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Sensory Basis of Food and Predator Detection in the Tadpoles of the Indian Skipper Frog *Euphlyctis cyanophlyctis* (Schneider, 1799) (Anura: Dicroglossidae): an Empirical Study

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Abstract: The majority of the amphibian larvae spend their early life stage in aquatic medium. In such system, they respond to a broad array of stimuli (tactile, chemical and visual cues) and exhibit suitable behavioural responses. The ability of detection of food and predator was studied in the tadpoles of the Indian skipper frog *Euphlyctis cyanophlyctis* in the laboratory setup by using a rectangular glass test tank with end compartments (stimulus zones) providing exclusively visual and and (or) chemical food (either boiled spinach or tadpoles of *Duttaphrynus melanostictus*) and predator (tadpoles of *Hoplobatrachus tigerinus*). A test tadpole *E. cyanophlyctis* 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 failed to detect either food or predator through visual cues. They spent almost an equal amount of time in the zone housing food or predator in a glass beaker as compared to the opposite zone, which was kept empty. However, they detected both food and predator solely through chemical cues. In tests with chemical food cues (either boiled spinach or tadpoles of *D. melanostictus*), they spent the majority of their time (67.25 % of total time) near chemical food cues rather than empty or visual food cues. In contrast, in tests with chemical predator cues (tadpoles of *H. tigerinus*), they spent the majority of their time (68.89 % of total time) away from the chemical predator cues. Therefore, the findings show that *E. cyanophlyctis* tadpoles detect both food and predator through chemical sensory mechanism rather than visual ones.

Key words: Anurans, food and predator detection, tadpole, visual and chemical cues

Introduction

Many anuran amphibians opportunistically breed either in temporary or permanent water bodies. Such water bodies consist of various types of tadpole species, including herbivorous, carnivorous and omnivorous. All tadpole species have to face one or the other risk, i.e., crowding, desiccation and predation; with all these difficulties, they successfully complete their early stage of life and come out of the

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water (Wilbur 1980, Laurila 2000, Benard 2004, Saidapur et al. 2009, Mogali et al. 2017, 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 behavioural responses (Hoff et al. 1999, Ferrari et al. 2010, Hettyey et al. 2012, Mogali et al. 2012, Carlson et al. 2015, Hemnani et al. 2022). Furthermore, the temporary waters where tadpole species reside are often turbid or contain aquatic vegetation that obscures visual cues to detect environmental changes. In such conditions, chemical cues are more useful to detect food and predators (Kiesecker et al. 1996; Hickman et al. 2004; Saidapur et al. 2009, Mogali 2018, Gazzola et al. 2022).

The tadpoles of the Indian skipper frog *Euphlyctis cyanophlyctis* (Schneider, 1799) (Anura: Dicroglossidae) are found in temporary or permanent water bodies in southern India (Hiragond & Saidapur 2001). The tadpoles of *E. cyanophlyctis* are basically bottom-dwellers and are omnivores feeding on detritus or algal matter as well as on some herbivorous tadpoles (Hiragond & Saidapur 2001). The visibility is generally low in such waters, due to shadows cast by vegetation and benthic areas that are typically covered by leaf litter and detritus. These water bodies are the home for both invertebrate (e.g., insects and its larvae) and vertebrate (e.g., tadpoles of *Hoplobatrachus tigerinus*, birds) predators. Our previous laboratory studies showed that most of the herbivorous tadpole species detected both food and predator solely through chemical cues (Veeranagoudar et al. 2004, Sugur et al. 2008, Saidapur et al. 2009, Mogali et al. 2012), whereas carnivorous tadpoles like *H. tigerinus* detected food through both visual and chemical cues (Saidapur et al. 2009). However, it is still unclear how the omnivore tadpole species detect both food and predators. Therefore, the present study was undertaken to find out the food and predator detection ability in tadpoles of *E. cyanophlyctis* in the laboratory experiments.

