*Brief communication*

# Sensory cues employed for the acquisition of familiarity-dependent recognition of a shoal of conspecifics by climbing perch (*Anabas testudineus* Bloch)

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In this study we showed that a freshwater fish, the climbing perch (*Anabas testudineus*) is incapable of using chemical communication but employs visual cues to acquire familiarity and distinguish a familiar group of conspecifics from an unfamiliar one. Moreover, the isolation of olfactory signals from visual cues did not affect the recognition and preference for a familiar shoal in this species.

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## 1. Introduction

Many shoaling fishes prefer to join a shoal of known individuals because living with familiar conspecifics enhances the benefits of social life due to reduced aggression, sharing of resources and more efficient transfer of information among the members of the group (Utne-Palm and Hart 2000; Price and Rodd 2006). Other benefits of being the member of a familiar shoal include increased survival, enhanced growth and improved performance in exploratory tasks (Höjesjö *et al.* 1998; Seppä *et al.* 2001; Bhat and Magurran 2006; Ioannou *et al.* 2011). A shoal is, however, a fission-fusion society and individual fishes often move from one shoal to another to maximize the benefits obtained (Pitcher and Parrish 1993). Although such an exchange of individuals between shoals could significantly affect the already existing dominance hierarchy and patterns of social interactions within a shoal, the newly joined individuals often acquire familiarity with the resident members of the group and *vice versa* in due course of time (Griffiths and Ward 2011).

Earlier studies have revealed experience to be a key factor leading to the acquisition of familiarity-dependent recognition of conspecific individuals or shoals (Griffiths and Ward 2011). Some fish could potentially learn certain phenotypes of conspecifics, with whom they have interacted, and all other fish possessing those specific traits could then be treated as familiar individuals (a general form of recognition). In contrast, other species may remember specific individuals based solely upon their previous experience (individual-specific recognition; Ward *et al.* 2009; Griffiths and Ward 2011). The time required for the development of familiarity-based recognition of conspecific groups typically differs across various species (Ward *et al.* 2009; Griffiths and Ward 2011).

Given the diversity of morphology, behaviour, mating systems and the nature of ecosystems inhabited, it becomes crucial to determine the sensory stimuli actually employed by piscine species to acquire familiarity with shoals of conspecifics (Webster *et al.* 2010). Such studies in sensory ecology have shown that many fish can recognize shoals of familiar individuals purely on the basis of odour cues

**Keywords.** Air-breathing fish; chemical communication; olfactory signals; sensory ecology; shoaling decision; social behaviour; social preference; visual cues



