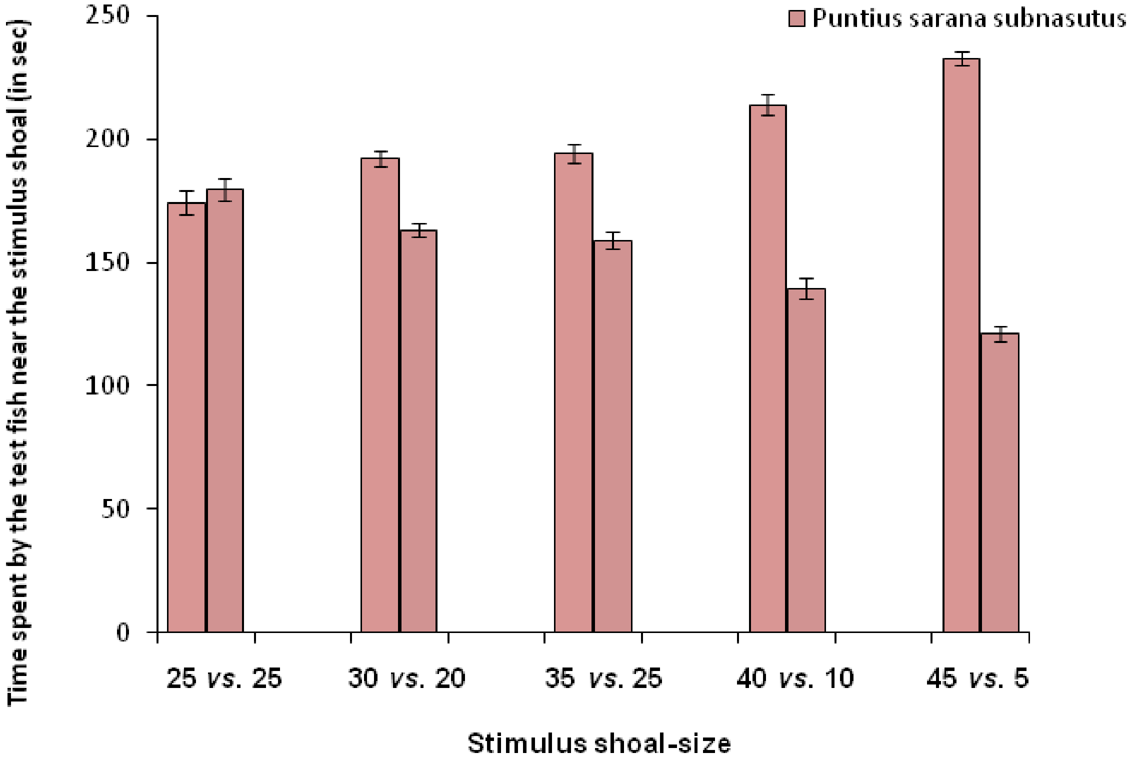
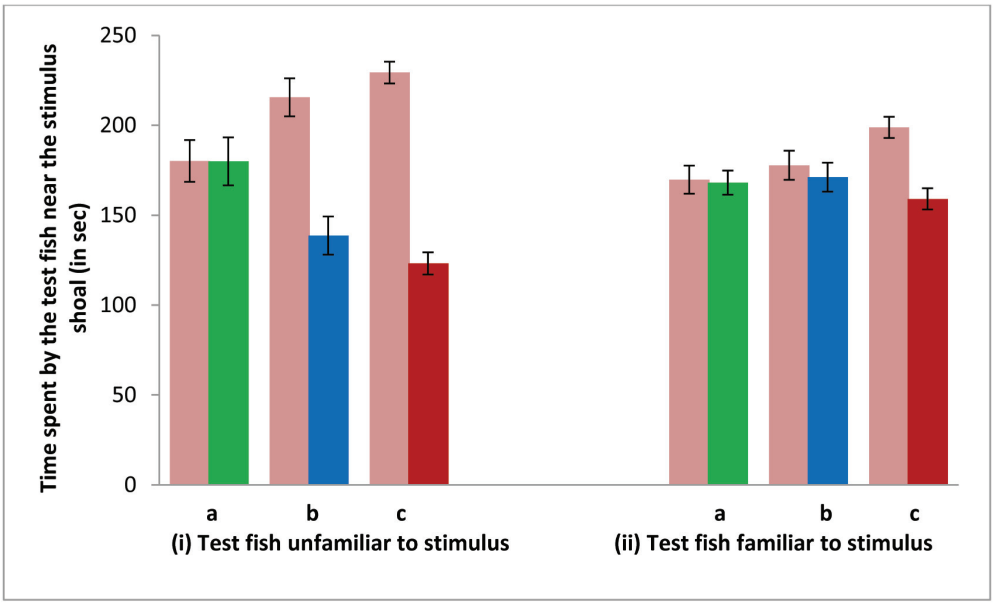


Figure 7(2) - Shoal-size discrimination in *Puntius sarana subnasutus* based on visual cues alone [Stimulus shoal : Siblings of test fish *Puntius sarana subnasutus* in varying numerical combination]

Experiments	Sibling Stimulus shoal group-size	Level of Significance
i	25 *vs.* 25	t = 0.550 p= 0.589
ii	20 *vs.* 30	t = 5.253 p= 0.000***
iii	15 *vs.* 35	t = 4.946 p= 0.000***
iv	10 *vs.* 40	t = 9.016 p= 0.000***
v	5 *vs.* 45	t =18.657 p= 0.000***

Table 7(1): Preference of *Puntius sarana subnasutus* to associate with larger group based on visual cues alone

Figure 7(3): Preference of *P. sarana subnasutus* to ■ sibling shoal and unfamiliar/ familiar (a) 🟩 conspecific (b) heterospecific (🟦 *Puntius parrah*), c) genetically unrelated 🟥 *Anabas testudineus* stimulus shoals at numerical combination of 25 *vs.* 25 based on visual cues alone

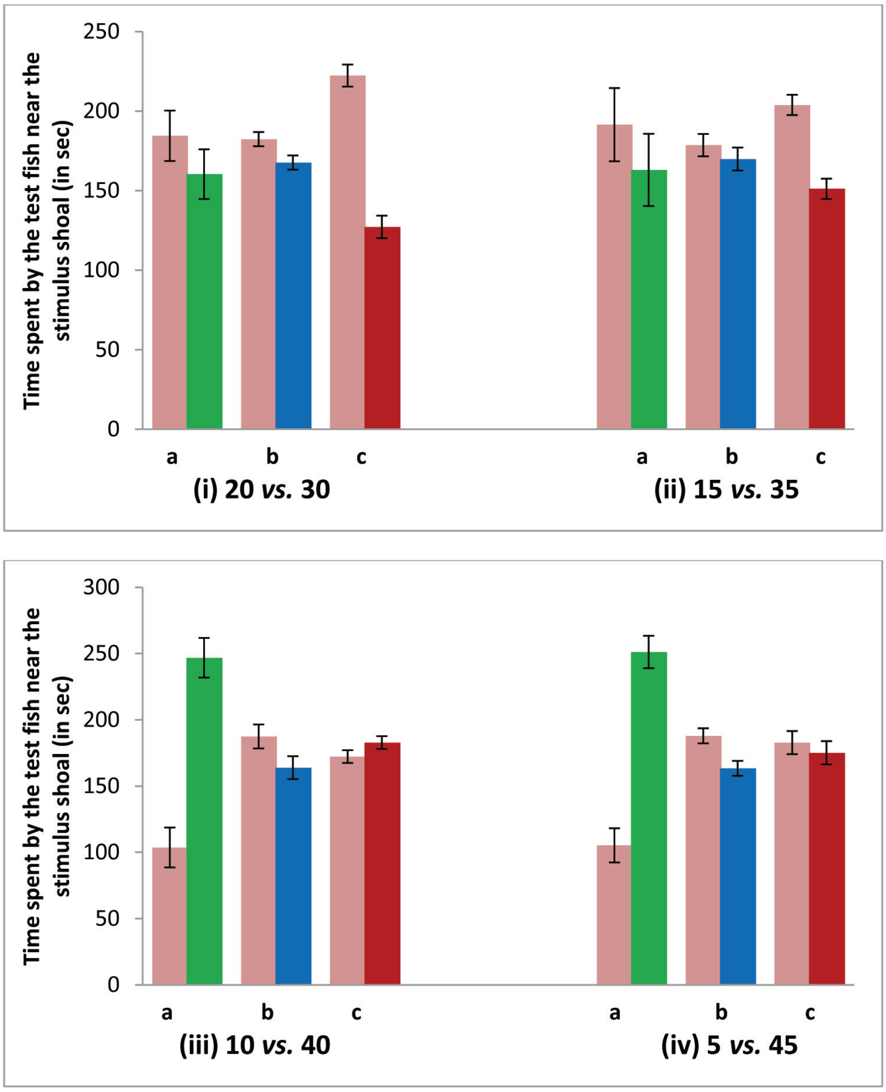


a) ■ 25 *P. sarana subnasutus* (Siblings of the test fish)
🟩 25 *P. sarana subnasutus* (Conspecifics of the test fish)

b) ■ 25 *P. sarana subnasutus* (Siblings of the test fish)
🟦 25 *P. parrah* (Heterospecific of the test fish)

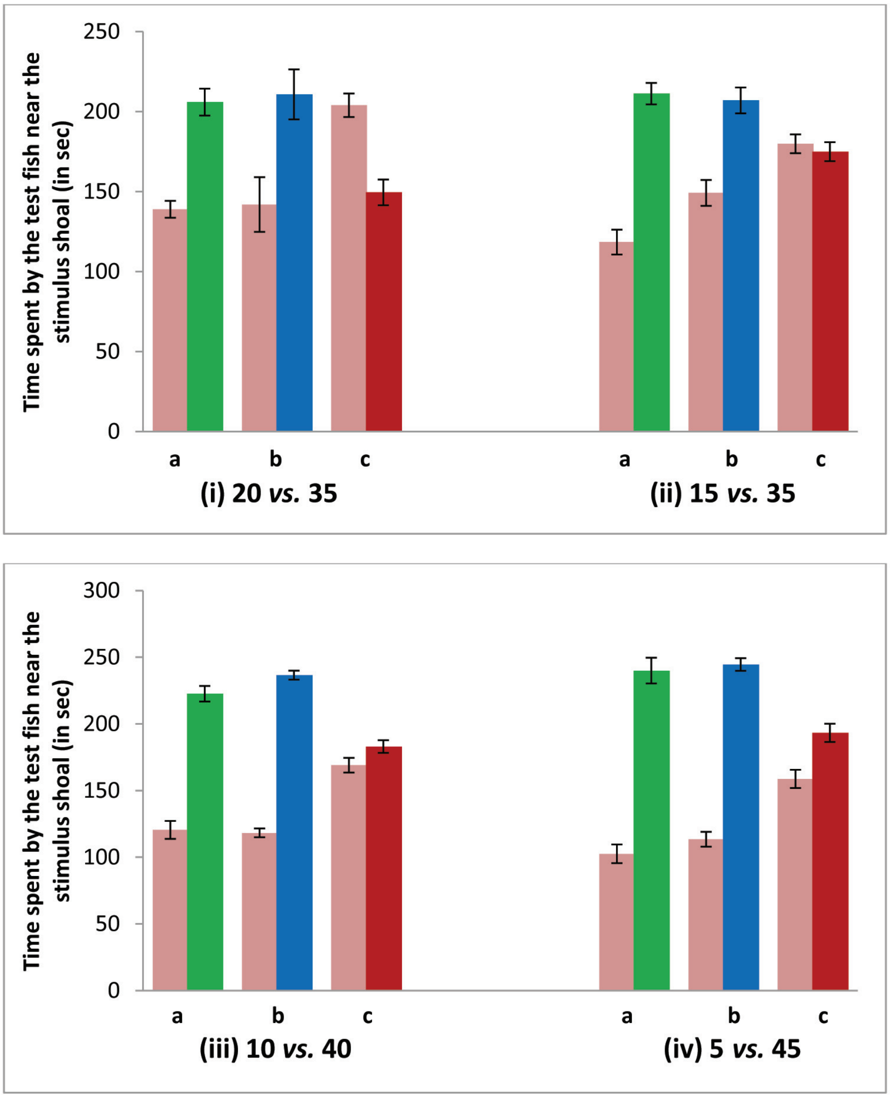
c) ■ 25 *P. sarana subnasutus* (Siblings of the test fish)
🟥 25 *Anabas testudineus* (Heterospecific of the test fish)

Figure 7(4): Ability of test fish (n=20) to discriminate their ■ siblings when alternate stimulus shoals composed of Unfamiliar (UF) 🟩 conspecifics (*Puntius sarana subnasutus*) and heterospecifics (🟦 *Puntius parrah* and 🟥 *Anabas testudineus*) in varying numerical combinations [Only Visual cues were available]



Stimulus shoals: ■ ***P. sarana subnasutus*** (Siblings of the testfish)
Exp - *(i) n=20, (ii) n=15, (iii) n=10, (iv) n=5*
🟩 ***P. sarana subnasutus*** (Conspecifics of the testfish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*
🟦 ***P. parrah*** (Heterospecific of the testfish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*
🟥 ***Anabas testudineus*** (Heterospecific of the testfish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*

Figure 7(5): Ability of test fish (n=20) to discriminate their ■ siblings when alternate stimulus shoals composed of Familiar (F) 🟩 conspecifics (*Puntius sarana subnasutus*); heterospecifics (🟦 *Puntius parrah* and 🟥 *Anabas testudineus*) in varying numerical combinations based on visual cues alone



Stimulus shoals: ■ ***P. sarana subnasutus*** (Siblings of the testfish)
Exp - *(i) n=20, (ii) n=15, (iii) n=10, (iv) n=5*
🟩 ***P. sarana subnasutus*** (Conspecifics of the testfish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*
🟦 ***P. parrah*** (Heterospecific of the testfish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*
🟥 ***Anabas testudineus*** (Heterospecific of the testfish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*

Test fish (n=20) (*P.sarana*)	Stimulus shoal (Un-familiar)	Stimulus shoal group-size				
		25 *vs.* 25	20 *vs.* 30	15 *vs.* 35	10 *vs.* 40	5 *vs.* 45
Individuals reared along with siblings	Sibling *vs.* Conspecific (*P.sarana subnasutus*)	t = 0.009 p=0.993	t = 0.767 p =0.453	t = 0.624 p =0.540	t = 4.780 p=0.001***	t = 5.905 p =0.001***
	Sibling *vs.* Hetrospecific *(P.parrah)*	t = 3.637 p=0.002**	t = 1.649 p =0.116	t = 0.621 p =0.542	t = 1.332 p =0.199	t = 2.184 p =0.042*
	Sibling *vs.* Hetrospecific *(A. testudineus)*	t = 8.755 p=0.001***	t = 7.251 p=0.001***	t = 4.095 p=0.001***	t = 1.096 p =0.287	t = 0.436 p =0.668

Table 7(2): Preference of *Puntius sarana subnasutus* to associate with siblings when larger group is composed of unfamiliar conspecifics (*Puntius sarana subnasutus*) and heterospecifics (*Puntius parrah* and *Anabas testudineus*)

Test fish (n=20) (*P.sarana*)	Stimulus shoal (Familiar)	Stimulus shoal group-size				
		25 *vs.* 25	20 *vs.* 30	15 *vs.* 35	10 *vs.* 40	5 *vs.* 45
Individuals reared along with conspecific/ heterospecific	Sibling *vs.* Conspecific (*P.sarana subnasutus*)	t = 0.123 p=0.903	t = 5.577 p=0.000**	t = 8.912 p=0.001***	t = 8.595 p=0.001***	t = 8.840 p=0.001***
	Sibling *vs.* Hetrospecific *(P.parrah)*	t =0.410 p=0.687	t = 9.614 p=0.001***	t = 4.396 p=0.001***	t =17.566 p=0.001***	t = 14.454 p=0.001***
	Sibling *vs.* Hetrospecific *(A. testudineus)*	t = 3.371 p=0.003**	t = 3.581 p =0.002**	t =0.474 p=0.641	t = 1.111 p=0.280	t = 2.518 p=0.021*

Table 7(3): Preference of *Puntius sarana subnasutus* to associate with siblings when larger group is composed of familiar conspecifics (*Puntius sarana subnasutus*) and heterospecifics (*Puntius parrah* and *Anabas testudineus*)

Discussion

Recognition in fish need not be restricted to one sensory modality. Different sensory modalities have different detection ranges. The useful range of a particular signal is determined both by intrinsic receptor sensitivity and signal disruption in the transmission medium (water, in this case). Acoustic signals can be detected over large distances due to the high velocity of sound in water (5 times faster than in air); however, their directionality is rapidly lost due to multiple reflection and refraction boundaries (e.g. surface of water, 3-D reef structure). Olfactory cues are slow and become distorted by water movement, meaning that odour plumes have to be slavishly tracked to their source. Visual cues are fast and highly directional but are limited in range due to the absorption and scattering properties of water. The attenuation of light causes a blurring of edges and a loss of contrast, and this property is wavelength specific, resulting changes in colour signals with distance and depth. Despite these challenges, many aquatic animals have very well developed visual sense (Collin et al., 2003; Kröger and Katzir, 2008) including colour vision, capabilities such as those described in damselfish (Siebeck et al., 2008).

Fish could not only smell but also see the two shoals; they could select the more preferred one with the aid of both these sensory modalities. In the present study we found that when both the stimulus shoals were siblings, the test fish is capable of recognizing the larger stimulus shoal (in number) based on visual cues alone. When only visual cues were available the upper contours of objects turned out to be the most important for shape discrimination (Guthrie and Muntz, 1993). The teleost eye is markedly myopic, according to Walls (1942), by as much as 15 diopters, and when in the rest position the lens is accommodated for near vision. It is adapted for seeing objects most clearly at distances of the order of 10cm. The distance at which any object of a certain size can be seen depends on the resolving power or visual acuity of the eye (other things being equal). This is best in good illumination and is diminished, though often, with a gain in absolute sensitivity in poor light. Eventhough there are no reliable data on the visual acuity of fish, if we assume a value similar to those found in man – an object 0.09mm long should be visible at 30cm and 0.3mm at a distance of 1m in clear water and good illumination.

Successful attempts have been made to prove the ability of fish to utilize numerical information in their choice. The live stimulus fish move, change location and orientation, modify their inter-individual distance, move into overlapping positions, i.e., dynamically alter numerous continuous variables including the total visible surface area, the linear dimensions and the density of the shoal which may be conveyed as potential visual cues to a fish. Further, several continuous variables, including shoal density, linear extent, or inter-fish distance, individually considered (Go´mez-Laplaza and Gerlai, 2011), as well as the overall swimming activity of the stimulus shoals had diverse effects on performance of the test fish. Some of these continuous variables are indeed found to affect the choice between numerically different shoals of angelfish (*Pterophyllum altum*), but the relevance of these continuous variables was also found to depend upon the numerical size of the contrasted shoals (Go´mez-Laplaza and Gerlai, 2013). Another very important non-numerical variable that determines the shoal-size discrimination based only on visual cues is the overall surface area of the shoal (shoals with larger surface area are preferred). The results of the present study indicate that the fish is capable of recognizing their siblings and group-size differences based on visual cues alone when the stimulus were situated very close to their field of vision. Because of the optical properties of water, physical nature of light and its complex interactions with the environment, a variety of different properties of visible objects can be recognized, such as brightness, hue, texture and contour as well as more subtle differences of degree such as patch size or pattern grain.

Mosquitofish were also shown to rely on this continuous variable: when the contrasted shoals presented to this species had similar surface areas, mosquitofish chose randomly both when numerically large or when numerically small shoals were contrasted (Agrillo et. al., 2008). Likewise, mosquitofish trained to discriminate sets of geometric figures, failed in the discrimination of the trained stimuli when the cumulative surface area of the geometric figures was matched. Very young guppies also failed the trained discrimination between two small sets of dots when the area of the contrasted dots was equal, although they did discriminate between large sets under these circumstances (Piffer et. al., 2013). Surface area appears to be an important non-numerical variable that has also been demonstrated to provide a basis for visually mediated discrimination between quantities in animal species and in different contexts.

Further, the results of the present study strongly support the argument that exposure to the conspecifics and heterospecifics during the early developmental stages have a profound effect on the formation of visually mediated recognition cues (Collin et al., 2003). In the absence of the visual cues during the early life time, the test fish exhibited no association preference towards heterospecifics *Puntius parrah* and *Anabas testudineus* when their number was equal and even larger than the alternate sibling stimulus shoal (Refer Chapter 6 of the thesis- 6A; Larvae *Puntius sarans subnasutus* reared with siblings only). Individually distinct cues are useful when animals interact repeatedly over time. Recognition of conspeciûcs is shown to be mediated through olfactory cues in a variety of taxa including fish (Olsen et al., 1998; Neff and Sherman, 2003; Mateo, 2006). In the absence of the olfactory cues (as the test fish was not previously exposed to the conspecifics the fish), a slight increase in the group size of conspecifics (20 siblings *vs.* 30 conspecifics and 15 siblings *vs.* 35 conspecifics) caused any change in the association preference of the test fish towards siblings. When the test fish was previously exposed to their conspecifics and heterospecifics, a slight increase in the number of the conspecifics and heterospecific *Puntius parrah* shifted the preference of the test fish towards associating with larger stimulus shoal of conspecific and heterospecific *Puntius parrah.* Since we provided only visual cues, the increased preference for larger heterospecific group may be because of the similarities in the colour and texture between the two species accompanied by the benefits from a larger group. Further, familiarity (most probably identified through colour preference) reinforced this larger group selection in spite of the genetic relatedness among siblings and conspecifics. The decreased preference towards a heterospecific stimulus shoal of *Anabas testudineus* may account for the importance of the homogeneity in the colouration of the fish shoal.

Studies have revealed that one trait that may influence the choice of shoal mate is the dazzling array of colours and pigment patterns found in fish species. Recognition based on colour raises an important question that, since fish cannot identify their own colour, how do they acquire a shoaling preference for fish of the same colour pattern i.e., whether the colour preference exhibited by a fish is innate or learned? Shoaling based on colour has previously been observed in mollies (*Poecilia sphenops*), where black and white morphs preferentially associated with fish of matching colour (Mc Robert and Bradner, 1998). Learning plays a significant role in behavioural ecology by allowing animals to optimally adjust behaviour to prevailing environmental conditions and maximize fitness benefits (Ward et al., 2005a). Engeszer et al. (2004) observed that fish raised in isolation showed no shoaling preference for any particular pattern. Fish raised in groups, however, showed a very strong shoaling preference for fish with the same pigment pattern as those they were reared with, regardless of their own phenotype. It is suggested that there is a role for learning and early experience in the acquisition of colour shoaling preferences in zebra fish. The test fish spent less time with the siblings compared to the time spent with siblings when the conspecifics/heterospecifics stimulus shoal was un-familiar.

Over the last few decades, basic recognition and numerical abilities have been demonstrated for several vertebrates including fish. Fish are still underrepresented in cognitive research, despite their high potential to answer questions concerning a wide array of cognitive abilities (Bshary et al., 2002). Single fish placed in an unknown environment show a strong tendency to join social companions and, if choosing between two shoals, they exhibit a preference for the larger one, an adaptive strategy that allows them to minimize the risks of predation. The mechanism behind this recognition may vary – a fish may depend in general on, olfactory, acoustical or visual cues, alone or in combination. In the present study when we used the visual cues as the sole recognition mechanism available for the fish, it successfully identified the group-size differences among stimulus shoals but failed to properly orient between siblings and conspecifics especially when they are familiar. Further research using more phenotypically different, distantly related species is necessary to find out the exact role of visual cues in sibling recognition and group-size discrimination.

Chapter - 8

Role of olfactory cues in shoaling preference and group-size discrimination

Aquatic organisms gain information about the status of their environment through various sensory modalities. For aquatic animals, chemical cues are of particular relevance owing to the properties of water as a solvent and a medium to disperse such cues, and owing to the limitations on vision at depth and in complex or turbid environment. Chemical stimuli include biochemical products released by conspecifics and other organisms, some of which may reveal the presence of food, mates and predators or spawning sites and help in parent – young interaction, social organization and homing. It is thought that that fishes sensed chemical signals only through gustatory sense and there is little role for olfaction in chemical communication. Although available information indicates that the chemical signals, including pheromones are more wide spread in the social interactions of fish than might have been suspected, their importance in modulating fish behaviour is only beginning to be fully appreciated.

Chemical cues are of enormous importance to shoaling fishes, which are strongly attracted towards the smell of conspecifics (Wyatt, 2003). Recent findings suggest that chemical cues may be of greater relative importance than visual cues in this context especially for larger range detection (Ward et al., 2007). Chemical cues allow the fish to discriminate between conspecifics with a high degree of specificity and play an important role in maintaining the patterns of social organization, from shoals to dominance hierarchies and territorial assemblages (Ward et al., 2002). Sibling discrimination by chemical cues has been demonstrated in several species of salmonoids (Olsen and Winberg, 1996). The juveniles of zebrafish are able to differentiate unfamiliar kin from non-kin, preferring the odour of unfamiliar full siblings to unfamiliar unrelated individuals (Gerlach and Lysiak, 2006). However, enhanced familiarity through repeated exposure also augmented olfactory preference for kin showing that zebrafish are capable of recognizing kin through past experience as well as by phenotype matching. Thus there are two general categories of kin recognition mechanisms, the first (indirect) mechanism based on ‘phenotype matching’ and the second mechanism based on ‘familiarity’ (Tang-Martinez, 2001). Here, an individual learns a template of its own phenotype and/or that of its familiar kin and later compares the phenotype of unfamiliar animals with this template. One of the most important templates involved in this type of recognition is the olfactory cues. The Major Histocompatibility Complex (MHC) genes are shown to be mainly responsible for the individual odour cues released into the water via urine (Apanius et al., 1997).

However, the use of these cues for recognition may be context–dependent and may rely upon the genetic make-up of the receiver. For example, in some situations, inbred animals may lose the ability to recognize kin because of inbreeding degeneration of the recognition mechanisms (Frommen et al., 2007b). In other cases, such individuals may show a more pronounced recognition of kin than their out-bred counterparts because it may be easier for a genetically homogenous phenotype to recognize a ‘like’ individual than it is for a heterogeneous phenotype. Furthermore, selection should be stronger on inbred individuals to avoid inbreeding since a further reduction of heterozygosity will produce even stronger inbreeding depression (Mazzi et al., 2004). The use of olfactory signals in social recognition of three spined sticklebacks has been further well documented by Ward et al. (2004 and 2006).

Two other major categories of olfactory cues that have powerful influences on animal behaviour and physiology are pheromones and food odours. Pheromones are renowned for their potency, specificity and the seemingly innate effects they induce (Sorensen, 1996), whereas food odours appear to be learned and are typically composed of large number of chemical constituents (Smith and Getz, 1994). The discovery that fish employ hormonal derivatives as pheromonal components has provided a particularly exciting possibility of gaining fundamental knowledge about olfactory processing, given that the structure and function of olfactory systems tends to be highly conserved across the taxa. The only vertebrate in which production, release, sensory detection and biological function of a pheromone have been thoroughly characterized is the goldfish (Sorensen et al., 1998).

The present study analysed the influence of olfactory cues in the shoal choice of *Puntius sarana subnasutus* in the absence of other cues. It examined whether they can assess differences in group-size without visual contact with the stimulus groups and whether individuals make association decisions based on odour cues of conspecifics and heterospecifics. Further, the role of familiarity in discriminating sibling odours from the odour of conspecifics/heterospecifics is also analysed.

The major objectives of the present study are:

i) Whether the fish is capable of estimating the group-size difference between two stimulus sibling shoals based on olfactory cues alone

ii) It is also designed to understand the unique role of olfactory cues in the sibling recognition ability of *Puntius sarana subnasutus*

iii) Whether familiarity (previous experience) with the shoal members influence the recognition ability and shoal-size discrimination capacity of *Puntius sarana subnasutus,* when only odour cues are available

Materials and method

Puntius sarana subnasutus and its conspecifics and heterospecific species *Puntius parrah* and *Anabas testudineus* were collected from ponds and canals associated with paddy fields of Irinjalakuda (10°25’, 10°18’47" N lat. and 76°17’19", 76°12’48"E long), Thrissur district, India, during January - March 2010. The fishes were acclimatized with laboratory conditions for two weeks in large cement tanks (175 x 90 x 90 cm) and fed *ad libitum* with commercially available tropical fish food (Marvel feeds, Aquarium systems, India). A pair of gravid fishes from each species were selected and induced to breed using GnRH analogue ovaprim. Healthy larvae of *Puntius sarana subnasutus* are selected and divided into four groups. The first group is reared along with 20 siblings in aquarium (45 x 23 x 23 cm). The second group is reared along with equal number of conspecifics *(Puntius sarana subnasutus*) in larger aquarium (85 x 32 x 32 cm). The third group of larvae is reared along with equal number of larvae of heterospecific species of *Puntius parrah* and the fourth group, along with equal number of larvae of *Anabas testudineus* (85 x 32 x 32 cm). All four groups of larvae have access to both olfactory and visual cues of their shoal mates.

Further, 100 larvae of all breeding pairs were reared separately for constituting the stimulus shoals. Experiments were conducted when the larvae were 8 months old (8 ± 2 cm long and weighing 14 ± 2 mg).

Experimental set-up: In a dichotomous choice test, test fish are given an opportunity to swim near the water scented with odour cues of siblings or heterospecifics. The testing arena consisted of a glass tank (60 x 80 x 30 cm) designed as a Y-maze as described in Shohet and Watt (2004) [Fig. 8(1)]. In one side, along width of the tank, two stimulus compartments A & B (15 x 20 x 30cm each) made of black opaque Plexiglas were placed. The middle pieces of the Plexiglas were plane without perforations while those in the right and left ends were perforated with minute pores having a diameter of 2mm. Any mixing of water between the stimuli compartments A & B were prevented carefully by sealing all gaps in the middle opaque plexiglas partition. Water flow is regulated at the rate of 200 ± 10 ml/ min into the stimulus compartments from the two glass tanks (45 x 23 x 23 cm) placed at an elevated position at the left and right corners of the experimental tank for housing siblings and heterospecifics. Compartment 'A' received water scented with sibling's odour and compartment 'B' received water scented with odour of heterospecifics. Odour scented water of sibling's and heterospecifics is prepared by holding fifteen individuals of siblings and heterospecifics overnight in separate tanks (Gerlach et al., 2008). Four such stimulus holding tanks of siblings and heterospecifics were prepared using offspring of each of the four breeding pairs used for induced breeding. The flow of water was regulated by inserting regulators in tubes connected to compartments 'A' and 'B'. To prevent the mixing of cues and to ensure constant water level inside the experimental tank, water was allowed to flow out through the holes drilled into the walls of the experimental tank.

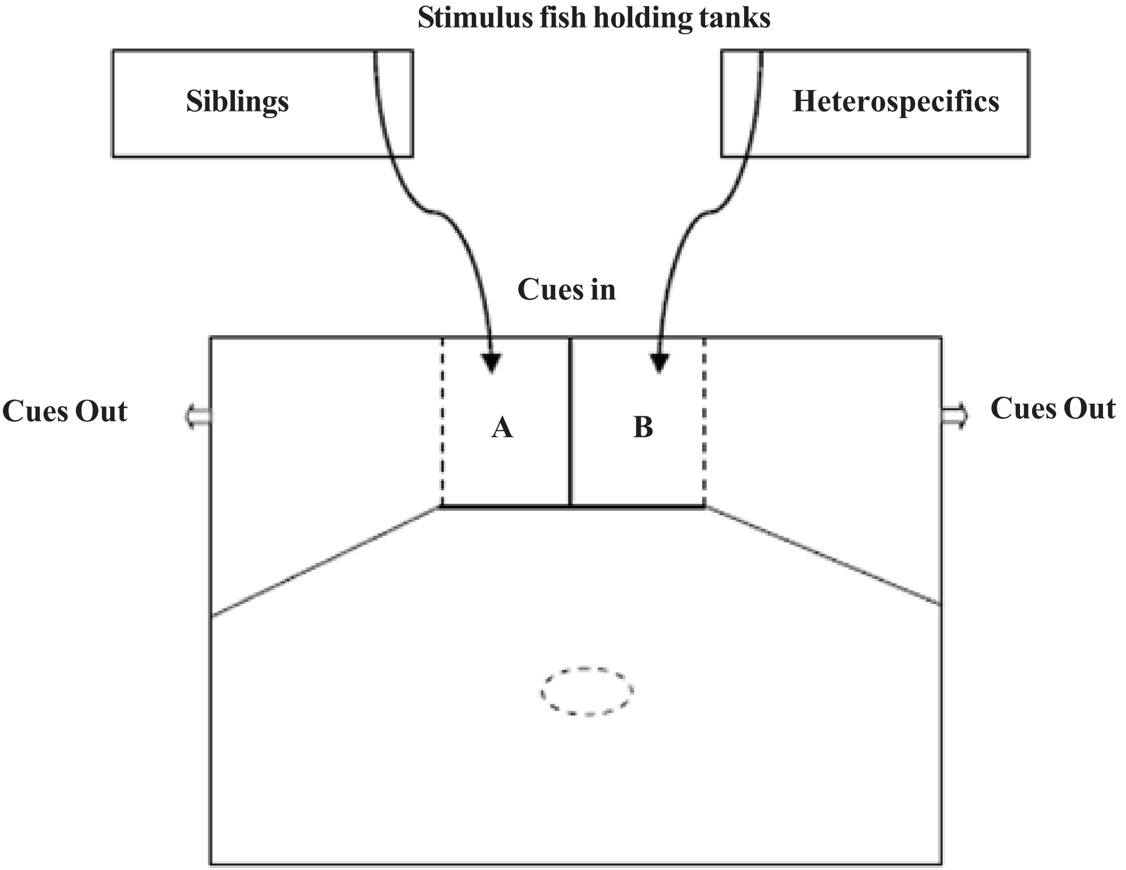


Figure 8(1): Schematic representation of the odour-cue choice tank: dashed lines indicate perforated walls of the compartment A&B, thick solid lines indicate opaque walls which provided a barrier to water flow and thin solid lines marks the preference zone

Using a solution of methylene blue, it is ascertained that the odour cue scented water from the stimulus tanks is not getting mixed up inside the experimental tank. The dye was allowed to flow at the same rate as that of the stimulus odour cue scented water and was detected to travel within 10 minutes from the inlet to the outlet situated near to the stimulus odour cue-receiving compartments in the experimental tank. The dye reached simultaneously in the stimulus odour cue-compartments A and B, and remained separate till the completion of the experiment. By observing the movement of dye particles into the central neutral arena of the experimental tank, we marked a preference zone on the base of the tank near to each odour cue compartments.

An 11W compact fluorescent lamp placed above the tank lighted the set up. A black curtain was tightened around the test tank in order to avoid the interference of external stimuli. Based on the dye experiments, we allowed ten minutes to elapse after opening the regulators in the water inflow tubes, so that olfactory cues provided by the stimulus fish would have enough time to spread over the stimulus odour cue compartments in the test tank. The test fish was always introduced individually into the centre of the experimental arena in a presentation cage made of clear, perforated acrylic sheets. The total duration of each experiment was sixteen minutes. Ten minutes were given to the test fish to assess the experimental arena and thereafter, the time they spent within the preference zone is recorded for six minutes using a stopwatch.

