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Sensory basis of food detection in tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae): an experimental approach

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Abstract

Sensory basis of food detection in tadpoles of *Polypedates maculatus* (Anura: Rhacophoridae): an experimental approach. The mechanism of food detection in tadpoles of *Polypedates maculatus* was experimentally tested. We used a rectangular glass test tank with stimulus zones in opposite ends to provide visual and/or chemical food. For visual cues, boiled spinach was placed inside a glass container, and for chemical cues boiled spinach was placed in a mesh cage. Each tadpole of *P. maculatus* (either at an early or medium developmental stage) was held at the center of the test tank for acclimation. The tadpole was released, and we recorded whether it approached or did not approach the caged food. Tadpoles of all stages failed to detect food using visual cues. Tadpoles of all stages detected food using chemical cues. In tests using chemical cues, they spent the majority of their time (69.3% by early stage tadpoles and 87.3% by medium-stage tadpoles) near the container with food than in the end with no containers or with only visual food cues. Tadpoles in medium stages spent more time near food (18.1% of total time) than tadpoles in early stages. These findings indicate that tadpoles of *P. maculatus* detect food by chemical sensory mechanisms rather than visual ones. Tadpoles in medium stages spent more time near food than tadpoles in early stages indicating that time spent foraging increases as tadpoles grow.

Keywords: Anuran larvae, Chemical cues, Developmental stage, Food detection behavior, Visual cues.

Resumo

Base sensorial da detecção de alimento em girinos de *Polypedates maculatus* (Anura: Rhacophoridae): uma abordagem experimental. O mecanismo de detecção de alimento em girinos de *Polypedates maculatus* foi testado experimentalmente. Utilizamos um aquário retangular de teste com zonas de estímulo em seus extremos opostos, fornecendo exclusivamente alimento visual e/ou químico. Para o estímulos visual, espinafre cozido foi colocado dentro de um recipiente de vidro, e para os estímulo químico, espinafre cozido foi colocado em uma gaiola de tela nas zonas opostas do aquários de testes. Cada girino de *P. maculatus* (na fase inicial ou média de

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desenvolvimento) era mantido no centro do aquário para a aclimatação, sendo depois liberado e deixado que se aproximasse ou se afastasse do alimento das gaiolas. Os girinos de ambas as fases não conseguiram detectar o alimento por meio de sinais visuais. Passaram quase o mesmo tempo na zona que abrigava o alimento no recipiente de vidro e na zona oposta, mantida vazia. Os girinos de ambas as fases detectaram o alimento unicamente por meio de estímulos químicos. Nos testes com estímulos químicos, os girinos passaram a maior parte do tempo (69,3% para girinos na fase inicial e 87,3% para girinos na fase média) perto do recipiente que liberava sinais químicos, em comparação com os recipientes vazios ou apenas com estímulos visuais na zona oposta do aquário. Curiosamente, o estudo também mostrou que os girinos na fase média estão mais associados ao alimento (18,1% do tempo total) do que os girinos de fase inicial. Essas descobertas evidenciaram que os girinos de *P. maculatus* detectam o alimento por mecanismos sensoriais químicos em vez de visuais. Além disso, os girinos de fase média permanecem mais associados ao alimento do que os girinos de fase inicial, o que indica claramente que a taxa de forrageio aumenta nos girinos de fase avançada.

Palavras-chave: Comportamento, Estágio de desenvolvimento, Estímulos químicos, Estímulos visuais, Larvas de anuros.

Introduction

Amphibians and especially anurans exhibit a great diversity of reproductive modes and reproduce in a great variety of habitats, including phytotelmata, forest litter, and burrows (Duellman and Trueb 1986, Haddad and Prado 2005). Tadpoles of the majority of anurans complete their development in different types of lotic and lentic water bodies (Saidapur *et al.* 2009, Mogali *et al.* 2011, 2012, 2020, 2021). 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 (Stauffer and Semlitsch 1993, Hoff *et al.* 1999, Mogali *et al.* 2015). Most anuran tadpoles are found in murky or turbid water with poor visibility. Anuran tadpoles in general are near-sighted and it is unlikely that they use vision to detect distant objects (Hoff *et al.* 1999). The perception of chemical cues seems to play a vital role in behavioral and physiological responses in tadpoles.

Tadpoles of most tropical anurans live in temporary water bodies that are rich in resources. They are consequently exposed to a wide range of larvae of invertebrate predators and to some vertebrate predators, mainly fishes (Saidapur 2001). Tadpoles feed on microorganisms, algae, and detritus depending upon their oral apparatus and prey availability (Sekar 1992, Hoff *et al.* 1999, Saidapur 2001, Santos *et al.* 2016, Protázio *et al.* 2020).