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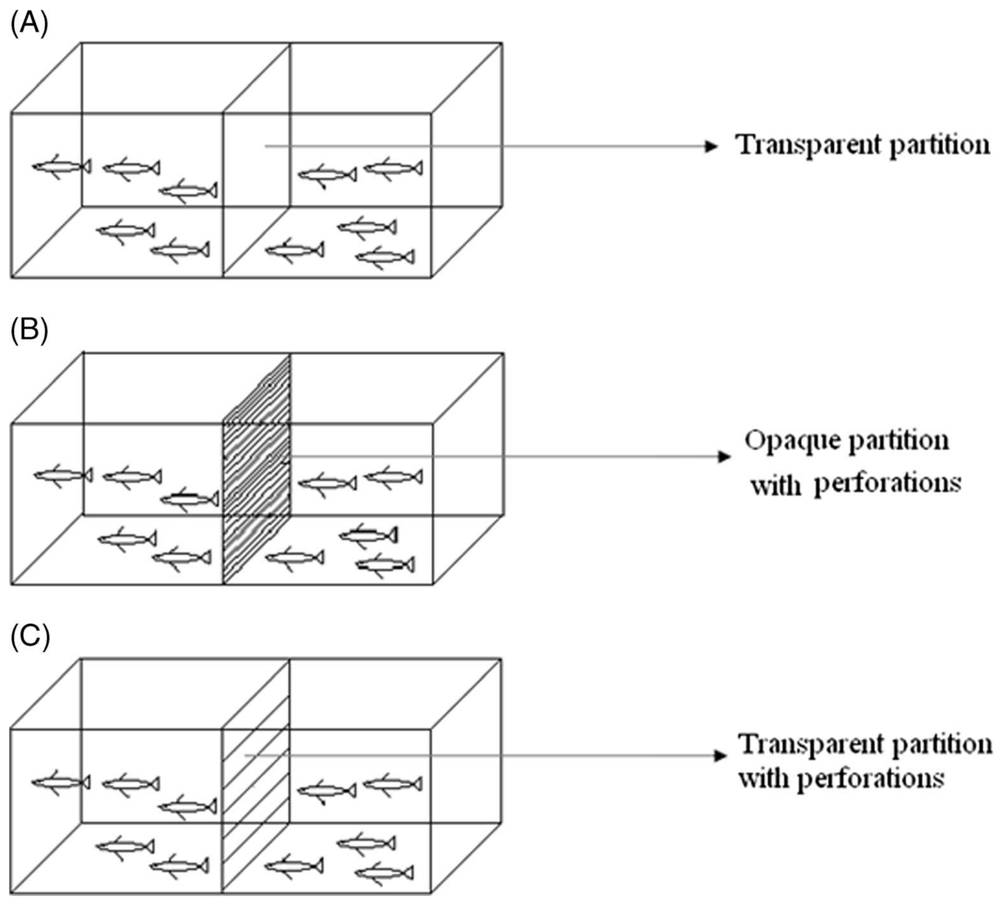


**Figure 1.** Digrammatic representation of the familiarization aquaria used for aquainting the subject climbing perch with different kinds of cues emitted by the stimulus shoal; visual cue alone (**A**), only olfactory cues (**B**), combination of olfactory and visual stimuli (**C**).

(fathead minnows; Brown and Smith 1994), while others employ self-referencing (sticklebacks; Morrell *et al.* 2007; Webster *et al.* 2010) or monitor visual cues of conspecifics (guppies; Griffiths and Magurran 1997) to attain familiarity-based recognition. Furthermore, certain species require inputs from more than one sensory modality to discriminate familiar individuals from unfamiliar ones (Ward and Mehner 2010) while some migratory species are even known to employ different sensory cues in different ecosystems in order to identify familiar conspecifics. For example, three-spined sticklebacks (*Gastero steusaculeatus;* Ward *et al.* 2004) and Nile tilapia (*Oreochro misniloticus*; Giaquinto and Volpato 1997) express a preference for known individuals only when familiar visual and olfactory cues are available in combination, whereas, the amphidromous Pacific blue-eye (*Pseudomugil signifier*) switches sensory modalities to locate familiar conspecifics with significant alterations of salinity in the habitat (Herbert-Read *et al.* 2010).

The climbing perch (*Anabas testudineus* Bloch), the subject species of the present study, is a highly priced food-fish of Southeast Asia. Natural populations of this species are, however, diminishing very rapidly in this region primarily due to overexploitation and other detrimental anthropogenic activities (Kohinoor *et al.* 2012). Although extensive research is currently being carried out to develop aquaculture programmes for this species, very few studies have addressed the nature of social behaviour in this species. According to Binoy and Thomas (2004, 2006) this fish has the ability to distinguish shoals containing familiar individuals from those with unfamiliar ones and this capacity could strongly influence its shoaling decisions. The actual sensory cues used by the species to recognize familiar individuals, however, remain unexplored although such knowledge would help us better understand the factors determining shoal formation, shoal cohesion or shoal fragmentation in this species. A better comprehension of these phenomena, in turn, is essential for designing effective conservation measures aiming at improvement of its natural populations and to enhance the growth and production in the pisciculture pond as well. The present study thus investigated the cues employed by this fish while acquiring familiarity with certain conspecific individuals,

