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Sensory Basis of Food and Predator Detection in the Tadpoles of the Bicolored Frog *Clinotarsus curtipes* (Jerdon, 1854) (Anura: Ranidae)

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Abstract: Most of the anuran tadpoles spend their early phase of life in aquatic medium. In such system, they respond to a wide variety of stimuli (e.g., tactile, chemical and visual cues) and exhibit appropriate behavioural responses. The ability to detection food and predator was studied in the tadpoles of the bicolored frog, *Clinotarsus curtipes* in the laboratory by using a rectangular glass test tank with end compartments (stimulus zones) providing exclusively visual and/or chemical food (boiled spinach) or predator (*Laccotrephes* sp.) cues. A test tadpole, *C. curtipes* (either starved or fed; Gosner stage 25) was held at the centre of the test tank for 5 min (acclimation) to perceive visual and/or chemical food or predator cues. Then it was released from the centre of the test tank and allowed to associate or stay away from the caged stimulus subjects (food or predator), which were placed at one end of the test tank either in a glass beaker (visual cues) or in a mesh cage wrapped with cheese cloth (chemical cues) for a period of 10 min. The test tadpoles were unable to detect both food and predator through visual cues. They spent almost an equal amount of time in the zone housing food or predator in a glass beaker or opposite zone, which was kept empty. However, they detected both food and predator solely through chemical cues. In tests with chemical food cues, they spent a significantly higher period of time (70.42% of total time) near chemical food cues rather than empty or visual food cues. In contrast, in tests with chemical predator cues, they spent the majority of their time (69.32% of total time) away from the chemical predator cues. The findings thus show that *C. curtipes* tadpoles detect both food and predator solely through chemical cues rather than visual cues.

Key words: Boiled spinach, chemical cues, Water scorpion, predator, tadpole, visual cues

Introduction

Most anurans usually deposit their eggs in temporary or permanent water bodies or streams with continuous or intermittent flowing water, where their larvae have to complete their early life stages (Wilbur 1980, Newman 1992, Eterovick & Barata 2006, Saidapur et al. 2009, Mogali et al. 2012, 2022). A number of studies have shown that anuran tadpoles respond to a wide variety of stimuli that include tactile, chemical, and visual cues and exhibit appropriate behavioral responses (Hoff et al. 1999, Saidapur et al. 2009). Often the anuran tadpoles are found in turbid water, or water filled with dense vegetation or leaf litter with poor visibility. Furthermore, anuran tadpoles in general are near-sighted

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and therefore it is unlikely that they use vision to detect objects at greater distances (Hoff et al. 1999, Mogali 2018). Some studies have documented the use of various stimuli among anuran tadpoles. For instance, tadpoles of *Anaxyrus americanus*, *Rana cascade*, *R. sylvatica* are reported to preferentially associate with their siblings and perceive the chemical cues emanating from them (Waldman 1991, Blaustein & Walls 1995). Anuran tadpoles are also known to avoid predators based on chemical cues emanating either from the body of the predators or from the wounds of its prey (Wilson & Lefcort 1993, Kiesecker et al. 1996, Mogali et al. 2012).

The tadpoles of the Bicolored frog, *Clinotarsus curtipes* are found in the gently flowing streams and isolated pockets of water throughout the year in southern Western Ghats of India (Hiragond & Saidapur 2001, Saidapur 2001). These are the largest tadpoles found in the Western Ghats (Daniels 1997). The larval period of *C. curtipes* ranges from 6–20 months (Hiragond 2002). The tadpoles of *C. curtipes* are basically bottom-dwellers and thrive on detritus and algal matter (Hiragond & Saidapur 2001). The visibility is generally low in such waters, due to shadows cast by dense vegetation, and the brownish-dark colour of the benthic areas that are typically covered by leaf litter and detritus (Mogali et al. 2021). These water bodies are home to several aquatic invertebrate predators, including the most dangerous water scorpions (Mogali et al. 2016, 2021). Thus, in such system, it is very important to know how these tadpoles detect their food and/or predator, whether through visual or chemical senses, hence the present experiment was conducted. In natural water bodies, as these tadpoles lived in the low illumination hence we hypothesized that they may detect both food and predator through chemical senses.