Materials and Methods

Tadpoles of *Euphlyctis cyanophlyctis* (Gosner stages 29-30), *Duttaphrynus melanostictus* (Gosner stages 29-30) and *Hoplobatrachus tigerinus* (Gosner stages 35-36) were collected from temporary ponds in the Karnatak University Campus, Dharwad (15.440407ºN, 74.985246ºE), Karnataka State, India, in June, 2014. Tadpoles of all species were immediately transported to the laboratory and each species was reared separately. Tadpoles of *E. cyanophlyctis* and *D. melanostictus* were reared separately in a glass aquarium (90 cm L × 30 cm W × 15 cm H) containing 25 L of aged tap water. Whereas, tadpoles of *H. tigerinus* were reared individually in a plastic tub (with diameter of 14 cm and 7 cm deep) with 500 mL of aged tap water to avoid cannibalism. Tadpoles of *E. cyanophlyctis* are omnivores, hence they were provided with boiled spinach and also tadpoles of *D. melanostictus*. Tadpoles of *D. melanostictus* are herbivores; hence they were given boiled spinach. Tadpoles of *H. tigerinus* are carnivores; thus, they were provided with tadpoles of *D. melanostictus* and *E. cyanophlyctis*. In all experimental trials, test tadpoles (*E. cyanophlyctis*) were of comparable body sizes (length 43.26 ± 0.93 mm, $\bar{x}$ ± SE; N = 25) and developmental stage (Gosner stages 29-30). The predators (*H. tigerinus*) were comparable in body size (length 45.80 ± 0.76 mm, $\bar{x}$ ± SE; N = 25) and developmental stage (Gosner stages 35-36).

A rectangular glass test tank (90 cm L × 30 cm W × 15 cm H) was used for conducting the trials (Fig. 1). 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 (either boiled spinach, 1 g or tadpoles of *D. melanostictus*, N = 4) or predator (tadpoles of *H. tigerinus*, N = 4) 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 would provide visual information, while food or predator placed in the mesh cage wrapped with cheese cloth would block visual cues but would 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 (*E. cyanophlyctis*) was used. A test tadpole (either starved or fed; Gosner stage 29-30) 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; after that the test tadpole was exposed to 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. Our assumption was that when test tadpoles detect food, they would spend more time in the zone near the one housing food. Whereas when test tadpoles detect predators or predation threats, they would spend more time

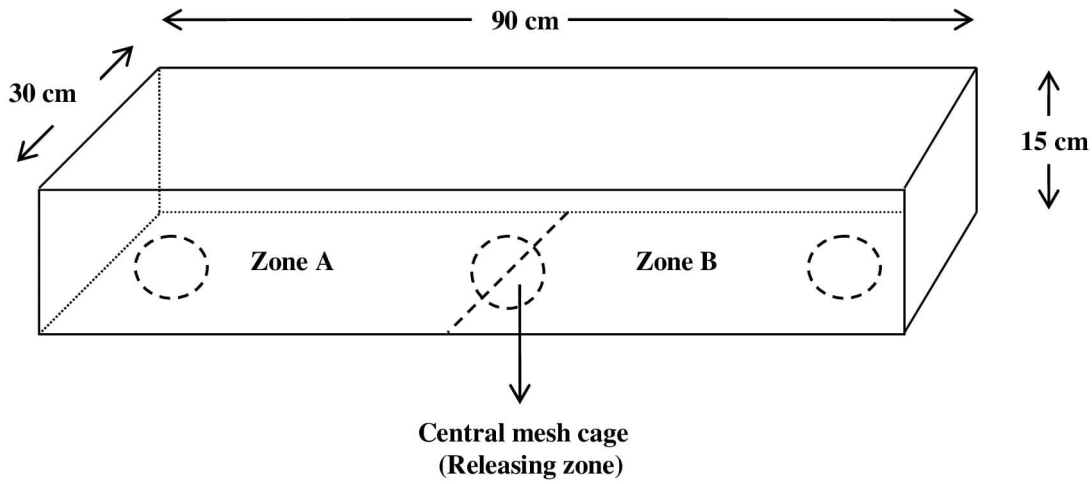

Fig. 1. The design of the test tank was used for determining the mechanism of food (boiled spinach or tadpoles of *Duttaphrynus melanostictus*) or predator (tadpoles of *Hoplobatrachus tigerinus*) detection by the tadpoles of *Euphlyctis cyanophlyctis*. The dotted central line visually divides the test tank into two zones. The circle in the centre of the test tank indicates the location of the release of the test tadpoles through a mesh cage. Circles in the end zones indicate areas where food or predator was kept either in a glass beaker or in a mesh cage wrapped with cheese cloth.