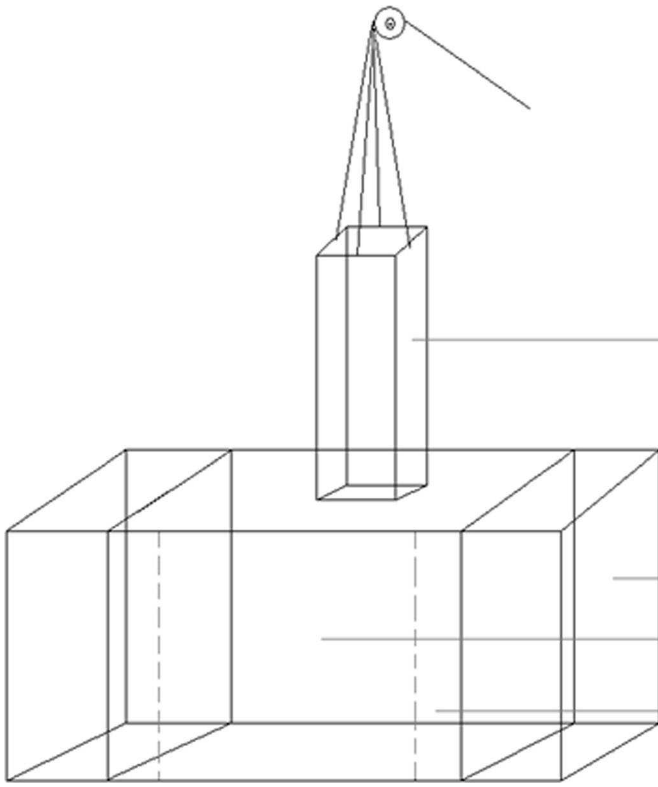


**Figure 2.** Digrammatic representation of the binary-choice apparatus used to test shoaling decisions made by climbing perch.

as shown by its ability to distinguish a familiar shoal of conspecifics from an equal-sized, but unfamiliar, one.

## 2. Materials and methods

### 2.1 *Subject species*

The climbing perch is an obligate air-breathing teleost fish that occurs in a variety of lentic and lotic freshwater ecosystems in India and other southeast Asian countries (Talwar and Jhingran 1991). The posteriorly compressed long body of this fish is olive green to dark brown dorsally but very pale ventrally. The eyes are golden red while the dorsal and caudal fins are grey, pectoral and anal fins pale yellow and the pelvic fin pale orange in colour in this species. The young are characterized by a large dark spot at the base of either side of the caudal fin and a small spot on the hind borders of the operculum. The transverse dark stripes on the hind part of the body and tail as well the stripe running from the eye to the operculum, along with the black blotches that typically characterize the young, often disappear with age (Yakupitiyage *et al.* 1998).

### 2.2 *Collection and familiarization*

The subject fish were collected from different *Kole* paddy fields in the Irinjalakuda region (10.30°–10.42°N, 76.20°–76.28°E) of Thrissur district, Kerala state, in southern India, and transferred to the laboratory. In order to avoid the confounding influence of the size of conspecific individuals on the shoaling decisions of the subject fish, individuals of size 62 ±38 mm (mean±SD, standard length) were alone used in the present study. Size-sorted fishes from each collection site were kept in groups of 20 individuals in separate aquaria (85×32×32 cm) for 15 days to acclimatize with the prevailing laboratory conditions. Subject fish could not be sexed due to the lack of sexual dimorphism (Zworykin 2012) in this species. Climbing perch, however, occur in mixed-sex populations in nature and do not show any reproductive activity under laboratory conditions even during monsoon, its breeding period in natural waterbodies (VV Binoy, pers. obs.).