The first set of experiment tested the sibling stimulus shoal-size discrimination ability of *Puntius sarana subnasutus* based on odour cues alone. Here, siblings of *Puntius sarana subnasutus* in different numerical combination (25 *vs.* 25, 20 *vs.* 30, 15 *vs.* 35, 10 *vs.* 40 and 5 *vs.* 45) were introduced into the stimulus shoal compartments keeping the total number of individuals in the stimulus shoal were always fifty. The second set of experiments tested the shoal choice of *Puntius sarana subnasutus* based on odour cues. Equal number of siblings of *Puntius sarana subnasutus* and either its conspecifics or heterospecific species *Puntius parrah* or *Anabas testudineus* were intoduced to the stimulus holding tanks. The water scented with their odour cues was then allowed to flow respectively to stimulus compartment 'A' and 'B' on either side of the test tank (25 siblings *vs*. 25 conspecific and heterospecifics). The last two sessions tested the group-size discrimination ability of *Puntius sarana subnasutus* siblings based on odour cues alone, when the alternate shoal is composed of either un-familiar conspecifics and heterospecifics or familiar conspecifics and heterospecifics in varying numerical combinations. The experiments were repeated in twenty test fish. Data were normalized using arcsine transformations. Since the correlation between the samples were significant, the data were analysed using parametric dependent sample two-tailed 't' test.

Result

The results of the present study show that group-size discrimination is possible in *Puntius sarana subnasutus* based on odour cues, in the absence of visual signals. The test fish spent significantly more time in the preference zone where the water conditioned with the odour cues of higher number of siblings is flowing.[Figure 8 (2); Table-8 (1)].

The test fish clearly discriminated the odour cues of their siblings from that of unfamiliar conspecifics and heterospecifics when presented with shoals composed of equal number of individuals (25 *vs*. 25). Here, the test fish exhibited association preference towards the odour cues of their siblings when the alternate stimulus shoal was composed of unfamiliar conspecifics. Further, the preference for sibling shoal was much stronger when the alternate shoal is composed of unfamiliar heterospecifics, *Puntius parrah*; or genetically distantly related, *Anabas testudineus* [Fig. 8(3) and Table- 8(2)]. However, familiarity with conspecifics and heterospecifics developed through coexistence during early rearing period, exerted profound influence on the associational preference of *Puntius sarana subnasutus.* No significant preference towards the odour of siblings was exhibited by the test fish when a binary choice was given between odour cues of siblings and that of familiar conspecific *Puntius sarana subnasutus;* heterospecifics *Puntius parrah;* or *Anabas testudineus* [Fig. 8(3) and Table- 8(3)].

Familiarity with group mates during early rearing period also influenced shoal size discrimination based on odour cues at later developmental stage of *Puntius sarana subnasutus*. This was demonstrated by the fact that when given a choice between shoal of siblings and shoal composed of varying number of **unfamiliar** conspecific *Puntius sarana subnasutus;* heterospecifics *Puntius parrah;* or genetically distantly related *Anabas testudineus*, the test fish always exhibited significantly more associational preference to sibling shoal, irrespective of the difference in the group size of the alternate shoal. [Fig. 8 (4) i,ii,iii and iv; Table 8(2)].

The response of the test fish changes, when given a choice between shoal of siblings and shoal composed of varying number of **familiar** conspecific *Puntius sarana subnasutus;* heterospecifics *Puntius parrah;* or genetically distantly related *Anabas testudineus.* The associational preference exhibited by the test fish to shoal composed of varying number of **familiar** conspecific-*Puntius sarana subnasutus;* heterospecifics-*Puntius parrah;* or genetically distantly related- *Anabas testudineus*, is not significantly different from that exhibited to smaller shoal size of the siblings [Fig. 8 (5) i, ii, iii, and iv; Table 8(3)].

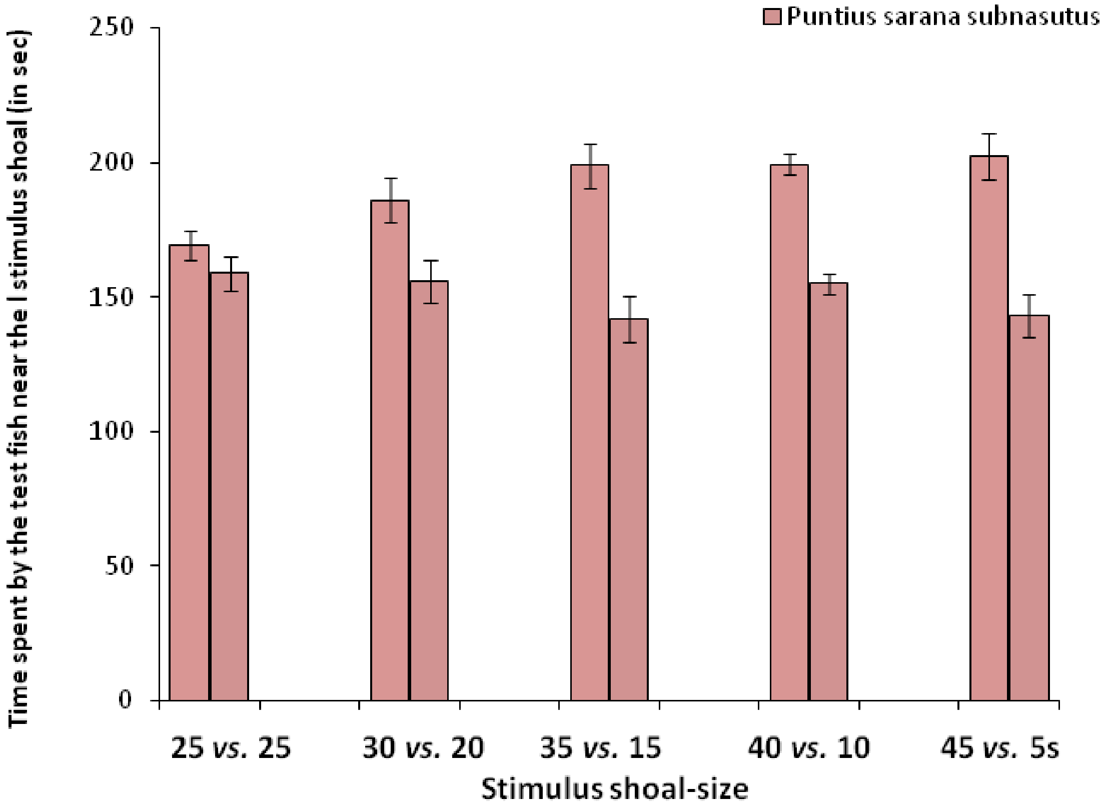
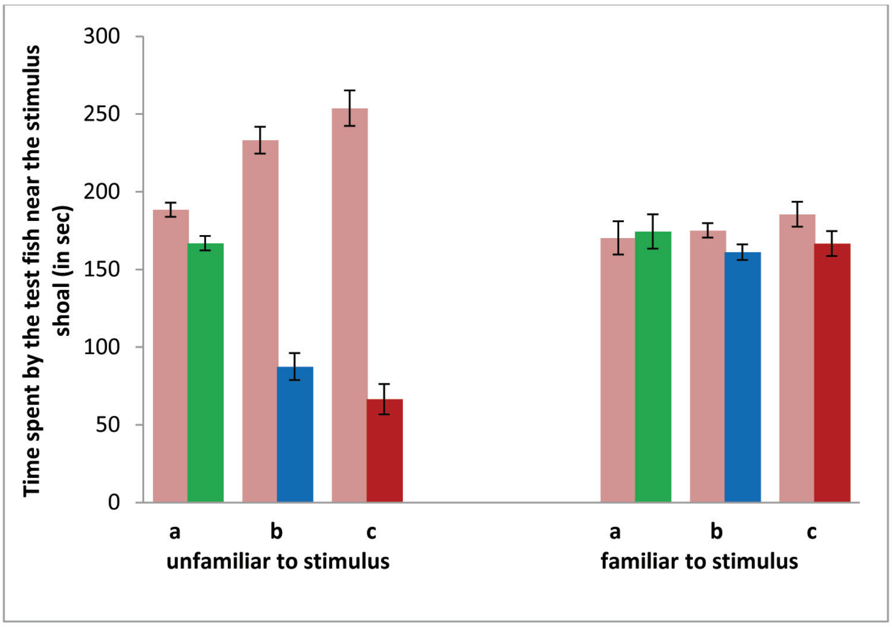


Fig. 8 (2): Shoal-size discrimination in *Puntius sarana subnasutus* when only olfactory cues are provided [Stimulus shoal : Siblings of test fish *Puntius sarana subnasutus* in varying numerical combination]

Experiments	Sibling Stimulus shoal group-size	Level of Significance
i	25 *vs.* 25	t =0.996, p =0.332
ii	20 *vs.* 30	t = 1.856, p =0.079*
iii	15 *vs.* 35	t = 3.394, p =0.003**
iv	10 *vs.* 40	t = 3.626, p =0.002**
v	5 *vs.* 45	t = 5.760 p= 0.001***

Table 8(1) : Preference of Puntius sarana subnasutus to associate with larger sibling group

Figure 8(3): Ability of the test fish to discriminate their ■ siblings when alternate stimulus are composed of un-familiar and familiar 🟩 conspecifics (*Puntius sarana subnasutus*); heterospecifics (🟦 *Puntius parrah*); and 🟥 *Anabas testudineus*) in equal number (25 vs 25) (Based on Olfactory cues alone)

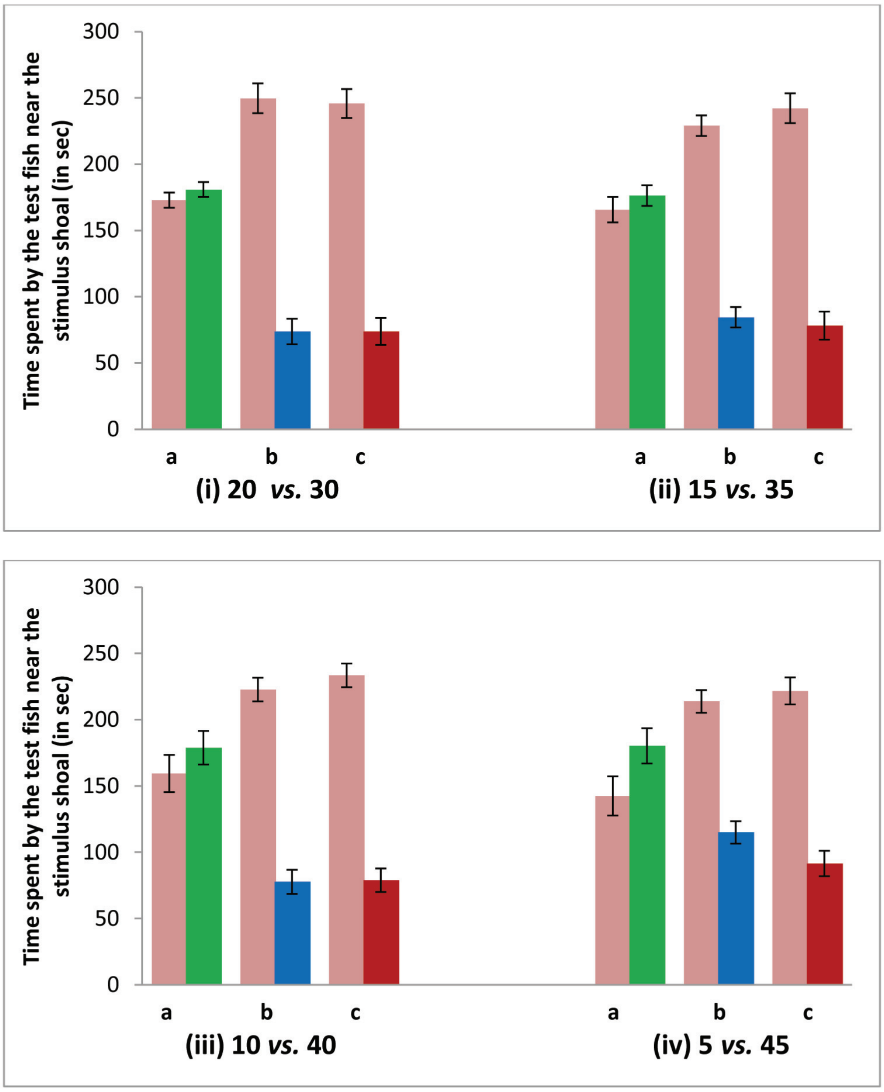


a) ■ 25 *P. sarana subnasutus* (Siblings of the test fish)
🟩 25 *P. sarana subnasutus* (Conspecifics of the test fish)

b) ■ 25 *P. sarana subnasutus* (Siblings of the test fish)
🟦 25 *P. parrah* (Heterospecific of the test fish)

c) ■ 25 *P. sarana subnasutus* (Siblings of the test fish)
🟥 25 *Anabas testudineus* (Heterospecific of the test fish)

Figure 8(4): Ability of test fish (n=20) to discriminate their ■ siblings when alternate stimulus shoals composed of Unfamiliar (UF) 🟩 conspecifics (*Puntius sarana subnasutus*) and heterospecifics (🟦 *Puntius parrah* and 🟥 *Anabas testudineus*) in varying numerical combinations [Only olfactory cues were available]



Stimulus shoals: ■ ***P. sarana subnasutus*** (Siblings of the test fish)

Exp - (i) n=20, (ii) n=15, (iii) n=10, (iv) n=5

🟩 ***P. sarana subnasutus*** (Conspecifics of the test fish)

Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*

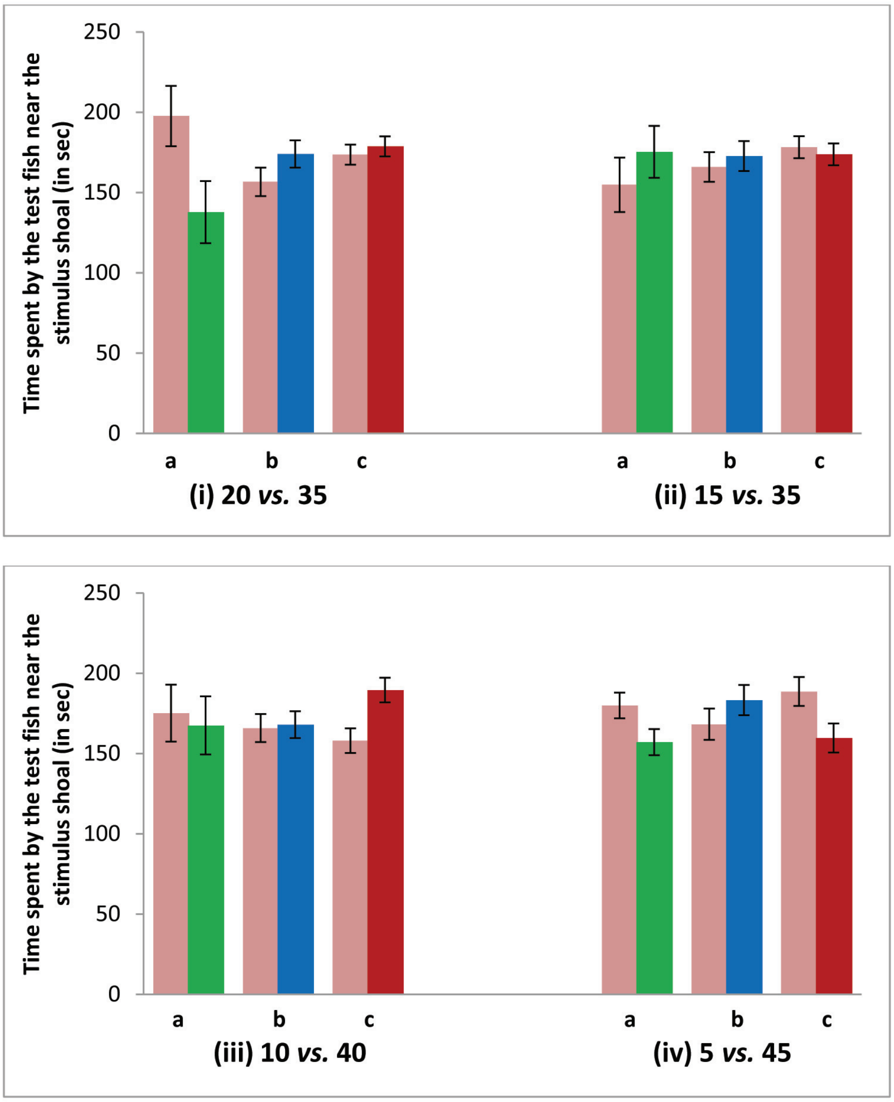
🟦 ***P. parrah*** (Heterospecific of the test fish)

Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*

🟥 ***Anabas testudineus*** (Heterospecific of the test fish)

Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*

Figure 8(5): Ability of test fish (n=20) to discriminate their ■ siblings when alternate stimulus shoals composed of Familiar (F) 🟩 conspecifics (*Puntius sarana subnasutus*) and heterospecifics (🟦 *Puntius parrah* and 🟥 *Anabas testudineus*) in varying numerical combinations [Only olfactory cues were available]



Stimulus shoals: ■ ***P. sarana subnasutus*** (Siblings of the test fish)
Exp - *(i) n=20, (ii) n=15, (iii) n=10, (iv) n=5*
🟩 ***P. sarana subnasutus*** (Conspecifics of the test fish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*
🟦 ***P. parrah*** (Heterospecific of the test fish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*
🟥 ***Anabas testudineus*** (Heterospecific of the test fish)
Exp - *(i) n=30, (ii) n=35, (iii) n=40, (iv) n=45*

Test fish (n=20) (*P.sarana subnasutus*)	Stimulus shoal (Un-familiar)	Stimulus shoal group-size				
		25 *vs.* 25	20 *vs.* 30	15 *vs.* 35	10 *vs.* 40	5 *vs.* 45
Individuals reared along with siblings	Sibling *vs.* Conspecific (*P.sarana subnasutus*)	t = 2.346 p=0.030*	t = 0.708 p=0.487	t = 0.716 p=0.483	t = 0.749 p= 0.463	t = 1.378 p= 0.184
	Sibling *vs.* Hetrospecific *(P.parrah)*	t = 8.535 p=0.000***	t = 8.477 p=0.001***	t = 9.316 p=0.001***	t = 6.941 p=0.001***	t = 5.860 p=0.000***
	Sibling *vs.* Hetrospecific *(A. testudineus)*	t = 8.955 p=0.000***	t = 8.181 p=0.001***	t = 7.529 p=0.001***	t = 8.774 p=0.00***	t = 6.618 p=0.000***

Table 8(2): Preference of *Puntius sarana subnasutus* to associate with siblings when larger group is composed of unfamiliar conspecifics (*Puntius sarana subnasutus*) and heterospecifics (*Puntius parrah* and *Anabas testudineus*)

Test fish (n=20) (*P.sarana*)	Stimulus shoal (Familiar)	Stimulus shoal group-size				
		25 *vs.* 25	20 *vs.* 30	15 *vs.* 35	10 *vs.* 40	5 *vs.* 45
Individuals reared along with conspecific and heterospecific	Sibling *vs.* Conspecific (*P.sarana subnasutus*)	t = 0.194 p =0.848	t = 1.601 p =0.126	t =0.662 p =0.516	t =0 .215 p =0.832	t = 1.514 p =0.146
	Sibling *vs.* Hetrospecific *(P.parrah)*	t = 1.455 p =0.162	t = 1.062 p =0.302	t = 0.379 p =0.709	t = 0.136 p =0.893	t = 0.813 p = 0.426
	Sibling *vs.* Hetrospecific *(A. testudineus)*	t = 1.179 p =0.253	t = 0.408 p =0.688	t = 0.330 p =0.745	t = 2.157 p =0.044	t = 1.630 p =0.120

Table 8(3): Preference of *Puntius sarana subnasutus* to associate with siblings when larger group is composed of familiar conspecifics (*Puntius sarana subnasutus*) and heterospecifics (*Puntius parrah* and *Anabas testudineus*)

Discussion

The study demonstrates that *Puntius sarana subnasutus* is able to identify kin based on odour cues alone. Associations with related individuals are known to convey significant benefits especially by increasing group cohesion, homogeneity within the shoals and reducing the aggressive bahaviour. Such a benefit was, for instance, suggested in studies of Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*) in which the mean frequency of aggressive interactions in shoals of related individuals was lower than in non-kin groups (Brown and Brown, 1993b). Additionally, the survival rate in a group composed of related pike (*Esox lucius* L.) was higher than in a group of unrelated individuals. In zebrafish (*Danio rerio*) fry in kin groups grew faster, although there was no reduction in aggressive behaviour (Gerlach et al., 2007). Shoaling with relatives may be further beneficial as it helps in the development of altruistic behaviour which in turn contributes to an individual's indirect fitness (Hamilton, 1964). Conversely, mixed species shoaling are also known to occur when the gain from a mixed group is greater, compared to the gains from a shoal composed of siblings and resource partitioning between species could reduce food competition in heterospecifics shoal (Ward et al., 2002).

The results suggest the importance of the odour cues in sibling recognition and the necessity of combinations of visual and chemical cues in group-size recognition. In the present study, when restricted for one sensory modality i.e., visual cues and provided only odour cues; the test fish discriminated the group size difference between stimulus sibling shoals. However, it failed to identify the group-size difference between siblings and conspecifics or that of heterospecifics. The benefits of joining with a phenotypically and genotypically similar sibling group may further be enhanced by an increase in the group-size. Many studies have shown the preference of fish to associate with a larger group mainly because of the anti-predator advantages (Rodgers et al. 2011). A study by Courtenay et al. (1997) demonstrated that the concentration of odours can affect preferences in salmonids. Their study, on the recognition of population-specific odours in juvenile coho salmon, found that individuals generally preferred water conditioned by members of their own population over that conditioned by non-members. However, when unequal number of fish were used in the conditioning tanks, water conditioned by the grater number of fish was preferred independent of population membership. A second experiment demonstrated that individuals preferred water conditioned with greater as opposed to lesser amounts of feces. The authors concluded that preferences are influenced by odour concentration and that this factor may mask factors such as population membership or genetics.

Sibling recognition by chemical cues has been demonstrated in several species of salmonids (Olsen and Winberg, 1996) and Arctic charr (Olsen et al., 2003). However, inbreeding depression has been shown to reduce individual fitness in many animal species (Crnokrak and Roff, 1999). One way to avoid inbreeding is to recognize kin using information from a variety of cues, then to avoid breeding with them. Previous research in three spined sticklebacks demonstrated that both inbred and out bred females prefer unfamiliar, unrelated males over familiar brothers (Frommen and Bakker, 2006). However, the test fish used in the present study were only 8 months old, just before their sexual maturity, the influence of sex based choice is completely eliminated from that of sibling preferences. A fish can recognize their siblings from un-familiar individuals either based on earlier experience with kin (phenotype matching) or some kind of self-reference (Mateo, 2004). Here, the fish was reared with siblings and heterospecics from the next day of hatching onwards. During this phase, they have an opportunity to become familiar with the olfactory cues of their kin, conspecifics and heterospcfic, which may be used later in life as a template to recognize relatives.

When one animal detects another; it may be capable of assessing all the available cues regarding the subject. Animals can use a suit of different cues to obtain information about their environment. In freshwater fish both the olfactory and visual senses are vital for survival as they mediate a number of fundamental behaviours including feeding, predator avoidance, grouping, migration, spawning and parental care (Hara, 1993). Still, the role of these different sensory modalities in regulating a particular behavioural pattern is context dependent. In short range detection visual cues are said to be more important. But over greater distances of a few body lengths, or owing limitations on vision at depth and in complex or turbid environment, the fish may gather information using their olfactory or gustatory senses (Ward et al., 2007). Fish are known to discriminate between conspecifics mainly through the products coded by the highly polymorphic loci of the major histocompatibility complex (MHC) (Olsen et al., 1998). In addition to being a part of the vertebrate immune system, the MHC affects an individual's odour profile. Their parallel role in the recognition of self and non-self had been studied for at least last 30 years (Boyse et al., 1987) suggested that, the MHC evolved due to the need for species recognition. MHC molecules are transmembrane molecules, which are shed from the cell surface and appear in the body fluids such as saliva, sweat and urine (Singer et al., 1997). These molecules can be accessed via the olfactory system and are used as signals of genetic relatedness and health. Differences in MHC loci are well known to influence the behavioural decisions in mice and fish (Reusch et al., 2001; Aeschlimann et al., 2003).

However, in the present study an increased preference with increase in odour concentration (number of individuals) was not observed when the alternate larger group is composed of either unfamiliar or familiar conspecifics or heterospecifics. The results were contradictory to the findings of Steck et al. (1999). In their study no preference for siblings was observed in juveniles of three-spined sticklebacks when they were tested singly where odour of non-familiar siblings and odour from non-familiar non-siblings was offered simultaneously on two sides. The same fish, however, showed a significant preference for the heavier of the two shoals from which the water with the odour had been taken. An MHC based recognition based on familiarity is still under investigation. When juvenile Arctic charr (*S. alpinus*) were given a choice between water scented by a full sibling whose MHC genotype was identical to their own and water scented by a full sibling whose MHC genotype was different, fish preferred water from MHC –identical siblings. The authors concluded that social learning cannot account for this result because test fish were reared in groups of siblings with variable MHC alleles. One possible explanation for familiarity based recognition suggests that juvenile charr acquired their kin template in a social context by selectively learning the kin odour corresponding to their own MHC genotype and could therefore later differentiate between all the MHC genotype that it had encountered during the critical period of development according to social context (Olsen et al., 1998). Social environment is implicated as an important factor in shaping the ontogeny and evolution of behavioural patterns. In the present study when the odour cues were from unfamiliar conspecifics/heterospecifics, the test fish significantly associated with their siblings. When the stimulus shoals were composed of sibling odours and familiar conspecifics/heterospecifics no such significant association was observed. The kin discrimination mechanism involves comparing odours gained from various sources with a learned or genetically dictated ‘recognition template’ (Brown et. al, 1993). The template may be learned during common rearing, but this odour must be consistent within families and therefore, genetically based, in order for individuals to recognize siblings of which they have had no prior experience. Individuals in these experiments were reared together from next day of hatching onwards.

Living in a medium where visual information often is limited but where chemical information abounds, fish throughout their long history have had both cause and opportunity to evolve chemo-sensory systems enabling individuals to respond adaptively to conspecifics and predator odours. However, the present study suggests that group-size recognition in *Puntius sarana subnasutus* depended more on visual cues than the odour cues while species discrimination is facilitated by odour cues. Association with a larger sibling group, being one of the most important anti-predator strategies, is a very essential behavioural pattern as far as the survival of the fish species is concerned. Factors such as turbidity by reducing the visual range (Alex and Thomas, 2012) or toxic chemicals disrupting the olfactory sensitivity of fishes (Tierney et al., 2010) may interfere with this vital behaviour pattern, adversely affecting the survival of fish. Therefore, it is necessary to control the aspects of water pollution especially industrial effluents and toxic chemicals that interfere with the clarity and quality of water affecting the growth of aquatic populations.

Chapter – 9

Effects of aquatic pollution on shoaling preference

In the past, basic amenities for living organisms such as air, land (soil) and water were pure, virgin, undisturbed, uncontaminated and basically most hospitable for living. But the situation is just reverse today because of the urban-industrial revolution and speedy exploitation of every bit of natural resources (De, 1996). Rapid industrialization has left with us an 'air unfit for breathing, water unfit for drinking', 'vegetables unfit for eating' and so on. Chemical fertilizers have done considerable damage to aquatic ecosystems in the past and continue to do so today. Pollution can negatively affect biosystems through destruction of ecosystems, shifts in ecosystems and bioaccumulation. All of these effects significantly impede ecosystem services that are important to us, such as providing potable water, preserving fisheries, protection from floods, and keeping herbivore levels low enough to prevent overconsumption of plant life (Maltby et al., 2010).

Untreated industrial waste has also rendered many aquatic environments toxic for organisms and unusable for living organisms. During the last 50 years, the amount of chemicals released by private households has also increased tremendously–this has mainly due to increased use of soaps, detergents, medicine, plastic etc. Even though the impact of households' pollutants is growing due to higher consumption, it is still negligible in comparison to industrial emissions. Shifts in ecosystems from pollution-intolerant to pollution-tolerant species can be caused by traces of pollutants (Hayes et al., 2002). Some organisms have the ability to ignore them or have better protections than others and are therefore safe while other species go extinct (Grant, 2010). Every organism accumulates pollutants during it's lifetime and if it is eaten, the pollutants are transferred to the predator. Hence, bioaccumulation especially targets the top predators of the food chain, including humans (Meador, 1993). Fishes are particularly susceptible to small quantities of these pollutants. They significantly damage certain physiological and biochemical processes when they enter the body of these animals, leading to stress.

Behavioural ecology represents a fusion of fields of behaviour, ecology, toxicology and conservation biology. When a chemical agent is introduced into the natural habitat of an organism, it produces a change in its behaviour without causing much alternation in corresponding anatomy or physiology

(Smith and Logan, 1997). As Warner et al., (1966) commented, "The behaviour (activities) of an organism represents the final integrated results of diversity of biochemical and physiological process. Thus single behavioural parameter is generally more comprehensive than physiological or biochemical parameter". Over the last few decades concern has been growing about the deleterious effects of intensive agricultural practices on freshwater ecosystems. Recent developments in ecotoxicology have shown that observations of behaviour, which represents a crucial step between biochemical and ecological responses to environmental contamination, can constitute a sensitive approach in sub- lethal toxicity monitoring (Saglio et al., 1996). To date, the majority of this data has resulted from observations focusing on a single behavioural end point related to swimming orientation, swimming capacity or spontaneous swimming activity (Bretaud et al., 2000). However, in fish, there are wide range of behavioural patterns that may be highly sensitive to changes in water quality parameters other than those directly related to swimming pattern.

The present study analysed how changes in the water quality, primarily increased turbidity, temperature and presence of detergents interfere with the sensory perception of the fish which in turn may affect their social behaviour and organization. In freshwater fishes, social cognition is achieved through a combination of visual and chemical cues (Brown and Smith, 1994). Vision is of primary importance to fishes in short range detection. However, over greater distances than a few body lengths, they may gather information using their olfactory and gustatory senses. These end points are of high ecological significance since they are involved in most aspects of intraspecific (social interactions, schooling, mating, paternal behaviours) and interspecific (feeding behaviour, predator avoidance) relations, as well as in the general orientation of the fish within its environment (migration, avoidance of risky areas).

9 (A): Effects of turbidity on the shoaling preferences of *Puntius sarana subnasutus*

Turbidity is one of the important physical characteristic of water that affects its optical property and cause light to be scattered and absorbed by particles rather than transmitted in straight lines through the water column. It is caused mainly by suspended matter or impurities that interfere with the clarity of water. These impurities may include clay, slit, finely divided inorganic and organic matter, soluble coloured organic compounds and plankton and other microscopic organisms. Typical sources of turbidity include waste discharges, run off from disturbed watersheds, excessive algae or aquatic weeds, humic acid and other compounds resulting from decay of organic substances, high iron concentrations which give water a rust-red colouration, air bubbles and particles from the treatment process (eg: lime softening) etc. Changes in turbidity can have direct and indirect effects on fish. At extremely high levels, turbidity can directly affect fish growth and survival, for example, by interfering with gill function or the quality of substrata for egg laying (Bash et. al., 2001). Turbidity limits the photic zone of the water column, diminishing the prey contrast and thus reducing the foraging success of fish (Utne-Palm, 1999; Sweka and Hartman, 2001) or it may reduce the time available for the prey to detect predators (Goldsborough and Kemp, 1998; Berger et al., 2004).

Community structure of aquatic organisms including fish may differ dramatically between clear-water and turbid-water (Meutter et al., 2005). Despite the generally poor quality of underwater images, fish depend to a great deal on vision as a source of sensory information (Guthrie and Muntz, 1993). Because of the physical nature of light and its complex interactions with the environment, a variety of different properties of visible objects can be recognized, such as, brightness, hue, texture, contour and more subtle differences of degree such as patch size or grain pattern. Agrillo et al. (2008), in a study have demonstrated that mosquito fish (*Gambusia holbrooki*) in selecting a larger shoal spontaneously use the sum of areas of the shoals and the overall quantity of the movements of individuals within the shoal. The present study analysed how turbidity of the water impose restrictions on shoaling preference in *Puntius sarana subnasutus*. The changes in preference of the test fish to associate with a larger group was tested by keeping the stimulus shoal in water having low, medium and high turbidity levels.

9 (B): Effects of water temperature in regulating on fish activity level and group-size discrimination in *Puntius sarana subnasutus*

Of all environmental factors that influence aquatic organisms, temperature is the most all-pervasive. For all aquatic animals, there is always a tolerable limit to environmental temperature while other factors may or may not be present to exert their effects. Fish are, for all practical purposes, thermal conformers, or obligate poikilotherms. That is, they are able to exert little significant influence on maintaining certain body temperatures thus fluctuate nearly in accordance with the temperature of their aquatic medium. Being cold-blooded animal, fish is affected by the temperature of the surrounding water which influences the body temperature, growth rate, food consumption, feed conversion and other body functions (Britz et al., 1997; Azevedo et al., 1998). Therefore, water temperature is a driving force in the fish life because its effects are more prominent than any other single factor. Growth and livability in fish are optimum within a defined temperature range (Gadowaski and Caddell, 1991). Although short-term changes, such as weather conditions, may influence a fish for a day or two, temperature has more predictable and seasonal effect. However, fish move into more favorable areas of a stream to regulate their body temperatures. In warmer environments fish have a longer growing season and faster growth rate but tend to have a shorter life span than in cool water. High water temperatures increase the metabolic rates, resulting in increased food demand. Although, fish can generally function in a wide range of temperatures, they do have an optimum range, as well as lower and upper lethal temperatures, for various activities (Beschta et al., 1987)

The functional properties of temperature acting on fish can be summarized as follows: temperature can act as a lethal agent that kills the fish directly; as a stressing agent that destroys the fish indirectly; as a controlling factor that sets the pace of metabolism and development; as a limiting factor that restricts activity and distribution, as a masking factor that interacts with other environmental factors by blocking or altering their potential expression, and as a directing agent in gradients that stimulate sensory perception and orientation activity. Each of these properties can be visualised as acting on two levels – on the individual fish and on the population of any one fish species. It is not surprising that sturdy species such as carp and gold fish have large thermal tolerance zones. Outside the thermal tolerance zone, premature death is inevitable and its onset is a function of both temperature and time of exposure (thermal resistance). Death occurs more rapidly when the temperature is farther from the threshold (Coutant, 1972). The thermal resistance equations allow prediction of fish survival (or death) in zones where human activity induces extreme high temperatures. For example 50% of the population of juvenile salmon and trout were perished while passing through warm mixing zones of thermal discharges to the Columbia River during their seaward migration (Becker et al., 1971). Adults may be able to detect and avoid such discharges. Young fish, however, might involuntarily pass into these flows; their survival would depend on the temperature of the discharge and howlong the fish remind in the hazardous zone.

A number of previous studies have shown that fish are indeed capable of choosing between shoals of different sizes (Blakeslee et al., 2009; Jordan et al., 2010). Here, the fish may display a rudimentary numerical ability or use non-numerical cues, namely the sum of areas of the shoals and the overall quantity of movements of the individuals within the shoal (Agrillo et al., 2008). Fish may prefer to associate with more active individuals because this in itself provides fitness benefits. There may be several reasons for this result. First, both larger and more active shoals might also have been more conspicuous to the test fish. A second possibility is that fish might simply have used shoal activity to gauge shoal size; over all activity level of a shoal may be perceptually easier for a fish to assess than actual number of individuals. A third explanation for associating with a more active fish may be the foraging advantages. Shoals containing more active fish may find food patches more rapidly, be actively feeding or be more confusing to predators. Water temperature has a significant role in controlling the activity level of a stimulus shoal (Pritchard et al., 2001) or social status. Freshwater fish have an optimum growing temperature in the range of 25-30^{o}C (Kausar and Salim, 2006) at which they grow quickly. The present study investigated how variations in the mean standard laboratory temperature caused changes in the shoal activity of the stimulus shoal and subsequent shoal size preference in *Puntius sarana subnasutus*.