The Indian tree frog, *Polypedates maculatus* (Gray, 1830), is widely distributed in India. In Southern India, populations breed only during the rainy season. Females deposit eggs in foam nests that are attached to vegetation such as bushes over water, under rocks, or adhered to walls of cement cisterns filled with water (Mohanty-Hejmadi and Dutta 1988, Girish and Saidapur 1999). Early embryonic development (up to stage 23 of Gosner 1960) occurs inside the foam nests, after which tadpoles drop into the water where they undergo further development and metamorphosis. Foam nests, including those of *P. maculatus,* supply adequate oxygen (Seymour and Loveridge 1994) and can maintain adequate temperatures for development (Dobkin and Gettinger 1985). Foam nests also protect eggs and tadpoles from predation in early developmental stages and prevent the eggs from desiccating (Heyer 1969, 1975, Downie 1990, Mogali 2018). Tadpoles of *P. maculatus* are bottom dwellers (in temporary and permanent water bodies) and forage on detritus and algal matter (Hiragond and Saidapur 2001). Visibility is generally low in these habitats because of turbid water and detritus. Little is known about tadpoles dwelling in environments with limited visibility (Fouilloux *et al.* 2022). In this context, we studied how tadpoles of *P. maculatus* detect their food using laboratory experiments.

Materials and Methods

Developmental stages are according to Gosner (1960). Four foam nests of the Indian tree frog, *Polypedates maculatus,* were located in temporary ponds during the early monsoon season (June 2017) on the Karnatak University Campus (15.440407° N, 74.985246° E), Dharwad, Karnataka State, India. The foam nests were attached to vegetation about 20 cm above the water. They were collected and placed separately in plastic tubs (32 cm diameter and 14 cm deep) with 1 L of dechlorinated, aged tap water and substratum collected from the same pond. The tadpoles emerged at stage 23 after 5–6 days. Tadpoles from all four foam nests were mixed to normalize genetic differences among the groups and were reared in a glass aquarium (75 × 45 × 15 cm) containing 15 L of aged tap water. When tadpoles reached the feeding stage (stage 25) they were provided with boiled spinach *ad libitum*. The mechanism of food detection in *P. maculatus* was studied at different developmental stages: early (stages 27–28) and medium (stages 35–36). In all experimental trials, tadpoles were of comparable body sizes and developmental stages (early stage tadpoles: total length 26.50 ± 0.65 mm ($\bar{x}$ ± SE); N = 25; medium stage tadpoles: total length 49.20 ± 1.20 mm ($\bar{x}$ ± SE); N = 25).

A rectangular glass tank (90 × 30 × 15 cm) was used for the food detection experiments (Figure 1). A central line perpendicular to the long axis of the test tank was drawn on the outside of the bottom, dividing it into two equal compartments referred to as stimulus zones A and B. The food (boiled spinach, 1 g) was placed either in a transparent glass container (9 cm diameter × 14 cm height) or within a mesh cage (10 cm diameter × 15 cm height) wrapped in cheesecloth. We assumed that food placed in the transparent glass container would block chemical cues but provide visual information, while food placed in the mesh cage wrapped with cheesecloth would block visual cues but allow diffusion of chemical cues in the water. Prior to each trial, the tank was filled with aged tap water to a height of 3 cm. The stimulus zones were reversed between the trials.

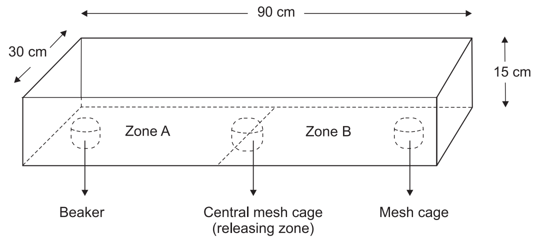

Figure 1. The design of the test tank for investigating the mechanism of food detection by the tadpoles (at early and medium stages of development) of *Polypedates maculatus*. The dotted central line visually divides the test tank into two zones (A and B). The central area was used to release test tadpoles. Containers in the opposite zones indicate areas where food was presented either in a transparent glass container or a mesh cage wrapped with cheesecloth.