In order to exclusively familiarize the subject climbing perch with visual cues from a shoal consisting of unfamiliar individuals, an aquarium of dimensions 56×28×28 cm (length×width×height) was divided into two equal-sized compartments (the familiarization aquarium) with the partition wall made of a transparent Plexiglas sheet without any perforations (figure 1A). The ends of this sheet were sealed to the walls of the aquarium to prevent the exchange of any chemical cues between the test individuals placed in the adjacent compartments, thus avoiding the potential development of any familiarity through olfactory cues. Another set of familiarization aquaria with partition walls made of opaque acrylic sheets with perforations (perforation diameter approximately 1 mm, 3–4 perforations/$cm^2$; figure 1B) were used for familiarizing test fish solely with olfactory cues of a test shoal placed in the adjacent chamber. Finally, familiarization aquaria with transparent but perforated acrylic partition sheets were used to familiarize the subject fish with a combination of visual and olfactory cues from the stimulus shoal (figure 1C). The side walls of all familiarization aquaria were covered with black paper while steel grids were placed on the top to prevent the fish from jumping out of the water. The water level was kept at a height of 25 cm, temperature at 25±1°C and the light period at 12 h: 12 h light : dark cycles.

Five climbing perch individuals were introduced into each side chamber of a familiarization aquarium and kept therein for 30 days to acquaint them with different types of cues of conspecific individuals living in the adjacent chamber. Individuals kept in familiarization aquaria with opaque perforated and transparent non-perforated partitions had access only to the olfactory and visual cues respectively of the individuals inhabiting the other side of the division wall while subjects kept in aquaria with transparent perforated partitions could use both visual and olfactory signals of the shoal present in the adjacent compartment for familiarization. As prior social experience could influence subsequent behaviour (Bhat and Magurran 2006), the possibility of the subject fish having encountered one another earlier was avoided by maintaining fish collected from distantly located sites (more than 3 km away) as matching shoals. The fish were fed twice (morning and evening) with food pellets with excess pellets being siphoned out 30 min after the initiation of each feeding session.

### 2.3 *Testing preference*

A binary-choice apparatus was used to test subject individuals in all the experiments described below (figure 2). This instrument consisted of an aquarium of dimensions 85×32×32 cm, divided in two side-chambers (16×32×32 cm) and a central chamber (53×32×32 cm) using Plexiglas sheets. The stimulus shoals were always presented in the side-chambers and this arrangement was kept undisturbed for 30 min so that the odour-producing chemicals (if present) could spread from both side chambers and diffuse into the central chamber (McLennan and Ryan 1999; Neff and Sherman 2005). The distribution and circulation of olfactory cues were confirmed by visualization using Methylene blue, as described in the dye tracer test (Neff and Sherman 2005). The subject fish were introduced individually in the central compartment of the apparatus in a transparent presentation cage (15×10×32 cm) made of perforated acrylic sheets (figure 2). They were then given 6 min each to assess the stimulus shoals, after which they were released by raising the presentation cage. The preference for a stimulus shoal was quantified by recording manually with a stopwatch for 6 min, the time during which any portion of the fish's body, excluding fins, was present in an area 5 cm from the side chambers where the stimulus groups had been presented. The subject fish were then returned to their respective home tanks. The water in the apparatus was changed and the experimental arena cleaned thoroughly after each trial. More importantly, the position of the stimulus shoals were interchanged after each trial in order to avoid any side bias that could have influenced the decisions made by the subject fishes.

### 2.4 *Tests with olfactory and visual cues*

The shoaling decision of individual climbing perch was recorded by presenting two stimulus shoals of five fishes each, in the two side-chambers of the apparatus, to the subject fish. The different combinations of cues (of stimulus shoals) available to the subject fish while making a shoal choice, in a binary-choice situation, included blank (empty chamber) vs. unfamiliar olfactory, blank vs. unfamiliar visual, unfamiliar olfactory vs. unfamiliar olfactory, unfamiliar olfactory vs. familiar olfactory, unfamiliar olfactory vs. unfamiliar visual, unfamiliar olfactory vs. familiar visual, unfamiliar visual vs. unfamiliar visual, and unfamiliar visual vs. familiar visual cues. The olfactory cues were always presented behind an opaque partition with perforations while a transparent Plexiglas divider without any perforation and sides sealed to the wall of apparatus was used to present the visual cues of the stimulus shoal alone to the subject fish. In experiments where an empty side chamber (blank) was used as a stimulus, a similar type of partition wall was used to separate the subject fish from both the stimulus shoal and the blank.