9 (C): Influence of surfactants on sibling recognition and group-size discrimination in *Puntius sarana subnasutus*

Chemical communication in fish plays a significant role in many aspects of survival, including shoaling, social organization, foraging, predator detection, parent-young interactions, mate selection and homing. Krause et. al, (2000) demonstrated that genetic relatedness (kinship) is one of the major factors that determine shoal formation in numerous fish species. The ability to recognize and discriminate a related individual (kin recognition) is thought to occur through chemosensory pathways (Quinn and Busak, 1985; Olsen et al., 2003; Coleman and Rosenthal, 2006) probably influenced by the major histocompatibility complex, MHC genes (Olsen et al., 1998). Differentiating kin from non-kin enables allocation of resources, evolution of altruistic behavior and avoidance of mating with close relatives (Gerlach and Lysiak, 2006).

Many studies have further highlighted the role of olfaction in the development of familiarity and or group recognition in fishes. A diet cue-based familiarity develop among domestic guppies (*Poecilia reticulata*) when individuals were fed with similar diet (Morrell et al., 2007). Sticklebacks discounted their social experience while choosing whom to associate with and preferred to shoal with previously encountered fish that smell the same as themselves (Ward et al., 2005b). The fishes are also shown to be discriminating group-size differences based on the concentration of water-borne odour cues (Refer chapter 8 of the thesis). Juvenile three-spined sticklebacks (*Gasterosteus aculeatus*) preferentially associated with larger shoals over smaller one, when the concentrations of odour cues are high (Steck et al., 1999). Courtenay et al. (2001) showed that coho salmon (*Oncorhynchus kisutch*) fry are sensitive to family specific odour cues.

The first experimental evidence to prove ability of fish to utilize odours to make association choices was provided by Wrede in 1932 (cited in Hemmings 1966). In teleostean fishes the olfactory epithelium is typically located in two blind sacs situated on the dorso-anterior aspect of head (Burne, 1909). These structures maintain open communication with the environment by way of one or two nares serving as avenues of incurrent and excurrent water exchange

(Kleerekoper, 1969; Finger, 1988). As an organ directly exposed to the aquatic medium, the olfactory receptors of the fish are prime targets for interactions with pollutants present in the external environment, leading to possible alterations of the natural chemo-sensory processes (Klaprat et al., 1992). Different combinations of chemicals along with other industrial effluents may reach in lakes, rivers and ponds through rains and wind, resulting in the risk of poisoning all aquatic fauna, including fishes (Adewoye, 2001).

Sodium Lauryl Sulphate (SLS) is a highly effective anionic surfactant used in many cleaning and hygiene processes requiring the removal of oily stains and residues. It is found in higher concentrations in industrial products including engine degreasers, floor cleaners and car wash soaps and in lower concentrations with tooth pastes, shampoos and shaving foams. Due to its ability to create lather, SLS is an important component in bubble bath formulations for its thickening effect (Belanger et al., 1995). Earlier experimental studies have shown that brief exposures to sub-lethal concentration of surfactants can cause various changes in physiological (Rosety-Rodriguez et al., 2002; Brunelli et al., 2008; Wadaan and Mubarak, 2009) and chemosensory behavioural (Bardach et al., 1965; Olsen and Hoglund, 1985; Ward et al., 2006; Ward et al., 2008) responses of fish. The present study assessed the effects of sub-lethal concentration of an anionic detergent, Sodium Lauryl Sulphate, on sibling recognition ability of *Puntius sarana subnasutus*. Further, with the aid of scanning electron microscope (SEM), analysed whether exposure to SLS inflicted any damages on the olfactory epithelium; disrupting the proper functioning of the chemosensory pathways of the fish.

Materials and methods

9 (A): Effects of turbidity on the shoaling preferences of *Puntius sarana subnasutus*

Puntius sarana subnasutus were collected from canals associated with paddy fields of Irinjalakuda (10°25’, 10°18’47" N lat. and 76°17’19", 76°12’48"E long.), Thrissur district, India, during January - March 2010 and acclimatized with laboratory conditions for two weeks in large cement tanks (175cm x 90cm x 90cm). A pair of healthy fish was induced to breed using ovaprim. On the next day of hatching, the larvae of *Puntius sarana subnasutus* were divided into two groups. The first group (80 larvae) is further divided into four subgroups of 20 siblings and allowed to grow in four different aquaria for eight months (60 x 23 x 23 cm). A second group of 200 larvae of the same breeding pair reared separately for constituting the stimulus shoals. Three samples of water were collected from different localities of the same canal [one from a less polluted (water having low turbidity) and other two from polluted and eutrophicated sites (water having medium and high levels of turbidity)]. The turbidity of water samples were measured by nephelometric method (Clesceri et al., 1998).

The experimental set-up consists of a central large (60 x 30 x 30cm) aquarium filled with clear water and two smaller (15 x 30x 30 cm) stimulus shoal aquaria containing turbid water placed on both sides of the central chamber [Figure 9(1)]. The changes in preference of the test fish to associate with a larger group was tested by keeping the stimulus shoals in water having low, medium and high turbidity levels (35.7NTU, 48.3NTU and 68.5NTU respectively).

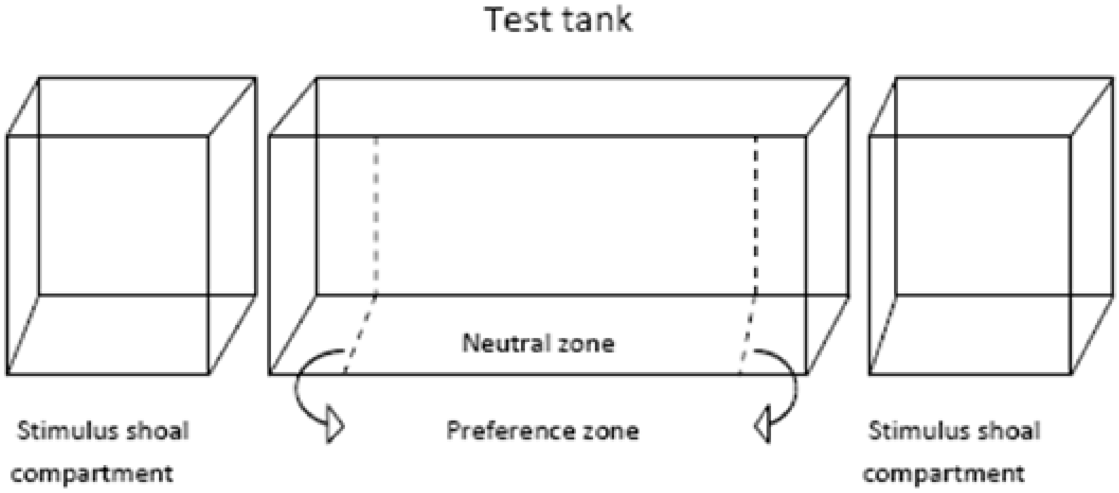


Figure 9(1): Experimental set-up for testing the shoaling preferences Of the test fish providing visual cues alone.

The removable opaque barriers were placed in between the side chambers and the central chamber. The test fish was introduced into the central chamber and allowed to acclimatize for ten minutes and after that the opaque screen was removed and then observed for further ten minutes. First four minutes were given to assess the stimulus shoals and the time spent by the test fish in the preference zone marked near each stimulus shoal was recorded for rest of six minutes. All experiments were repeated using twenty different individual fish. Three types of dichotomous preference tests were conducted using water samples having turbidity levels of 35.7, 48.3 and 68.5 NTU. The basic protocol was same in all the experiments. To test the ability for group-size recognition of *Puntius sarana subnasutus* in turbid water, two sibling stimulus shoals were exposed in five different numerical combinations (Ratio- 1:1, 1:1.5, 1:2.3, 1:4 and 1:9 respectively). The data were analyzed using non-parametric two-tailed 't' test [SPSS 11.0.1 statistical package].

9 (B): Effects of water temperature in regulating on fish activity level and group-size discrimination in *Puntius sarana subnasutus*

Adult *Puntius sarana subnasutus* were collected from the ponds of Irinjalakuda (10°25', 10°18'47" N lat. and 76°17'19", 76°12'48"E long), Thrissur district, India, during January - March 2010 . A pair of healthy fish was induced to breed using ovaprim. On the next day of hatching, the larvae of *Puntius sarana subnasutus* were divided into two groups. The first group (80 larvae) is further divided into four subgroups of 20 siblings and allowed to grow in four different aquaria for eight months (60 x 23 x 23 cm). A second group of 200 larvae of the same breeding pair reared separately for constituting the stimulus shoals. The larvae were fed with *artemia* nauplius *ad libitum*. Experiments were conducted on eight months old larvae.

Puntius sarana subnasutus has an increased preference to associate with larger sibling stimulus shoal (Refer chapter 3 of the thesis). The present study investigated whether the fish take shoal activity into account when making a shoaling decision. Here, the shoal activity of the larger stimulus shoal was manipulated by changing the water temperature. The fish was expected to spend more time with a larger group that is more active in terms of higher swimming speed.

a) Quantification of fish activity (Pilot study)

This experiment is designed to test whether changes in mean standard water temperature has any detectable effect on the activity level of *Puntius sarana subnasutus*. The fish was tested in cold water (18; 20; 22; 24^0C) or in warm water (28, 30, 32, 34^0C). The time taken by the fish to reach near fish bait (earth worm) hung on a string at the one end of the aquarium opposite to the releasing area of the test fish is taken as the indication of the activity of the fish. The test aquarium (60 x 23 x 23 cm) was filled with water at room temperature (26^0C). Then water at appropriate temperature was allowed to flow in and out from the stimulus tanks at a rate of 500ml/sec from a thermal bath placed above the experimental tanks. At each temperature [in cold water (18, 20, 22, 24^0C) or in warm water (28, 30, 32, 34^0C)] we repeated the experiment in twenty fish. After testing five individuals, the whole water in the experimental set-up was renewed.

The test fish were always introduced individually into the centre of the experimental arena in a presentation cage made of clear, perforated acrylic sheets. Ten minutes were given to the test fish to acclimate with the water temperature and to assess the experimental arena. Thereafter, the fish was slowly released by raising the box. Each fish was observed for six minutes and the time taken by the fish make the first bite of the bait was recorded. Those fish that failed to detect bait within the prescribed time was removed from the analysis. A significant decrease in fish activity was observed when the temperature reached at 18^0C or rose up to 32^0C ($p < 0.05$).

b) Determination of shoal preference

This experiment was conducted to determine whether activity level of the stimulus shoal has an overriding influence on the size of the shoal in the context of shoal choice in *Puntius sarana subnasutus*. The central test tank and one of the stimulus tanks were filled with water at room temperature. The other stimulus tank received either warm or cool water (depending on the experiment) from a water bath placed above the stimulus chamber. A preference zone (almost equal to the length of the test fish) was marked within the test tank adjacent to the side of each stimulus tank (Fig. 9(2). The test fish was always introduced individually into the central neutral zone of this aquarium. Smaller sibling stimulus shoal was introduced to the side compartment containing water at the room temperature (26^0C) and the larger shoal was introduced to the compartment containing either warm water (32^0C) or cold water (18^0C).

Removable opaque barriers were placed in between the side chambers and the central chamber. The experimental fish was introduced into the central chamber and allowed to acclimatize for ten minutes and after that the opaque screen was removed and observed it for further ten minutes. First four minutes were given to assess the stimulus shoals and the time spent by the test fish in the preference zone marked near each stimulus shoal was recorded for rest six minutes. All experiments were repeated using twenty different individual fish.

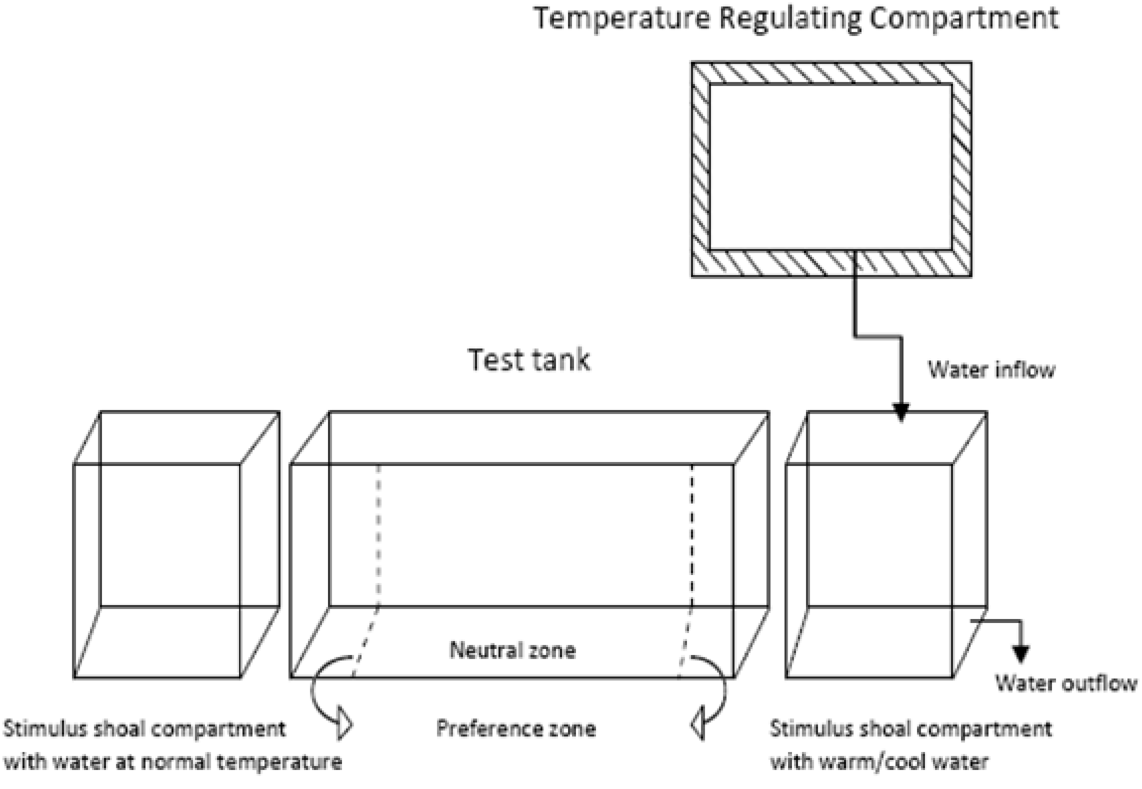


Fig 9(2). Experimental set-up for testing the shoaling preference of fish at different water temperature (18^0C) and (32^0C) **providing visual cues only**

The shoaling preferences of *Puntius sarana subnasutus* is tested in a dichotomous choice test keeping sibling shoals in stimulus shoal compartments in the following order: 25 *vs.* 25, 15 *vs.* 35 and 5 *vs.* 45 (Ratio- 1:1, 1:2.3, and 1:9 respectively). To determine the effect of temperature on activity level of the stimulus shoal and subsequent shoal choice by the test fish, the larger stimulus shoal was kept either in cool water (18^0C) or in warm (32^0C) water and the smaller shoal in water kept at room temperature.

9 (C): Influence of surfactants on sibling recognition and group-size discrimination in *Puntius sarana subnasutus*

Puntius sarana subnasutus and another heterospecific species *Puntius parrah* were collected from ponds and canals associated with paddy fields of Irinjalakuda (10°25’, 10°18’47" N lat. and 76°17’19", 76°12’48"E long.), Thrissur district, India, during January - March 2010. The fishes were acclimatized with laboratory conditions for two weeks in large cement tanks (175 x 90 x 90 cm) and fed *ad libitum* with commercially available tropical fish food (Marvel feeds, Aquarium systems, India). A pair of healthy fish was induced to breed using ovaprim. On the next day of hatching, the larvae of *Puntius sarana subnasutus* were divided into two groups. The first group (80 larvae) is further divided into four subgroups of 20 siblings and allowed to grow in four different aquaria for eight months (60 x 23 x 23 cm). A second group of 200 larvae of the same breeding pair reared separately for constituting the stimulus shoals. Similarly produced larvae of *Puntius parrah* were used as heterospecifics. Experiments were conducted when the larvae were 8 months old (8 ± 2 cm long and weighing 14 ± 2 mg).

The anionic surfactants SLS (CH_3-$(CH_2)_{110}$-SO_3-Na) with a purity greater than 99% was purchased from SIGMA and was dissolved in de-ionized water to form a stock solution, which was then added directly to the experimental tanks to obtain the desired concentrations as follows. 96hr LC-50 value 8.5 mg /l was determined according to methods outlined in Sprague (1971 & 1976). Five fishes from each of the four breeding pair of *Puntius sarana subnasutus* were transferred to the treatment tanks while another five from each breeding pair were kept as controls. The experimental fish were treated for 168h in a sub-lethal concentration (2.8 mg /l) of SLS, which is one-third of 96hr LC-50 value 8.5 mg /l (Konar, 1969).

In a dichotomous choice test, test fish were given an opportunity to swim near the water scented with odour cues of siblings or heterospecifics taken in equal number 25 *vs.* 25 (ratio 1:1). The experimental arena (60 x 80 x 30 cm) is designed like a Y-maze as described in Shohet and Watt (2004) (Refer chapter 8 of the Thesis). The experiment was repeated in twenty treated fish and twenty control fish. Sibling recognition ability of the fish was measured after 72hrs (3 days), 120hrs (5 days) and 168hrs (7 days) of exposure to SLS. Data were normalized using arcsine transformations. Since the correlation between the samples were significant, the data were analyzed using parametric dependent sample two-tailed 't' test.

After the behavioural analysis two fish from control and treatment groups were sacrificed by spinal transection. Their olfactory pits were superficially cleansed with 70% ethanol to remove any debris, olfactory epithelia was dissected out and immediately fixed in 3% gluteraldehyde (in 0·1 Ì phosphate buffer, pH 7·5) at 4°C for 24 h, washed thoroughly in phosphate buffer at room temperature to remove the traces of gluteraldehyde. The tissues were then run through the cycle of 1% osmium tetraoxode (in 0·1 Ì phosphate buffer at pH 7·5) (35 min)—1 % aqueous (pre-centrifuged) tannic acid (15 min)—0·1 Ì phosphate buffer (pH 7·5) (5 min) for osmium deposition (Singh, 1991) as an alternative to the gold sputtering· With the help of graphite glue, the specimens were mounted on the brass strubs; examined under scanning electron microscope (SEM centre, NIT, Calicut) and photographed 10 kV 32.4mm X 500SE.

Results:

9 (A): Effects of turbidity on the shoaling preferences of *Puntius sarana subnasutus*

The results of the present study suggest that *Puntius sarana subnasutus* is capable of detecting larger group of shoal mates at lower and intermediate turbid water while it failed to recognize the shoal-size difference in highly turbid water. As the number of shoal mates increased in the stimulus shoal, the fish successfully associated with the larger shoal kept in 35.7 and 48.3 NTU turbid water [Fig. 9(3&4)]. However, they failed to discriminate the shoal size difference in 68.5 NTU turbid water, [Fig. 9 (5)], and significantly associated with one of the sibling shoal when the ratio was 1:1 and two smaller group of siblings when the ratio was 1:1.5. When the ratio was 1:2.3, 1:4 and 1:9 at 68.5 NTU, it randomly associated with either of the stimulus shoal [Table: 9(1)].

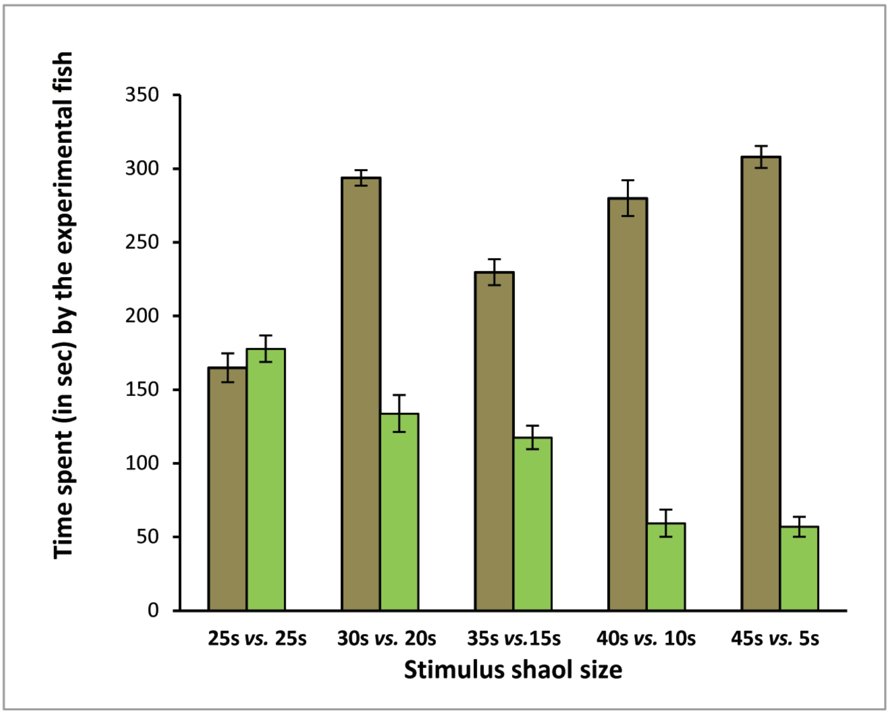


Figure 9(3) Preference of *Puntius sarana subnasutus* for larger group (🟫) and smaller group(🟩) of stimulus shoals kept in low turbid warer (35.7 NTU) n = 20 Mean time (spent in sec) ± SE

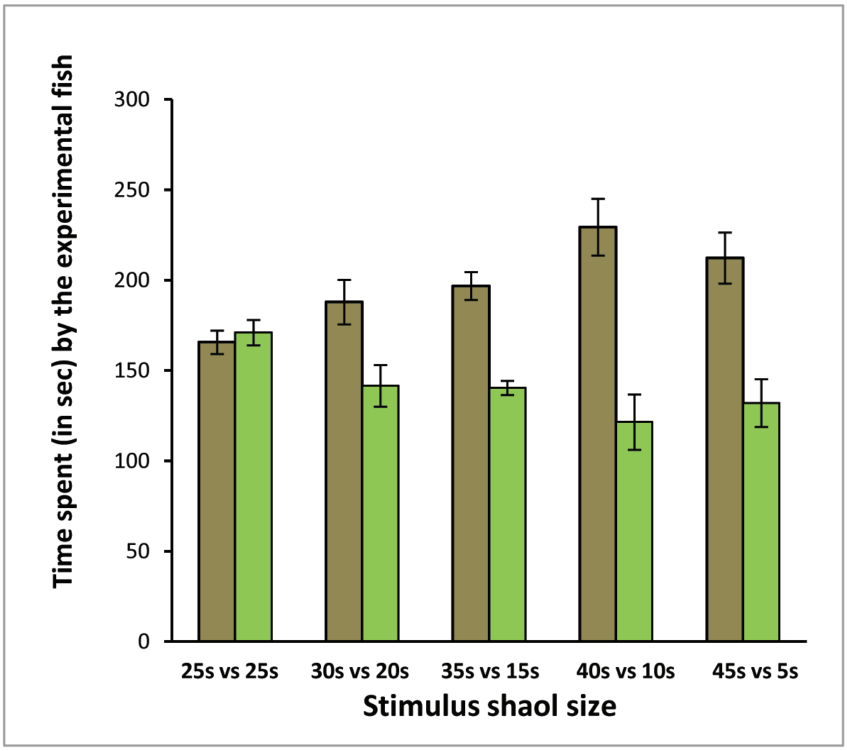


Figure 9(4) Preference of *Puntius sarana subnasutus* for larger group (🟫) and smaller group (🟩) of stimulus shoals kept in medium turbid water (48.3 NTU) **n = 20 Mean time (spent in sec) ± SE.**

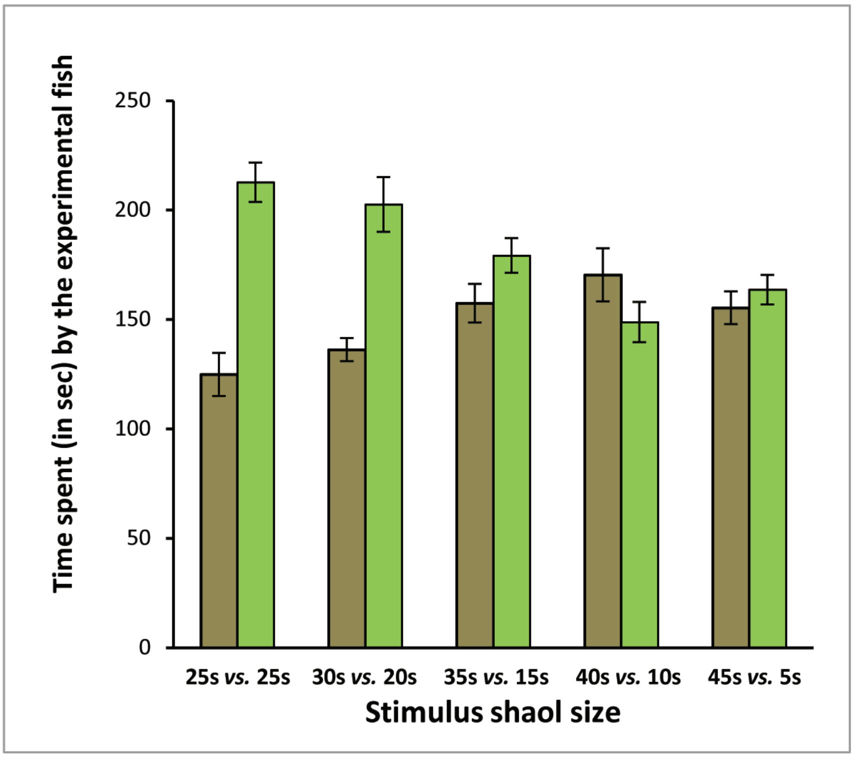


Figure 9(5) Preference of *Puntius sarana subnasutus* for larger group (🟫) and smaller group (🟩) of stimulus shoals kept in high turbid water (68.5 NTU) **n = 20 Mean time (spent in sec) ± SE .**

Group size	**Turbidity of water sample (NTU)**		
	35.8	**48.3**	**68.5**
25s *vs.* 25s	t = 0.705; p=0.49	t = 0.408; p=0.688	t = 6.234;p=0.001***
30s *vs.* 20s	t = 1.841; p=0.081	t = 1.971; p=0.064	t = 3.405; p=0.003**
35s *vs.* 15s	t = 6.849; p=0.001***	t = 3.902; p=0.001**	t = 1.444; p=0.165
40s *vs.* 10s	t = 10.774; p=0.001***	t = 3.690; p=0.002**	t = 0.847; p=0.408
45s *vs.* 5s	t = 20.077; p=0.001***	t = 2.959; p=0.008**	t = 0.592; p=0.561

Table 9(1): Preference to associate with the larger group of stimulus shoal in Puntius sarana subnasutus at different turbidty levels of water

9 (B): Effects of water temperature in regulating on fish activity level and group-size discrimination in *Puntius sarana subnasutus*

Variations in water temperature significantly affected the association preferences of *Puntius sarana subnasutus*. When the stimulus shoals and test fish were kept in water at normal laboratory temperature (26^{0}C) and the stimulus shoal size is equal (25 *vs.* 25) the fish exhibited no significant association preference for any particular shoal. However, given a choice between stimulus shoal consisting of 15 *vs.* 35 & 5 *vs.* 45, the test fish significantly associated with the larger shoal [Fig. 9(6), Table: 9(2)]. However no significant association preference was observed when one of the stimulus shoals is kept in water at 18^{0}C or 32^{0}C and the alternative shoal and test fish are kept in water at 26^{0}C. When one of the sibling stimulus shoals is kept at 32^{0}C; the test fish associated with the alternate shoal kept at 26^{0}C irrespective of their number. This preference was highly significant when the number of individuals in the stimulus shoal is equal (25 *vs.* 25). When the shoal-size differed by twenty (15 *vs.* 35) or even thirty five (5 *vs.* 45), the test fish did not exhibited any significant preference towards the larger shoal [Fig. 9(7), Table: 9(3)]. Similarly, the test fish also decreased their preference for stimulus shoal kept at 18^{0}C even if they were composed of larger number of individuals. However this decrease in preference was not as significant as the preference pattern exhibited in warm water [Fig. 9(8), Table: 9(4)]. At 18^{0}C, the fish significantly associated with the stimulus group kept at 26^{0}C when the number of individuals in the stimulus shoal is the same or slightly larger (25 *vs.* 25 and 15 *vs.* 35). The test fish randomly moved between the stimulus shoals without exhibiting any particular association preference to a specific stimulus shoal when the shoal size was and 5 *vs.* 45.

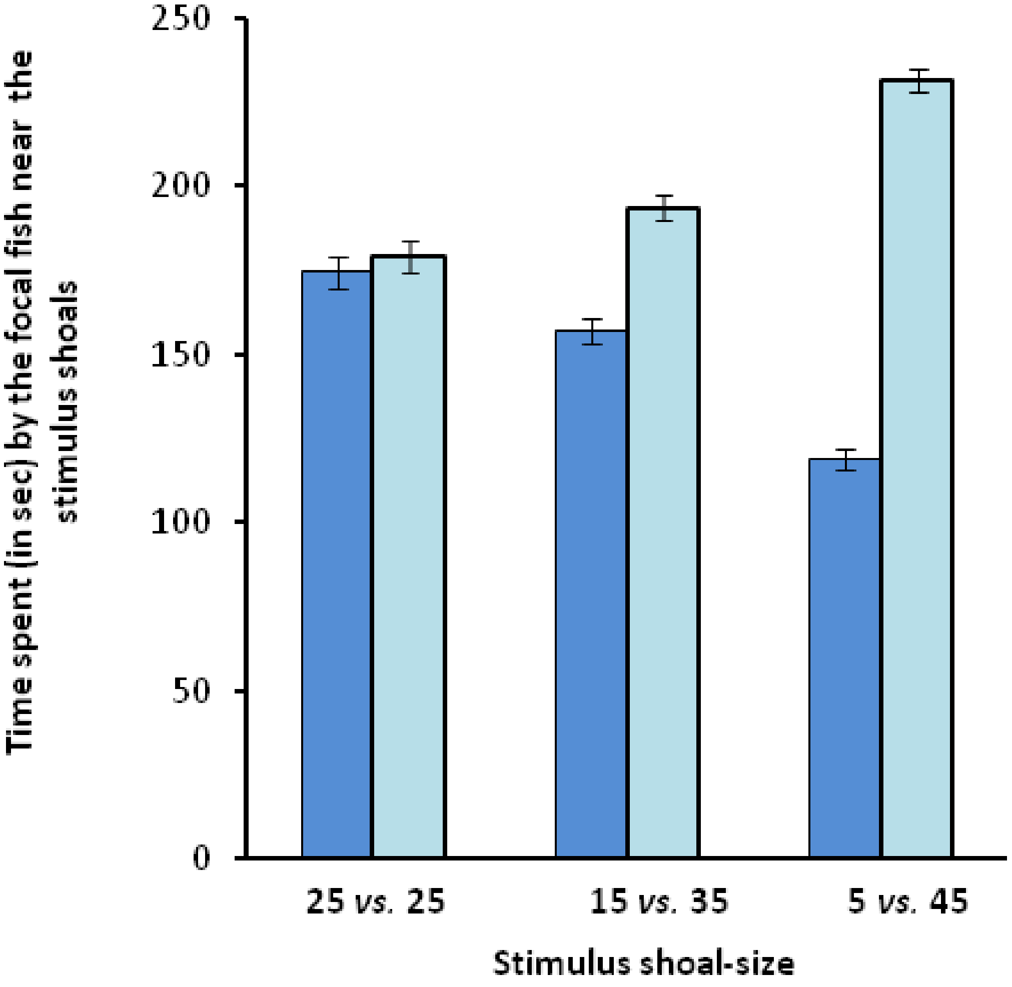


Figure 9(6): Association preference of *Puntius sarana subnasutus* to the sibling stimulus shoals when both the stimulus shoal kept at 26^{o}C

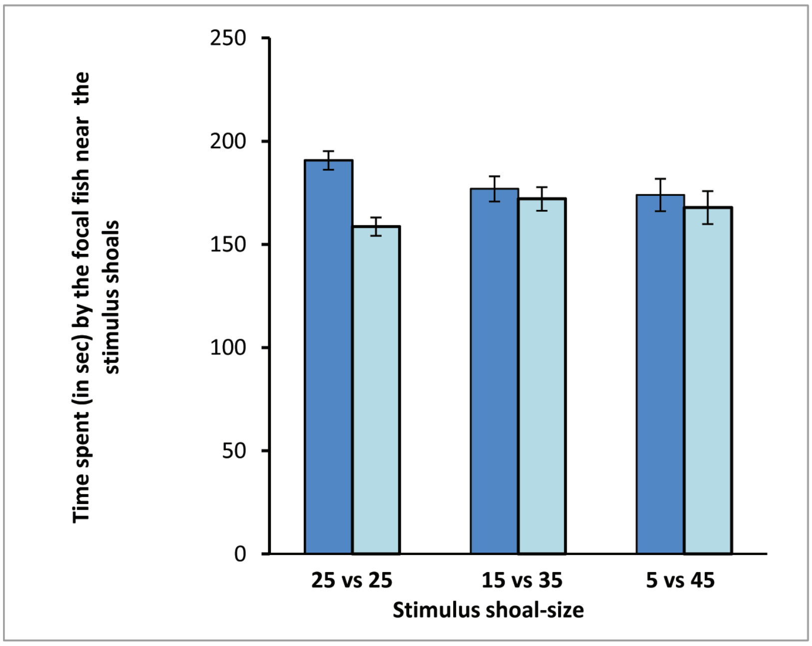


Figure 9(7): Association preference of Puntius sarana subnasutus to the sibling stimulus shoals when one stimulus shoal (■) kept at 26°C and the alternate stimulus shoal (□) kept at 32°C

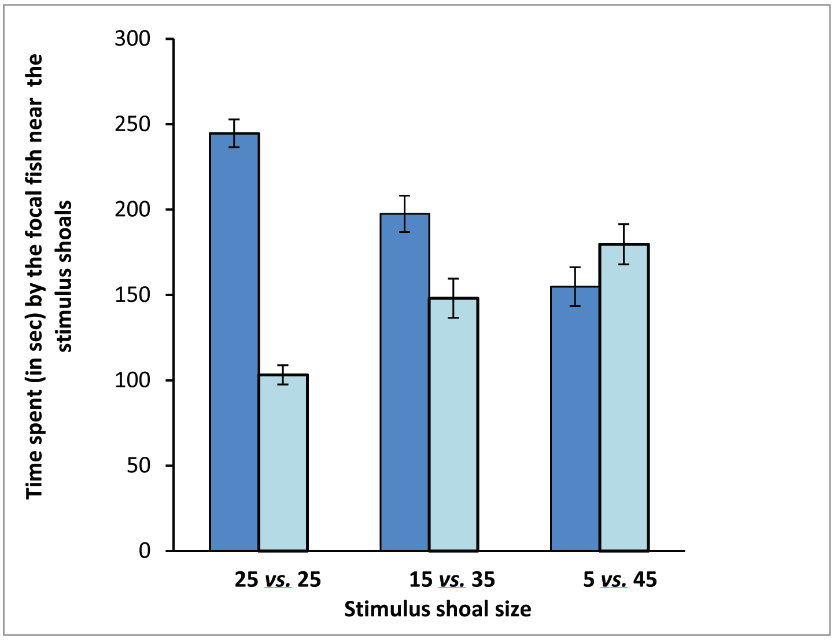


Figure 9(8): Association preference of Puntius sarana subnasutus to the sibling stimulus shoals when one stimulus shoal (■)kept at 26°C and the alternate stimulus shoal (□) kept at 18°C

A) Table 9(2) - Both stimulus shoal kept at 26°C

Group size		**t Value**	**p Value**
Stimulus 1 Laboratory temperature (26°C)	**Stimulus 2** Laboratory temperature (26°C)		
25	25	t=0.474	p=0.641
15	35	t=4.973	p=0.001***
5	45	t=19.141	p=0.001***

B) Table 9(3) - Larger stimulus shoal kept at 32°C

Group size		**t Value**	**p Value**
Stimulus 1 Laboratory temperature (26°C)	**Stimulus 2** High temperature (32°C)		
25	25	t=5.710	p=0.001***
15	35	t=0.427	p=0.675
5	45	t=1.02	p=0.320

C) Table 9(4) - Larger stimulus shoal kept at 18°C

Group size		**t Value**	**p Value**
Stimulus 1 Laboratory temperature (26°C)	**Stimulus 2** Low temperature (18°C)		
25	25	t=10.984	p=0.001**
15	35	t=2.31	=0.032*
5	45	t=0.290	p=0.212

9 (C): Influence of surfactants on sibling recognition and group-size discrimination in *Puntius sarana subnasutus*

It is observed in preliminary experiments that the test fish preferentially oriented toward water taken from their home tank compared to water taken from a tank that contained no fish (t=9.356, p=0.001). Throughout the experiment, the control fish spent significantly more time near the compartment having water scented with the odour of their siblings [Figure 9(9) - 72h- t=8.955, p=0.001; 120h- t=8.596, p= 0.001; and 168h- t=8.689, p=0.001] while fish treated in SLS exhibited decreased preference for siblings over time [Figure 9(10)]. After 72h of exposure to SLS, however, the fish successfully discriminated the sibling odour cues and associated with it (t=5.312, p=0.001) even though the total time spent in exploration was less compared to that of the control fish. Upon 120h exposure, the treated fish further reduced their exploratory activity but still spent significantly more time near the compartment containing sibling odour cue (t=2.630, p=0.017). No significant association preference for either stimulus was observed in fish exposed to SLS for 168h (t=1.104, p=0.284) and the fish spent very less time in exploration. The treated fish, in all experiments spent more time motionless in the central arena and visited the compartment containing odour cue of heterospecific, *Puntius parrah*, more frequently, indicating clearly the disruption in their ability for sibling recognition (Figure 9(9) & 9(10).

