

A tidal wave six and half feet in height, swept over the shores of Karachi five hours after the 'quake' shock. It is believed that the 'quake' caused the tidal wave, which took sometime to travel to Karachi.

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## Sensory basis of food perception in tadpoles of the frog, *Sphaerotheca breviceps*

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**The mechanism of food detection was studied in tadpoles of the frog *Sphaerotheca breviceps* using a rectangular glass tank, the two ends of which served as stimulus zones and housed the food (boiled spinach), providing either visual (food inside a glass beaker) or chemical (food inside a mesh cage wrapped with cheese cloth) cues or both. Each test tadpole (starved for 24 h before the trials) was placed in a centrally kept mesh cage for 5 min to enable perception of food cues. Each trial lasted for 10 min and the time spent by test tadpoles in each stimulus zone was recorded. The tadpoles showed no bias towards any particular side of the apparatus or trial procedure (end bias tests). In tests with visual cues at one end of the test tank also, the tadpoles moved randomly as in end bias tests. In contrast, in tests with chemical cues in one stimulus zone, the tadpoles spent majority of their time near chemical cues of food rather than in the zone that was chemically blank or provided only visual cues. In tests with food in open space in one zone and in the mesh cage in the opposite zone (both providing water-borne chemical cues), the tadpole distribution was random. The findings thus show that *S. breviceps* tadpoles detect food by chemical sensory mechanism rather than visual ones.**

**Keywords:** Food detection, food cues, frog, tadpole.

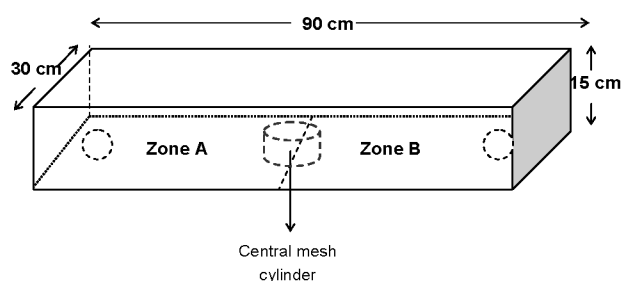
FORAGING is important in all living organisms for optimum growth, maintenance and reproduction. In most anurans the transitory tadpole stage is designed to exploit the benefits of the aquatic medium in order to gain an optimal size before metamorphosis and taking to terrestrial life. It is basically a food-gathering and growing phase in an aquatic environment. A number of studies have shown that anuran tadpoles respond to chemical, visual or tactile stimuli to elicit appropriate responses<sup>1</sup>. Often the anuran larvae are found in turbid/murky water or water filled with dense vegetation with poor visibility. Further, the tadpoles in general are near-sighted and therefore it is unlikely that they use vision to detect objects at greater distances<sup>1</sup>. Some studies have documented the use of chemical cues in the detection of various stimuli among anuran tadpoles. For instance, tadpoles of *Bufo americanus*<sup>2</sup>, *Rana cascade*, *Rana sylvatica*<sup>3</sup> and *Bufo scaber*<sup>4</sup> are reported to preferentially associate with their siblings

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(sibs) and perceive chemical cues emanating from the sibs. Amphibian tadpoles are also known to avoid predators based on the chemical cues emanating from the body of the predator or from the damaged conspecifics, the so-called alarm cues<sup>5-10</sup>. However, little is known about the foraging behaviour and mechanism of food detection among anuran tadpoles. The only study by Veerangoudar *et al.*<sup>11</sup> reported that the tadpoles of *Rana temporalis* which live in lotic waters detect food based on chemical cues in the laboratory studies. The present study was undertaken to find out the mechanism of food detection in tadpoles of the frog, *Sphaerotheca breviceps* that live in small ephemeral water bodies/puddles generally with turbid waters.

Tadpoles of *S. breviceps* (Gosner stages 22 and 23) were collected from rain-filled puddles on the Karnatak University campus, Dharwad (15°27'N, 75°3'E) and placed in an aquarium (75 × 45 × 15 cm) containing aged tap water. On reaching the feeding stage (Gosner stage 25), they were fed with boiled spinach. Tadpoles of stages 28–30 were used for the present study.