### 2.5 *Tests with combinations of the cues*

The influence of familiar visual cues in combination with familiar olfactory cues, unfamiliar olfactory cues with familiar visual cues, familiar olfactory cues with unfamiliar visual cues and the separation of familiar olfactory cues from familiar visual cues on the recognition of a familiar shoal were also addressed in this study. In the first case, a shoal, whose olfactory and visual cues were familiar to the subject fish, was presented behind a transparent partition with perforations while a shoal familiar by visual cues alone (behind a transparent partition with perforations), a shoal familiar solely by the olfactory cues (behind a transparent partition with perforations), a shoal with familiar visual and olfactory cues (behind a transparent partition without any perforation) were the stimuli in subsequent experiments. A shoal of five unfamiliar conspecifics was the second stimulus in all these three sets of experiments.

Three experiments keeping empty side chambers (blank) as stimuli were also conducted to check whether utilization of different combinations of partition walls, which alters the spatial property of the experimental arena, have any impact on the time spent by climbing perch in the preference zones. The combination of division walls used in these experiments were opaque perforated vs. opaque perforated, opaque perforated vs. transparent perforated and transparent perforated vs. transparent non-perforated walls.

Twenty-five individual fish were tested in each experiment and the individuals used in a particular experiment were never used in any other experiment. All the fish were released at the site of their collection, after the experiments were completed.

## 3. Results

The subject fish in the central chamber of the test aquaria did not exhibit any significant preference for either side of the aquaria when different combinations of partition walls – opaque perforated vs. opaque non-perforated (paired *t*-test, $t_{24}$=−1.31, P>0.05), opaque perforated vs. transparent perforated ($t_{24}$=−0.58, P>0.05) and transparent perforated vs. transparent non-perforated ($t_{24}$=−0.91, P>0.05) were tested against one another in blank experiments (ANOVA, carried out on the time spent in the preference zones of the right–left chambers, $F_{2,72}$=0.13, P>0.05). The mean of the data pooled from these three sets of experiments was thus used as the blank value in subsequent experiments (figure 3).

The climbing perch were unable to reach a definitive decision, in terms of the time spent in the preference zone next to either side of the test aquaria, when tested with olfactory cues of an unfamiliar stimulus shoal against blank (paired *t*-test, $t_{24}$=−1.21, P>0.05), odour of another unfamiliar shoal ($t_{24}$=0.42, P>0.05) or olfactory cues of a familiar shoal ($t_{24}$=1.84, P>0.05).The subject fish exhibited a significant preference for an unfamiliar shoal, visible to them but without any access to olfactory cues, over blank ($t_{24}$=−6.15, P<0.001), and over shoals represented solely by familiar ($t_{24}$=−4.70, P<0.001) or unfamiliar ($t_{24}$ =−4.85, P<0.001) odour cues.

The subject fish spent significantly more time near a shoal of individuals with familiar visual cues over one of unfamiliar individuals ($t_{24}$=−3.58, P<0.01) but failed to make a choice when a pair of shoals with unfamiliar visual cues were presented to them ($t_{24}$=0.59, P>0.05). It may be noted that a separation of olfactory cues from visual cues of a familiar shoal never affected the preference of the subject fish for that group ($t_{24}$=−3.67, P<0.01).