Analysis of olfactory epithelium using SEM provided evidences for the structural changes in the olfactory epithelium due to treatment with SLS. The olfactory lamellae as well as the mucous secreting cells were severely damaged (Figure 9(11): 1-3). Thus, anionic surfactant SLS significantly reduced the sibling recognition ability of *Puntius sarana subnastutus* and altered the structure of olfactory epithelium while the control fish with intact olfactory epithelium exhibited a strong preference for sibling odour cues.

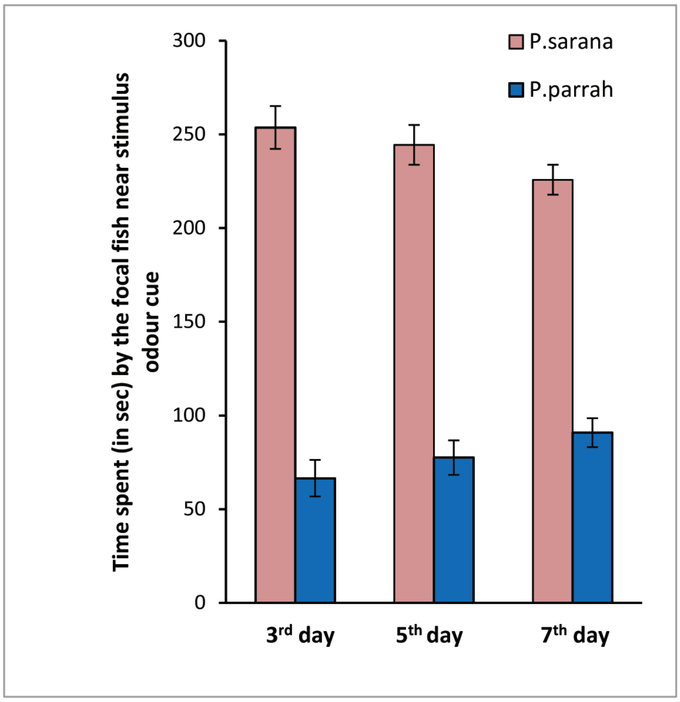


Figure 9(9): Preference of *Puntius sarana subnasutus* (control group) to the stimulus odour cues of their siblings (🟥) and heterospecific *Puntius parrah* (🟦) n = 20 Mean time (spent in sec) ± SE

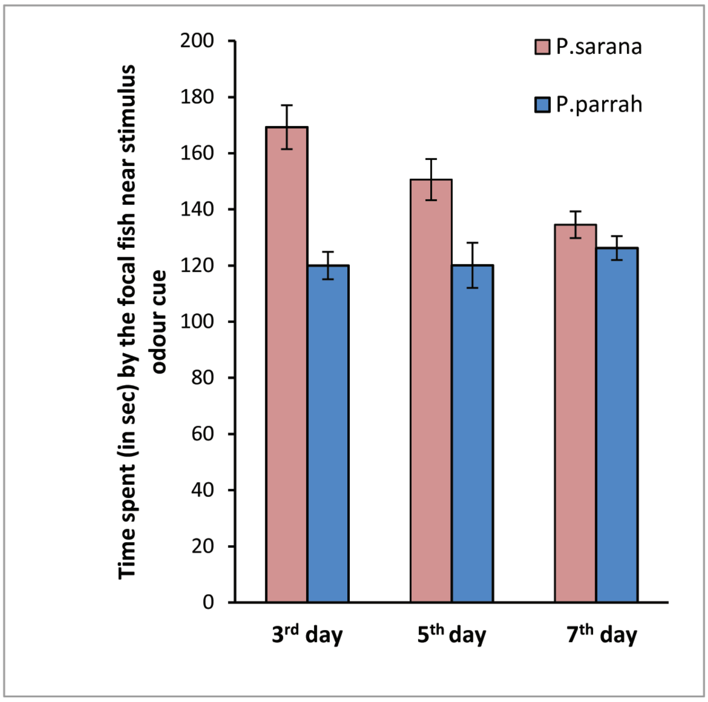
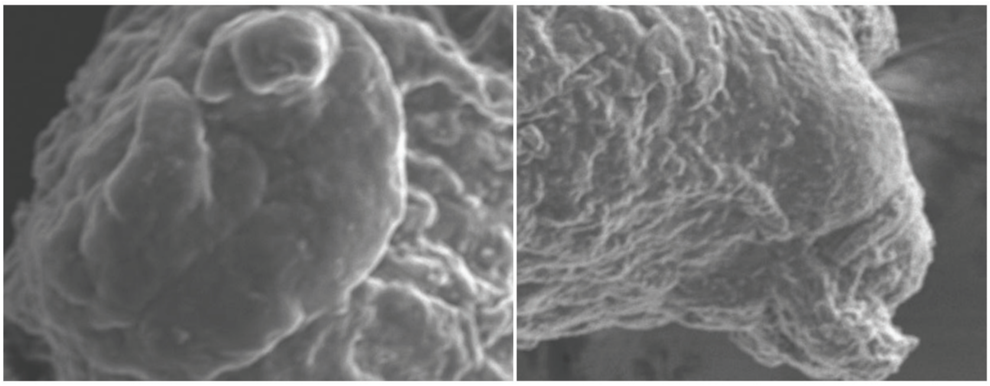
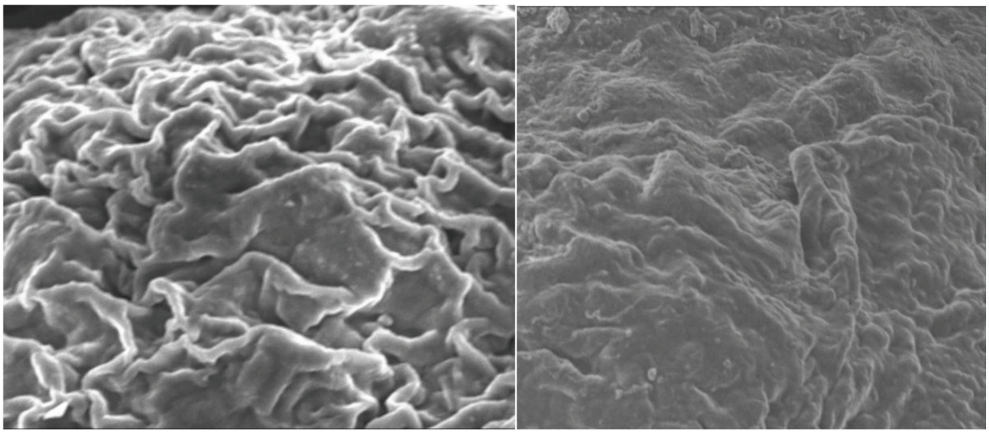


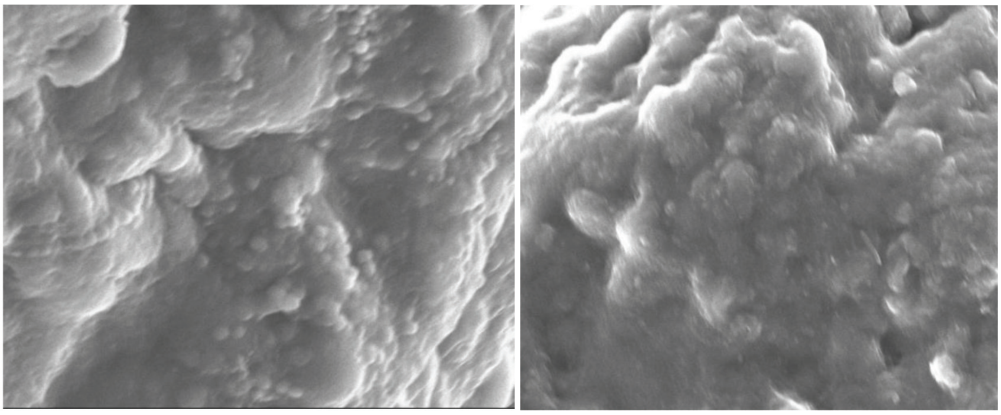
Figure 9(10) Preference of *Puntius sarana subnasutus* (Sodium Lauryl Sulphate treated group) to the stimulus odour cues of their siblings (🟥) and heterospecific *Puntius parrah* (🟦) n = 20 Mean time (spent in sec) ± SE



1: a) Intact lamellae of control fish   1: b) Damaged lamellae of treated fish



2: a) olfactory epithelial surface of control fish   2: b) Olfactory epithelial surface of treated fish



3: a) Intact Mucus secreting cells of control fish   3:b) Damaged Mucus secreting cells of treated fish

Figure 9(11): Olfactory epithelium of control fish and Sodium Lauryl Sulphate treated fish

Discussion

(A) Effect of turbidity on shoal choice preference

The results of the present study clearly indicate that turbidity acts through visual effects, perhaps by altering the contrast between individual and the background. The behavioural repertoire of most fish species depends on vision, and visual ability of fish will be affected by the physical conditions of their environment such as illumination, wave length and turbidity. It is reported that turbidity limits the visual ability of fish which interferes with its social behaviour (Berg and Northcote, 1985). This can have varying effects on fish growth and survival, depending on a range of factors such as ambient light levels and depth, relative visual sensitivities of predators and prey. Earlier studies on foraging have shown that although the efficiency of prey capture decreased at higher turbidity, the fish exhibited some degree of feeding activity at 45 NTU turbidity level when some underwater observations were made (Madej et al., 2004).

In the present study, it has been shown that 35.7, 48.3 NTU turbidity did not interfere much with natural shoal recognition ability of the fish. However, the fish failed to exhibit normal exploratory behaviour and shoal recognition at the 68.5 NTU turbidity. It failed to discriminate the group size differences between the stimulus shoals when placed in highly turbid water. This clearly indicates that a long term exposure to highly turbid water adversely affect the social behavioural patterns and the survival of fish even though, low and moderate turbidity levels are tolerable. Here the increased level of turbidity may affect the visual range of the fish perhaps by altering the contrast between the shoal mates and the back ground. Studies on predator avoidance (Meager et al., 2006) have shown that most fish species evade predators by using escape responses involving a high energy burst of swimming. Visual escape responses require both perception of the predator and a decision to escape, based on the level of perceived risk. The time available for prey to detect predators and decide on an appropriate response is limited by predator attack speed. Similarly, turbidity may limit the time prey have to evade a predator, by reducing visual distance. The combined effects of predator attack speed, social recognition and turbidity are therefore likely to be complex.

Environmental factors such as turbidity and habitat complexity affect many aspects of aquatic food webs and social interaction. Contrast degradation theory (De Robertis et al., 2003) predicts that increased turbidity decreases the visibility of objects that are visible at longer distances more than that of objects that are visible at short distances. Consequently, turbidity should disproportionately decrease social recognition in fish which select their shoal mates based on colour or group-size. The shoaling fish have been shown to discriminate between individuals on a very general basis such as species, body length, colour, relatedness, familiarity and group size. Changes in the light environment can induce colour pattern changes in fishes (Eg; for background matching) and also affect the perception of colour and its use as a behavioural signal. Kelly et al., (2010) studied the ability of western rainbowfish (*Melanotaenia australis*) to adapt to changes in water quality via changes in their colour patterns and shoaling behaviour, specifically a simulated increase in the tannin content of water. Rainbow fish in the tannin filter treatments increased the production orange and black pigmentation over the two week treatment period and displayed grater inter-individual shoaling distances than those in the other group. Fish in neutral filter treatment reduced their orange and black clour but showed similar shoaling behaviour to control group (Kelly et al., 2010)

Many aspects social recognition is achieved by a combination of visual and chemical cues (Ward et al., 2004). High turbidity limits the range of visual recognition and the fish fail to discriminate their shoal mates. A shoaling fish effectively counter predator attack through avoidance, dilution, abatement, evasion, confusion, detection, inspection etc. In shoals, information about the type and quality of food source, number of companions, threat and alternative feeding sites are collectively acquired and decisions are taken surprisingly rapidly (Pitcher and Parrish, 1993). The behavioural plasticity conferred by shoaling may therefore be seen as one of its major general advantages. Turbidity, by reducing the visual range interferes with this vital behavioural pattern, adversely affecting the survival of fish. Therefore, it is necessary to control water pollution especially industrial effluents and eutrophication that interferes with the clarity of water affecting the growth of aquatic populations.

(B) Effect of temperature on shoal choice preference

Climate change is one among the major environmental and anthropogenic stresses faced by fisheries management and aquaculture which is likely to exacerbate the difficulties of achieving sustainable practices. Changes in temperature affect aquatic life. Temperature determines which organisms will thrive and which will diminish in numbers and size. For each organism there is a thermal death point. Also there is a range of temperature that produces optimal abundance. The effects of temperature on life of a poikilotherm are profound. High and low temperatures that are lethal to individual organism of a species determine the distribution and abundance of populations. However, more often the distribution and abundance of populations is determined by less than lethal temperatures interacting with other environmental factors that either tend to favour or act adversely on reproduction and growth.

Shoaling in fishes is an indispensable strategy for escaping from predators. It has been demonstrated that most of the fish species preferred to associate with a larger active group primarily to maximize the anti predator advantages of shoaling (Turesson and Bronmark, 2007). How the fish could recognize the group size difference while making decision about group membership is still in debate. Buckingham et al. (2007) suggest one possibility that fish might use ratio of the number of individuals in a shoal to determine differences in group sizes in a manner consistent with Weber's law. Weber's law states that the ease of any numerical comparison is based on the ratio between the stimuli compared; as the ratio becomes smaller the comparison becomes more difficult. For example, female green swordtails (*Xiphophorus hellerii*) significantly associated with the larger shoal when the ratio was 2:1 but showed no preference when the ratio was 1.5:1 (Buckingham et al., 2007). Agrillo and Dadda (2007) found that in female topminnows (*Fundulus notatus*) when groups differed in size by one individual, choice of the larger shoal was significantly above that predicted by chance up to a maximum of three fish in the larger shoal (i. e., 2 *vs.* 1 and 3 *vs.* 2 but not 4 *vs.* 3 or larger). The fish when presented with two shoals with 1:2 numerical ratio, choice was significantly above chance in 4 *vs.* 2 but not in 8 *vs.* 4 combinations. The discrepancy in these group-size recognition patterns based on number, points to the involvement of certain other factors in these particular behavioural pattern. For shoals composed of related individuals there is an overall reduction in aggressive behavior. For example, in different salmonoid species the level of aggression was lower when groups were composed of kin (Brown and Brown, 1993a). The tendency of a fish to associate with a larger group directly correlates with the anti-predator function of shoaling. It is established that opercular rate, which typically increases under risk (Barreto et al., 2003) and may be indicative of a fish's preparedness to flee (Hawkins et al., 2004), was lower in larger groups, even in the absence of an overt predation threat. In a larger group in addition to increased vigilance, there are benefits related to dilution and predator confusion (Pitcher and Parrish, 1993). Time devoted to predator avoidance is the time lost from other activities such as foraging. For this reason, membership of a larger shoal provides advantages over and above the slight differences in phenotypic characters.

In the present study, the preference for a larger shoal is reversed or lost when manipulated the shoal activity by changing the temperature. Since fish are cold blooded, their body temperature is essentially the temperature of the surrounding water (Morvan et al., 1998), so that their entire physiology, including food consumption, feed conversion and other body functions, is influenced by environmental temperature. Although fish can generally function in a wide range of temperatures, they do have an optimum range, as well as lower and upper lethal temperatures. Jauncey and Ross (1982) have reported that most of the fish species cease to feed at temperature (below 16^0C). Increasing temperature decreased the growth rate in Tasman seafish (*Cheilodactylus spectabilis*) as the individuals struggled to maintain cardiac function and respiration in the face of increased metabolic demands at higher temperature (Neuheimer et al., 2011). It is well recognized in many species that heat distress suffered by animals will reduce the rate of animal feed intake and result in poor growth performance and activity (Rowlinson, 2008). In the present study, the poor swimming movements of individual fish as a result of alternations in temperature greatly reduced the association preference of the test fish towards them. Here, the test fish avoided such a group in spite of a number of anti predator advantages offered from a larger group. Even though smaller in size, shoals containing more active fish may, for example, find food patches more rapidly, or more confusing to predators (Pritchard et al., 2001). Further, the decreased activity of stimulus shoal as an indicator of parasitic load (Ward et al., 2005a).

Most of the changes in water temperature result from land use activity. An exception is the release of cold bottom water from stratified artificial impoundments such as the dams that may alter the flora and fauna for many miles downstream. Higher temperatures diminish the solubility of dissolved oxygen and thus decrease the availability of oxygen for respiration; elevated temperatures increase the metabolism, respiration and oxygen demand of fish and other aquatic life, approximately doubling the respiration for a rise of every 10°C. Hence the demand for oxygen is increased under conditions where oxygen supply is lowered. The solubility of many toxic substances increases as well as intensify as temperature rises. Higher temperatures act against desirable fish life by favoring the growth of sewage fungus and the putrefy sludge deposits, and finally even with adequate dissolved oxygen. There is a maximum temperature that each species of fish or other organism can tolerate. Higher temperatures results in death. The maximum temperatures that adult fish can tolerate may with the species of fish, prior acclimatization, oxygen availability and the synergistic effects of other pollutants. If the water is cooler, fish become lethargic and tend to be inactive.

Apart from these, the study demonstrates some disparity in the patterns by which the fish responded to variations in temperature. The fish reduced their activity significantly for an increase of +6^{0}C from the mean standard laboratory temperature while only a decrease of 8^{0}C from the mean standard laboratory temperature reduced the fish activity significantly. Further, the stimulus shoal kept in water at 32^{0}C was found to be more inert and motionless and hence the test fish significantly associated with the alternate shoal at 26^{0}C even though the shoal size is smaller. However, no such stringent preference was observed when the stimulus shoal was kept in water at 18^{0}C; rather the fish passively moved between the stimulus shoals. This clearly indicates that slight increase in temperature could have more impact on the survival of fish than slight decreases in temperature. It is very likely that within a few years climatic changes may induce several negative impacts on physiology of fish in localities where temperature increase, through limiting oxygen transport (FAO, 2008). This would have significant impacts on distribution and probably, abundance of both freshwater and marine species. Future studies in this regard are necessary to mitigate the difficulties in achieving sustainable practices.

(C) Effect of surfactants on shoal choice preference

Accurate and up-to-date information about environment is crucial for successful thriving in a habitat. Fishes are remarkably sensitive to dissolved water-borne molecules. Fish detect chemical stimuli through at least two different channels for chemoreception, olfaction (smell) and gustation (taste) (Hara, 1993). Even though there are many studies which point out the importance of olfaction in social recognition, especially in the identification of siblings, conspecifics and heterospecifics (Quinn and Busak, 1985; Olsen et al., 1998; Olsen et al., 2003; Coleman and Rosenthal, 2006), only a few studies (Bardach et al., 1965; Olsen and Hoglund, 1985; Ward et al., 2006; Ward et al., 2008) have focused on the effect of alterations in these basic mechanisms due to various chemical pollutants in the aquatic habitat.

Waldman (1987, 1991) proposed that individual recognition might develop following a simple sequence of events. Phenotypic signals which may be chemical and visual in nature are exchanged between individuals. When an individual acquires such a signal, it compares this to a template. This template, against which an individual compares these signals, may be genetically hard-wired or alternatively it may be learned during a sensitive period of development. If the signal matches the template, recognition is said to have occurred. It is well known that a variety of secretions and excretions like mucus, urine and feces act as chemical cues in fish and can convey information on age, sex, kinship and dominance (Thom and Hurst, 2004). For social animals, other group members are a source of information about the environment, both via active signaling and communication and through their reactions to external stimuli. Many of the benefits of shoaling are maximized when it is composed of siblings (Gerlach and Lysiak, 2006).

Surfactants that get into the aquatic environment exert a number of hazardous toxic effects on the inhabitants. In the present study, *Puntius sarana subnasutus* treated with SLS failed to discriminate the odour cues from their siblings and heterospecifics. Bardach et al., (1965) have reported that the exposure to surfactants affected the receptor function in catfish (*Ictalurus natalis*). Olsen and Hoglund (1985) found that surfactants reduced chemo attraction of juvenile Arctic charr (*Salvelinus alpinus*) to conspecifics chemical cues. Conspicuous alterations on the gill epithelium of ornate wrasse (*Thalassoma pavo*) were observed after 96 and 192h exposure to SLS. It has been suggested that the fall of surface tension induced by surfactants is the main cause of death since, under such conditions, the access of dissolved oxygen is limited (Prat and Giraud, 1964).

In freshwater fishes, social recognition is achieved using a combination of visual and chemical cues (Brown and Smith, 1994). The present study investigated the species discrimination ability of *Puntius sarana subnasutus* between sibling and heterospecific shoals providing only the odour cues. Chemical cues are of particular relevance to aquatic animals owing to the properties of water as a solvent and a medium to disperse such cues because of the limitations of vision at depth and in complex or turbid environment (Ward et al., 2007). Here, the control group successfully identified and preferentially oriented towards their sibling odour cues while the SLS treated fish exhibited variations in this specific recognition patterns. They failed to recognize the odour cues from siblings and randomly associated either with siblings or heterospecifics. Further, as the duration of exposure increased the ability for discrimination between siblings and heterospecifics was almost found to be absent in the treated group. This indicates the deleterious effects of SLS on the olfactory recognition abilities of the fish.

SLS may probably damage the olfactory epithelium of the fish disrupting its basic mechanism to identify and associate with their siblings. The SEM analysis of the olfactory epithelium of the SLS treated fish revealed defective olfactory lamellae and mucus secreting cells. This in turn may account for the decreased preference for sibling odour cues and the overall reduction in the exploratory activities of the treated fish compared to the control fish.

The olfactory receptors of fish are extremely sensitive to chemical odour cues. This greater sensitivity of olfactory receptors holds the possibility that certain chemicals in very minimal quantity might induce damages to the olfactory epithelium. It is now generally accepted that odorant molecules binds to a receptor protein in the ciliary plasma membrane, enabling them to activate a G protein. The electrical signals thus generated can be tapped, amplified and displayed at various levels of olfactory system (Hara, 1993). SLS by damaging the olfactory epithelium interfered with this basic mechanism of recognition. High levels of chemical pollutants in the aquatic environment may limit the olfactory range of recognition in fishes and in turn they may fail to discriminate shoal mates properly. Considering the broad range of toxicological affects of surfactants on aquatic life, special attention should be given to reduce the excessive use these chemicals in industries and human settlements adjacent to aquatic habitats.

Chapter 10

Consolidated discussion

In nature, the composition of fish shoals is typically nonrandom and the shoaling decisions appear to be based on the relative value of costs and benefits associated with group membership. By choosing companions according to characteristics such as body size, colour, species or even familiarity, individuals enhance the benefits of grouping. As shoaling is an indispensable antipredatory behavior, a proper knowledge of the development of this behaviour is crucial for understanding the fluctuations in fish stocks.

Onset of shoaling

The present study reveals the social grouping strategies and recognition mechanisms in *Puntius sarana subnasutus*. Because fish vary tremendously in the ontogenic timing of shoaling behaviour, as the prelude to the study, the onset of shoaling behaviour is tested in laboratory bred offspring of *Puntius sarana subnasutus,* so as to confirm whether the fish begins to shoal immediately on hatching or whether this behaviour arises during later stages of development. The larvae are relatively sessile during the first two days of hatching and remained immobile without showing any shoaling behaviour. Nevertheless, with the progress of development the fish preferred to move towards the group and subsequently preferred to join with the group from the tenth day of hatching onwards.

Shoal size determination

For group living animals, individual fitness varies as a function of group size, and hence shoal choice of an individual reflects a dynamic trade-off between the costs and benefits of membership. The results of the present study indicate that juveniles of *Puntius sarana subnasutus* generally prefer to join the larger group and that this preference is stronger when the larger shoal is composed of phenotypically and genotypically similar individuals.

Group size *vs.* genotypic and phenotypic similarity

When siblings of *Puntius sarana subnasutus* (offspring of a single breeding pair) were given a choice between phenotypically and genotypically similar siblings and phenotypically (larger body-sized) and genotypically different individuals (conspecifics- offspring of a different breeding pair of

Puntius sarana subnasutus), the test fish preferred to associate with the sibling shoal when their number is fairly large. However, the fish associated with the large body-sized conspecific stimulus shoal when the number of individuals in the sibling stimulus shoal was very small. Here, the fish, in spite of the oddity effect, preferred the larger group of conspecifics.

Early rearing environment and shoaling preference

An important feature for animals that live in social groups is kin recognition ie., they must be able at least to discriminate between individuals of their own and other species. The benefits of grouping may vary accordingly, whether they are joining with siblings / conspecifics / heterospecifics. The early social environment may have a disproportionate effect on the recognition of kin and conspecifics later in life because some species imprint on parental or sibling phenotypes. Further the fish are able to discriminate heterospecifics individuals and form mixed groups depended on context. Familiarity as this phenomenon is termed (associating with individuals on the basis of previous experience with them) is dependent on the potential for repeated interactions.

Early social ambience and age have considerable influence on shoaling preferences of *Puntius sarana subnasutus*. When measured the shoaling preference of the larvae reared along with their siblings, the test fish preferred sibling shoal when the size of the shoal is equal or nearly equal to the conspecific shoal. Duration of rearing with siblings (3 months or 8 months) has no significant change in the preference of the test fish. However, the behaviour of the larvae of *Puntius sarana subnasutus* changed, when the size of the conspecific shoal increased. Similar treatment groups when exposed to heterospecific *Puntius parrah,* failed to exhibit any preference to a shoal of *Puntius parrah*, even when the number of heterospecific shoal to sibling shoal increased. However larvae reared with siblings for 8 months, showed a tendency to prefer both shoals irrespective of whether the shoal is composed of heterospecifics or siblings. When the stimulus shoal is genetically distantly related *Anabas testudineus*, the test fish exhibited very clear preference for sibling shoal in all five numerical ratios, irrespective of the age of the fish at the time of testing.

When the larvae were reared in groups of siblings and equal number conspecific heterospecific *Puntius parrah* or genetically distantly related *Anabas testudineus,* the test fish treated them as 'familiars'. Contrary to the increased preference exhibited towards siblings during the earlier experiments, the fish preferred familiar conspecifc shoals to that of sibling shoal irrespective of the rearing density and stimulus shoal size ratio. The tendency of the test fish to associate with the familiar conspecific stimulus shoal on a slight increase in their number further indicates the influence of familiarity on shoaling preference.

Such a preference was not observed familiar congeneric stimulus shoal composed of *Puntius parrah*. Here the test fish associated with the familiar group of *Puntius parrah* only when their number is comparatively very large compared to the number of siblings. When the size of the familiar heterospecific *Puntius parrah* stimulus shoal is equal or nearly equal to the sibling shoal, the larvae preferred to shoal with their siblings. However, the test fish exhibited no significant association preference to the larger shoal composed of familiar individuals of genetically more distantly related *Anabas testudineus*. Results of the present study suggest that individuals learn their species identity from social environment and that this affects their social preference. Nevertheless, the number of individuals in the immediate social environment environment during earlier rearing condition failed to produce any marked effect on the species recognition and group-size discrimination ability of the fish.

Isolation and shoal choice

Isolation from the next day of hatching onwards did not cause any marked effect on the sibling recognition abilities of *Puntius sarana subnasutus* compared to the control individuals reared in groups of ten individuals. Isolated fish (both isolated for a period of one month and three months) effectively identified their siblings from conspecifics when the number of individuals in the stimulus shoals were the same. However, *Puntius sarana subnasutus,* a fish which exhibits strong preference to associate with the larger group, exhibited disruption in shoal size discrimination, when reared in total isolation. Further, those individuals isolated for a period of 45 days from the next day of hatching and then reared in group for one month behaved similar to the individuals those reared in total isolation for three months. They failed to discriminate the larger sibling group from the smaller sibling shoal presented to them in all numerical combinations. On the other hand, the individuals reared in group for a period of 45 days from the day of hatching and then reared in isolation for a period of one month behaved as that of controls and identified the group size differences between the sibling stimulus shoals. The fish spent significantly more time with the larger sibling stimulus group. One month of isolation immediately after hatching affected the individuals more severely and they failed to restore their group-size discrimination ability even after returning to a social group. This result, coupled with early cross rearing experiments, strongly support the argument that there exists a critical period during which the visually and olfactory mediated shoaling preference is acquired, after which the fish fails to regain this ability. Social behaviour in *Puntius sarana subnasutus* provides a fascinating system for studying learning and the neurological and molecular basis of imprinting and social memory. The study also indicate the need of providing a natural, stress-free rearing environment for induced bred larvae/juveniles that are employed for culturing and fish stock replenishing programmes.

Sensory cues in kin recognition and shoal-size discrimination

The present study shows that the fish employ olfactory or visual cues alone or in combination for shoal recognition and shoal size discrimination. Vision is of primary importance in fish for short-range detection. This is indicated by the fact that the test fish clearly recognized the group-size differences between the stimulus shoals of their siblings in various numerical combinations, even when visual cues alone are available. When the test fish was exposed to unfamiliar (previously not encountered) conspecifics and heterospecific stimulus shoals, the fish successfully identified their siblings and spent significantly more time with them based only on visual cues. At the same time, a variation in the preferences is observed when the heterospecifics were familiar to the test fish (test fish reared along with heterospecifics). Here, the fish exhibited a significant association with the stimulus shoal composed of their siblings only when the alternate group was consisted of genetically more unrelated fish (*Anabas* *testudineus*). Group-size discrimination based only on olfactory cues is possible in *Puntius sarana subnasutus* when both the stimulus shoals were composed of siblings in different numerical combinations. The fish spent more time near the chamber containing water scented with the odour cues of the larger group of siblings indicating their ability for shoal size discriminations based on the concentration of odour cues. Further, *Puntius sarana subnasutus* clearly discriminated the odour cues of their siblings from that of heterospecifics or conspecifics when the number of individuals in the stimulus shoal was the same. However, such a preference based on the concentration of odour cues was not observed when the larger shoal is composed of either conspecific *Puntius sarana subnasutus* or heterospecifics *Puntius parrah* or *Anabas testudineus.* Early exposure during the time of development to heterospecifics *Puntius parrah* or *Anabas testudineus* larvae also did not cause any change in this behaviour. The present study suggests that group-size recognition in *Puntius sarana subnasutus* depended more on visual cues than the odour cues while species discrimination is more facilitated by odour cues. In the context of kin recognition, *Puntius sarana subnasutus* dependent more on visual cues for group-size discrimination while olfactory cues are necessary for sibling / conspecific / heterospecific discrimination.

Aquatic pollution and shoaling preference

Associating with a sibling shoal or with a larger group, being one of the most important anti-predator strategies, is a very essential behavioural pattern as far as the survival of the fish species is concerned. Factors such as turbidity that interferes with the visual range, variations in water temperature that affect the activity level of the fish shoal, toxic chemicals that disrupt the olfactory sensitivity of fishes may adversely affect this vital behaviour pattern. Therefore, it is necessary to control pollution of water with industrial effluents and toxic chemicals that interfere with the clarity and quality of water affecting the normal behavioural development of fish populations.

An understanding of shoaling dynamics can help to elucidate the evolutionary implications of group living. Furthermore, understanding shoaling behaviour is important in fisheries, as shoaling fish are among the most heavily exploited species in the world’s oceans. Commercial fisheries have exploited the susceptibilities of shoaling fish through various fishing techniques. In many cases and most often exploitation of fish shoals has not been sustainable and has resulted in stock collapses. An understanding of the structure and dynamics of fish shoals based on the mechanisms and constraints the fish shoal encounters, is fundamental in fisheries management, and may become the key for avoiding over-exploitation and facilitate conservation of fish stocks.

References

Abbas, H. and Authman, M.M.N. (2009) Effects of accumulated selenium on some physiological parameters and oxidative stress indicators in tilapia fish (*Oreochromis spp*). *Am Eurasian J Agric Environ Sci.,* 5(2): 219-225.

Abramson, G. and Kuperman, M. N. (2001) Social games in a social network, *Phys Rev*., E 63: 030901.

Adar, E., Lotem, A. and Barnea, A. (2008) The effect of social environment on singing behavior in the zebra finch (*Taeniopygia guttata*) and its implication for neuronal recruitment. *Behav Brain Res.,* 187: 178–184.

Adewoye, S. O. (2001) A comparative study on the behavioral responses of *Clariasgariepinus* on exposure to soap and detergent effluents. *Adv Appl Sci Res.,* 1(1): 89-95.

Aeschlimann, P.B., Häberli, M.A., Reusch, T.B.H., Boehm, T. and Milinski, M. (2003) Female sticklebacks Gasterosteus aculeatus use self-reference to optimize MHC allele number during mate selection. *Behav Ecol Sociobiol.,* 54:119–126.

Agrillo, C. and Dadda, M. (2007). Discrimination of the larger shoal in the poeciliid fish *Girardinus falcatus*. *Ethol Ecol Evol.*, 19(2): 145–157.

Agrillo, C., Dadda, M. and Bisazza, A. (2007) Quantity discrimination in female mosquitofish. *Anim Cog.,* 10: 36-70.

Agrillo, C., Dadda, M., Serena, G. and Bisazza, A. (2008) Do fish count? Spontaneous discrimination of quantity in female mosquitofish. *Anim Cogn.,* 11: 495–503.

Agrillo, C., Dadda, G., Serena, G. and Bisazza, A. (2009) Use of number by fish. *PLoS ONE* , 4: (3) e4786.

Albert, A.Y.K., Millar, N.P. and Schluter, D. (2007) Character displacement of male nuptial colour in threespine sticklebacks (*Gasterosteus aculeatus*). *Biol J Linnean Soc.,* 91: 37–4.

Alex, J. and Thomas, K. J. (2011) Factors influencing shoaling preferences in *Puntius sarana subnasutus, Curr Sci.*, 100: 633-634.

Alex, J and Thomas, K. J. (2012). The influence of turbidity on shoaling preferences of *Puntius sarana subnasutus*. *J Inland Fish Soc India.*, 43 (2): 79-81

Altizer, S., Nunn, C. L., Thrall, P. H., Gittleman, J. L., Antonovics, J., Cunningham, A. A., Dobson, A. P., Ezenwa, V., Jones, K. E., Pedersen, A. B., Poss, M. and Pulliam, J. R. C. (2003) Social organization and parasite risk in mammals: Integrating theory and empirical studies. *Annu Rev Ecol Evol Syst.,* 34: 517 - 547.