A rectangular glass tank (90 × 60 × 15 cm; Figure 1) was used for conducting the 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 was placed either in a glass beaker or within a mesh cage wrapped with cheese cloth or in the open in either one or both stimulus zones, separated from each other by 70 cm. We assumed that food placed in the beaker would block chemical cues but provide visual information, while the food placed in the mesh cage wrapped with cheese cloth would block visual cues but allow diffusion of chemical cues in the water. The food placed in the open without any barrier would provide both visual and chemical cues. Prior to each trial, the test tank was filled with aged tap water (2.5 cm height). The stimulus zones were reversed between the trials. In each trial a single test tadpole, starved for 24 h was used. A test tadpole was placed in an open-ended mesh cage (8 cm dia-



**Figure 1.** Design of test tank used for determining mechanism of food perception by the tadpoles of *Sphaerotheca breviceps*. The dotted central line visually divides the tank into two zones. Circles in the end zones indicate areas where the food was kept either in a glass beaker or a mesh cylinder wrapped with cheese cloth or in the open.

meter × 10 cm height) kept in the centre of the test tank and allowed to explore food cues for 5 min before its release. It was then released by gently lifting the cage and allowing it to move freely in the test tank. The trial period was set at 10 min. The time spent by the tadpole in each stimulus zone during a trial period was recorded. After each trial the test tank was washed and water was renewed. A test tadpole was used only once.

End bias tests were conducted to check whether the tadpoles show bias towards any end of the choice tank, or for any of the containers used for placing the food (open-ended cylindrical mesh cage wrapped with cheese cloth, glass beaker). 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 tank and the other zone blank; (3) with a mesh cage wrapped with cheese cloth in one zone with the other zone blank, and (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. In each set, 25 trials were conducted.

Tests involving detection of food based on visual or chemical cues or both by *S. breviceps* tadpoles were as follows. In tests for food detection based on visual cues, a beaker containing 2 g of boiled spinach 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 ( $n = 30$  trials). In the tests for food detection based on chemical cues, an open-ended mesh cage wrapped with cheese cloth with 2 g boiled spinach was placed at one zone of the test tank and another mesh cage wrapped with cheese cloth but devoid of food was placed at the other zone ( $n = 30$  trials). Association choice of test tadpoles was then recorded.

Tests were also conducted with both visual and chemical cues of food to know the sensory basis of food detection in tadpoles, with the expectation that these tests would also lend support to the findings of the above tests in which visual or chemical cues of food were provided one at a time. Three sets of tests were conducted, viz. (1) trials with a beaker containing 2 g boiled spinach (visual food cues) in one stimulus zone and a mesh cage wrapped with cheese cloth containing food (chemical food cues) placed in the other zone; (2) trials with a beaker containing food (2 g boiled spinach) placed in one stimulus zone (visual food cues) and food was in the open (both visual and chemical food cues) at the other zone, and (3) trials with 2 g boiled spinach placed in the mesh cage wrapped with cheese cloth (chemical food cues) in one stimulus zone and food placed in the open (both visual and chemical food cues) at the other zone.

Association choice of test tadpoles was then recorded. Each set consisted of 30 trials. Wilcoxon matched-pairs signed ranks test was used for comparing the time spent

by tadpoles in each of the two stimulus zones. We tested the time spent by the test tadpoles in a particular stimulus zone of the choice tank from a hypothetical random time expected (300 s) under the null hypothesis that tadpoles would spend an equal amount of time in each zone of the test tank. Only one score was used in comparison with the expected mean, not to violate the independence of the data. All tests were two-tailed. Each of the four end bias tests showed that the tadpoles have no bias towards any particular stimulus zone or the containers used to house the food. Therefore, data on all end bias tests were pooled and analysed and are presented in Table 1. The differences were judged significant if  $P < 0.05$ .

Soon after their release from the central mesh cage, the test tadpoles moved randomly in the tank and in about 5 min time their distribution became stabilized. In some trials, they took 2–4 min to make their first move after being released from the cage. The pooled data of end bias tests showed that the tadpoles moved randomly in the test tank. They showed no bias towards any sides of the test tank or the food containers used ( $Z = -0.631$ ,  $P = 0.528$ ; Table 1).