In the final set of experiments, the test fish preferred to join a shoal of conspecifics with familiar visual, but unfamiliar olfactory, cues over a group of individuals with both unfamiliar visual and olfactory cues ($t_{24}$=−4.19, P<0.01). No significant preference for any of the stimulus shoals was observed when one shoal provided a blend of familiar olfactory and unfamiliar visual cues while the other was totally unfamiliar ($t_{24}$=0.33, P>0.05).

## 4. Discussion

Chemical signalling is believed to be one of the most effective media of communication in a spatially complex aquatic habitat and many fish species particularly utilize odour cues to achieve familiarity-based recognition of conspecific individuals (Webster *et al.* 2010). The climbing perch, which inhabits tropical aquatic water bodies with high levels of biotic and abiotic complexity, however, failed to reach a shoaling decision when olfactory signals alone, whether familiar or unfamiliar, were available to detect the presence of a shoal of conspecific individuals. In fact, this fish exhibited a distinct preference for unfamiliar shoals, which could be accessed visually, over shoals that could be perceived by familiar or unfamiliar odour cues alone. These results, taken together, along with the demonstrated preference for a visually familiar shoal over a shoal with unfamiliar visual cues suggest that climbing perch cannot utilize olfactory signals to acquire familiarity-dependent recognition of conspecific shoals. The species can, nevertheless, utilize visual cues for this purpose, as has also been reported earlier in guppies and zebrafish (Griffiths and Magurran 1997; Engeszer *et al.* 2004; Spence and Smith 2007).

The climbing perch is thus unusually different from species like the Nile tilapia and stickleback (Griffiths and Ward 2011; Giaquinto and Volpato 1997), which require a blend of visual and olfactory cues to distinguish a familiar shoal; the separation of familiar olfactory cues from familiar visual cues thus did not affect the preference for familiar shoals in this species. It is perhaps significant that the failure of this fish to utilize olfactory signals and its dependence instead on visual cues to recognize familiar shoals is not unique to this particular situation. We have, for example, shown earlier that olfactory cues announcing the presence of anaquatic predatory fish, the striped snakehead or murrel (*Channa striatus*) or those from a skin extract of a conspecific individual, which was a probable source of alarm pheromones, failed to influence the exploratory behaviour of this species in a novel open-field situation (Binoy 2008). The presence of a large eye-spot as a visual stimulus was, nevertheless, capable of inducing a fear response in individuals in a similar experimental situation (Binoy and Thomas 2003).