Materials and Methods

Four egg clutches (average number of eggs in a single clutch is 500) of *Clinotarsus curtipes* were collected from a stream in the southern Western Ghats near Anmod village (latitude 15.430888ºN, longitude 74.373601ºE, altitude 2188.32 ft), Karnataka State, India, in September 2013. Egg clutches were immediately transported to the laboratory and each clutch was reared separately in a plastic tub (32 cm diameter and 14 cm deep) containing 5 litres of aged tap (dechlorinated) water. Hatching time occurred (almost synchronously) eight days later, at Gosner stage 19 (Gosner 1960). After hatching, tadpoles of all clutches were mixed to normalize genetic differences among the groups. Mixed tadpoles were reared in a glass aquarium (90 cm L × 30 cm W × 15 cm H) containing 25 litres of aged tap water. When tadpoles reached the feeding stage (Gosner stage 25; Gosner 1960) then they were provided with boiled spinach *ad libitum*. Adult individuals of *Laccotrephes* sp. (N = 100; predators) were also collected from the same site from where the egg clutches of *C. curtipes* were obtained and were reared individually, to avoid cannibalism, in small plastic tubs (14 cm diameter and 7 cm deep) with 500 ml of aged tap water. Predators were exclusively fed with tadpoles of *C. curtipes*. In all experimental trials, test tadpoles (*C. curtipes*) were of comparable body sizes (length 21.50 ± 0.60 mm, $\bar{x}$ ± SE; N = 25) and developmental stage (Gosner stage 25). The predators (*Laccotrephes* sp.) were comparable in body size (length 60.80 ± 0.70 mm, $\bar{x}$ ± SE; N = 25).

A rectangular glass test tank (90 cm L × 30 cm W × 15 cm H; Fig. 1) was used for conducting trials. A central line perpendicular to the long axis of the test tank was drawn at the bottom on the outer surface dividing it into two equal compartments, referred to as the stimulus zones A and B. The food or predator was placed either in a transparent glass beaker or within a mesh cage wrapped with cheese cloth in either one or both stimulus zones. We assumed that food or predator placed in the beaker would block chemical cues but provide visual information, while food or predator placed in the mesh cage wrapped with cheese cloth would block visual cues but allow diffusion of chemical cues in the water. Prior to each trial, the test tank was filled with aged tap water to a height of 3 cm. The stimulus zones were reversed between the trials. In each trial, a single test tadpole (*C. curtipes*) was used. A test tadpole (Gosner stage 25) was placed in an open-ended mesh cage (10 cm diameter × 15 cm height) kept in the centre of the test tank and allowed to acclimate as well as perceive food or predator cues (visual/chemical) for 5 min. The test tadpole was then released by gently lifting the mesh cage without disturbance and allowing it to move freely in the test tank. The trial period was set at 10 min. The time spent by the test tadpole in each stimulus zone during the trial period was recorded with the help of a stopwatch. Our assumption was that when test tadpoles detect food they would spend more time in the zone near the one housing food. When test tadpoles detect predators or predation threats they would spend more time in the zone away from the one housing predators. On the other hand, failure to detect such a threat will result in a random movement of test tadpoles in the test tank regardless of the food or predator cues (visual

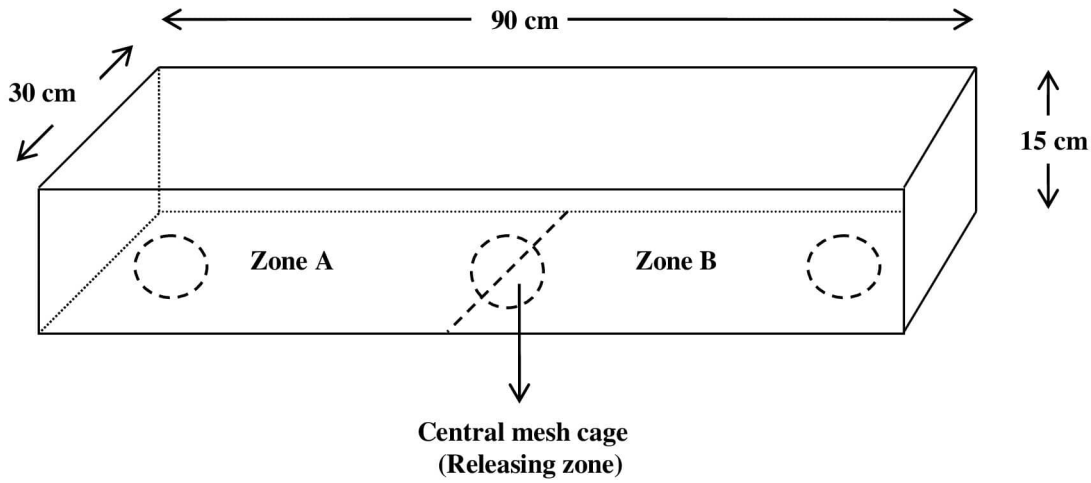

Fig. 1. The design of the test tank was used for determining the ability of food or predator detection by the tadpoles of *Clinotarsus curtipes*. The dotted central line visually divides the test tank into two zones. The circle(s) in the center of the test tank indicates the location of the release of the test tadpoles; whereas that in the end zones areas where food or predator was kept.