Apanius, V., Penn, D., Slev, P. R., Ruff, L. R. and Potts, W. K. (1997) The nature of selection on the major histocompatibility complex. *Crit Rev Immunol.*, 17: 179–224.

Arnold, K. E. (2000) Kin recognitioin rainbowfish (*Melanotaenia eachamensis*): sex, sibs and shoaling. *Behav Ecol Sociobiol.*, 48: 385-391.

Azevedo, P. A., Cho, C. Y., Leeson, S. and Bureau, D. P. (1998) Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). *Aquat Living Resour.,*11(4): 227-238.

Barber, I. and Wright, H.A. (2001) How strong are familiarity preferences in shoaling fish? *Anim Behav.,* 61: 975–979.

Barber, I., Downey, L. C., and Braithwaite, V. A. (1998). Parasitism, oddity and the mechanism of shoal choice. *J Fish Biol.*, 53: 1365-1368.

Barber, I., Hoare, D. and Krause, J. (2000) Effects of parasites on fish behavior: a review and evolutionary perspective. *Rev Fish Biol Fisher.*, 10: 131-165.

Bardach, J. E., Fujiya, M. and Holl, A. (1965) Detergents: effects on the chemical senses of the fish, Ictalurus natalis (le Sueur). *Science.*, 148: 1605–1607.

Barreto, R. E., Luchiari, A. C. and Marcondes, A. L. (2003) Ventilatory frequency indicates visual recognition of an allopatric predator in naïve Nile tilapia. *Behav Process.*, 60: 235-239.

Bash, J., Berman, C. and Bolton, S. (2001) Effects of turbidity and suspended solids on salmonids. *Center for Streamside Studies*, University of Washington, Seattle, WA.

Becker, C. D., Coutant, C. C. and Prentice, E. F. (1971) Experimental drifts of juvenile salmonids through effluent discharges at Hanford. *Part II. 1969 Drifts and Conclusions*. USAEC Rept. BNWL-1529, Battelle Northwest, Richland, Washington.

Beecher, M. D. (1982) Signature systems and kin recognition. *Am Zool.*, 22: 447–490.

Begon, M., Harper, J. L. and Townsend, C. R. (1996) *Ecology*, 3rd ed. / Blackwell Science.

Behrmann-Godel, J., Gerlach, G. and Eckmann, R. (2006) Kin and population recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): can assortative shoaling drive population divergence? *Behav Ecol Sociobiol*, 59: 461–468.

Belanger, S. E., Meiers, E. M. and Bausch, R. G. (1995) Direct and indirect ecotoxicological effects of alkyl sulfate and alkyl ethoxysulfate on macroinvertebrates in stream mesocosms. *Aquat Toxicol.*, 33: 65-87.

Berg, L. and Northcote, T. G. (1985) Changes in territorial, gill-flaring, and feeding-behaviour in juvenile coho salmon (*Oncorhynchus kisutch*) following short-term pulses of suspended sediment. *Can J Fish Aquat Sci.,* 42(8): 1410-1417.

Berger, J., Swenson, J. E. and Persson, I. L. (2001) Recolonizing carnivores and naive prey: conservation lessons from Pleistocene extinctions. *Science*, 291: 1036—1039.

Berger, R., Bergstrom, L., Graneli, E. and Kautsky, L. (2004) How does eutrophication affect different life stages of *Fucus vesiculosus* in the Baltic Sea? A conceptual model. *Hydrobiologia.,* 514(1-3): 243-248.

Bertram, B. C. R. (1978) Living in groups: predators and prey. In: *Behavioural Ecology: An Evolutionary Approach* (eds. Krebs, J.R., Davies, N.B.), Blackwell, London, pp. 64– 96.

Beschta, R. L., Bibly, R. E., Brown, G. W., Holtby, L. B. and Hofstra, T.D. (1987) Stream temperature and aquatic habitat. In: *Streamside Management: Forestry and Fishery Interactions* (eds. Salo, E. O., and T. W. Cundy) University of Washington, Institute of Forest Resources. Contribution No.57. pp: 191-232.

Beukema, J. J. (1968) Predation by the three-spined stickleback (*Gasterosteus aculeatus* L.): the influence of hunger and experience. *Behaviour,* 31: 1-126.

Bhat, A. and Magurran, A. E. (2006) Benefits of familiarity persist after prolonged isolation in guppies. *J Fish Biol.,* 68: 759-766.

Biju, C. R., Thomas, R. K. and Ajithkumar, C. R. (1999) Fishes of Parambikulam Wildlife Sanctuary, Palakkad District, Kerala. 96: 82-87.

Binoy, V. V. and Thomas, K. J. (2004) The climbing perch (*Anabas testudineus* Bloch), a freshwater fish, prefers larger unfamiliar shoals to smaller familiar shoals. *Curr Sci*., 86: 207-211.

Bisazza, A. (2010) Cognition. In: *Ecology and evolution of poeciliid fishes* (eds. Evans J, Pilastro A, Schlupp I), Chicago University Press, Chicago, pp. 165–73.

Blakeslee C., Mc Robert, S. P., Brown, A.C. and Clotfelter, E. D. (2009) The effect of body colouration and group-size on social partner preferences in female fighting fish (*Betta splendens*). *Behav process*., 80(2): 157-161.

Blumstein, D. T. (2002) Moving to suburbia: ontogenetic and evolutionary consequences of life on predator-free islands. *J Biogeogr*., 29: 685—692.

Bonga, W. S. E. (1997) The stress response in fish. *Physiol Rev.,* 77: 591-625.

Boyd, R. and Richerson, P. J. (1985) Culture and the evolutionaryprocess. Chicago, IL: University of Chicago Press.

Boyse, E. A., Beauchamp, G. K. and **Yamazaki, K.** (1987) *The genetics of body scent. Trends Genet.,* 3: 97–102.

Bradner, J. and McRobert, S. P. (2001) The effect of shoal size on patterns of body colour segregation in mollies. *J Fish Biol.,* 59: 960–967.

Bretaud, S., Toutant, J. P. and Saglio, P. (2000). Effects of carbofuran, diuron and nicosulfuron on acetylcholinesterase activity in goldfish (*Carassius auratus*). *Ecotox Environ Saf.*, 47: 117-124.

Britz, P. J., Hecht, T. and Mangold, S. (1997) Effect of temperature on growth, feed consumption and nutritional indices of *Haliotis midae* fed a formulated diet. *Aquaculture.*, 152: 191-203.

Brown, J. A. and Colgan, P. W. (1986). Individual and species recognition in centrachid fishes: evidence and hypotheses. *Behav Ecol Sociobiol.*, 19: 373-379.

Brown, G. E. and Brown, J. A. (1992) Do rainbow trout and Atlantic salmon discriminate kin? *Can J Zool.*, 70: 1636-1640.

Brown, G. E. and Brown, J. A. (1993a) Do kin always make better neighbours? The effects of territory quality. *Behav Ecol Sociobiol.,* 33: 225-231.

Brown, G. E. and Brown, J. A. (1993b) Social dynamics in salmonid fishes: do kin make better neighbours? *Anim Behav.,* 45: 863–871.

Brown, G. E. and Smith, R. J. F. (1994) Fathead minnows use chemical cues to discriminate natural shoalmates from unfamiliar conspecifics. *J Chem Ecol.,* 20: 3051-3061.

Brown, G. E. and Brown, J. A. (1996). Kin discrimination in salmonids. *Rev Fish Biol Fisher.*, 6: 201 – 219.

Brown, G. E., Brown, J. A. and Crosbie, A. M. (1993) Phenotype matching in juvenile rainbow trout. *Anim Behav.*, 46: 1223–1225.

Brown, C. and Laland, K. N. (2003) Social learning in fishes: a review. *Fish Fish.,* 4: 280-288.

Brunelli, E., Talarico, E., Corapi, B., Perrotta, I. and Tripepi, S. (2008) Effects of a sublethal concentration of sodium lauryl sulphate on the morphology and Na^+/K^+ ATPase activity in the gill of the ornate wrasse (*Thalassoma pavo*). *Ecotox Environ Saf.*, 71(2): 436-445.

Bshary, R., Wickler, W. and Fricke, H. (2002) Fish cognition: a primate's eye view. *Anim Cogn.,* 5: 1–13.

Buckingham, J. N., Wong, B. B. M. and Rosenthal, G. G. (2007) Shoaling decisions in female

swordtails: how do fish gauge group size? *Behaviour,* 144: 1333–1346.

Burne, R. H. (1909) The anatomy of the olfactory organ of teleostean fishes. *Proc Zool Soc Lond.,* 2: 610-663.

Buske, C. and Gerlai, R. (2011) Shoaling develops with age in Zebrafish (*Danio rerio*). *Prog Neuropsychopharmacol Biol Psychiatry.,* 35: 1409–15.

Butler, M. J., MacDiarmid, A. B. and Booth, J. D. (1999) The cause and consequence of ontogenetic changes in social aggregation in New Zealand spiny lobsters. *Mar Ecol Prog Ser.*, 188: 179–191.

Cacioppo, J. T. and Hawkley, L. C. (2009) Perceived social isolation and cognition. *Trends Cogn Sci.*, 13(10): 447-454.

Carlin, N. F., Halpern, R., Ho¨lldobler, B. and Schwartz, P. (1987) Early learning and the recognition of conspecific cocoons by Carpenter Ants (Camponotus spp). *Ethology*, 75: 306–316.

Cavagna, A., Cimarelli, A., Giardina,I., Orlandi, A., Parisi, G., Procaccini, A., Santagati, R. and Stefanini, F. (2008) New statistical tools for analyzing the structure of animal groups. *Math Biosci.*, 214: 32-37.

Chapman, B. B., Morrell, L. J., Benton, T. G. and Krause, J. (2008). Early interactions with adults mediate the development of predator defences in guppies. *Behav Ecol.*, 19: 87–93.

Chambers, R. C., Witting, D. A. and Lewis, S. J. (2001) Detecting critical periods in larval flatfish populations. *J Sea Res.*, 45: 231-242.

Charlesworth, D., and Charlesworth, B. (1987) Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Evol Syst.,* 18: 237–268.

Chivers, D. P., Brown, G. E. and Smith, R. J. F. (1995) Familiarity and shoal composition in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Can J Zool.,* 73: 955-960.

Clesceri, L.S., Greenberg, A. E. and Eaton, A. D. (1998) Measurement of Turbidity. In: *Standard methods for the examination of water and wastewater* (eds. Clesceri, L.S, Greenberg, A. E. and Eaton, A. D.), United Book Press, Inc., Baltimore, Maryland, pp 2-10.

Coleman, W. S. and Rosenthal, G. G. (2006) Swordtail fry attend to chemical and visual cues in detecting predators and conspecifics. *PLoS ONE.*, 1:e118.

Collin, S. P., Marshall, N. J. and Collin, S. P. (2003). Sensory Processing in Aquatic Environments, New York: Springer, pp 446.

Coolen, I., van Bergen, Y., Day, R. L. and Laland, K. N. (2003) Heterospecific use of public information by fish in a foraging context. *Proc R Soc., B.*, 270: 2413–2419.

Courtenay, S. C., Quinn, T. P., Dupuis, H. M. C., Groot, C. and Larkin, P. A. (1997) Factors affecting the recognition of population specific odours by juvenile coho salmon. *J Fish Biol.*, 50: 1042–1060.

Courtenay, S. C., Quinn, T. P., Dupuis, H. M. C., Groot, C. and Larkin, P. A. (2001) Discrimination of family specific odours by juvenile coho salmon: roles of learning and odour concentration. *J Fish Biol.*, 58: 107-125.

Coutant, C.C. (1972) Biological aspects of thermal pollution. II. Scientific basis for water temperature standards at power plants. *CRC Crit Rev in Envir Control.*, 3: 1-24.

Crnokrak, P. and Roff, D. A. (1999) Inbreeding depression in the wild. *Heredity,* 83: 260–270.

Croft, D. P., Arrowsmith, B. J., Bielby, J., Skinner, K., White, E., Couzin, I. D., Magurran, A. E., Ramnarine, I. and Krause, J. (2003) Mechanisms underlying shoal composition in the Trinidadian guppy (*Poecilia reticulata*). *Oikos*, 100: 429–438.

Croft, D. P., James, R., Thomas, P., Hathaway, C., Mawdsley, D., Laland, K. N. and Krause, J. (2006) Social structure and co-operative interactions in a wild population of guppies (Poecilia reticulata). *Behav Ecol Sociobiol.,* 59: 644–650.

Cushing, D. H. (1990) Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Adv Mar Biol,* 26: 249-293.

D'Ettorre, P. and Heinze, J. (2005) Individual recognition in ant queens. *Curr Biol.*, 15: 2170–2174.

Dahanukar, N., Raut, R. and Bhat, A. (2004) Distribution, endemism and threat status of freshwater fishes in the Western Ghats of India, *J Biogeogr.,* 31: 123-136.

Dahanukar, N. (2011) *Puntius sarana ssp. subnasutus*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org>.

Dale, J., Lank, D. B. and Reeve, H. K. (2001) Signaling individual identity versus quality: a model and case studies with ruffs, queleas, and house finches. *Am Nat.*, 158: 75–86.

Davidson, D. L. and Morris, D. W. (2001) Density-dependent foraging effort of deer mice (*Peromyscus maniculatus*). / Funct. Ecol. 15: 575/583.

Davis, H. and Perusse, R. (1988). Numerical competence in animals: Definitional issues, current evidence, and a new research agenda. *Behav Brain Sci.*, 11: 561-615.

Davis, R. E. and Kassel, J. (1975) The ontogeny of agonistic behavior and the onset of sexual maturation in the paradise fish, *Macropodus opereularis* (Linnaeus). *Behav Biol.,* 14: 31-39.

Dawkins, R. (1982) The extended phenotype. San Francisco, CA:W. H. Freeman

De Robertis, A., Ryer, C. H., Veloza, A. and Brodeur, R. D. (2003) Differential effects of turbidity on prey consumption of piscivorous and planktivorous fish. *Can J Fish Aquat Sci.,* 60(2): 1517-1526.

De, A. K. (1996) Environmental Chemistry, IIIrd edition, Newage International Publishers, New Delhi; 167-282.

Demski, L. S. and Beaver, J. A. (2001). Brain and cognitive function in teleost fishes. In: *Brain evolution and cognition.* (eds. Roth, G. and M. F. Wulliman,), John Wiley and Sons, Inc., New York, pp. 297-332.

Despland, E. and Hamzeh S. (2004) Ontogenetic changes in social behaviour in the forest tent caterpillar, *Malacosoma disstria*. *Behav Ecol Sociobiol.*, 56: 177–184.

Douglas, R. H. and Hawryshyn, C. W. (1990). Behavioral studies of ûsh vision: an analysis of visual capabilities. In The Visual System of Fish (ed. R. H. Douglas and M. B. A. Djamgoz), London: Chapman and HallDouglas & Hawryshyn, pp. 373–418.

Dugatkin, L. A. (1992) Sexual selection and imitation: females copy the mate choice of others. *Am Nat.*, 139: 1384–1389.

Dugatkin, L. A. and Wilson, D. S. (1992) The prerequisites for strategic behaviour in bluegill sunfish, *Lepomis macrochirus*. *Anim Behav.*, 44: 223-230.

Dunbar, R. I. M. (1995) Neocortex size as a constraint on group-size in primates. *J Hum Evol.*, 20: 469-493.

Ehrlich, P. R. and Ehrlich, A. H. (1973) Co evolution: heterospecific shoaling in Caribbean reef fishes. *Amer Na.*, 107: 701-711.

Engeszer, R. E., Ryan, M. J. and Parichy, D. M. (2004). Learned social preferences in zebra fish. *Curr Biol.,* 14: 881-884.

Engeszer, R. E., Da Barbiano, L. A., Ryan, M. J. and Parichy, D. M. (2007a) Timing and plasticity of shoaling behavior in zebra fish, *Danio rerio*. *Anim Behav.,* 74: 1269-1275.

Engeszer, R. E., Patterson, L. B., Rao, A. A. and Parichy, D. M. (2007b) Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish*, 4: 21 - 40.

Engeszer, R. E., Wang, G., Ryan, M. J., and Parichy, D. M. (2008) Sex-specific perceptual spaces for a vertebrate basal social aggregative behavior. *Proc Natl Acad Sci.*, 105: 929-933.

Eschmeyer, W. N. and Fong, J. D. (2014) Catalog of fishes – Icthyology, Institute for biodiversity science and sustainability, California Academy of Sciences (http://research. calacademy.org/research/ichthyology/catalog/fishcat.asp)

Evans, J. P. and Kelley, J. L. (2008) Implications of multiple mating for offspring relatedness and shoaling behaviour in juvenile guppies. *Biol Lett.*, 4(6): 623–626.

Feigenson, L., Carey, S. and Spelke, E. (2002) Infants' discrimination of number vs continuous extent. *Cogn Psychol.,* 44: 33-66.

Feigenson, L., Dehaene, S. and Spelke, E. (2004) Core systems of number. *Trends Cogn Sci.,* 8: 307-314.

Feneron, R. and Jaisson, P. (1995) Ontogeny of nestmate brood recognition in a primitive ant, Ectatomma tubercalutum Olivier (Ponerinae). *Anim Behav.*, 50: 9–14.

Ferguson, M. M. and Noakes, D. L. G. (1981). Social grouping and genetic variation in common shiner, *Notropis cornutus* (Cyprinidae). *Environ Biol fish.*, 6: 357-360

Finger T.E. (1988) Organization of chemosensory systems within the brains of bony fishes. In: *Sensory Biology ofAquatic Animals* (eds, Atema, J. et al.), Springer, pp. 339-363.

Fitzgerald, G. J. and Morrissette, J. (1992) Kin recognition and choice of shoal mates by threespine sticklebacks. *Ethol Ecol Evol.,* 4: 273-283.

Fleming, I. A. and Einum, S. (1997) Experimental tests of genetic divergence of farmed from wild Atlantic salmon due to domestication. *ICES J Mar Sci.,* 54: 1051-1063.

Food and Agriculture Organisation of the United Nations (2008) Climate change, energy and food. High-level conference on food security: the challenges of climatic change and bioenergy, Rome, 3-5 June.

Forsgren, E., Kvarnemo, C. and Lindstrom, K. (1996) Mode of sexual selection determined by resource abundance in two sand goby populations. *Evolution*, 50: 646–654.

Fowler, E.V., Peters, J. M., Gatton, M. L., Chen, N. and Cheng, Q. (2002) Genetic diversity of the DBLalpha region in Plasmodium falciparum var genes among Asia-Paciûc isolates. *Mol Biochem Parasitol.,* 120: 117–126.

Freon, P. and Misund, O. (1999) Dynamics of pelagic fish distribution and behaviour: Effects on fisheries and stock assessment. Blackwell publishing Ltd, Oxford.

Frommen, J. G. and Bakker, T. C. M. (2006) Inbreeding avoidance through non random mating in sticklebacks. *Biol Lett.,* 2: 232–235

Frommen, J. G., Luz, C. and Bakker, T. C. M. (2007a) Kin discrimination in sticklebacks is mediated by social learning rather than innate recognition. *Ethology*., 113: 276–282.

Frommen, J. G., Mehlis, M., Brendler, C. and Bakker, T. C. M. (2007b) Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*) familiarity, kinship and inbreeding, *Behav Ecol Sociobiol.,* 61: 533

Frommen, J. G., Hiermes, M. and Bakker, T. C. M. (2009) Disentangling the effects of group size and density on shoaling decisions of three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav Ecol Sociobiol.* 63: 1141-1148.

Gadowaski, D. M. and Caddell, S. M. (1991) Effects of temperature on early-life-history stages of California halibut *Paralichthys californicus*. *Fish Bull*., 89: 567-576.

Gallagher, J. E., Herz, M. J., and Peeke, H. V. S. (1972). Habituation of aggression: The effects of visual social stimuli on behavior between adjacently territorial convict cichlids. *Behav Biol.,* 7: 359-368.

Gallego, A. and Heath, M. R. (1994) The development of schooling behaviour in Atlantic herring *Clupea harengus*. *J Fish Biol.,* 45: 569-588.

Gaskin, T., Futerman, P. and Chapman, T. (2002) Increased density and male-male interactions reduce male longevity in the medûy, *Ceratitis capitata*. *Anim Behav*., 63: 121- 129.

Gerlach G. and Lysiak N. (2006) Kin recognition and inbreeding avoidance in zebrafish , *Danio rerio*, is based on phenotype matching. *Anim Behav.*, 71(6): 1371 – 1377

Gerlach G., Schardt U., Eckmann R., and Meyer A. (2001) Kin structured subpopulations of Eurasian perch (*Perca fluviatilis*) *Heredity,* 86: 213-221

Gerlach, G., Hodgins-Davis, A., Mac Donald, B. and Hannah, R. C. (2007). Benefits of kin association: related and familiar zebra fish larvae (*Danio rerio*) show improved growth. *Behav Ecol Sociobiol*, 61: 1765-1770.

Gerlach, G., Hodgins-Davis, A., Avolio, C. and Schunter, C. (2008) Kin recognition in zebraûsh: a 24-hour window for olfactory imprinting. *Proc Rl Soc.,* 275: 2165-2170.

Giles, N. and Huntingford, F. A. (1984) Predation risk and interpopulation variation in anti-predator behaviour in the three spined sitickleback. *Anim Behav*., 32: 264-275.

Giraldeau, L. A. (1998) The ecology of information use. In: *Behavioural Ecology: an Evolutionary Approach* (eds J.R. Krebs and N.B. Davies). Blackwell Science, Oxford, pp.42-68.

Glasser, F. and Oswald, M. (2001) High stocking densities reduce Oreochromis niloticus yield: model building to aid the optimisation of production. *Aquat Living Resour.,* 14: 319-326.

Go´mez-Laplaza, L. M. and Gerlai, R. (2011) Spontaneous discrimination of small quantities: Shoaling preferences in angelfish (*Pterophyllum scalare*). *Anim Cogn*., 14: 565–574.

Go´mez-Laplaza, L. M. and Gerlai, R. (2013) Quantification abilities in angelfish (*Pterophyllum scalare*): The influence of continuous variables. *Anim Cogn.,* 16: 373–383.

Godin, J. G. J., Classon, L. J. and Abrams, M. V. (1988). Group vigilance and shoal size in a small characin fish. *Behaviour.,* 104: 29–40.

Goldsborough, W. J. and Kemp, W. M. (1988) Light Responses of a Submersed Macrophyte - Implications for Survival in Turbid Tidal Waters. *Ecology.,* 69(6): 1775-1786.

Goldschmidt, T., Bakker, T. M. C. and Feuth-De Bruijn, E. (1993) Selective copying in mate choice of female sticklebacks. *Anim Behav*., 45: 541–547.

Gonda, A., Herczeg, G. and Merilä, J. (2009) Habitat-dependent and –independent plastic responses to social environment in the ninespine stickleback (*Pungitius pungitius*) brain. *P Roy Soc Lond B*., 276: 2085–2092.

Gramapurohit, N. P., Veeranagoudar, D. K., Mulkeegoudra, S.V., Shanbhag, B. A. and Saidapur, S. K. (2006) Kin recognition in Bufo scaber tadpoles: ontogenetic changes and mechanism. *J Ethol.,* 24: 267–274.

Grandel, H., Kaslin, J., Ganz, J., Wenzel, I. and Brand, M. (2006) Neural stem cells and neurogenesis in the adult zebrafish brain: Origin, proliferation dynamics, migration and cell fate. *Dev Biol.*, 295(1): 263-277.

Grant, A. (2010) Detecting ecological effects of pollutants in the aquatic environment In: *Ecology of Industrial Pollution*, chapter 7, Cambridge University Press, pp 147-161.

Grant, J. W. A. and Green, L. D. (1996) Mate copying versus preference for actively courting males by female Japanese medaka (*Oryzias latipes*). *Behav Ecol.*, 7: 165–167.

Griffiths, S. W. (1997). Preferences for familiar fish do not vary with predation risk in the European minnow. *J Fish Biol.*, 51: 489-495.

Griffiths, S. W. (2003) Learned recognition of conspecifics by fishes. *Fish Fish.*, 4: 256–268.

Griffiths, S. W. and Magurran, A. E. (1997a) Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proc R Soc Lond B Biol Sci.,* 264:547–551.

Griffiths, S. W. and Magurran, A. E. (1997b) Familiarity in schooling fish: how long does it take to acquire? *Anim Behav.*, 53: 945–949.

Griffiths, S. W. and Magurran, A. E. (1998). Sex and schooling behaviour in the Trinidadian guppy. *Anim Behav.*, 56: 689-693.

Griffiths, S. W. and Magurran, A. E. (1999) Schooling decisions in guppies (*Poecilia reticulata*) are based on familiarity rather than kin recognition by phenotype matching. *Behav Ecol Sociobiol.,* 45: 437–443.

Grifûths, S.W., Brockmark, S., Ho¨jesjo¨, J. and Johnsson, J. I. (2004) Coping with divided attention: the advantage of familiarity. *Proc R Soc Lond B.,* 271: 695–699.

Gruendel, A. D. and Arnold, W. J. (1969). Effects of early social deprivation on reproductive behavior of male rats. *J Comp Physiol Psych.,* 67: 244-272.

Guthrie, D. M. and Muntz, W. R. A. (1993). Role of vision in fish behavior. In: *Behavior of Teleost Fishes*, 2nd edn (ed T. P. Pitcher), London: Chapman and Hall pp. 89-121.

Hager, M. C. and Helfman, G. S. (1991) Safety in numbers—shoal size choice by minnows under predatory threat. *Behav Ecol Sociobiol.,* 29: 271–276.

Hain, T. J. A. and Neff, B. D. (2006) Promiscuity drives selfreferent kin recognition. *Curr Biol*. 16: 1807–1811.

Hain, T. J. A. and Neff, B. D. (2007) Multiple paternity and kin recognition mechanisms in a guppy population. *Mol Ecol.*, 16: 3938-3946

Hamilton, W. D. (1964) The genetical evolution of social behavior I and II. *J Theor Biol.,* 7: 1-52.

Hansen, M. M., Mensberg, K. L. D., Rasmussen, G. and Simonsen, V. (1997) Genetic variation within and among Danish brown trout (*Salmo trutta* L.) hatchery strains, assessed by PCR RFLP analysis of mitochondrial DNA segments. *Aquaculture.,* 153: 15-29.

Hara, T. J. (1993) Role of olfaction in fish behavior. In: *Behavior of Teleost Fishes*, 2nd edn (ed T. P. Pitcher), London: Chapman and Hall, London. pp 152–176

Harcourt, J. L., Biau, S., Johnstone, R. and Manica, A. (2010) Boldness and information use in three-spined sticklebacks. *Ethology*., 116: 440–447

Hatchwell, B. J., Ross, D. J., Fowlie, M. K., McGowan A. (2001). Kin discrimination in cooperatively breeding long-tailed tits. *Proc R Soc Lond Biol Sci.,* 268: 885–890.

Hauser, L. and Ward, R. D. (1998) Population identification in pelagic fish: the limits of molecular markers. In: *Carvalho G.R., editor. Advances in Molecular Ecology*, NATO Science Series, Series A: Life Sciences. Vol. 306. Amsterdam: IOS Press; pp. 191-224.

Hauser, L., Carvalho, G. R. and Pitcher, T. J (1998). Genetic population structure in the Lake Tanganyika sardine, *Limnothrissa miodon*. *J Fish Biol Supplement A.,* 53: 413-429.

Hawkins, L. A., Amstrong, J. D. and Magurran, A. E. (2004) Predator-induced hyper ventilation in wild and hatchery Atlantic salmon fry. *J Fish Biol.,* 65: 88-100.

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C. and Vonk, A. (2002). Feminization of male frogs in the wild. *Nature,* 419: 895-896.

Heiligenberg, W. F. and Kramer, U. (1972) Aggressiveness as a function of external stimulation. *J Comp Physiol.,* 77: 332-340.

Helfman, G. S. and Schultz, E. T. (1984) Social transmission of behavioural traditions in a coral reef fish. *Anim Behav.,* 32 (2): 379-384.

Hemmings, C. C. (1966) Olfaction and vision fish schooling. *J Exp Biol.,* 45: 449-464.

Hensor, E., Couzin, I. D., James, R and Krause, J. (2005) Modelling density-dependent fish shoal distributions in the laboratory and field. *Oikos*, 110: 344- 352.

Herskin, J. and Steffensen, J. F. (1998). Energy savings in sea bass swimming in a school: measurements of tail beat frequency and oxygen consumption at different swimming speeds. *J Fish Biol.*, 53: 366–376.

Hesse, S., Bakker, T. C. M., Baldauf, S. A. and Thunken, T. (2012) Kin recognition by phenotype matching is family- rather than self-referential in juvenile cichlid ûsh. *Anim Behav*., 84: 451-457.

Heth, G., Todrank, J. and Johnston, R. E. (1998) Kin recognition in golden hamsters: evidence for phenotype matching. *Anim Behav*., 56:409–417.

Hoare, D. J. and Krause, J. (2003) Social organisation, shoal structure and information transfer. *Fish Fish.*, 4(3): 269-279.

Hoare, D.J., Krause, J., Peuhkuri, N. and Godin, J. G. J. (2000a) Body size and shoaling in fish (Review). *J Fish Biol*., 57: 1351-1366.

Hoare, D. J., Ruxton, G. D., Godin, J. G. J. and Krause, J. (2000b) The social organization of free- ranging fish shoals. *Oikos,* 89: 546-554.

Hoare, D. J., Couzin, I. D., Godin, J. G. J., and Krause, J., (2004). Context Dependent group size choice in fish. *Anim Behav.,* 67: 155-164.

Ho¨jesjo¨, J., Johnsson, J. L., Petersson, E. and Ja¨rvi, T. (1998). The importance of being familiar: individual recognition and social behavior in sea trout (*Salmo trutta*). *Behav Ecol.*, 9: 445–451.

Holmes, W. G. and Sherman, P. W. (1982) The ontogeny of kin recognition in two species of ground squirrels. *Am Zool.*, 22: 491-517.

Hoppitt, W. J. E. and Laland, K. N. (2008) Social processes inûuencing learning in animals: a review of the evidence. *Adv Stud Behav.,* 38: 105–165.

Houston, A. I. and McNamara, J. M. (1999) Models of adaptive behaviour. / Cambridge Univ. Press

Hunter, J. R. and Coyne, K. M. (1982) The onset of schooling in northern anchovy larvae, *Engraulis mordax*. *CalCOFI Rep.*, 23: 246-251.

Immelmann, K. (1972) Sexual and other longterm aspects of imprinting in birds and other species. *Adv Study Behav.*, 4: 147–174.

Insel, T. R., and Fernald, R. D., (2004). How the brain processes social information: searching for the social brain. *Annu Rev Neurosci.,* 27: 697-722.

Isingrini M, Lenoir A and Jaisson P. (1985) Preimaginal learning as a basis of colony-brood recognition in the ant *Cataglyphis cursor*. *Proc Natl Acad Sci USA.*, 82: 8545–8547.

Jaffe, K. and Chacon, G. (1995) Nonlinear trends in the evolution of the complexity of nervous systems, group size and communication systems: a general feature in biology. *Behav Brain Sci.*, 18: 386.

Jauncey, K. and Ross, **B**. (1982) The effects of varying dietary protein levels on the growth, feed conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, 27: 43-54.

Jordan, L. A., Avolio, C., Herbert-Reed, J. E., Krause J, Rubenstein, D. I. and Ward, A. J. W. (2010) Group structure in a restricted entry system is mediated by both resident and joiner preferences. *Behav Ecol Socio Biol.*, 64:1099-1106

Kausar, R and Salim, M. (2006) Effect of water temperature on the growth performance and feed conversion ratio of *Labeo rohita, Pakistan Vet. J.,* 26(3): 105-108.

Keenleyside, M.H.A. (1955). Some aspects of the schooling behaviour of fish. *Behaviour*, 8: 183–248

Kelly, J., Chan, S., Cummins, G., Phillips, B. and Shand, J. (2010) Effects of changes in water quality on colour pattern expression and shoaling behaviour in rainbow fish. The university of western Australia, Crawley, Australia.

Kemp, D. J., Reznick, D. N. and Grether, G. F. (2008) Ornamental evolution in Trinidadian guppies (*Poecilia reticulata*): insights from sensory processing-based analyses of entire colour patterns. *Biol J Linnean Soc.,* 95: 734–747.

Kent, A, Hawkins, S. J. and Doncaster, C. P. (2003) Population consequences of mutual attraction between settling and adult barnacles. *J Anim Ecol*., 72: 941-952.

Klaprat, D. A., Evans, R. E. and Hara, T. J. (1992) Environmental contaminants and chemoreception in fishes. In: *Fish chemoreception*. Fish and fisheries series 6. Hara TJ, editor. Chapman & Hall, London, pp 321.

Kleerekoper, H. (1969) Olfaction in Fishes. Bloomington: Indiana University Press. 222pp

Konar, S. K. (1969) Two organophosphorus insecticides DDVP and phosphamidon as selective toxiciants. *T Am Fish Soc*., 98: 430.

Kotrschal, A., Rogell, B., Maklakov, A.A., and Kolm, N. (2012). Sexspecific plasticity in brain morphology depends on social environment in the guppy, *Poecilia reticulata*. *Behav Ecol Sociobiol.,* 66: 1485–1492.

Kozak, G. M. and Boughman, J. W. (2008) Experience influences shoal member preference in a species pair of sticklebacks. *Behav Ecol*., 19 (3): 667-676

Krause, J. (1993) The inûuence of hunger on shoal size choice by three-spined sticklebacks, *Gasterosteus aculeatus*. *J Fish Biol*., 43: 775–780.

Krause, J. and Godin, J. G. J. (1994) Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae)—effects of predation risk, fish size, species composition and size of shoals. *Ethology*, 98: 128–136.

Krause, J., Godin, J. G. J. (1996) Influence of prey foraging posture on flight behavior and predation risk: predators take advantage of unwary prey. *Behav Ecol.*, 7: 264-271.

Krause, J. and Ruxton, G. D. (2002) Living in groups. Oxford series in ecology and evolution, (eds. May, R. M. and Harvey, P. H.). Oxford Univ. Press, Oxford.

Krause, J., Godin, J. G. J. and Brown, D. (1996a) Phenotypic variability within and between fish shoals. *Ecol.,* 77: 1586-1591.

Krause, J., Godin, J.G. J. and Brown, D. (1996b) Size-assortativeness in multi-species fish shoals. *J Fish Biol.*, 49: 221-225.

Krause, J., Godin, J. J. and Rubenstein, D. (1998). Group choice a function of group size differences and assessment time in fish: the influence of species vulnerability to predation. *Ethology*, 104: 68-74.

Krause, J., Hartmann, N. and Pritchard, V. L. (1999) The influence of nutritional state on shoal choice in zebra fish, *Danio rerio*. *Anim Behav.,* 57: 771-775.

Krause, J., Butlin, R. K., Peuhkuri, N. and Pritchard, V. L. (2000) The social organization of fish shoals: A test of the predictive power of laboratory experiments for the field. *Biol. Rev. Camb. Philos. Soc.*, 75: 477–501.

Kröger, R. H. and Katzir, G. (2008). Comparative anatomy and physiology of vision in aquatic tetrapods. In: *Sensory Evolution on the Threshold: Adaptations in Secondarily Aquatic Vertebrates* (ed. J. G. M. Thewissen and S. Nummela), Berkeley, CA: University of California Press. pp.121 -147.

Kruijt, J. P., Ten Cate, C. J. and Meeuwissen, G. B. (1983) The influence of siblings on the development of sexual preferences of male zebra finches. *Dev Psychobiol.,* 16: 233-239.

