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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, $t = 3.759$, $n = 20$, $P = 0.0006$).

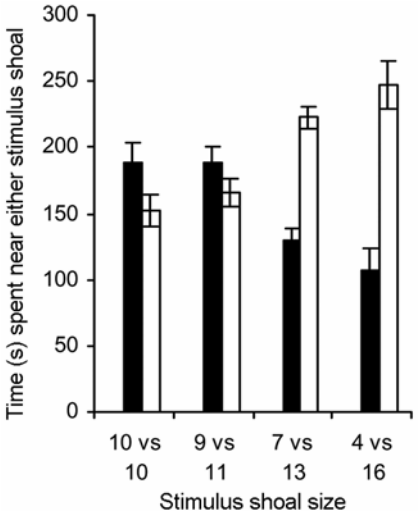

Figure 1. Preference of individual *Puntius sarana subnasutus* for phenotypically similar smaller/larger sibling stimulus shoals. Mean time (spent in sec) ± SE near smaller group (■) and larger group (□) of siblings shoals; n/N = 20/80.

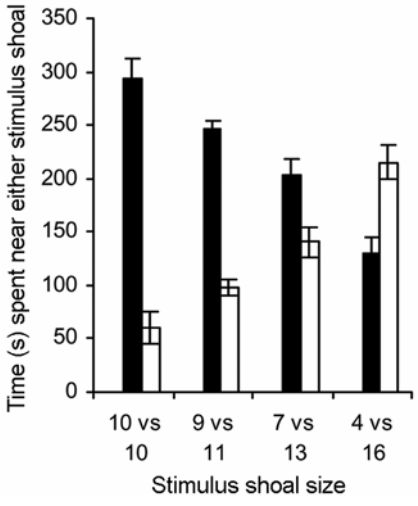

Figure 2. Preference of individual *P. sarana subnasutus* for similar-sized sibling/dissimilar-sized larger, non-sibling stimulus shoals. Mean time (spent in seconds) ± SE near sibling (■) and non-sibling (□) shoals; n/N = 20/80.

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.

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