Table 1. Association choice of *Clinotarsus curtipes* tadpoles in response to visual/chemical cues of food (boiled spinach)

Tests	Mean time (s) spent ± SE		
	Zone A	Zone B	Z[#] and P values
End-bias	300.84 ± 10.69	299.16 ± 10.69	Z = -0.037; P = 0.970
Blank (A) vs. Visual (B)	306.36 ± 23.81	293.64 ± 23.81	Z = -0.143; P = 0.886
Blank (A) vs. Chemical (B)	177.44 ± 12.42	422.56 ± 12.42	Z = -4.292; P < 0.05[*]
Visual (A) vs. Chemical (B)	175.20 ± 11.86	424.80 ± 11.86	Z = -4.345; P < 0.05[*]

[#]Wilcoxon matched-pairs signed-ranks test. [*]Significantly different

and/or chemical). After each trial, the test tank was washed and water was renewed. For food detection experiments a 24 h starved test tadpoles of comparable body sizes were used; whereas for predator detection experiments, well-fed (boiled spinach) test tadpoles of comparable body sizes were used. Each test tadpole, food item or predator was used only once. No food was provided to the test tadpoles or the predators during the trials. All trials were conducted under natural photoperiod and temperature. The daily temperature of the testing room in the laboratory fluctuated between 27–28 ºC. The following tests were conducted.

End-bias tests were conducted to check whether the test tadpoles show bias towards any end of the test tank or for any of the containers used for placing the food or predator (glass beaker, open-ended cylindrical mesh cage wrapped with cheese cloth). These tests involved four sets of trials:

(1) With both stimulus zones of the test tank without any containers.

(2) With only one stimulus zone, with a glass beaker containing water to the level that matched the water in the test tank and the other zone blank.

(3) With a mesh cage wrapped with cheese cloth in one zone with the other zone blank.

(4) With a beaker and a mesh cage wrapped with cheese cloth placed at the opposite ends of the test tank.

The time spent by a tadpole in each zone in a given trial was recorded. All trials were conducted separately for starved and fed test tadpoles. In each set, 25 trials were conducted (4 types of end-bias tests × 2 types of test tadpoles × 25 trials for each set = total of 200 end-bias tests). For all trials, tadpoles of *C. curtipes* are at Gosner stage 25 only. The duration of Gosner stage 25 in this species is very long i.e., minimum 20 days (Our Personal Observations). All experimental trials were carried out in the laboratory during the daytime between 0900 and 1600 h with uniform light entering all sides of the test tank. Daily 20 trials were carried out. The duration of the experiment was 18 days.

Tests involving detection of food or predator based on visual and/or chemical cues were as follows. In tests for food or predator detection based

Table 2. Association choice of *Clinotarsus curtipes* tadpoles in response to visual/chemical cues from a predator (*Laccotrephes* sp.)

Tests	Mean time (s) spent ± SE		
	Zone A	Zone B	Z[#] and P values
End-bias	297.23 ± 11.20	302.77 ± 11.20	Z = -0.422; P = 0.673
Blank (A) vs. Visual (B)	289.12 ± 12.46	310.88 ± 12.46	Z = -1.014; P = 0.310
Blank (A) vs. Chemical (B)	415.96 ± 8.64	184.04 ± 8.64	Z = -4.372; P < 0.05[*]
Visual (A) vs. Chemical (B)	418.12 ± 9.26	181.88 ± 9.26	Z = -4.373; P < 0.05[*]

[#]Wilcoxon matched-pairs signed-ranks test. [*]Significantly different

on visual cues, a beaker containing either 1 g of boiled spinach or predator (*Laccotrephes* sp.; n = 1) was placed at one end of the test tank and the other end was provided with a beaker containing water matched to the level in the tank (n = 25 trials for each food/predator). In tests for food or predator detection based on chemical cues, an open-ended mesh cage wrapped with cheese cloth either with 1 g boiled spinach or predator (n = 1) was placed at one zone of the test tank; another mesh cage wrapped with a cheese cloth but devoid of food or predator was placed at the other zone (n = 25 trials for each food/predator). In tests for food or predator detection based on both visual and chemical cues, a beaker containing either 1 g boiled spinach or a single predator (visual food or predator cues) in one stimulus zone and a mesh cage wrapped with cheese cloth containing either 1 g boiled spinach or a single predator (chemical food or predator cues) placed in the other zone (n = 25 trials for each food/predator). Association choice of test tadpoles was then recorded. In all of the above tests, data on the time spent by test tadpoles in stimulus zones A or B were compared by the Wilcoxon matched-pairs signed-ranks test (SPSS software ver. 16.0).