Kuo, Z. Y. (1960). Studies on the basic factors in animal fighting: V. Inter-species coexistence in fish. *J Genet Psych.,* 97: 181-194

Kuroyanagi, S. Tashiro, H. and Sugiyama, N. (2010) A numerical study of primordial magnetic field amplification by inflation-produced gravitational waves**. *Phys Rev.*, D81. 023510.**

Lachlan, R. F., Crook, S. L. and Laland, K. N. (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Anim Behav.*, 56(1): 181-190

Laland, K. N. (2004) Social learning strategies. *Learn Behav*., 32: 4–14

Laland, K.N. and Williams, K. (1997) Shoaling generates social learning of foraging information in guppies. *Anim Behav*., 53: 1161-1169

Landeau, L. and Terborgh, J. (1986) Oddity and the 'Confusion effect' in predation. *Anim Behav.,* 34: 1372-1380

Larkin, P.A. and Walton, A. (1969) Fish school size and migration. *J Fish Res Board Can.,* 26: 1372-1374.

Larsson, M. (2012) Why do fish school? *Curr Zool.,* 58(1): 116 - 128.

Le Vin, A. L., Mable, B. K. and Arnold, K. E. (2010) Kin recognition via phenotype matching in a cooperatively breeding cichlid, Neolamprologus pulcher. *Anim Behav*., 79: 1109-1114

Lorenzi, M. C., Cometto, I. and Marchisio, G. (1999) Species and colony components in the recognition odor of young social wasps: their expression and learning (*Polistes biglumis* and *P. atrimandibularis*; Hymenoptera: Vespidae). *J Insect Behav*., 12: 147–158.

Maaswinkel, H. and Li, L. (2003) Olfactory input increases visual sensitivity in zebraûsh: a possible function for the terminal nerve and dopaminergic inter plexiform cells. *J Exp Biol.,* 206: 2201-2209.

Mackinson, S. (2000) An adaptive fuzzy expert system for predicting structure, dynamics and distribution of herring shoals. *Ecol Model*., 126: 155-178.

Mackinson, S., Sumaila, U. A. and Pitcher,T. J. (1997) Bioeconomics and catchability: fish and fishers behaviour during stock collapse. *Fish Res.,* 31: 11-17.

Madej, M. A., Wilzbach, M., Cummins, K., Ellis, C. and Hadden, S. (2004) The Significance of Suspended Organic Sediments to Turbidity, Sediment Flux, and Fish-Feeding Behavior. Paper presented at the *Redwood Region Forest Science Symposium*, 15-17 March, Rohnert Park, CA.

Magurran, A. E. (1986) Predator inspection behaviour in minnow shoals: Differences between populations and individuals. *Behav Ecol Sociobiol*., 19: 267-273.

Magurran, A. E. (1989). The inheritance and development of minnow anti-predator behavior. *Anim Behav.*, 39: 834-842.

Magurran, A. E. and Pitcher, T. J. (1987) Provenance, shoal size and the sociobiology of predator-evasion behaviour in minnow shoals. *Proc R Soc Lond B*., 229: 439-465.

Magurran, A. E. and Seghers, B. H. (1990). Population differences in predator recognition and attack cone avoidance in the guppy. *Anim Behav.,* 40: 443-452.

Magurran, A. E., Seghers, B. H., Shaw, P. W. and Carvalho, G. R. (1994) Schooling preferences for familiar ûsh in the guppy, *Poecilia reticulata*. *J Fish Biol*., 45: 401–406.

Makris, N. C., Ratilal, P., Jagannathan, S., Gong, Z., Andrews, M., Bertsatos, I., Godø, O. R., Nero, R. W. et al. (2009). ”Critical Population Density Triggers Rapid Formation of Vast Oceanic Fish Shoals”. *Science,* 323 (5922): 1734–1737

Maltby, L., Paetzold, A. and Warren, P. H. (2010) Sustaining industrial activity and ecological quality: the potential role of an ecosystem services approach. *Ecology of Industrial Pollution*, chapter 16, Cambridge University Press.

Marshall, T. C., Slate, J., Kruuk, L. E. B. and Pemberton, J. M. (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Mol Ecol.*, 7: 639-655

Martínez-porchas, M., Martínez-córdova, L.R. and Ramos-enriquez, R. (2009) Cortisol and Glucose: Reliable indicators of fish stress? *PanamJAS.*, 4(2): 158-178

Masuda, R. and Tsukamoto, K. (1999) School formation and concurrent developmental changes in carangid fish with reference to dietary conditions. *Environ Biol Fish.,* 56: 243-252

Mateo. J. M. (2004) Recognition systems and biological organization: the perception component of social recognition. *Ann Zool Fenn.*, 41: 729–745.

Mateo, J. M. (2006). The nature and representation of individual recognition cues in Belding's ground squirrels. *Anim Behav.*, 71, 141–154.

Mathis, A. and Chivers, D. P. (2003). Overriding the oddity effect in mixed-species aggregations: group choice by armored and nonarmored prey. *Behav Ecol.*, 14: 334-339.

Mazzi, D., Künzler, R., Largiadèr, C. R. and Bakker T. C. M. (2004) Inbreeding affects female preference for symmetry incomputer-animated sticklebacks. *Behav Genet.,* 34: 417–424.

Mc Robert, S. P. and Bradner, J. (1998) The influence of body coloration on shoaling preferences in fish. *Anim Behav.*, 56: 611-615.

McCann, L. I. and Carlson C., C. (1982) Effect of cross-rearing on species identification in zebra fish and pearl danios. *Dev Psychobiol.* 15: 71–74.

Meador, J.P. (1993) Toxic Metals in Pilot Whales (*Globicephala melaena*) from Strandings in 1986 and 1990 on Cape Cod, Massachusetts. *Can J Fish Aquat Sci.,* 50 (12): 2698-2706

Meager, J. J., Domenici, P., Shingles, A., and Utne-Palm, A. C. (2006) Escape responses of juvenile Atlantic Cod (*Gadus morhua L.*): the effects of turbidity and predator speed. *J Exp Biol.*, 209: 4174-4184

Menon, A.G.K. (1963) Sub-species of the cyprinid fish *Puntius sarana* (Ham) with the description of P. *s. spilurus* (Gunther) from Ceylon. *Spolia Zeylanica* 30(1): 65-70.

Menon, A.G.K. (1999) *Check list - fresh water fishes of India.* Records of the Zoological Survey of India, Occasional Paper No.175

Metcalfe, N.B. and Thomson, B.C., (1995) Fish recognize and prefer to shoal with poor competitors. *Proc R Soc Lond B.,* 259: 207–210.

Meutter, Van de F., De Meester, L. and Stoks, R. (2005) Water turbidity affects predator-prey interactions in a fish-damselfly system. *Oecol*., 144: 327-336.

Miller, M. and Gerlai, R. (2012) From Schooling to Shoaling: Patterns of Collective Motion in Zebrafish (*Danio rerio*). *PLoS ONE*., e48865 7 (11): 1-6

Mirza, R. S. and Chivers, D. P. (2001) Chemical alarm signals enhance survival of brook charr (*Salvelinus fontinalis*) during encounters with predatory chain pickerel (*Esoxiger*). *Ethology,* 107: 989–1005.

Morrell, J. L., Hunt, L. K., Croft, P. D. and Krause, J. (2007) Diet, familiarity, and shoaling decisions in guppies. *Anim Behav*., 74: 311-319.

Morvan, C. L., Troutaud, D and Deschaux, P. (1998) Differential effects of temperature on specific and nonspecific immune defences in fish. *J Exp Biol*., 201: 165–168

Naish, K. A., Carvalho, G. R. and Pitcher, T. J. (1993) The genetic structure and micro distribution of shoals of *Phoxinus phoxinus*, the European minnow. *J Fish Biol Supplement A*., 43: 75-89

Neff, B. D. and Sherman, P. W. (2003). Nestling recognition via direct cues by parental male bluegill sunûsh (*Lepomis macrochirus*). *Anim Cogn*., 6: 87–92.

Neuheimer, A. B., Thresher, R. E., Lyle, J. M. and Semmens, J. M. (2011) Tolerance limit for fish growth exceeded by warming waters. *Nature Climate Change*, 1: 110-113.

Nøttestad, L. and Axelsen, B. E. (1999). Herring schooling manoeuvres in response to killer whale attacks”. *Can J Zool.,* 77 (10): 1540–1546

Nowbahari, E. (2007) Learning of colonial odor in the ant *Cataglyphis niger* (Hymenoptera: Formicidae). *Learn Behav*., 35: 87–94.

Obukhov, D. K. (1996). Developments of the CNS of Sturgeon fishes grown under different ecological condition. *Proceedings* ofthe *International Congress* on the *Biology of Fishes*. San Francisco state University, Physiology Section, American Fisheries Society pp 149-155

Olsen, K. H. (1989) Sibling recognition in juvenile Arctic charr, *Salvelinus alpines*. *J Fish Biol.,* 34: 571-581.

Olsen, K. H. (1992) Kin recognitionin fish mediated by chemical cues. In: *Fish chemo reception* (Hara, T. J. ed). Chapman and Hall, London, pp 229-248.

Olsen, K. H. and Hoglund, L. B. (1985) Reduction by a surfactant of olfactory mediated attraction between juveniles of Arctic charr (*Salvelinus alpinus* L.). *Aquat Toxicol*., 6: 57–69.

Olsen, K. H. and Winberg, S. (1996) Learning and sibling odour preference in juvenile arctic charr, *Salvelinus aplinus* (L.), *J Chem Ecol,* 22: 773.

Olsen, K.H., Javri, T. and Lof, A. C. (1996) Aggressiveness and kinship in browntrout (*Salmo trutta*) *Parr Behav Ecol.,* **7** (4): 445-450.

Olse´n, K. H., Grahn, M., Lohm, J. and Langefors, A. (1998). MHC and kin discrimination in juvenile Arctic charr, *Salvelinus alpinus* (L.). *Anim Behav.*, 56(2): 319–327.

Olsen, K. H., Grahn, M. and Lohm J. (2002) Influence of MHC on sibling discrimination in Arctic charr, *Salvelinus aplinus* (L.). *J Chem Ecol.,* 28: 783-795.

Olsen, K. H., Grahn, M. and Lohm, J. (2003) The inûuence of dominance and diet on individual odours in MHC identical juvenile Arctic charr siblings. *J Fish Biol*., 63: 855–62.

Overholtzer, K. L. and Motta, P. J. (2000) Effects of mixed-species foraging groups on the feeding and aggregation of juvenile parrotfishes. *Environ Biol Fishes.,* 58: 345-354.

Pavlov, D. S. and Kasumyan, A. O. (2000) Patterns and mechanisms of schooling behavior in fish: A review. *J Ichthyol*., 40: S163–S231.

Penn, D. J. (2002) The scent of genetic compatibility: sexual selection and the major histocompatibility complex. *Ethology,* 108: 1-21.

Pethiyagoda, R. (1991) Freshwater fishes of Sri Lanka. The Wildlife Heritage Trust of Sri Lanka, Colombo. pp 362.

Pethiyagoda, R., Meegaskumbura, M. and Maduwage, K. (2012) A synopsis of the South Asian fishes referred to *Puntius* (Pisces: Cyprinidae). *Ichthyol Explore Freshwaters*., 23(1): 69-95.

Peuhkuri, N. (1997) Size-assortative shoaling in fish: the effect of oddity on foraging behaviour. *Anim Behav.,* 54: 271-278.

Peuhkuri, N. and Seppae, P. (1998) Do three-spined sticklebacks group with kin? *Ann Zool Fenn.,* 35: 21-27.

Peuhkuri, N., Ranta, E., Juvonen, S. K. and Lindstrom, K. (1995) Schooling affects growth in three-spined stickleback, *Gasterosteus aculeatus. J Fish Biol.,* 46: 221-226.

Piffer, L., Petrazzini, M. E. M. and Agrillo, C. (2013) Large number discrimination in newborn fish. *PLoS ONE*., 8(4): e62466.

Pitcher, T. J. (1997). Fish shoaling behavior is a key factor in the resilience of ûsheries: shoaling behavior alone can generate range collapse in ûsheries. *Proc 2nd World Fish Cong.,* 2: 143-148.

Pitcher, T. J. and Magurran, A. E. (1983) Shoal size, patch profitability, and information exchange in foraging gold fish. *Anim Behav,* 31: 546-555.

Pitcher, T. J. and Parrish, J. K. (1993) Functions of shoaling behaviour in teleosts. In: *Behaviour of Teleost Fishes* (ed. Pitcher, T. J.), Chapman and Hall, London, pp. 363- 439.

Pitcher, T. J., Magurran, A. E. and Winfield, I. (1982) Fish in larger shoals find food faster. *Behav Ecol Sociobiol.*, 10: 149-15.

Piyapong, C., Butlin, R. K., Faria, J.J., Scruton, K.J., Wang, J. and Krause, J. (2011) Kin assortment in juvenile shoals in wild guppy populations. *Heredity*, 106: 749–756.

Pollen, A. A., Dobberfuhl, A. P., Igulu, M. M., Renn, S. C. P., Scace, J. G., Shumway, C. A. and Hofmann H. A. (2007) Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. *Brain Behav Evol.*, 70: 21-39

Pouyaud, L., Desmarais, E., Chenuil, A., Agnese, J. F. and Bonhomme, F. (1999). Kin cohesiveness and possible in breeding in the mouthbrooding tilapia *Sarotherodon melanotheron* (Pisces Cichlidae). *Mol Ecol.*, 8: 803-812.

Powell, D. K., Tyler, P. A. and Peck, L. S. (2001) Effect of sperm concentration and sperm ageing on fertilisation success in the Antarctic soft-shelled clam *Laternula elliptica* and the Antarctic limpet *Nacella concinna*. *Mar Ecol Prog Ser.*, 215: 191-200.

Prat, J. and Giraud, A. (1964) The pollution of water by detergents. O. E. C. D., Paris. p. 86.

Pritchard, V. L., Lawrence, J, Butlin, R.K. and Krause, J. (2001) Shoal choice in zebra fish, *Danio rerio*: the influence of shoal size and activity. *Anim Behav.,* 62: 1085-1088

Pyanov, A. I. (1993) Fish learning in response to trawl fishing. ICES. *J Mar Sci.*, 196: 12-16.

Queiroz, H. and Magurran, A. E. (2005). Safety in numbers? Shoaling behaviour of the Amazonian red-bellied piranha. *Biol Lett.,* 1: 155-157.

Queller, D. C., Ponte, E., Bozzaro, S. and Strassmann, J. E. (2003) Single-gene greenbeard effects in the social amoeba *Dictyostelium discoideum*. *Science,* 299: 105–106.

Quinn, T. P. and Fresh, K. (1984) Homing and straying in chinook salmon *(oncorhynchus tshawytscha)* from Cowlitz River Hatchery. Washington. *Can J Fish Aquat Sci.,* 41: 1078-1082.

Quinn, T. P. and Busack, C. A. (1985) Chemosensory recognition of siblings in juvenile salmon (*Oncorhynchus kisutch*). *Anim Behav*., 33: 51-56.

Quinn, T. P. and Hara, T. J. (1986). Sibling recognition and olfactory sensitivity in juvenile coho salmon (*Oncorhynchus kisutch*). *Can J Zool.,* 64: 921-925.

Radakov, D. V. (1973) Schooling in the ecology of fish. Israel Program for Scientific Translation, translated by Mill H. Halsted Press, New York.

Rahman, A. K. A. (1989) Freshwater fishes of Bangladesh. Zoological Society of Bangladesh. Department of Zoology, University of Dhaka. pp 364.

Ranta, E. and Lindstorm, K. (1990) Assortative schooling in three-spined sticklebacks. *Ann Zool Fennici,* 27: 67-75.

Ranta, E. and Kaitala, V. (1991) School size affects individual feeding success in three-spined sticklebacks (*Gasterosteus aculeatus* L.). *J Fish Biol*., 39: 733-737.

Ranta, E. and Juvonen, L. K. (1993) Interference affects food-finding rate in schooling sticklebacks. *J Fish Biol*., 43: 531-535.

Reader, S. M. and Laland, K. N. (2002) Social Intelligence, innovation and enhanced brain size in primates. *Proc Natl Acad Sci U S A.,* 99(7): 4436-4441.

Reusch, T. B. H., Häberli, M. A., Aeschlimann, P. B. and Milinski, M. (2001) Female sticklebacks count alleles in a strategy of sexual selection explaining MHC polymorphism. *Nature.,* 414: 300–302.

Rodgers, G. M., Ward, J. R., Askwith, B. and Morrell, L. J. (2011) Balancing the dilution and oddity effects: Decisions depend on body size. *PLoS ONE* 6(7): e14819

Rosenthal, G. G. and Ryan, M. J. (2005) Assortative preferences for stripes in danios. *Anim Behav.,* 70: 1063-1066.

Rosety-Rodríguez, M., Ordoñez, F. J., Roldan, S., Rosety, J. M., Rosety, M., Ribelles, A., Carrasco, C. and Rosety, I. (2002) Acute effects of sodium dodecyl sulphate on the survival and on morpho-histochemical characteristics of the trunk kidney of juvenile turbot *Scophthalmus maximus L. Eur J Histochem*., 46: 179-184.

Rowlinson, P. (2008) Adapting Livestock Production Systems to Climate Change – Temperate Zones. Livestock and Global Change conference proceeding. May 2008, Tunisia.

Ruhl, M., Kürschner, W. M. and Krystyn, L. (2009) Triassic–Jurassic organic carbon isotope stratigraphy of key sections in the western Tethys realm (Austria). *Earth Planet Sc Lett*., 281: 169–187.

Ryer, C. H. and Olla, B. L. (1999). Light-induced changes in the prey consumption and behaviour of two juvenile plankivorous fish. *Mar Ecol-Prog Ser*., 181: 41-51.

Saglio P., Trijasse, S. and Azam, D. (1996) Behavioural effects of water borne carbofuran in gold fish. *Arch Environ Contam Toxicol.,* (31): 232-238.

Scace, J., Dobberfuhl, H. E. and Shumway, C. (2006). Complexity and the evolution of the social brain. *Proceedings of .6th International Congress*. Evolution and Ecology II: 1-8

Schausberger, P. (2007) Kin recognition by juvenile predatory mites: prior association or phenotype matching? *Behav Ecol Sociobiol*., 62: 119–125.

Schein, M. W. (1963) On the irreversibility of imprinting. *Z Tierpsychol*., 22: 50-103.

Schlupp, I. and Ryan, M.J. (1997). Male Sailûn mollies (*Poecilia latipinna*) copy the mate choice of other males. *Behav Ecol*., 8: 104-107.

Schutz, F. (1965) Sexuelle pragung bei anatiden. *Z Tierpsychol*., 22: 50-103

Schwassmann, H. O. and Kruger, L. (1965) Organization of the visual projection upon the optic tectum of some freshwater fish. *J Comp New.,* 124: 113-l26.

Sekuler, R. and Blake, R. (1994) Perception. McGraw-Hill, New York, NY.

Shaji, C.P. and Easa, P.S. (1995) Freshwater fish diversity in Wayanad, Kerala, South India. *Journal of Zoological Society of Kerala.,* 5(1&2): 30-36.

Shaw, E. (1960) The development of schooling behavior in fishes. *Physiol Zool.,* 33: 79–86.

Shaw, E. (1962). Environmental conditions and the appearance of sexual behavior in the platyfish. In*:* "*Roots of Behavior*." (eds. E. Bliss), New York: Harper.

Shaw, E. (1978) Schooling Fishes. *Am. Sci.,* 66: 166-175.

Sherman, P. W., Reeve, H. K. and Pfennig, D. W. (1997) Recognition systems. In: *Behavioral ecology: an evolutionary approach,*4th edn (ed. J. R. Krebs & N. B. Davies), pp. 69–96, 4th edn. Oxford, UK: Blackwell Scientific.

Shohet, A. J. and Watt, P. J. (2004) Female association preferences based on olfactory cues in the guppy, *Poecilia reticulate*. *Behav Ecol Sociobiol*., 55: 363-369.

Siebeck, U. E., Wallis, G. M. and Litherland, L. (2008). Colour vision in reef fish. *J Exp Biol*., 211: 354 -360.

Singer, A. G., Beauchamp, G. K. and Yamazaki, K. (1997) Volatile signals of the major histocompatibility complex in male mouse urine. *Proc Natl Acad Sci U S A.,* 94: 2210-2214.

Singh, R. Í. (1991) Laboratory workshop in neuroanatomy with training in light and electron microscopy instruction manual. TIFR, Bombay, pp 16-17.

Smith, B. H. and Getz, W. M. (1994) Nonpheromonal olfactory processing in insects. *Annu Rev Entomol.,* 39: 351–375

Smith, E. H. and Logan, D. T. (1997) Linking environmental Toxicology, ethology and conservation. In: *Behavioural approaches to conservations in the wild*. (eds. Clemons, J. T. and Buchholz. R), Cambridge: Cambridge University Press, pp 277-302.

Smith, M. J. and Harper, D. (2003) Animal signals. Oxford: Oxford University Press.

Snekser, J. L., McRobert, S. P. and Clotfelter, E. D. (2006). Social partner preferences of male and female fighting fish (*Betta splendens*). *Behav Proc.,* 72: 38-41.

Snekser, J. L., Ruhl, N., Bauer, K. and McRobert, S.P. (2010) The influence of sex and phenotype on shoaling decisions in zebrafish. *Int J Comp Psychol*., 23: 70-81.

Sorensen, P.W. (1996) Behavioral responsiveness to pheromones provides fundamental and unique insight into olfactory function. *Chem Senses*., 21(2): 245-256.

Sorensen P. W and Hobson K. A. (2005) Stable isotope analysis of amphidromous Hawaiian gobies suggests their larvae spend a substantial period of time in freshwater river plumes. *Environ Biol Fishes*., 74: 31–42.

Sorensen, P.W., T.A. Christensen, and N.E. Stacey. (1998) Discrimination of pheromonal cues in fish: emerging parallels with insects. *Curr Opin Neurobiol*., 8(4): 458-467.

Sørensen, C., Bohlin, L. C., Øverli, O. and Nilsson, G. E. (2011) Cortisol reduces cell proliferation in the telencephalon of rainbow trout (*Oncorhynchus mykiss*). *Physiol Behav*., 102: 518–523

Soria, M., Gerlotto, F. and Freon, P. (1993) Study of learning capabilities of tropical clupeoids using an artificial stimulus. *ICES J Mar Sci.*, 196: 105-116.

Spence, R. and Smith, C. (2007) The role of early learning in determining shoaling preferences based on visual cues in the zebrafish, *Danio rerio*. *Ethology*., 113: 62–67.

Sprauge, J. B. (1971) Measurement of pollutant toxicity to fish, III. Sublethal effect and safe concentrations. *Water Res*., 5: 245-246.

Sprague, J. B. (1976) The ABC´s off pollutant bioassay using fish. Cairn J Jr, Dickson KL, editors. Biological methods for the assessment of water quality. American Society for Testing and Materials. Philadelphia.

Steck, N., Wedekind, C. and Milinski, M. (1999) No sibling odor preference in juvenile three-spined stickleback, (*Gasterosteus aculeatus*). *Behav Ecol.*, 10: 493-497.

Street, N. G., Magurran, A. E. and Pitcher, T. J. (1984) The effects of increasing shoal size on handling time in goldûsh *Carassius auratus*. *J Fish Biol.*, 25: 561-566.

Svensson, P. A., Barber, I. and Forsgren, E. (2000) Shoaling behaviour of the two spotted goby. *J Fish Biol.*, 56: 1477-1487.

Svendsen, J. C., Skov, J., Bildsoe, M. and Steffensen J. F. (2003) Intra-school positional preference and reduced tail beat frequency in trailing positions in schooling roach under experimental conditions. *J Fish Biol.,* 62: 834–846.

Swaney, W., Kendal, J., Capon, H., Brown, C. and Laland, K (2001) Familiarity facilitates social learning of foraging behaviour in the guppy. *Anim Behav.*, 62: 591–598

Sweka, J. A. and Hartman, K. J. (2001) Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling. *Can J Fish Aquat Sci.,* 58: 386–393.

Talwar, P. K. and Jhingran, A. G. (1991) Inland fishes of India and adjacent countries. vol 1. A.A. Balkema, Rotterdam. pp 541.

Tang-Martinez, Z. (2001) The mechanisms of kin discrimination and the evolution of kin recognition in vertebrates: a critical re-evaluation. *Behav Process.*, 53: 21–40.

Theodorakis, C.W. (1989) Size segregation and the effects of oddity on predation risk in minnow school. *Anim Behav.*, 38: 496-502.

Thom, M. D. and Hurst, J. L. (2004) Individual recognition by Scent. *Annls Zool Fennici.*, 41:765-787.

Thomas, K. R., Biju, C. R. and Ajithkumar, C. R. (2000) Fish fauna of Chimmony and Peechi-Vazhani Wildlife sanctuaries, Kerala, India. *J Bombay Nat Hist Soc*., 97(2): 289-292.

Tibbetts, E. A. (2002) Visual signals of individual identity in the wasp Polistes fuscatus. *Proc R Soc Lond B Biol Sci.,* 269: 1423–1428.

Tierney, K. B., Baldwin, D. H., Hara, T. J., Ross, P. S., Scholz, N. L. and Kennedy, C. J. (2010) Olfactory toxicity in fishes. *Aquat Toxicol*., 96: 2–26.

Tinbergen, N. (1953). "Social Behavior in Animals." London: Methuen.

Turesson, H. and Bronmark, C. (2007) Predator-prey encounter rates in freshwater piscivores: Effects of prey density and water transparency. *Oecologia.,* 153: 281–290.

Turner, G. F. and Pitcher, T. J. (1986) Attack abatement: a model for group protection by combined avoidance and dilution. *Am Nat.,* 128: 228-240.

Utne-Palm, A. C. (1999) The effect of prey mobility, prey contrast, turbidity and spectral composition on the reaction distance of *Gobiusculus flavescens* to its planktonic prey. *J Fish Biol.,* 54(6): 1244-1258.

Utne-Palm, A. C. W. (2001) Response of naïve two-spotted gobies *Gobiusculus flavescens* to visual and chemical stimuli of their natural predator, cod *Gadus morhua*. *Mar Ecol Prog Ser.,* 218: 267–274

Utne-Palm, A. C. and Hart, P. J. B. (2000) The effects of familiarity on competitive interactions between threespined sticklebacks. *Oikos.,* 91: 225–232.

Viscido, S., Parrish, J., and Grunbaum, D. (2004) Individual behavior and emergent properties of fish schools: a comparison of observation and theory. *Mar Ecol Prog Ser.,* 273: 239-249.

Wadaan, M. A. M. and Mubarak, M. (2009) Blood chemistry changes as an evidence of the toxic effects of anionic surfactant sodium dodecyl sulfate. *Asian J Sci Res.,* 2:113-118.

Waldman, B. (1981) Sibling recognition in toad tadpoles: the role of experience. *Z Tierpsychol*. 56: 341–358.

Waldman, B. (1987) Mechanisms of kin recognition. *J Theor Biol.,* 128: 159–185.

Waldman, B. (1988) The ecology of kin recognition. *Annu Rev Ecol Syst.,* 19: 543-571.

Waldman, B. (1991) Kin recognition in amphibians. In "*Kin recognition*". (ed Hepper, P. G.) Cambrige University Press. London. pp. 162–219.

Waldman, B. (1999) Kin recognition in amphibians. In: *Kin Recognition* (ed. P.G. Hepper). Cambridge university press, Cambridge, pp. 162-219.

Walls, G. L. (1942) The vertebrate eye and its adaptive radiation. Bloomfield Hills, MI: Cranbrook institute of science pp 785

Warburton, K. (2003) Learning of foraging skills by fish. *Fish Fish*., 4(3): 203–215.

Warburton, K. and Lees, N. (1996). Species discrimination in guppies: learned responses to visual cues. *Anim Behav*., 52: 371-378.

Ward, A. J and Hart, P. J. B (2003) The Effects of kin and familiarity on interactions between fish. *Fish Fish.*, 4: 348-358.

Ward, A. J. W and Hart, P. J. B (2004) Foraging benefits of shoaling with familiars may be klepto-parasitised by outsiders. *Anim Behav*., 69: 329-325.

Ward, A.J.W. and Hart, P.J.B. (2005) Foraging benefits of shoaling with familiars may be exploited by outsiders. *Anim Behav*., 69: 329–335.

Ward, A. J. W., Axford, S. and Krause, J. (2002) Mixed-species shoaling in fish: the sensory mechanisms and costs of shoal choice. *Behav Ecol Sociobiol.,* 52: 182-187.

Ward, A. J. W., Hart, P. J. B. and Krause, J. (2004) The effect of habitat- and diet-based cues on association preferences in three-spined sticklebacks. *Behav Ecol*., 15(6): 925-929.

Ward, A. J. W., Duff, A. J., Krause, J. and Barber, I. (2005a) Shoaling behaviour of sticklebacks infected with a microsporidian parasite. *Environ Biol Fish.,* 72: 155-160.

Ward, A. J. W., Holbrook, R. I., Krause, J. and Hart, P. J. B. (2005b) Social recognition in sticklebacks: the role of direct experience and habitat cues. *Behav Ecol Sociobiol*., 57: 575–583.

Ward, A. J. W., Duff, A. J. and Currie, S. (2006) The effects of the endocrine disrupter 4-nonylphenol on the behaviour of juvenile rainbow trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci.,* 63: 377–382.

Ward, A. J. W., Webster, M. M. and Hart, P.J.B. (2007) Social recognition in wild fish populations. *Proc. R. Soc. Lond. B*., 274: 1071–1077.

Ward, A. J. W., Duff, J. A., Horsfall, S. J. and Currie, S. (2008) Scents and scents-ability: pollution disrupts chemical social recognition and shoaling in fish. *Proc R Soc Lond Ser B Biol sci.,* 274: 101–105.

Ward, A. J. W., Webster, M. M., Magurran, A. E., Currie, S. and Krause, J. (2009) Species and population differences in social recognition between fishes: a role for ecology? *Behav Ecol.,* 20: 511-516.

Warner, R. R. (1990) Male versus female influences on mating-site determination in a coral-reef fish. *Anim Behav*., 39: 540–548.

Warner, R. E., Peterson, K. K and Borgman. L. (1966) Behavioral pathology in fish: A quantitative study of sub-lethal pesticide toxication, *J Appl Ecol.,* 3: 223.

Webster, M. M. and Hart, P. J. B. (2006) Kleptoparasitic prey competition in shoaling fish:

effects of familiarity and prey distribution. *Behav Ecol.,* 17: 959-964.

Webster, M. M., Goldsmith, J., Ward, A. J. W. and Hart, P. J. B. (2007) Habitat-specific

chemical cues influence association preferences and shoal cohesion in fish. *Behav Ecol Sociobio.,* 62: 273–280.

Weetman, D., Atkinson, D. and Chubb, J. C. (1999) Water temperature influences the shoaling decisions of guppies, *Poecilia reticulata*, under predation threat. *Anim Behav.*, 58: 735–741.

Welty, J. L. C. (1934) Experiments on groups of fishes. *Physiol Zool B.*, 7: 85-128.

Whiten, A. and Ham, R. (1992) On the nature and evolution of imitation in the animal kingdom: reappraisal of a century of research. *Adv Study Behav.*, 21: 239–283.

Whitlock, K. E., Newton, L. A., Rivard, M. V. and Boyce, M. L. (2003) Cellular basis of olfactory imprinting in the zebraûsh, *FASEB J.*, 17: A381.

Winberg, S. and Olsen, K. H. (1992) The influence of rearing conditions on the sibling odor preference of juvenile arctic charr, *Salvelinus alpinus* L. *Anim Behav.*, 44: 157–164.

Wisenden, B. D. and Smith, R. J. F. (1998) A re-evaluation of the effect of shoalmate familiarity on the proliferation of alarm substance cells in fathead minnows. *J Fish Biol.*, 53: 841-846

Wisenden, B. and Dye, T. (2009) Young convict cichlids use visual information to update olfactory homing cues. *Behav Ecol Sociobiol.*, 63: 443-449.

Witte, K. and Ryan, M. J. (1998) Male body length influences mate choice copying in the sailfin molly, *Poecilia latipinna*. *Behav Ecol.*, 9: 534–539.

Wong, B. M. and Rosenthal, G. G. (2005) Shoal choice in swordtails when preferences conflict. *Ethology.,* 11: 179-186.

Wyatt, T. D (2003) Pheromones and animal behaviour: communication by smell and taste. Cambridge University Press, Cambridge.

Yamamoto, N. and Ito, H. (2000) Afferent sources to the ganglion of the terminal nerve in teleosts. *J Comp Neurol.*, 428, 355-375.

Youson, J. H. (1988) First metamorphosis. In: *Fish physiology.* (eds.W. S. Hoare and D. J. Randall) Volume 11B, Academic Press, New York, pp. 135-196.

List of Publications

PAPER PUBLICATIONS

1. Alex N.J. & Thomas K. J. (2014) Heterospecific familiarity on the shoaling preferences of *Puntius sarana subnasutus*. ***Journal of experimental zoology, India*** (accepted)

2. Alex N.J. & Thomas K. J. (2013) Onset of shoaling preference in *Puntius sarana subnasutus*. ***International Journal of Education and Research*** Vol. 1 No. 1 pp 1-10

3. Alex N.J. & Thomas K. J. (2012) Influence of genetic relatedness and shoal size on shoaling preferences in juvenile *Puntius sarana subnasutus* (Hamilton Valenciennes). ***Indian Journal of experimental biology***, Vol. 50 pp 583-586

4. Alex N. J. & Thomas K. J. (2011) Influence of turbidity on the shoaling preferences of *Puntius sarana subnasutus*. ***Journal of inland Fishery Society of India***, Vol. 43 (2) pp 79-81.

5. Alex N. J. & Thomas K. J. (2011) Factors influencing shoaling preferences in *Puntius sarana subnasutus,* ***Current Science***, Vol.100 pp 633-634

6. Alex N. J. & Thomas K. J. (2011) Effect of sub-lethal concentration of methyl parathion on the behavioural activities of climbing perch (*Anabas testdineus* : Bloch). ***Advances in applied science research***, vol. 2(5) pp 167-172

PAPER PRESENTATIONS

1. Jilna Alex and John Thomas K: Influence of water temperature in regulating fish activity level and group-size discrimination in Puntius sarana subnasutus – National Seminar on *"Lakes, Rivers and Wetland Ecosystems – A climatechange perspective"* organized by Department of P.G. studies and Research in Geology, MES Ponnani College, Ponnani, Kerala, March: 19-21, 2014.

2. Jilna Alex and John Thomas K:Technique of induced breeding in *Puntius sarana subnasutus* – National Seminar on *"Forest – A Biodiversity Rich Biome: Current status and Future Implications"* organized by Department Zoology, St. Joseph's College, Irinjalakuda, Kerala, March: 1&2, 2012.

3. Jilna Alex and John Thomas K: Genetic relatedness and Group-size in Shoaling Preferences of *Puntius sarana subnasutus* – National Seminar on *"Biodiversity: Utilization and Management for Natural Harmony"* Jointly organized by Departments of Botany and Zoology, K.K.T.M. Gvt. College, Pullut, Kodungallur, Kerala, February: 8 & 9, 2012.

4. Jilna Alex and John Thomas K: Onset of shoaling preference in *Puntius sarana subnasutus* - Colloquim on *"Ethology from Organism Down to Ethobiomolecules"* 34th Annual Conference of Ethological Society of India, CABB, University of Kerala, December: 16-18, 2010.

5. Jilna Alex and John Thomas K: Effect of sub-lethal concentration of methyl parathion on the behavioural activities of climbing perch (*Anabas testudineus* : Bloch) – National Symposium on *"Biotechnological and Biorational Approaches for Pest Management in Agriculture"* 33rd Annual Conference of Ethological Society of India UAS, Dharwad, November: 6-7, 2009.

6. Rejitha V., Jilna Alex and Peter, M.C.S. Handling of Fe by Intestinal and Gill epithelia of Climbing perch (*Anabas testudineus)* kept in Fe II iron-rich water – Society of Reproduvtive Biology and Endocrinology, 2007.