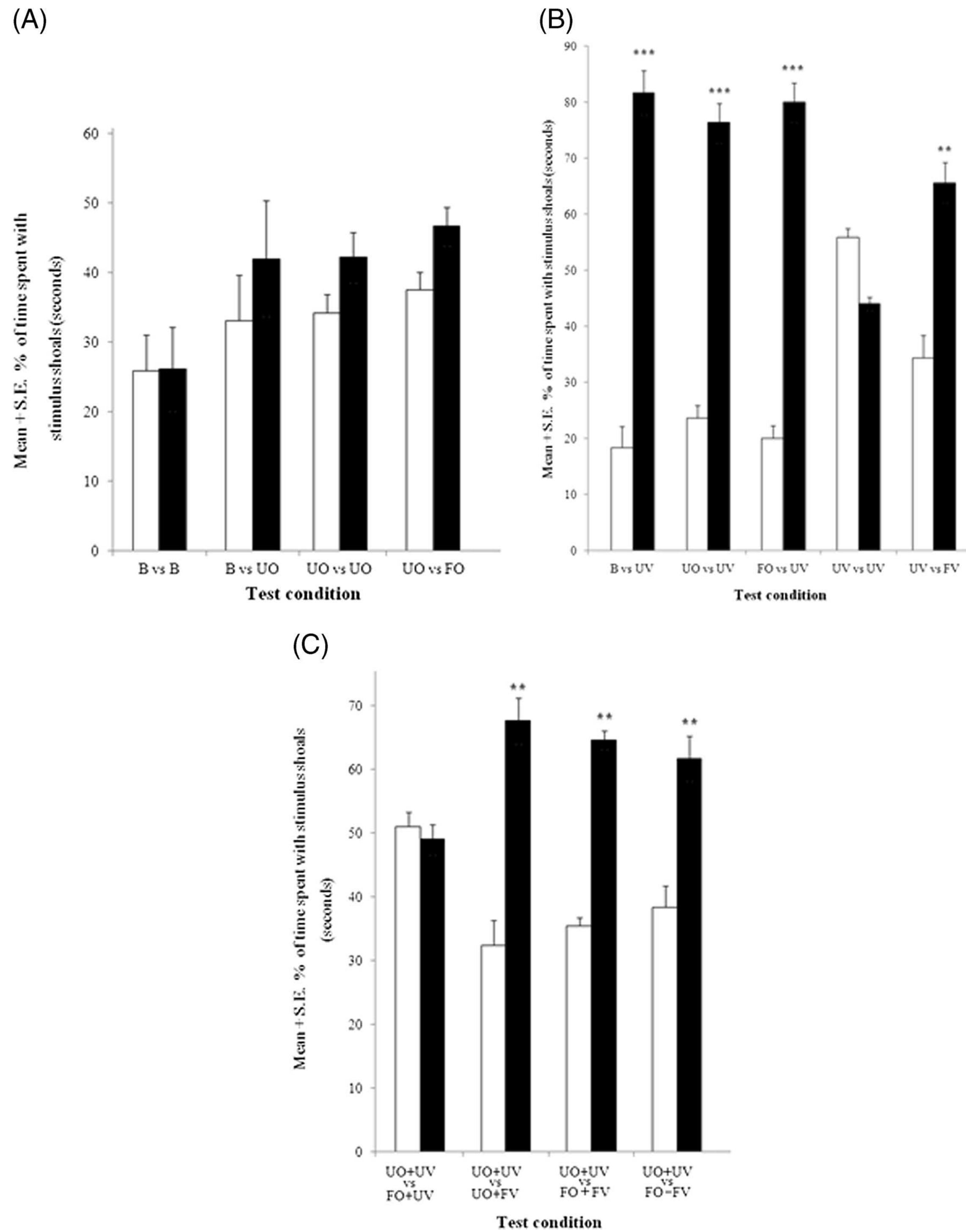


**Figure 3.** Influence of the aquired familiarity with olfactory (**A**), visual (**B**) and combinations of conspecific cues (**C**) on shoaling decisions in climbing perch. B: blank; UO: unfamiliar olfactory cues; FO: familiar olfactory cues; UV: unfamiliar visual cues; FV: familiar visual cues.

Being an obligatory air-breathing fish, individual climbing perch have to breathe in air multiple times a day in synchrony with the other shoal members. Air-breathing fish are found to reduce the enhanced risk of aerial predation during surfacing by orchestrating air-gulping, which disables a bird predator to eye-lock an individual fish in order to attack it (Chapman and Chapman 1994). In such contexts, the visual cues of a conspecific individual could constitute more dependable stimuli than would olfactory ones to achieve behavioural synchronization and, therefore, to acquire familiarity. The presence of large eyes and a large optic lobe in the brain, coupled with a poorly developed olfactory lobe (Bersa 1997), supports this hypothesis of the dependency of the species on visual cues, rather than on olfactory ones, to perform several specific functions. Further studies are, however, essential to discover and analyse the exact visual cues utilized by this species to acquire familiarity with conspecific shoals.

The climbing perch survives in different kinds of ecosystems, ranging from lakes and ponds with remarkably low turbidity to murky and foul-smelling sewage, where the visibility is exceedingly low (Munshi and Hughes 1991). Additionally, this species is famous for its migratory urge and its consequent ability to change its habitat during the Asian monsoons (Sokheng *et al*. 1999). The cross-ecosystem variation in sensory stimuli and the sensory adaptations that allow the species to successfully negotiate contrasting habitats, following migration, raise important questions to be addressed by future research.

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