in the zone away from the one housing predators. On the other hand, failure to detect food or predator (visual and/ or chemical cues) would result in a random movement of test tadpoles in the test tank. After each trial, the test tank was washed and water was renewed. For food detection experiments, 24-h-starved test tadpoles of comparable body sizes were used; whereas for predator detection experiments, a well-fed test tadpole of comparable body sizes were used. A test tadpole or food 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 varied between 25–26 ºC. The following tests were conducted.

End-bias tests were conducted to check whether the test tadpoles showed 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, viz. (1) with both stimulus zones of the test tank without any containers; (2) with only one stimulus zone with glass beaker containing water to the level that matched the water in the test tank and the other zones kept blank, and (3) with a mesh cage wrapped with cheese cloth in one zone with the other zones kept 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. Since for food detection experiments, we used starved (for 24 h) test tadpoles and for predator detection experiments, we used fed test tadpoles as a consequence we carried out the above-mentioned end-bias tests separately by using both starved and fed test tadpoles. In each set, 25 trials were conducted (4 types of end-bias tests × 2 types of test tadpoles, starved and fed × 25 trials for each set = a total of 200 end-bias tests).

Tests involving detection of food or predator based on visual or chemical cues or both by tadpoles of *E. cyanophlyctis* were conducted as follows. In tests for food or predator detection based on visual cues, a beaker containing either 1 g of boiled spinach/ tadpoles of *D. melanostictus* n = 4 or predator (*H. tigerinus*; n = 4) 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 having either 1 g boiled spinach or 4 tadpoles of *D. melanostictus* or predator (n = 4) were placed at one zone of the test tank and 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 4 tadpoles of *D. melanostictus* or 4 predators (visual food or predator cues) in one stimulus zone and a mesh cage wrapped with cheese cloth containing either 1 g boiled spinach or 4 tadpoles of *D. melanostictus* or 4 predators (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 using the Wilcoxon matched-pairs signed-ranks test (SPSS software ver. 16.0).

Results

Sensory basis of food detection in tadpoles of *Euphlyctis cyanophlyctis*

In the end-bias tests, test 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 presented in Table 1 (Z = -0.453; P = 0.651).

In the experiments using boiled spinach as food, 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 (boiled spinach) was visible through the glass beaker at one end of the test tank (Z = -0.411; P = 0.681; Table 1). In trials involving food (boiled spinach) 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 = -4.265; P = 0.000; Table 1). In tests involving food (boiled spinach) 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 = -4.086; P = 0.000; Table 1).

In the experiments using tadpoles of *Duttaphrynus melanostictus* as food, 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 (tadpoles of *D. melanostictus*) was visible through the glass beaker at one end of the test tank (Z = -0.304; P = 0.761; Table 1). In trials involving food (tadpoles of *D. melanostictus*) providing only chemical cues, the test tadpoles spent significantly more time in the zone housing food inside the mesh cage wrapped with cheese cloth as compared to the chemically blank zone housing only the mesh cage wrapped with cheese cloth without any food (Z = -4.086; P = 0.000; Table 1). In tests involving food (tadpoles of *D. melanostictus*) 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 as compared to the zone providing visual cues of food through the glass beaker (Z = -4.114; P = 0.000; Table 1).

In tests involving only visual cues of food (boiled spinach), in none of the 25 trials, test tadpoles went near the beaker or hit it when food was kept in a transparent glass beaker. Also, in tests involving only visual cues of food (tadpoles of *D. melanostictus*), in none of the 25 trials, test tadpoles went near the beaker or hit it when food was kept in a transparent glass beaker. In contrast, in tests in-

Table 1. Association choice of tadpoles of *Euphlyctis cyanophlyctis* (24 h starved) in response to visual/ chemical cues of food (either boiled spinach or tadpoles of *Duttaphrynus melanostictus*). #Wilcoxon matched-pairs signed-ranks test. *Significantly different. For each test, 25 trials in total, new test tadpoles were used. Four different types of end-bias tests were carried out (25 × 4 = 100 trials). For food detection experiments, for each test, 25 trials in total, new test tadpoles were used (25 × 6 = 150 trials).