Reprints

Chutiwat, S., *Palaeobotanist*, 2001, **50**, 27–34.
13. D'Arrigo, R. D. *et al*., *Geophys. Res. Lett*., 2006, **33**, L04709.
14. Shah, S. K., Bhattacharyya, A. and Chaudhury, V., *Dendrochronologia*, 2007, **25**, 57–64.
15. Fritts, H. C., *Tree-Rings and Climate*, Cambridge University Press, Cambridge, 1976.
16. Stokes, M. A. and Smiley, T. L., *An Introduction to Tree-Ring Dating*, University of Chicago Press, Chicago, 1968, p. 73.
17. Holmes, R. L., *Tree-Ring Bull*., 1983, **43**, 69–78.
18. Cook, E. R., Briffa, K. R., Shhiyatov, S. and Mazepa, V., In *Methods of Dendrochronology: Applications in the Environmental Science* (eds Cook, E. R. and Kairiukstis, L. A.), Kluwer, Dordrecht, 1990, pp. 104–162.
19. Cook, E. R., Dissertation, University of Arizona, USA, 1985.
20. Cook, E. R. and Kairiukstis, L. A. (eds), *Methods of Dendrochronology: Applications in the Environmental Sciences*, Kluwer, Dordrecht, 1990, p. 394.
21. Dai, A. G., Trenberth, K. E. and Quian, T., *J. Hydrometeorol*., 2004, **5**, 1117–1130.
22. Priya, P. B. and Bhat, K. M., *IAWA*, 1999, **20**, 181–192.
23. Bhattacharyya, A., Eckstein, D., Shah, S. K. and Chaudhary, V., *Curr. Sci*., 2007, **93**, 1159–1164.
24. Biondi, F. and Waikul, K., *Comput. Geosci*., 2002, **30**, 303–311.
25. Ram, S., Borgaonkar, H. P. and Sikder, A. B., *Quaternary Int*., 2010, **212**, 70–75.
26. Namias, J., *J. Climate Appl. Meteorol*., 1983, **22**, 30–39.

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Factors influencing shoaling preference in *Puntius sarana subnasutus*

Shoaling is a common phenomenon among fishes, and over half of the estimated 28,000 species of fishes[1] form shoals at some point during their life[2]. The decision of an individual to join a group may depend on a range of factors, such as group size[3], body size[4], colour[5], species[6], familiarity[7] and kinship[8]. Grouping based on such attributes is thought to reduce predation risk by minimizing phenotypic oddity or through coordinating antipredator behaviour and reducing competition within group members[9].

For effective grouping, individuals need to be able to recognize group members that possess suitable phenotypic cues. In freshwater fishes, social recognition is known to be achieved using a combination of visual and chemical cues[10]. Here, we evaluate the influence of kinship and body size of individuals on the shoal choice of *Puntius sarana subnasutus* by giving an opportunity to single individuals to shoal either with similar-sized full sibs or with phenotypically dissimilar, larger non-sibs. We examined the decision of individuals when preference to larger shoal conflicted with inclination for assorting based on kinship and phenotypic similarity.

P. sarana subnasutus were collected from canals associated with paddy fields at Irinjalakuda (10°25′–10°18′47″N lat. and 76°17′19″–76°12′48″E long.), Thrissur District, Kerala, India, during January–March 2008. Using Gonadotropin-releasing hormone (GnRH) analogue Ovaprim, gravid pairs were induced to breed and eight-month-old (standard length = 7.7 ± 2.15 cm and body mass = 11 ± 2 mg) sub-adults of the same breeding pair were selected as the sibling group. Individuals of the same age and size group selected from the young ones of another breeding pair formed the non-sibling group. *P. sarana subnasutus*, less than one-year-old (standard length = 16.3 ± 2 cm and body mass = 26.75 ± 4.12 mg), collected from the canal, formed the larger non-siblings group.

Shoaling preferences were tested in 70 l aquaria (85 cm × 32 cm × 32 cm), which was divided into two stimulus compartments (measuring 16 × 32 × 32 cm each) on the right and left, and a central compartment (measuring 53 × 32 × 32 cm). The test fish were introduced individually into the central compartment in a presentation cage. Ten minutes were given to the fish for assessment of stimulus shoal and thereafter, the movements of the fish were recorded for 6 min using a stopwatch.

We conducted three types of choice experiments and the basic protocol was the same in all of them. To test the influence of kinship on shoal preference (experiment 1), two stimulus shoals – one with siblings and the other with non-siblings of similar body size and equal number (10 versus 10; 1 : 1 ratio) were presented in the side chambers. In experiments 2 and 3, the number of individuals in stimulus shoals was altered in the following numerical combinations: 10 versus 10, 9 versus 11, 7 versus 13, and 4 versus 16 (ratios 1 : 1, 1 : 1.2, 1 : 1.9 and 1 : 4 respectively)[7]. In experiment 2, we tested the preference of the fish to larger shoals by providing similar-sized sibling stimulus shoal on either side. To determine the trade-off point of sibling preference against shoal size and larger body size (experiment 3), the test fish was given two different stimuli; one with similar-sized siblings and the other with larger non-siblings. The data were analysed using non-parametric two-tailed t-test (SPSS 11.0.1 statistical package).

In the first experiment, the test fish displayed an overall significant preference for stimulus shoals composed of siblings ($t = 7.041$; $n = 20$; $P < 0.0001$). When the test fish was presented to sibling stimulus shoals in the above-mentioned combinations, the fish preferred to remain with the larger group. The fish did not exhibit any preference for a particular stimulus shoal when the ratio was 1 : 1 and 1 : 1.2 (10 versus 10; $t = 1.928$, $n = 20$, $P = 0.0614$, and 9 versus 11; $t = 1.5025$, $n = 20$, $P = 0.413$). However, at higher ratios (1 : 1.9 and 1 : 4), the fish exhibited a significant preference for larger shoals (7 versus 13;

$t = 7.907$, $n = 20$, $P < 0.0001$, and 4 versus 16; $t = 5.621$, $n = 20$, $P < 0.00011$; Figure 1).

When the trade-off point of sibling preference against body size and shoal size was tested, the fish showed varying preference to different ratios (Figure 2). Preference to shoal with kin was shown only when the number of individuals in the kin-stimulus shoal was either 10 or 9 (1 : 1, $t = 9.592$, $n = 20$, $P < 0.0001$, and 1 : 1.2, $t = 13.123$, $n = 20$, $P < 0.0001$). At lower combinations when the number of individuals in the kin-stimulus shoal was less (7 or 4), the fish preferred to associate with larger shoal of larger-sized, non-siblings (1 : 1.9, $t = 2.946$, $n = 20$, $P = 0.00551$, and 1 : 4, $t = 3.759$, $n = 20$, $P = 0.0006$).

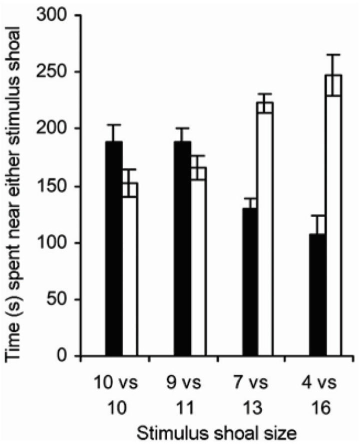

Figure 1. Preference of individual *Puntius sarana subnasutus* for phenotypically similar smaller/larger sibling stimulus shoals. Mean time (spent in sec) ± SE near smaller group (■) and larger group (□) of siblings shoals; n/N = 20/80.

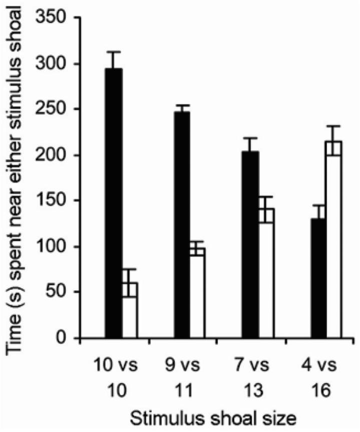

Figure 2. Preference of individual *P. sarana subnasutus* for similar-sized sibling/dissimilar-sized larger, non-sibling stimulus shoals. Mean time (spent in seconds) ± SE near sibling (■) and non-sibling (□) shoals; n/N = 20/80.

In the present study *P. sarana subnasutus* showed specific preference for shoals composed of siblings over shoals composed of non-siblings, when the number of individuals in the stimulus shoals remained the same. However, it showed a significant preference for the larger shoal composed of siblings, indicating that the test fish was able to discriminate a larger shoal. Association with the smaller/larger shoals may be based on the odour or visual cues[11]. Usually a larger group of fish can confer lower predation risk by dilution effect, early predator detection ('many eyes'), group defence and coordinated group manoeuvres[2]. These factors may increase the chances of survival and provide better opportunities for foraging under predation pressure. When the trade-off between preferences for the sibling group and larger-sized non-siblings was tested (10 : 10 ratio), the test fish associated with the sibling group in spite of the overall increase in the surface area of the larger stimulus shoal. This finding is at variance with earlier studies on the shoaling preference in fish based on surface area. Mosquito fish (*Gambusia holbrooki*) spontaneously use non-numerical cues, namely the cumulative areas of the shoals and the overall quantity of the movements of individuals within the shoal[12]. Our results suggest that genetic relatedness influences the shoaling preference in *P. sarana subnasutus*. However, the fish traded-off the preference to siblings when the number of individuals in the stimulus sibling shoal was low, indicating the overriding influence of shoal size on shoaling preference in this species. Future studies are needed to elucidate the trade-off between kin and shoal-size preference in the context of shoaling decisions in fish.

1. Nelson, J. S., In *Fishes of the World*, John Wiley, New York, 2006, 4th edn.
2. Pitcher, T. J. and Parrish, J. K., In *Behaviour of Teleost Fishes* (ed. Pitcher, T. J.), Chapman and Hall, London, 1993, pp. 363–439.
3. Krause, J., Godin, J. G. J. and Brown, D., *J. Fish Biol.*, 1996, **49**, 221–225.
4. Wong, B. M. and Rosenthal, G. G., *Ethology*, 2005, **111**, 179–186.
5. McRobert, S. P. and Bradner, J., *Anim. Behav.*, 1998, **56**, 611–615.
6. Barber, I., Downy, L. C. and Braithwaite, V. A., *J. Fish Biol.*, 1998, **53**, 1365–1368.
7. Binoy, V. V. and Thomas, K. J., *Curr. Sci.*, 2004, **86**, 207–211.
8. Behrmann, G. J., Gerlach, G. and Eckmann, R., *Behav. Ecol. Sociobiol.*, 2006, **59**, 461–468.
9. Ward, A. J. W., Axford, S. and Krause, J., *Behav. Ecol. Sociobiol.*, 2002, **52**, 182–187.
10. Brown, G. E. and Smith, R. J. F., *J. Chem. Ecol.*, 1994, **20**, 3051–3061.
11. Ward, A. J. W., Holbrook, R. I., Krause, J. and Hart, P. J. B., *Behav. Ecol. Sociobiol.*, 2005, **57**, 575–583.
12. Agrillo, C., Dadda, G., Serena, G. and Bisazza, A., *Anim. Cogn.*, 2008, **11**, 495–503.

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SHORT COMMUNICATION

INFLUENCE OF TURBIDITY ON THE SHOALING PREFERENCES OF *PUNTIUS SARANA SUBNASUTUS*

Received : 18.07.2011; Accepted : 21.10.2011

Turbidity is one of the important physical characteristic of water that affects its optical property. At extremely high levels, turbidity can directly affect fish growth and survival, for example, by interfering with gill function or the quality of substrata for egg laying (Bash *et. al.*, 2001). By limiting the photic zone, turbidity can also reduce the foraging success by diminishing the prey contrast (Sweka and Hartman, 2001) or it may reduce the time available for the prey to detect predators (Berger *et al.*, 2004).

Fishes are social organisms that form social aggregations called shoals (Shaw, 1978). One of the major factors influencing shoaling decision of individual fish is the group size (Wong and Rosenthal, 2005; Alex and Thomas, 2011). Agrillo *et al.* (2008), in a study has demonstrated that mosquito fish (*Gambusia holbrooki*) in selecting a larger shoal spontaneously use the sum of areas of the shoals and the over all quantity of the movements of individuals with in the shoal. Despite the generally poor quality of under water images, fish depend to a great deal on vision as a source of sensory information (Guthrie and Muntz, 1993). In the present study, we have analysed how turbidity of the water impose restrictions on shoaling preference in *P. sarana subnasutus.*

Fish were collected from canals associated with paddy fields of Irinjalakuda (10^{o}25’, 10^{o}18’47” N lat. and 76^{o}17’19”, 76^{o}12’48”E long.), Thrissur district, India, during January - March 2010 and acclimatized with laboratory conditions for two weeks. Three samples of water were collected from different localities of the same canal (one from a less polluted and other two from polluted and eutrophicated sites). The turbidity of water samples were measured by nephelometric method (Clesceri *et al.*, 1998). Shoaling preferences were tested in a 30 liter aquarium (55 x 23 x 23cm), which was divided into two stimulus compartments (measuring 15 x 23 x 23 cm each) on the right and left and a central compartment (measuring 25 x 23 x 23cm). The test fish were introduced individually into the central compartment using a presentation cage. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, the movement of the fish was recorded for six minutes using a stopwatch.

Three types of dichotomous preference tests were conducted with the test fish. To test the ability for group-size recognition of *Puntius sarana subnasutus* in turbid water, two stimulus shoals were given in five different numerical combinations (25 vs 25, 20 vs 30, 15 vs 35, 10 vs 40, 5vs 45, Ratio- 1:1, 1:1.5, 1:2.3, 1:4 and 1:9 respectively) in each stimulus compartment. The experiments were replicated thrice in water samples having turbidity levels of 35.7, 48.3 and 68.5 NTU respectively. The data were analyzed using non-parametric two-tailed ‘t’ test [SPSS 11.0.1 statistical package]. Our results suggest that *Puntius sarana subnasutus* is capable of detecting and recognizing their shoal mates at lower and intermediate turbid water while it failed to recognize the shoal-size difference in highly turbid water. The fish successfully associated with the larger shoal both in 35.7 and 48.3 NTU turbid water as the number of shoal mates increased in the stimulus shoal (Fig. 1 A and B). However, it failed to discriminate the shoal size difference in 68.5 NTU turbid water (Fig. 1 C) but randomly associated with either of the stimulus shoal (Table:1).

The behavioural repertoire of most fish species depends on vision. It is reported that turbidity limits the visual ability of fish which interferes with its social behaviour (Berg and Northcote, 1985). Earlier studies on foraging have shown that although the efficiency of prey capture decreased at higher turbidities, the fish exhibited some degree of feeding activity at 45 NTU (Madej *et al.*, 2004). In the present study, the fish failed to discriminate the group size differences between the stimulus shoals when placed in highly turbid water (68.5 NTU) probably due to the reduced contrast between the shoal mates and the back ground. This clearly indicates that a long term exposure to highly turbid water adversely affects the social behavioural patterns and the survival of fish even though low and moderate turbidity levels are tolerable.

In many aspects social recognition is achieved by a combination of visual and

Table 1: Association preference in *Puntius sarana subnasutus* at different levels of water turbidy

Group size	Turbidity of water sample (NTU)		
	35.8	48.3	68.5
25s vs 25s	t = 0.705; p=0.49	t = 0.408; p=0.688	t = 3.405; p=0.003**
30s vs 20s	t = 1.841; p=0.081	t = 1.971; p=0.064	t = 1.444; p=0.165
35s vs 15s	t = 6.849; p=0.000***	t = 3.902; p=0.001**	t = 6.234; p=0.000***
40s vs 10s	t = 10.774; p=0.000***	t = 3.690; p=0.002**	t = 0.847; p=0.408
45s vs 5s	t = 20.077; p=0.000***	t = 2.959; p=0.008**	t = 0.592; p=0.561

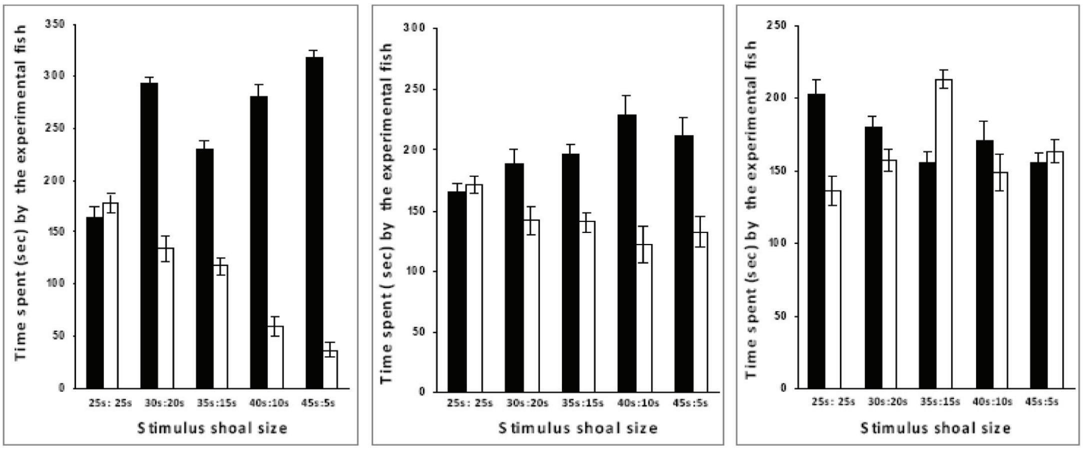

Fig. 1 (A) Fig. 1 (B) Fig. 1 (C)

Fig. 1 Preference of *Puntius sarana subnasutus* for larger group (s-■) / smaller group (□) of stimulus shoals in 35.7 NTU (A), 48.3 NTU (B) and 68.5 NTU (C) turbid water n = 20 Mean time (spent in sec) ± SE chemical cues (Ward *et al.*, 2004). High turbidity limits the range of visual recognition and the fish fail to discriminate their shoal mates. In shoals, information about the type and quality of food source, number of companions, threat and alternative feeding sites are collectively acquired and decisions are taken surprisingly rapidly (Pitcher and Parrish, 1993). The behavioural plasticity conferred by shoaling may therefore be seen as one of its major general advantages. Turbidity, by reducing the visual range interferes with this vital behavioural pattern, adversely affecting the survival of fish. Therefore, it is necessary to control the aspects of water pollution especially industrial effluents and eutrophication that interferes with the clarity of water affecting the growth of aquatic populations.

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References:

Alex, J. and Thomas, K. J. 2011. Factors influencing shoaling preferences in *Puntius sarana subnasutus*. *Curr. Sci.*, **100** (5): 633-634.

Agrillo, C., Dadda, G., Serena, G. and Bisazza, A. 2008. Do fish count? Spontaneous discrimination of quantity in female mosquito fish. *Anim. Cogn.*, **11**: 495-503.

Bash, J., Berman, C. and Bolton, S. 2001. Effects of turbidity and suspended solids on salmonids. Center for Streamside Studies, University of Washington, Seattle, WA.

Berg, L. and Northcote, T. G. 1985. Changes in territorial, gill-flaring, and feeding-behaviour in juvenile coho salmon (*Oncorhynchus kisutch*) following short-term pulses of suspended sediment. *Can J Fish Aquat Sci.*, **42**(8): 1410-1417.

Berger, R., Bergstrom, L., Graneli, E. and Kautsky, L. 2004. How does eutrophication affect different life stages of *Fucus vesiculosus* in the Baltic Sea? A conceptual model. *Hydrobiologia.*, **514**(1-3): 243-248.

Clesceri, L.S., Greenberg, A. E. and Eaton, A. D. 1998. Measurement of Turbidity, pp 2-10. *In* Clesceri, L.S, Greenberg, A. E. and

Eaton, A. D. (ed.), *Standard methods for the examination of water and wastewater.* United Book Press, Inc., Baltimore, Maryland.

Guthrie, D. M. and Muntz, W. R. A. 1993. Role of vision in fish behavior, pp 363-439. *In* Pitcher, T. J. (ed.), *Behaviour of Teleost Fishes.* Chapman and Hall, London.

Madej, M. A., Wilzbach, M., Cummins, K., Ellis, C. and Hadden, S. 2004. *The Significance of Suspended Organic Sediments to Turbidity, Sediment Flux, and Fish-Feeding Behavior.* Paper presented at the Redwood Region Forest Science Symposium, 15-17 March, Rohnert Park, CA.

Pitcher, T. J. and Parrish, J. K. 1993. Functions of shoaling behaviour in teleosts, pp 363-439. *In* Pitcher, T. J. (ed.), *Behaviour of Teleost Fishes.* Chapman and Hall, London.

Shaw, E.1978. Schooling Fishes. *Am. Sci.*, **66**: 166-175.

Sweka, J. A. and Hartman, K. J. 2001. Effects of turbidity on prey consumption and growth in brook trout and implications for bioenergetics modeling. *Can J Fish Aquat Sci.,* **58**: 386–393.

Ward, A. J. W., Hart, P. J. B. and Krause, J. 2004. The effect of habitat- and diet-based cues on association preferences in three-spined sticklebacks. *Behav. Ecol.*, **15**(6): 925-929.

Wong, B. M. and Rosenthal, G.G. 2005. Shoal choice in swordtails when preferences conflict. *Ethol.*, **111**: 179-186.

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HETEROSPECIFIC FAMILIARITY ON SHOALING PREFERENCES OF *PUNTIUS SARANA SUBNASUTUS*

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ABSTRACT – The ability of fish to recognize and preferentially associate with familiar conspecifics has been well documented in a series of laboratory experiments. The present study investigated the influence of heterospecific familiarity and shoal size on sibling preferences of *Puntius sarana subnasutus* during selection of a particular shoal. The fish preferentially associated with sibling shoal than another larger shoal composed of unfamiliar, *Anabas testudineus*. However, *Puntius sarana subnasutus* reared along with *Anabas testudineus* from the day of hatching onwards preferred to shoal with *Anabas testudineus* depending on the group size of the shoal. When the stimulus shoals were equal in number, the test fish always selected the sibling shoal regardless of their familiarity with the heterospcific *Anabas testudineus* stimulus shoal. But when the group-size of the latter increased, the test fish shifted their preference towards the larger, familiar heterospecific group. Our results indicate the overriding influence of familiarity and group-size on the sibling preferences of *Puntius sarana subnasutus*. The development of familiarity from larval stages onwards provides a good promise for composite fish culturing.

Key words : *Puntius sarana subnasutus*, *Anabas testudineus*, conspecifics, heterospecific, familiarity, shoal-size.

INTRODUCTION

Fish often organize themselves into loose social aggregations called shoals during some part or whole of their life. Individual fish make shoal choices on the basis of behavioural and phenotypic characters of the members of the shoal (Wright *et al*, 2006). The decision of with who to associate is among the most fundamental of the choices an individual can make, influencing its survival. In spite of several advantages ascribed to shoaling (Pitcher and Parrish, 1993), there are also certain associated costs such as competition for food (Krause and Ruxton, 2002) and spread of infectious disease (Cote and Poulin, 1995). The relative trade off between the two determines the shoal selection of an individual fish. Such a balance is potentially represented in the large degree of variations in shoal size observed in the wild (Croft *et al*, 2003).

Group size of a stimulus shoal is a well known factor influencing group preference and most of the fishes prefer the larger of the two shoals (Krause and Ruxton, 2002). This might be because of an increased vigilance of group members, the dilution effects or better foraging opportunities and the higher confusion effect produced in the predator (Pitcher and Parrish, 1993). However, fish may sometimes exhibit strong shoaling preference for their siblings, even when there is slight increase in the number and size of a conspecific stimulus shoal (Alex and Thomas, 2011). Further, fishes are also known to preferentially select shoals of conspecifics over shoals of heterospecifics (Krause and Godin, 1994) and shoals of individuals of the same colour (Mc Robert and Bradner, 1998).

A number of recent studies have reported the preference for unrelated familiar individuals in a range of taxa (Krause and Ruxton, 2002; Ward *et al*, 2003). However, the role of familiarity in shoal choice has been investigated mostly employing conspecific groups, differing in size of other characteristics, despite the fact that mixed species group are common in a range of taxa, including cyprinid fishes (Allan and Pitcher, 1986). In the present study, we investigated the shoaling preferences of siblings of *Puntius sarana subnasutus* reared along with an equal/larger group of heterospecific *Anabas testudineus* from the day hatching onwards. We also investigated how the familiarity developed between these two species during the early developmental stage affect choice of shoal mates in later life.

MATERIALS AND METHODS

In order to avoid the influence of familiarity among breeding pairs, *Puntius sarana subnasutus* and *Anabas tesudineus* were collected from two different ponds in Irinjalakuda (located in Avittathur and Konthulapuram respectively), Thrissur district, India, during January - March 2009 (10°25', 10°18'47" N lat. and 76°17'19", 76°12'48"E long.). The ponds were separated by more than 6km. The fishes were acclimatized with laboratory conditions for two weeks in large cement tanks (175cm x 90cm x 90cm). The tanks were provided with sand substratum and water level was maintained at 60 cm. The temperature in the laboratory was constant at 26^{0}C, with a constant light:dark cycle of 12:12. Under laboratory conditions, they were fed *ad libitum.* with commercially available tropical fish food (Marvel feeds, Aquarium systems, India). A pair of gravid fishes were selected from both species were induced to breed using GnRH analogue Ovaprim. Fish larvae were fed with *Artemia* nauplius *ad libitum*.

In order to study the influence of early social ambience on shoaling preference of *Puntius sarana subnastutus* siblings, the larvae of *Puntius sarana subnastutus* were reared in groups of twenty siblings in five small aquaria (45x23x23 cm) from the day of hatching onwards. Similarly, a second set of larvae from the same parents were also divided into five subgroups of 10 and reared with equal number of *Anabas tesudineus*. The four walls of the aquarium were covered with black paper isolating the fish from ambient cues. Apart from these experimental groups, about hundred larvae of each species (both siblings and heterospecifics) were reared separately in groups of twenty for providing respective unfamiliar stimulus shoals.

When the larvae were 3months old, shoaling preference was tested in a 70 liter aquarium (85cm x 32cm x 32cm). The aquarium was divided into two stimulus compartments using perforated clear Plexiglas sheets (measuring 16 x 32 x 32 cm each) on the right and left and a central compartment (measuring 53 x 32 x 32cm) (Fig.1). Ten centimeters in front of each side compartments, was marked as preference zone (almost equal to the length of the test fish so that the preference is recorded only when the whole body of the fish is within the preference zone), leaving a neutral zone of 33cm in the middle of the tank. An 11w compact fluorescent lamp placed above the tank lighted the set up. Three sides of the experimental aquarium were also covered with black paper to prevent interference of external stimuli. Additionally, a black curtain was tightened around the test tank. Two stimulus shoals were introduced into the side chambers. The test fish were always introduced individually into the central compartment in a presentation cage made of transparent, perforated acrylic sheets (15cm x 10cm x 27cm). The total duration of the experiment was sixteen minutes. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, the time spent by the fish within the preference zone was recorded for six minutes using a stopwatch, sitting behind the black screen and looking through a horizontal slit on the screen. Total of twenty individuals (four fishes from each of five groups of *Puntius sarana subnastutus*) were tested.

We conducted mainly two types of choice experiments and the basic protocol was same in all experiments. In our previous study we had already found that *Puntius sarana subnasutus* preferred to associate with larger sibling shoal when both stimulus shoals were composed of their siblings (Alex and Thomas, 2011). In the present study (Experiment I), we analysed whether this preference changed when the larger shoal is composed of heterospecifics. Here, the test fish were given an opportunity to swim near familiar sibling stimulus shoals and unfamiliar heterospecifics. The two stimulus groups were presented in five different ratios i.e., 25siblings vs. 25heterospecifics, 20siblings vs. 30 heterospecifics, 15siblings vs. 35 heterospecifics, 10siblings vs. 40 heterospecifics, 5siblings vs. 45 heterospecifics (ratio Ratio- 1:1, 1:1.5, 1:2.3, 1:4 and 1:9) respectively on either stimulus compartments. In the second set of experiments we tested how the familiarity with the heterospecifics from hatching periods onwards could affect the shoaling preference of the fish exhibited in experiment I. For this we repeated the first experiment using larvae reared along with the heterospecifics from the day of hatching onwards. We also switched over the positions of the stimulus shoals (in half of both experiments) to eliminate the risk of side biases and repeated the preference experiment. Since the correlation between the samples were significant, we analysed the data using parametric dependent sample two-tailed 't' test.

RESULTS

In experiment I, in which *Puntius sarana subnasutus* were given a choice between their familiar siblings and unfamiliar *Anabas testudineus*, none of the fish selected the latter stimulus shoal. The fish exhibited strong preference for siblings shoal irrespective of the difference in group-size ratios [(25S vs 25A: t =16.66, p=0.001; 20S vs 30A: t =23.26, p=0.001; 15S vs 35A: t =20.43, p=0.001; 10S vs 40A: t =10.8, p=0.001; 5S vs 45A: t =9.63, p=0.001); (S-Sarana; A- Anabas)]. Even an increase in the number of *Anabas testudineus* stimulus shoal failed to draw the attention of the test fish towards them. However, in experiment II, familiarity of *Puntius sarana subnasutus* with *Anabas testudineus* during the period of early development onwards, considerably changed its associational preference. Even though, the test fish significantly associated with the sibling group when stimulus group were equal in number (25S vs 25A: t =5.11, p=0.001), they spent more time with higher ratios *Anabas* compared to the test fish in experiment I(20S vs 30A: t =1.94, p=0.067; 15S vs 35A: t =3.018, p=0.006;

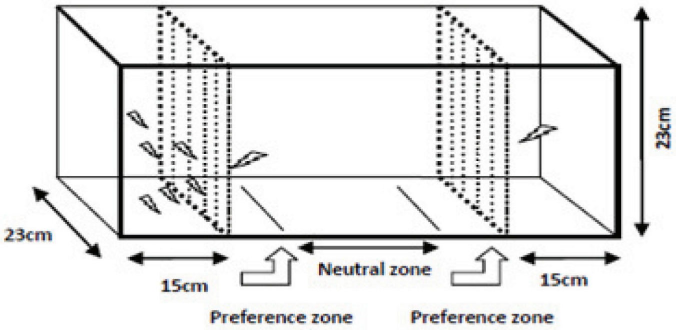

Fig. 1 : The experimental setup used to measure shoaling preferences.

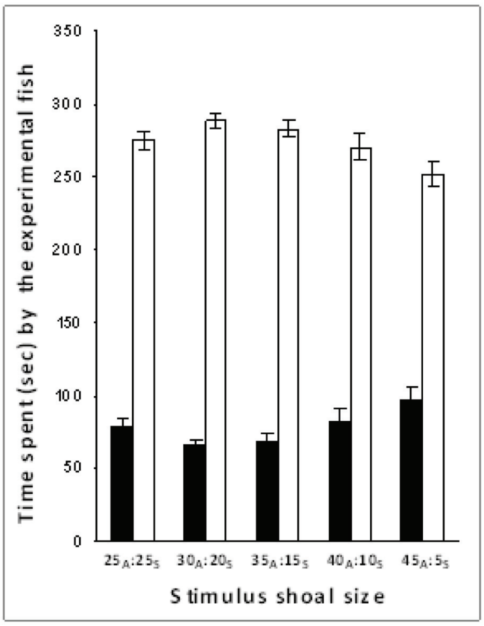

Fig. 2 : Preference of *Puntius sarana subnasutus* for siblings (S-□) and heterospecific *Anabas testudineus* (A- ■) stimulus shoals n = 20 Mean time (spent in sec) ± SE.

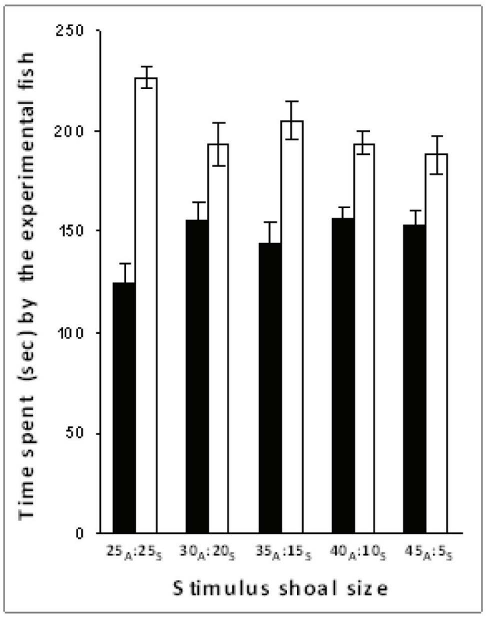

Fig. 3 : Preference of *Puntius sarana subnasutus* for siblings (S-□) and heterospecific *Anabas testudineus* (A- ■) stimulus shoals n = 20; Mean time (spent in sec) ± SE.

10S vs 40A: t =3.039, p=0.003; 5S vs 45A: t =2.29, p=0.034).

DISCUSSION

Despite the increased sibling preference exhibited by *Puntius sarana subnasutus* (Alex and Thomas, 2011), in the present study, the fish showed some degree of association preference towards the heterospecific group when they were familiar. Without the influence of familiarity with heterospecifics, they associated with familiar siblings and increased their preference for siblings with respect to increase in number of individuals in the stimulus shoal. Apart from the common benefits of shoaling (Pitcher and Parrish, 1993), association with a sibling group offers some more advantages. Griffiths and Amstrong (2002) reported significantly greater levels of resource sharing occurring between siblings than non-kin and reduced level of competition when they were related. For shoals composed of familiar individuals also there exist similar benefits (Chivers *et al*, 1995). In the present study, *Puntius sarana subnasutus* preferentially assorted with familiar *Anabas testudineus* regardless of their heterospecificity when they were reared together from the day of hatching onwards.

The behavioural patterns of fish are the result of innate (‘built-in’) patterns of maturation (developmental changes) and of learning processes (imprinting and trial-and-error learning) (Kieffer and Colgan, 1992). Social environment is also implicated as an important factor in shaping the ontogeny, evolution of brain architecture and subsequent learning. A kind of behavioural modification as a result of learning from the hatching period onwards may account for the development of familiarity between different species of fishes. Shoaling with familiars may lead to more stable dominance hierarchies, and thus, to a reduction of aggressive behaviour between the members of a shoal (Gómez-Laplaza, 2005). In addition, shoaling with familiar individuals facilitates the evolution of altruistic behaviour (Utne-Palm and Hart, 2000). Sticklebacks, for instance, preferentially joined individuals who had proven to be cooperative in the past (Milinski *et al*, 1990). Furthermore, groups of familiar individuals have shown an improved antipredator behaviour similar to shoals of related individuals. Recent work in sticklebacks have shown that adult, nonreproductive individuals prefer to shoal with familiar fish (Ward *et al*. 2005) and with familiar siblings (Frommen and Bakker 2004).

The mechanisms employed by individuals to discriminate familiars were also based on odour cues (Brown and Smith, 1994) or visual cues (Griffiths and Magurran, 1997a) and said to be developed gradually. In the guppy it took over 12 day period (Griffiths and Magurran, 1997b) and there is an upper limit of forty individuals that the fish can become familiar with (Griffiths and Magurran, 1997a). Whatever may be the mechanism, there is much experimental evidence to show that once this familiarity has been established, the association preferences can be maintained for long periods of time, even when regular reinforcement is not given (Griffiths and Magurran, 1997b). In the present study even though the overall preference for *Anabas testudineus* increased in the case of test fish reared in familiar with *Anabas testudineus*, the test fish traded off their preference for siblings only when the latter stimulus shoals were larger. Thus the familiarity when enforced with an increase in group-size overrode the effect of genetic relatedness.

Associations with familiar heterospecifics can also provide a number of general benefits offered from a sibling shoal. Guppies showed an active preference for sword tails when juveniles of the former species were raised with the latter, potentially suggesting imprinting as a mechanism (Warburton and Lees, 1996). Familiarity developed from the day of hatching onwards helped the test fish to recognize the heterospecific stimulus shoal which were composed of unfamiliar individuals of the same species. Advantages of familiarization with heterospecifics can also be applied in the field of composite fish culturing.