Results

Food detection in *Clinotarsus curtipes* tadpoles

In the end-bias tests, tadpoles (starved) moved freely throughout the test tank. They showed no bias towards any particular side of the test tank. The placement of the glass beaker or mesh cage made no difference to the test tadpoles. They moved randomly throughout the test tank. Hence, data from all sets of end-bias tests were pooled and are presented in Table 1 (Z = -0.037; P = 0.970).

In tests involving food providing only visual cues, there was no significant difference in the time spent by the test tadpoles between the stimulus zones housing beakers with or without food, even though the food was visible through the glass beaker at one end of the test tank (Z = -0.143; P = 0.886; Table 1). In trials involving food providing only chemical cues, the test tadpoles spent significantly more time in the zone housing food inside the mesh cage wrapped with cheese cloth compared to the chemically blank zone housing only the mesh cage wrapped with cheese cloth without any food (Z = -4.292; P < 0.05; Table 1). In tests involving food providing both visual as well as chemical cues, the test tadpoles spent a significantly greater amount of time in the zone that housed food in the mesh cage wrapped with cheese cloth compared to the zone providing visual cues of food through the glass beaker (Z = -4.345; P < 0.05; Table 1).

Predator detection in *Clinotarsus curtipes* tadpoles

In the end-bias tests, tadpoles (fed) moved freely throughout the test tank. They showed no bias towards any particular side of the test tank. The placement of the glass beaker or mesh cage made no difference to the test tadpoles. They moved randomly throughout the test tank. Hence, data from all sets of end-bias tests were pooled and are presented in Table 2 (Z = -0.422; P = 0.673).

In trials with the visual cues of predators at one end zone, the test tadpoles moved randomly and freely throughout the test arena. The time spent by test tadpoles near or away from the predators was comparable (Z = -1.014; P = 0.310; Table 2). In trials with water-borne chemical cues of predators, the test tadpoles spent a significantly greater amount of time away from them (Z = -4.372; P < 0.05; Table 2). In trials with both visual as well as chemical cues of predators, the test tadpoles spent a significantly greater amount of time in the zone housing predators inside the glass beaker compared to the zone providing water-borne chemical cues of predators (Z = -4.373; P < 0.05; Table 2).

Discussion

The tadpoles of *C. curtipes* are unable to detect both food and predator through visual cues. When food or predator was kept in a transparent glass beaker, they neither approached the food nor avoided the predators or their area. They moved randomly throughout the test tank. However, tadpoles responded to food or predator chemical cues. They moved towards the food placed in a mesh cage wrapped with cheese cloth. Our results are in conformity with an earlier study on *Indosylvirana temporalis* (Veeranagoudar et al. 2004), *Sphaerotheca breviceps* (Sugur et al. 2008), and *Polypedates maculatus* (Mogali et al. 2022) tadpoles. However, in tests with chemical predator cues, *C. curtipes* tadpoles moved away from the predators placed in a mesh cage wrapped with cheese cloth. They also show reduced movements in the predator zone and they suddenly returned to the predator-free area. Our results are in conformity with earlier study on *Bufo melanostictus* (Saidapur et al. 2009), *S. breviceps* (Saidapur et al. 2009), *I. temporalis* (Mogali et al. 2012), and *P. maculates* tadpoles (Mogali 2018). Thus, blocking visual food or predator cues did not limit the detection of food or predator that is exclusively based on water-borne chemical cues. These findings show that *C. curtipes* tadpoles have a sense of chemical perception. The ability to detect food or predator based on chemical cues may evolve especially under poor visibility conditions, such as in turbid water or shadows from vegetation or benthic area that is naturally covered by leaf litter and detritus. It may also help tadpoles to forage or avoid predators at night hours. Indeed, herbivorous tadpoles like *C. curtipes* do forage and avoid predators during night hours (Our Personal Observations). The present study shows conclusively that the tadpoles of *C. curtipes* detect both food and predator using chemical senses. A failure to detect food or predator based on visual cues by the tadpoles of *C. curtipes* supports the general view that anuran tadpoles have poor vision.

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