Tests	Time (s) spent Mean (Min-Max ± SD)		
	Zone A	Zone B	Z# and P values
End-bias (pooled)	297.30 (100-544 ± 81.88)	302.70 (56-500 ± 81.88)	Z = -0.453; P = 0.651
Food (boiled spinach)			
Blank (A) vs. Visual (B)	309.12 (193-463 ± 63.33)	290.88 (137-407 ± 63.33)	Z = -0.411; P = 0.681
Blank (A) vs. Chemical (B)	187.28 (92-312 ± 64.65)	412.72 (288-508 ± 64.65)	Z = -4.265; P = 0.000*
Visual (A) vs. Chemical (B)	196.56 (69-372 ± 72.59)	403.44 (228-531 ± 72.59)	Z = -4.086; P = 0.000*
Food (tadpoles of *D. melanostictus*)			
Blank (A) vs. Visual (B)	296.28 (166-412 ± 62.23)	303.72 (188-434 ± 62.23)	Z = -0.304; P = 0.761
Blank (A) vs. Chemical (B)	203.80 (103-338 ± 64.54)	396.20 (262-497 ± 64.54)	Z = -4.086; P = 0.000*
Visual (A) vs. Chemical (B)	198.88 (85-344 ± 67.82)	401.12 (256-515 ± 67.82)	Z = -4.114; P = 0.000*

Table 2. Association choice of tadpoles of *Euphlyctis cyanophlyctis* (fed) in response to visual/ chemical cues from a predator (tadpoles of *Hoplobatrachus tigerinus*). #Wilcoxon matched-pairs signed-ranks test. *Significantly different. For each test, 25 trials in total, new test tadpoles were used. Four different types of end-bias tests were carried out (25 × 4 = 100 trials). For predator detection experiments, for each test, 25 trials in total, new test tadpoles were used (25 × 3 = 75 trials).

Tests	Time (s) spent Mean (Min-Max ± SD)		
	Zone A	Zone B	Z# and P values
End-bias (pooled)	301.39 (120-512 ± 69.79)	298.61 (88-480 ± 69.79)	Z = -0.071; P = 0.943
Blank (A) vs. Visual (B)	312.84 (242-407 ± 43.30)	287.16 (193-358 ± 43.30)	Z = -1.186; P = 0.236
Blank (A) vs. Chemical (B)	415.00 (279-564 ± 73.51)	185.00 (36-321 ± 73.51)	Z = -4.212; P = 0.000*
Visual (A) vs. Chemical (B)	412.08 (250-512 ± 68.18)	187.92 (88-350 ± 68.18)	Z = -4.184; P = 0.000*

volving only chemical cues of food (boiled spinach), in 15 out of 25 trials, tadpoles were seen hitting the mesh cage with their snouts presumably trying to reach the food that was not seen, but its presence was sensed using chemical cues. Also, in tests involving only chemical cues of food (tadpoles of *D. melanostictus*), in 14 out of 25 trials, tadpoles were seen hitting the mesh cage with their snouts presumably trying to reach the food (prey tadpoles) that was not seen, but its presence was sensed using chemical cues. In tests involving both visual and chemical cues of food (boiled spinach), in 15 out of 25 trials, tadpoles were seen hitting the mesh cage with their snouts presumably trying to reach the food but none of the tadpoles neither went near the beaker nor hit the beaker. Further, in tests involving both visual and chemical cues of food (tadpoles of *D. melanostictus*), in 13 out of 25 trials, tadpoles were seen hitting the mesh cage with their snouts presumably trying to reach the food but none of the tadpoles neither went near the beaker nor hit the beaker.

Sensory basis of predator detection in the tadpoles of *Euphlyctis cyanophlyctis*

In the end-bias tests, test 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 (Z = -0.071; P = 0.943; Table 2).