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REFERENCES

Alex J and Thomas K J (2011) Factors influencing shoaling preferences in *Puntius sarana subnasutus*. *Curr. Sci*. **100,** 633-634.

Allan J R and Pitcher T J (1986) Species segregation during predator evasion in cyprinid fish shoals. *Freshwater. Biol.* **16,** 653–659.

Brown G E and Smith R J F (1994) Fathead minnows use chemical cues to discriminate natural shoalmates from unfamiliar conspecifics. *J. Chem. Ecol*. **20,** 3051-3061.

Chivers D P, Brown G E and Smith R J F (1995) Familiarity and shoal composition in fathead minnows (*Pimephales promelas*): implications for antipredator behaviour. *Can. J. Zool*. **73**, 955-960.

Cote I and Poulin R (1995) Parasitism and group size in social animals: a meta-analysis. *Behav. Ecol.* **6**, 159-165.

Croft D P, Arrowsmith B J, Bielby J, Skinner K, White E, Couzin I D, Magurran A E, Ramnarine, I and Krause J (2003) Mechanisms underlying shoal composition in the Trinidadian guppy, *Poecilia reticulata*. *Oikos* **100**, 429–438.

Frommen J G and Bakker T C M (2004) Adult three-spined sticklebacks prefer to shoal with familiar kin. *Behav*. **141,** 1401–1409.

Gómez-Laplaza L M (2005) The influence of social status on shoaling preferences in the freshwater angelfish (*Pterophyllum scalare*). *Behav.* **142,** 827–844**.**

Griffiths S W and Magurran A E (1997a) Schooling preferences for familiar fish vary with group size in a wild guppy population. *Proc. R. Soc. Lond. [Biol]*. **264,** 547–551.

Griffiths S W and Magurran A E (1997b) Familiarity in schooling fish: how long does it take to acquire? *Anim. Behav*. **53**, 945–949.

Griffiths S W and Armstrong J D (2002) Kin-biased territory overlap and food sharing among Atlantic salmon juveniles. *J. Anim. Ecol.* **71**, 480–486.

Kieffer J D and Colgan P W (1992) The role of learning in fish behaviour. Reviews In: *Fish Biology and Fisheries* **2,** 125-143.

Krause J and Godin J G J (1994) Shoal choice in the banded killifish (*Fundulus diaphanus*, Teleostei, Cyprinodontidae): effects of predation risk, fish size, species composition, and size of shoals. *Ethol*. **98,** 128-136.

Krause J and Ruxton G D (2002) *Living in groups.* Oxford university press, New York.

Mc Robert S P and Bradner J (1998) The influence of body coloration on shoaling preferences in fish. *Anim. Behav.* **56,** 611-615.

Milinski M D, Külling D and Kettler R (1990) Tit for tat: sticklebacks (*Gasterosteus aculeatus*) "trusting" a cooperating partner. *Behav Ecol.* **1**, 7–11.

Pitcher T J and Parrish J K (1993) Functions of shoaling behaviour in teleosts. In: *Behaviour of Teleost Fishes* (Ed Pitcher T J) pp 363- 439, Chapman and Hall, London.

Utne-Palm A C and Hart P J B (2000) The effects of familiarity on competitive interactions between threespined sticklebacks. *Oikos* **91**, 225–232.

Warburton K and Lees N (1996) Species discrimination in guppies: learned responses to visual cues. *Anim. Behav*. **52,** 371–378.

Ward A J W, Axford S and Krause J (2003). Cross-species familiarity in shoaling fishes. *Proc. R. Soc. Lond. B.* **270**, 1157–1161.

Ward A J W, Holbrook R I, Krause J and Hart P J B (2005) Social recognition in sticklebacks: the role of direct experience and habitat cues. *Behav. Ecol. Sociobiol*. **57,** 575–583.

Wright D, Ward A J W, Croft D P and Krause J (2006) Social Organization, Grouping, and Domestication in Fish. *Zebrafish*. **3**, 141-155.

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ONSET OF SHOALING PREFERENCE IN PUNTIUS SARANA SUBNASUTUS

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ABSTRACT

Fishes are social organisms and form social groups in some stage of their life cycle. Loose aggregations of fishes forming a social assembly are called shoals. Fish that join with a shoal usually gains a wide variety of benefits. In the present study, the onset of shoaling behaviour in a freshwater fish, *Puntius sarana subnasutus* was tested using induced breeding technique. In dichotomous choice test, 10-days-old (mean body length = 9 mm) larvae and 20, 30 and 40-days-old juveniles (Mean body length 2.8, 3.1, 4.6 cm respectively) were given the opportunity to swim near shoals of ten fish or a single fish. The larvae on their completion of fin-ray development and start of scale formation to attainment of sexual maturity is said to be juveniles. When introduced into the experimental arena, the larvae as well as the juveniles demonstrated shoaling behavior, swimming near a group of fish rather than a single fish. The fish clearly exhibited an increasing preference to associate with a group corresponding to an increase in their age and the juveniles when become 40-days old, showed little preference for a single fish. The significance of developmental process in shoaling preference of fishes is discussed.

KEY WORDS: Shoaling preference, Larvae, Juveniles, *Puntius sarana subnasutus*

INTRODUCTION

Shoaling behavior in fish has been a focus of behaviuoral studies for over fifty years (Keenleyside, 1955). Shoaling confers a number of individual selective advantages to the group members, mainly related to predator avoidance and better foraging opportunities. The “many eyes” advantages of shoaling predict the benefits of better inspection of surroundings and continual monitoring of the predator. It also forms a “confusion” effect whereby the hunting efficacy of predators is reduced due to disorientation and the inability to target an individual fish (Pitcher and Parrish, 1993). Further, dense groups can be perceived as a large active object and therefore repel predators through mimicry (Breder, 1959). Besides these actual predation advantages, inspection can also have an effect on making success, with inspectors able to advertise their fitness qualities (Evans et al., 2002). Shoaling allows the fish to exploit more feeding patches more rapidly, possibly with the advantages from a better predator inspection, the fish can access patches associated with higher risk. This in turn increases the intensity and efficiency of feeding (Pitcher and parish, 1993; Sackley and Kaufman, 1996). Shoaling also provides better hydrodynamic advantage by reducing the amount of energy required to move for shoaling fishes as opposed to solitary fish (Wright et al., 2006).

However, there are certain costs associated with shoaling. The individuals may experience higher degree of competition when they join a shoal (Krause, 1994) and increase the risk of parasitic infection (Dugatkin et al., 1994). The relative trade-off between these benefits and costs can determine the choice of joining with a particular shoal. An individual fish can take decisions about joining a shoal based on the characteristics of the existing group such as group size (Wong and Rosenthal, 2005), body size (Krause and Ruxton, 2002) colour (Mc Robert and Bradner, 1998), kinship (Frommen et al., 2007; Alex and Thomas, 2011) etc. In the present study we tried to analyse the influence of age on the shoaling behavior of larvae and juveniles of *Puntius sarana subnasutus* and their ability to discriminate difference in group size of two stimulus shoals is tested.

MATERIALS AND METHOD

Puntius sarana subnasutus were collected from canals associated with paddy fields of Irinjalakuda (10°25', 10°18'47" N lat. and 76°17'19", 76°12'48"E long), Thrissur district, India, during January - March 2009 and acclimatized with laboratory conditions for two weeks in large cement tanks (175cm x 90cm x 90cm). The tanks were provided with sand substratum and water level was maintained at 60 cm. The temperature in the laboratory was constant at 26^0C, with a constant light:dark cycle of 12:12. Under laboratory conditions, they were fed *ad libitum* with a range of commercially available tropical fish food (Marvel feeds, Aquarium systems, India). Gravid pairs were selected and induced to breed using GnRH analogue Ovaprim. Healthy larvae were reared in the laboratory in four glass tanks (45cm x 23cm x 23cm) in a well-aerated condition and fed with *artemia* nauplius larvae.

EXPERIMENTAL PROCEDURE

We tested the ability of the larvae and juveniles to discriminate group size difference between two stimulus shoals. In a dichotomous choice test, 10-days-old larvae (mean body length = 9 mm) and 20-30-40-days old juveniles (Mean body length 2.8, 3.1, 4.6 cm respectively) were given an opportunity to swim near either a single sibling fish or a group of 10 siblings. Shoaling preferences were tested in 30 liter a aquarium (45x23x23 cm), which was divided into two stimulus shoal compartments (measuring 15x23x23 cm each) and a central compartment (measuring 15x23x23 cm) (Fig.1). Perforated clear Plexiglas sheets separated compartments. Ten centimeters in front of each side compartments was marked as preference zone (almost equal to the length of the test fish so that the preference is recorded only when the whole body of the fish is within the preference zone), leaving a neutral zone of in the middle of the tank. An 11w compact fluorescent lamp placed above the tank lighted the set up. Three sides of the aquarium were covered with black paper to prevent interference of external stimuli. Additionally, a black curtain was tightened around the test tank. Two stimulus shoals were introduced into the side chambers. The test fish were always introduced individually into the central compartment in a presentation cage made of transparent, perforated acrylic sheets (15cm x 10cm x 27cm). The total duration of the experiment was sixteen minutes. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, the movements of the fish were recorded for six minutes using a stopwatch, sitting behind the black screen and looking through a horizontal slit on the screen. We also switched the positions of the stimulus shoals (in half of the experiments) to eliminate the risk of side biases and repeated the preference experiment.

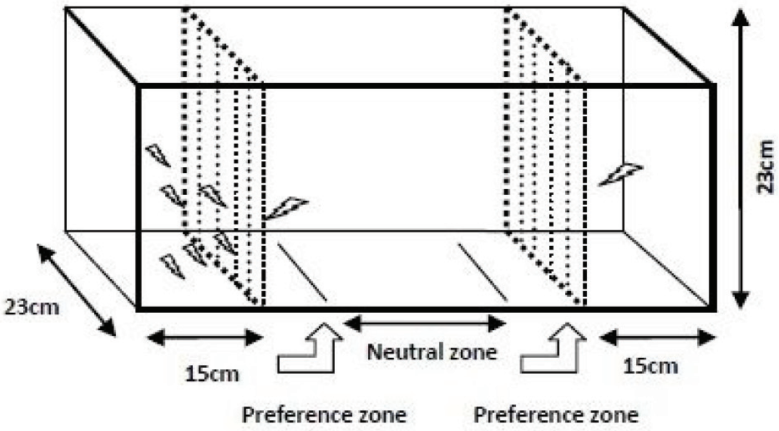

Figure 1 : Diagram showing the experimental setup

Data were analyzed using the parametric dependent sample two-tailed ‘t’ test [SPSS 11.0.1 statistical package].

RESULTS

The larvae as well as the juveniles always preferred to shoal with a group of ten individuals rather than a single fish indicating the overriding influence of group-size on the shoaling behavior and their ability to discriminate shoals of different size. The fish clearly exhibited an increasing preference to associate with a group corresponding to an increase in their age. Even though 10-days-old larvae and 20 and 30- days old juveniles explored near the single fish, they discriminated the group size difference between the stimulus shoals and spent significant time with the larger group (t = 4.56, p = 0.000; t = 5.57, p = 0.000; t = 6.91, p = 0.000 respectively). However, 40-days old juveniles showed very little preference for the single fish (t = 12.84, p = 0.000) Fig. 2.

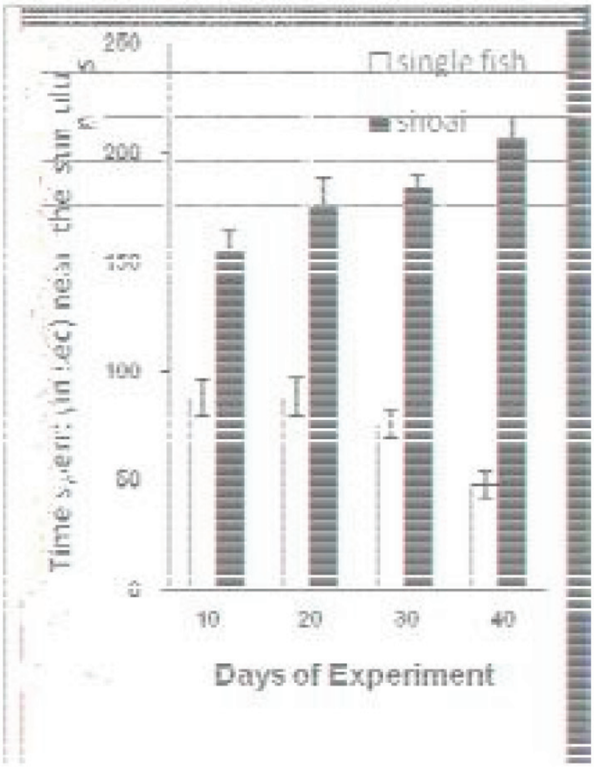

Figure 2 : Preference of juvenile *Puntius sarana subnasutus* for Single (□) / ten (■) sibling stimulus shoals n = 20

Mean time (spent in sec) ± SE

DISCUSSIONS

Fishes are able to discriminate among shoals of different size, based on the circumstances (Krause, et al., 1998, Hoare et al., 2004 and Wong and Rosenthal, 2005) and always preferred to associate with the larger shoal probably due to antipredator advantages (Harger and Helfman, 1991), better foraging opportunity (Pitcher et al., 1982) and better transmission of information (Lachlan, 1998). In the present study also, the fish performed a better discrimination of group-size differences and selected the larger group to shoal. The preference increased correlated with an increase in age of the fish, clearly exemplifying a close association between the age and learning capacity of the fish. Many experiments have clarified the genetic and learned basis of shoaling behaviour (Giles and Huntingford, 1984; Magurran, 1989; Magurran and seghers, 1990). Learning is a complex ontogenic process that allows animals to acquire, store and subsequently use information about the environment. This information complements genetic prowess, allowing animals to fine tune their behavior according to circumstances. The increased preference exhibited by the juveniles to associate with a group may be aroused when their innate ability to form a shoal at the larval stage is supplemented from the learned advantages of associating with a group. This increased social interaction might have been led to subsequent changes in the brain.

Scace et al., (2006) in a study explored the social organization on the brains and behaviours of cichlid fishes using the Insel and Fernald (2004) framework for processing of social information. The social behavior of cichlid fish relied on visual stimuli -> social meaning -> social motivation -> social behavior. In fish, the telencephalon is involved in a variety of social and cognitive behaviours (Demski and Beaver, 2001) and the telencephalic size is correlated with social complexity, social learning, enhanced visual cognition and innovation (Reader and Laland, 2002). In the present study, the increasing preference for a larger group with the increase in age may be correlated with the increase in size of brain of the fish. In 50-52 days old sturgeon fishes the central nervous system acquires definite structure and form stabilization of conditional reflex reactions (Obukhov, 1996). Further studies are necessary to determine the role of brain size of larval and juvenile fishes in differentiating the group-size and the role of other factors such as surface area, contour length, density or extent of shoal movement in aiding this particular behavior.

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REFERENCES

1. Alex, J. & Thomas, K. J., (2011). Factors influencing shoaling preferences in *Puntius sarana subnasutus, Current Science*, 100, 633-634
2. Breder, C. M., (1959). Studies on social groupings in fishes. *Bulletin of the American Museum of Natural History,* 98, 1-27
3. Demski, L. S., & Beavver, J. A., (2001). Brain and cognitive function in teleost fishes. In: *Brain and cognition.* (eds. Roth, G. and M. F. Wulliman,), John Wiley and Sons, Inc., New York, pp. 297-332
4. Dugatkin, L. A., Fitzgerald, G. J., & Lavoie, J., (1994). Juvenile three-spined stickle backs avoid parasitized conspecifics. Environmental Biology of Fishes, 39, 215-218
5. Evans, J. P., Kelly, J. L., & Ramnarine, I. W., (2002). Female behavior mediates male courtship under predation risk in the guppy (*Poecilia reticulate*) *Behavioural Ecology and Socio Biology,* 52, 496-502

6. Frommen, J. G., Mehlis, M., Brendler, C., & Bakker, T. C. M., (2007). Shoaling decisions in three-spined sticklebacks (Gasterosteus aculeatus) familiarity, kinship and inbreeding. *Behavioural Ecology and Socio Biology,* 61, 533-539
7. Giles, N., and Huntingford, F. A., (1984). Predation risk and interpopulation variation in anti-predator behaviour in the three spined sitickleback. Animal Behaviour, 32, 264-275
8. Harger, M. C., & Helfman, G. S., (1991). Safety in numbers - shoal size choice by minnows under predatory threat. *Behavioural Ecology and Socio Biology,* 29, 271-276
9. Hoare, D. J., Couzin, I. D., Godin, J. G. J., & Krause, J., (2004). Context Dependent group size choice in fish. *Animal Behaviour,* 67, 155-164
10. Insel, T. R., & Fernald, R. D., (2004). How the brain processes social information: searching for the social brain. *Annuval Review of Neuroscience,* 27, 697-722
11. Keenleyside, M.H.A., (1955). Some aspects of the schooling behaviour of fish. *Behaviour*, 8, 183-248
12. Krause J., & Ruxton, G. D., (2002). Living in groups. New York: Oxford university press.
13. Krause, J., (1994). The influence of food competition and predation risk on size-assortative shoaling in juvenile chub (*Leuciscus cephalus*). *Ethology,* 96, 105-116
14. Krause, J., Godin, J. G. J., & Brown, D., (1998). Body length variation within multi-species fish shoals: the effects of shoal size and number. *Oecology,* 114, 67-72

15. Lachlan, R. F., Crook, S. L., & Laland K. N., (1998). Who follows whom? Shoaling preferences and social learning of foraging information in guppies. *Animal Behaviour*, 56(1), 181-190

16. Magurran, A. E., (1989). The inheritance and development of minnow anti-predator behavior. *Animal Behaviour*, 39, 834-842

17. Magurran, A. E., & Seghers, B. H., (1990). Population differences in predator recognition and attack cone avoidance in the guppy. *Animal Behaviour*, 40, 443-452

18. Mc Robert, S. P., & Bradner, J., (1998). The influence of body coloration on shoaling preferences in fish. *Animal Behaviour*, 56, 611-615

19. Obukhov, D. K., (1996). Developments of the CNS of Sturgeon fishes grown under different ecological condition. *Proceedings of Intnatonal Congress*

20. Pitcher, T. J., & Parrish, J. K., (1993). Functions of shoaling behaviour in teleosts. In: *Behaviour of Teleost Fishes* (ed. Pitcher, T. J.), Chapman and Hall, London, 363-439

21. Pitcher, T. J., Magurran, A. E., & Winfield, I., (1982). Fish in larger shoals find food faster. *Behavioural Ecology and Socio Biology*, 10, 149-151

22. Reader, S. M., & Laland, K. N., (2002). Social Intelligence, innovation and enhanced brain size in primates. *Proceedings of the Naional Academy of Science, U. S. A.,* 99(7), 4436-4441

23. Sackley, P. G., & Kaufman, L. S., (1996). Effect of predation on foraging height in a planktivorous coral reef fish, Chromis nitidia. *Copeia*, 3, 726-729

24. Scace, J., Dobberfuhl., Higgins, E., & Shumway, C., (2006). Complexity and the evolution of the *social brain. Proceedings of .6th International Congress.*

25. Wong, B. M., & Rosenthal, G.G., (2005). Shoal choice in swordtails when preferences conflict. *Ethology,* 111: 179-186

26. Wright, D., Nakamichi, R., Krause, J., & Butlin, R. K. (2006). QTL Analysis of behavioural and morphological differentiation between wild and laboratory zebra fish (*Danio rerio*). *Behavioural Genetics,* 12, 1-4.

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Influence of genetic relatedness and shoal size on shoaling preferences in juvenile *Puntius sarana subnasutus* (Hamilton Valenciennes)

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When presented with stimulus shoals of siblings and conspecifics in equal number, *P. sarana subnasutus* were able to discriminate their siblings and preferred to associate with them. Given a choice between large shoal and a small shoal consisting of siblings, the juvenile fish preferred to associate with larger stimulus group to the smaller one. However, juveniles traded off their preference for sibling shoal with large non-sibling conspecific stimulus group, regardless of the possible benefits gained from associating with sibling shoals. The results reveal the ability of fish to discriminate their siblings during their early developmental stage and the overriding influence shoal size in the context of shoaling preference in *P. sarana subnasutus*.

Keywords: Juvenile fish, *Puntius sarana subnasutus*, Shoaling, Social preference

Fish are strikingly social organisms and more than 25% of the approximately 28000 species of teleosts form social groups throughout their lives and over 50% school as juveniles[1]. Fish exhibit shoaling preferences for groups consisting of conspecifics[2] or familiar individuals[3]. Individuals often assort with respect to characteristics such as body-size[4], colour[5], species[6], and relatedness[7,8]. Another important attribute that influences shoaling decision of individual fish is the group size. The number of individuals in a stimulus group plays a role in the shoal choice preferences of fish[9,10]. In these studies, in spite of density-dependent fitness pay offs such as competition, most of the individuals preferred to join with a larger group. The fish may prioritize their benefits and decide to join with a particular shoal[11].

Recognition involves the production of a cue or signal by one individual or a group and evaluation of that by another individual[12] in the context of decision making. Fish are able to recognize their kin[13] and differentially respond to kin and unrelated conspecifics. The ability to discriminate kin from non-kin provides several benefits, especially in the context of mate choice[14]. Individuals who recognize kin are able to avoid reproduction with related individuals and thus prevent the disadvantages of inbreeding. Further, shoaling with kin reduces competition and enhances antipredator behaviours[15]. In the present study, influence of kin recognition and shoal size has been investigated on shoaling preference of juveniles of *Puntius sarana subnasutus* (Hamilton Valenciennes).

Materials and Methods

Puntius sarana subnasutus were collected from the ponds of Irinjalakuda, Thrissur district, India (10°25, 10°18 47 N lat. and 76°17 19, 76°12 48 E long.) during January-March 2009. In order to avoid relatedness among breeding pairs, two ponds located in Avittathur and Konthilapuram, which were separated by more than 6 km were selected. The fish were acclimatized with laboratory conditions for two weeks in large cement tanks (175 × 90 × 90 cm). The tanks were provided with sand substratum and water level was maintained at 60 cm. The temperature in the laboratory was constant at 26 °C, with a constant light:dark cycle of 12:12 h. Under laboratory conditions, the fish were fed *ad libitum* with commercially available tropical fish food (Marvel feeds, Aquarium Systems, India).

Five pairs of gravid fish were selected from fish collected from Avittathur pond and two pairs from fish collected from Konthilapuram pond and induced to breed using GnRH analogue Ovaprim. Fish larvae

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were fed with *Artemia* nauplius *ad libitum.* Fifty larvae from each of the seven breeding pairs were selected and reared in separate tanks. The larvae of fish collected from Avittathur breeding pairs were used for testing preference of juveniles to sibling (Experiment I) and conspecific (Experiment II) shoals. Another 50 larvae of the five Avttathur breeding pairs were reared separately from the test group in order to provide the respective stimulus sibling groups of experiment I. The larvae produced from fish collected from Konthilapuram pond were used as stimulus conspecifics for assessing the trade off point for shoal size preference to siblings and conspecifics (Experiment II).

Shoaling preferences were tested in a 30 liter aquaria (55 × 23 × 23 cm), which was divided into two stimulus compartments using perforated clear Plexiglas sheets (measuring 15 × 23 × 23 cm each) on the right and left and a central compartment (measuring 25 × 23 × 23 cm) (Fig. 1). Seven centimeters in front of each side compartments was marked as preference zone (almost equal to the length of the sibling fish so that the preference is recorded only when the whole body of the fish is within the preference zone), leaving a neutral zone of 11 cm in the middle of the tank. An 11 w compact fluorescent lamp placed above the tank lighted the set up. Three sides of the aquarium were covered with black paper to prevent interference of external stimuli. Additionally, a black curtain was tightened around the test tank. Two stimulus shoals were introduced into the side chambers. The test fish were always introduced individually into the central compartment in a presentation cage made of transparent, perforated acrylic sheets (15 × 10 × 27 cm). The total duration of the experiment was 16 min. Ten minutes were given to the test fish to assess the stimulus shoals and thereafter, the time spent by the fish within the preference zone was recorded for six minutes using a stopwatch, sitting behind the black screen and looking through a horizontal slit on the screen.

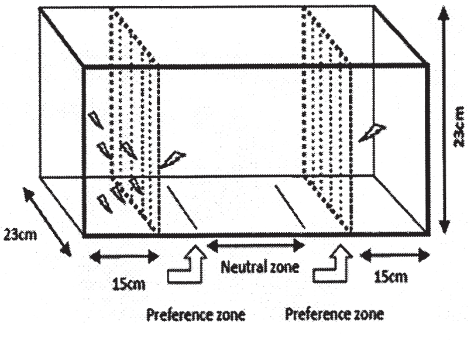

Fig. 1—The experimental setup used to measure shoaling preferences; perforated clear Plexiglas separated the right and left stimulus shoals

Mainly two types of choice experiments were conducted and the basic protocol was same in all experiments. To test the influence of shoal size on shoaling preference of juveniles of *P. sarana subnasutus* to siblings, (Experiment 1) two sibling shoals in five different numerical combinations (25 siblings *vs.* 25 siblings, 20 siblings *vs.* 30 siblings, 15 siblings *vs.* 35 siblings, 10 siblings *vs.* 40 siblings and 5 siblings *vs* 45 siblings; ratio: 1:1, 1:1.5, 1:2.3, 1:4 and 1:9 respectively) were presented on either side. In the second set of experiments the juveniles were tested for their ability to recognize siblings over conspecifics and the influence of group-size over sibling preference by providing two stimulus shoals—one containing siblings and the other containing non sibling conspecifics—in the same ratios as above. The experiment was repeated in 20 individuals (four fish from each of the five Avittathur breeding pairs). The positions of the stimulus shoals (in half of the experiments) were switched to eliminate the risk of side biases and repeated the preference experiment.

Two-tailed non parametric '*t*' test was used for analyzing the data, n=20 [SPSS 11.0.1 statistical package].

Results

The juveniles always preferred to shoal with the larger stimulus shoals indicating the overriding and of shoal size on shoaling preference, and their ability to discriminate shoals of different size. In the first experiment, when the number of siblings were equal in both the stimulus shoals, the fish did not show any preference for a particular group (Fig. 2, 25s *vs* 25s: t_{19} = 1.06, P = 0.512). Nevertheless, as the number of individuals in the sibling stimulus shoal increased, they selected the larger group (Fig. 2, 20s *vs* 30s: t_{19} = 2.48, P = 0.023, 15s *vs* 35s: t_{19} = 2.89, P = 0.009, 10s *vs* 40s: t_{19} = 3.24, P = 0.004 and 5s *vs* 45s: t_{19} = 6.87, P = 0.000). In the second experiment, the juveniles exhibited a strong preference for their siblings when they were simultaneously presented with the siblings and conspecifics stimulus shoals in equal number (Fig. 3, 25s *vs* 25c: t_{19} = 5.84, P = 0.000). Similar, but non-significant tendency for siblings was also found

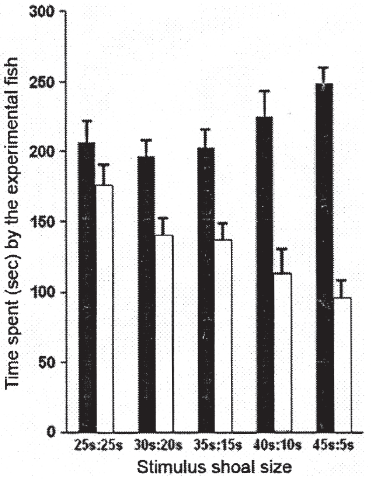

Fig. 2—Shoal size preference of juvenile *P.sarana subnasutus* for sibling stimulus shoals n = 20; Mean time (spent in sec) ± SE

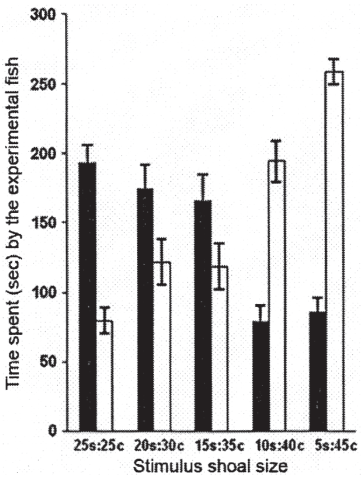

Fig. 3—Shoal size preference of juvenile *P.sarana subnasutus* for sibling (s-■) and conspecific (c-□) stimulus shoals; n = 20 Mean time (spent in sec) ± SE

at slightly increased group size of conspecific stimulus shoal (Fig. 3, 20s *vs* 30c: t_{19} = 1.63, P = 0.120; 15s *vs* 35c: t_{19} = 1.408, P = 0.175). However, this preference for siblings diminished when the number of individuals in the conspecific shoal was further increased (10s *vs* 40c: t_{19} = 4.89, P = 0.000, 5s *vs* 45c: t_{19} = 8.98, P = 0.000).

Discussion

Density-dependent variables have long been established as an important area of ecological research, but the effects of the local density of kin on grouping behaviour of juveniles were not well understood. It is well known that living in groups can provide both potential benefits (greater protection from predators, improved foraging efficiency etc.) and involve costs (increased competition for resources, contagious disease etc.) for the members of the group[16]. Hoare *et al.*[4] have shown that when the risk of predation is high, banded killifish (*Fundulus diaphanus*) preferred to join larger shoals; on the other hand, when there is the possibility to get food they tend to move toward a smaller group. In the present study, the juveniles preferred a larger group when both stimulus shoals consisted of siblings. Association with a sibling group usually offers many benefits to the individual, although, there are some disadvantages. The proportions of violent agonistic acts are less in pure sibling groups, decreasing the risk of physical injury, which in turn may increase their survival rate until reproduction[17]. However, in a sibling shoal the individual may experience inbreeding cost, increased risk of parasitic load, disease transmission, etc.[14]. Thus, shoal choice is not random process but influenced by a range of factors. The ability to discriminate which shoal is larger will be useful for the juveniles as well, since they could join the larger or the smaller shoal depending on the context.

Further, when the choice was between an equal number of siblings and conspecific shoals, the juveniles preferred to shoal with their siblings. Fishes have been shown to discriminate between individuals based on their relatedness. Recognition usually develops following a simple sequence of events either visually or through olfaction. Studies have established a high degree of similarity between MHC genotypes of related individuals which affects the odour of its carrier allowing detection and recognition between individuals[18]. It is suggested that as a part of the nascent learning during a sensitive period of development or by the innate recognition ability, individuals gather information and record it as a template in the brain[19]. Once acquired, such recognition templates have the ability to persist without reinforcement over a considerable period[20]. Individuals exchange their phenotypic chemical and visual signals and compare this to the template. If the signal matches the template, recognition takes place[21].

Even though the juveniles successfully identify their siblings from their conspecifics, this recognition ability never prevented them from selecting a larger group of conspecifics from a smaller group of siblings. The present results indicate the tendency in *P.sarana subnasutus* for orienting themselves towards a larger group develops during the juvenile stage itself. It is reported that most freshwater fishes are social and live in large aggregates, gaining benefits such as hydrodynamic advantages during locomotion or increased predator confusion, collective vigilance, social information transfer and foraging benefits[22]. Barber and Wright[9] found that conspecific familiarity could influence the shoal choice in adult *Phoxinus phoxinus,* when shoals differ in size. In the present study, the failure of the fish to exhibit a preference for their conspecifics for a slight increase in conspecific shoal size may be due to their unfamiliarity with the conspecifics. Here the genetic relatedness played a prominent role in shoal selection. Many studies have shown that fish gains many benefits while in a sibling group[13,17]. However, the fish traded off the preference for their siblings when the difference in stimulus shoal size of siblings and conspecifics became much larger. Further studies using other less related heterospecific species are necessary to ascertain whether this preference for a larger conspecific group is due to the overriding effect of larger shoal size over genetic relatedness. Similarly, the exact criteria for the selection of a relatively larger group are also yet to be established. It may be based on the continuous variable such as surface area, contour length, density or amount of shoal movement[23]. Further studies are needed to clarify which factor induced the fish to trade off its preference for a related group, when the number of individuals in an unrelated group increases.

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References:

1 Shaw E, Schooling Fishes. *American Scientist* 66 (1978) 166.

2 Barber I, Downy L C & Braithwaite V A, Parasitism, oddity and the mechanism of shoal choice, *J Fish Biol,* 53 (1998) 1365.

3 Binoy V V & Thomas K J, The climbing perch (*Anabas testudineus* Bloch), a freshwater fish, prefers larger unfamiliar shoals to smaller familiar shoals, *Curr Sci,* 86 (2004) 207.

4 Hoare D J, Couzin T D, Godin G J & Krause J, Context-dependent groupsize choice in fish, *Anim Behav,* 67 (2004) 155.

5 Mc Robert S P & Bradner J, The influence of body coloration on shoaling preferences in fish, *Anim Behav,* 56 (1998) 611.

6 Krause J, Butlin R K, Peuhkuri N & Pritchard V L, The social organization of fish shoals: a test of the predictive power of laboratory experiments for the field, *Biolog Rev Cambridge Philosophical Society,* 75 (2000) 477.

7 Frommen J G, Mehlis M, Brendler C & Bakker T C M, Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*) familiarity, kinship and inbreeding, *Behav Ecol Sociobiol,* 61 (2007) 533.

8 Alex J & Thomas K J, Factors influencing shoaling preferences in *Puntius sarana subnasutus, Curr Sci,* 100 (2011) 633.

9 Barber I & Wright H A, How strong are familiarity preferences in shoaling fish? *Anim Behav,* 61 (2001) 975.

10 Wong B M & Rosenthal G G, Shoal choice in swordtails when preferences conflict, *Ethology,* 111 (2005) 179.

11 Krause J & Godin J G J, Shoal choice in the banded killifish (*Fundulus diaphanus,* Teleostei, Cyprinodontidae): Effects of predation risk, fish size, species composition, and size of shoals, *Ethology,* 98 (1994) 128.

12 Sherman P W, Reeve H K & Pfenning, D W, Recognition systems, in *Behavioural ecology: An evolutionary approach,* 4th ed., edited by J R Krebs & N B Davies (Blackwell science, Oxford) 1997, 69.

13 Ward A J W & Hart P J B, The effects of kin and familiarity on interactions between fish, *Fish Fish,* 4 (2003) 348.

14 Poulin R, Parasitism and shoal size in juvenile sticklebacks: conflicting selection pressures from different ectoparasites? *Ethology,* 105 (1999) 959.

15 Grand T C & Dill L M, The effect of group size on the foraging behaviour of juvenile coho salmon: Reduction of predation risk or increased competition, *Anim Behav,* 58 (1999) 443.-

16 Olsen K H & Winberg S, Learning and sibling odour preference in juvenile arctic char, *Salvelinus aplinus* (L.), *J Chem Ecol,* 22 (1996) 773.

17 Penn D J, The scent of genetic compatibility: sexual selection and the major histocompatibility complex genes, *Ethology,* 108 (2002) 1.

18 Olsen K H, Grahn M & Lohm J, Influence of MHC on sibling discrimination in Arctic charr, *Salvelinus aplinus* (L.), *J Chem Ecol,* 28 (2002) 783.

19 Courtenay S C, Quinn T P, Dupuis H M C, Groot C & Larkin P A, Discrimination of family specific odours by juvenile coho salmon: roles of learning and odour concentration, *J Fish Biol,* 58 (2001) 107.

20 Waldman B, Mechanisms of kin recognition, *J Theor Biol,* 128 (1987) 159.

21 Pitcher T J & J K Parrish, Functions of shoaling behaviour in teleosts, in *Behaviour of teleost fishes,* edited by T J Pitcher (Chapman and Hall, London) 1993, 363.

22 Agrillo C, Dadda G, Serena G & Bisazza A, Use of number by fish, *PLoS ONE,* 4 (2009) 4786.