In tests involving food providing only visual cues, there was no significant difference in the time spent by the 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 ( $Z = -0.363$ ,  $P = 0.716$ ). In trials involving food providing only chemical cues, the 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 ( $Z = -3.135$ ,  $P = 0.002$ ). In tests involving food providing both visual as well as chemical cues, the tadpoles spent 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 ( $Z = -2.623$ ,  $P = 0.009$ ). Likewise, the tadpoles spent majority of their time in the zone where food was kept in the open without a barrier than near the other zone displaying food in glass beaker ( $Z = -3.56$ ,  $P = 0.00$ ). However, when food was provided in a mesh cage wrapped with cheese cloth at one end and in the open at the other end, tadpoles moved randomly between both zones and there was no difference in the time spent by the tadpoles between the two zones ( $Z = -0.378$ ,  $P = 0.705$ ).

The tadpoles of *S. breviceps* are found in rain-filled puddles or ephemeral water bodies that are generally turbid or murky with low visibility. Therefore, the ability of these tadpoles to detect food based on visual perception may be limited. It is widely believed that amphibian tadpoles are near-sighted<sup>1</sup>. If so, it would limit the visual detection of food or prey. In the present study, tadpoles of *S. breviceps* were tested to understand the mechanism of food detection in clear water within a limited area of the test tank. Yet *S. breviceps* tadpoles ignored the food placed in a glass beaker, indicating the ineffectiveness of visual food cues in attracting the tadpoles towards the food. However, the tadpoles responded to invisible chemical food cues and moved towards the food placed in a mesh cage covered with cheese cloth. In fact, a few 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. Thus, blocking visual food cues did not limit detection of food that is exclusively based on water-borne chemical cues. These findings show that *S. breviceps* tadpoles have a strong sense of chemical perception.

Further, a random distribution of tadpoles in the test tank regardless of whether the food was placed in the open or within the mesh cage covered with cheese cloth in opposite stimulus zones also strengthens the view that the tadpoles are guided to food by water-borne chemical cues emanating from the food. These findings are in conformity with those reported on the tadpoles of *R. temporalis*<sup>11</sup>. The ability to detect food based on chemical cues may evolve especially under poor visibility conditions, such as in murky/turbid water filled with dense vegetation. It may also help tadpoles to forage at night. Indeed, herbivorous tadpoles like *S. breviceps* do forage during the night hours (our unpublished observations). The present study shows conclusively that the tadpoles of *S. breviceps* perceive and locate food using chemical sense. A failure to detect food based on visual cues by the tadpoles of *S. breviceps* supports the general view that anuran tadpoles may have poor vision.

**Table 1.** Mean time spent by tadpoles of *Sphaerotheca breviceps* in the two stimulus zones of the test tank

Test	Time spent (s) $\pm$ SE		
	Zone A	Zone B	Z <sup>#</sup> and P values
End bias	316.13 $\pm$ 21.45	283.86 $\pm$ 21.45	Z = -0.631 P = 0.528
Visual (A) vs blank (B)	318.56 $\pm$ 35.99	280.53 $\pm$ 36.18	Z = -0.363 P = 0.716
Chemical (A) vs blank (B)	428.95 $\pm$ 32.41	170.44 $\pm$ 32.25	Z = -3.135 P = 0.002*
Visual (A) vs chemical (B)	198.83 $\pm$ 30.08	401.18 $\pm$ 30.08	Z = -2.623 P = 0.009*
Visual (A) vs visual and chemical (B)	147.04 $\pm$ 28.02	453.01 $\pm$ 26.85	Z = -3.566 P = 0.000*
Visual and chemical (A) vs chemical (B)	298.07 $\pm$ 43.28	301.97 $\pm$ 43.28	Z = -0.378 P = 0.705

<sup>#</sup>Wilcoxon matched-pairs signed-ranks test; \*Significantly different.

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Rafael Campos-Ramos, Hortencia Obregón-Barboza and Alejandro M. Maeda-Martínez

**Survival, growth and composition of *Poecilia latipinna* fry fed enriched *Artemia nauplii***

C. Arulvasu and N. Munuswamy

**Aerobic Gram-positive heterotrophic bacteria *Exiguobacterium mexicanum* and *Microbacterium* sp. in the gut lumen of *Artemia franciscana* larvae under gnotobiotic conditions**

César Orozco-Medina, Alejandro López-Cortés and Alejandro M. Maeda-Martínez