In trials with the visual cues of predators (tadpoles of *H. tigerinus*) 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 = -1.186; P = 0.236; Table 2). In trials with water-borne chemical cues of predators (tadpoles of *H. tigerinus*), the test tadpoles spent a significantly greater amount of time away from them (Z = -4.212; P = 0.000; Table 2). In trials with both visual as well as chemical cues of predators (tadpoles of *H. tigerinus*), 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 = -4.184; P = 0.000; Table 2).

In tests involving only visual cues of predator (tadpoles of *Hoplobatrachus tigerinus*), test tadpoles moved randomly and freely throughout the test tank and in 10 out of 25 trials, test tadpoles went near the beaker and also hit it even though the predator was kept in a transparent glass beaker. Those results indicated that the tested tadpoles ignored the visual cues of the predator. In contrast, in tests involving only chemical cues of the predator, in none of the 25 trials, test tadpoles went near the mesh cage or hit it when the predator was kept in a mesh cage wrapped with cheese cloth. The latter results indicated that the tested tadpoles avoided the predator or its area. In tests involving both visual and chemical cues of the predator, in none of the 25 trials, test tadpoles went near the mesh cage or hit it when the predator was kept in a mesh cage wrapped with cheese cloth, suggesting avoidance of the predator or its area.

Discussion

Evolution of good sensory perception of chemical cues is apparently a very important aspect of anuran larvae in the context of their life history (Hoff et al. 1999, Lannoo 1999). Certainly, the microhabitats in which tadpoles reside often have poor visibility conditions due to turbidity, shadow cast by vegetation, decayed leaf-litters or phytoplankton. Furthermore, the tadpoles appear to be near-sighted (Hoff et al. 1999). Therefore, the only other option is the use of chemical cues for detection of food and predators. This mechanism can significantly aid foraging vis-à-vis detection of food/ predators in the night hours or in relatively low-lit areas of the bottom. In the present study, tadpoles of *E. cyanophlyc-*

tis were tested to understand the mechanism of food or predator detection in clear water within a limited area of the test tank. The tadpoles of *E. cyanophlyctis* failed to detect both food and predators through visual cues. When food or the predator were kept in a transparent glass beaker, they neither went near the food nor avoided the predators or their area. They moved randomly throughout the test tank. However, tadpoles responded to invisible chemical food or predator cues. They moved towards the food placed in a mesh cage wrapped with cheese cloth. Indeed, more tadpoles were seen hitting the mesh cage with their snouts presumably trying to reach the food that was not seen, but its presence was sensed using chemical cues. Our results are in conformity with an earlier study on *Sphaerotheca breviceps* (Sugur et al. 2008), *Rana temporalis* (Veeranagoudar et al. 2004) and *Polypedatus maculates* tadpoles (Mogali et al. 2022). In contrast, *E. cyanophlyctis* tadpoles moved away from the predators placed in a mesh cage wrapped with a cheese cloth. Indeed, the majority of the tadpoles did not go near the predators. They also showed reduced movements in the predator zone and they suddenly returned to the predator-free area. Our results are in conformity also with earlier studies on tadpoles of *Rana lessonae* and *R. esculenta* (Stauffer & Semlitsch 1993), *Bufo boreas* (Kiesecker et al. 1996), *Mannophryne trinitatis* (Jowers et al. 2006), *R. temporalis* (Mogali et al. 2012) and *P. maculates* (Mogali 2018). Thus, blocking visual food or predator cues did not limit detection of food or predator that was exclusively based on water-borne chemical cues. These findings show that tadpoles of *E. cyanophlyctis* have a strong sense of chemical perception. The ability to detect food or predators 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 (Stauffer & Semlitsch 1993). It may also help tadpoles to forage or avoid predators at night hours. Indeed, omnivorous tadpoles like *E. cyanophlyctis* do forage and avoid predators during the night hours (our personal observations). The present study shows conclusively that the tadpoles of *E. cyanophlyctis* detect both food and predators using chemical senses. A failure to detect food or predator based on visual cues by the tadpoles of *E. cyanophlyctis* supports the general view that anuran tadpoles have poor vision.

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