In each trial, a single tadpole (*P. maculatus*) was tested. A tadpole starved for 24 hours (either at an early or medium stage of development) was placed in an open-ended mesh cage (10 cm diameter × 15 cm height) wrapped with cheesecloth. The mesh cage was placed in the center of the tank for 5 min to allow the tadpole to acclimate as well as to perceive visual and/or chemical food cues. The tadpole was then released by gently lifting the mesh cage without disturbance, allowing it to freely move in the tank. The time spent by the tadpole in each stimulus zone during the trial period was recorded for 10 min. Our assumption was that when tadpoles detected food, they would spend more time in the zone with food than in the opposite zone without food. Failure to detect food would result in random movements of tadpoles in the tank. After each trial, the tank was washed and the water was changed. Each tadpole and container of food was used only once. All trials were conducted under natural photoperiod and temperature. The daily temperature of the testing room in the laboratory varied between 25–26°C.

End-bias tests were conducted to check whether the tadpoles showed bias toward either end of the test tank or for either type of container used for placing the food (a glass container and an open-ended cylindrical mesh cage wrapped with cheesecloth) (Sugur *et al.* 2008). These tests involved four sets of trials: (1) with no containers at either end of the test tank; (2) with a glass container containing water level with that in the test tank at one end and the other end with nothing; (3) with a mesh cage wrapped with cheesecloth in one end and nothing in the other end; and (4) with a glass container and a mesh cage wrapped with cheesecloth placed at the opposite ends of the tank. The time spent by a tadpole in each zone in a given trial was recorded. We carried out the end-bias tests separately using early and medium developmental stages of tadpoles. In each set, 25 trials were conducted (4 types of end-bias tests × 2 types of test tadpoles, early and medium stages of development × 25 trials for each set = total of 200 end-bias tests).

Tests involving detection of food based on visual or chemical cues by the tadpoles were conducted as follows. In tests for food detection based on visual cues, a glass container containing 1 g of boiled spinach was placed at one end of the test tank and the opposite end was provided with a glass container containing water level with that in the tank ($N = 25$ trials). In tests for food detection based on chemical cues, an open-ended mesh cage wrapped with cheesecloth containing 1 g of boiled spinach was placed at one end of the tank and a similar cage but devoid of food was placed at the opposite end ($N = 25$ trials). In tests for food detection based on both visual and chemical cues, a glass container containing 1 g of boiled spinach (visual food cues) was place in one end of the tank, and a mesh cage wrapped with cheesecloth containing 1 g of boiled spinach (chemical food cues) was placed in the other end ($N = 25$ trials). The amount of time spent in each stimulus zone by a tadpole was recorded and analyzed using the Wilcoxon matched-pairs signed-ranks test (SPSS software ver. 16.0).

Results

Mechanism of Food Detection at Early Stages of Development

In the end-bias tests, tadpoles of early stages moved freely throughout the tank, showing no bias toward any particular zone of the test tank ($Z = -0.331$; $p = 0.741$; Table 1). The placement of the glass container or mesh cage made no difference to space use by the tadpoles.

In tests involving food providing only visual cues, there was no significant difference in the time spent by tadpoles between the stimulus zones with glass containers with or without food, even though the food was visible through the glass container at one end of the tank ($Z = -0.414$; $p = 0.679$; Table 1). In trials involving food providing only chemical cues, tadpoles spent significantly more time in the zone with food inside the mesh cage wrapped with cheesecloth compared to the mesh cage without food ($Z = -4.347$; $p = 0.000$; Table 1). In tests involving food providing both visual and chemical cues, tadpoles spent a significantly greater amount of time in the zone with food in the mesh cage wrapped with cheesecloth compared to the zone providing visual cues of food through the glass container ($Z = -4.346$; $p = 0.000$; Table 1).

In the 25 trials with only visual cues of food in glass containers, no tadpoles approached the container or touched it. In contrast, in 16 of 25

Table 1. The amount of time spent by tadpoles of *Polypedates maculatus* (at early stages of development; stages 27–28) in response to visual/chemical cues of food, boiled spinach. #Wilcoxon matched-pairs signed-ranks test. *Significantly different.

Tests	Mean time spent ± SE (s)		*Z* and *p* values#
	Zone A	Zone B	
End-bias	302.16 ± 8.07	297.84 ± 8.07	*Z* = -0.331; *p* = 0.741
Blank (A) vs. Visual (B)	294.92 ± 16.22	305.08 ± 16.22	*Z* = -0.414; *p* = 0.679
Blank (A) vs. Chemical (B)	184.44 ± 9.39	415.56 ± 9.39	*Z* = -4.347; *p* = 0.000*
Visual (A) vs. Chemical (B)	176.40 ± 8.69	423.60 ± 8.69	*Z* = -4.346; *p* = 0.000*

trials involving only chemical cues of food, tadpoles touched the mesh cage with their snouts, presumably sensing chemical cues from food that could not be seen but was sensed through chemical cues. In 17 of 25 trials involving both visual and chemical cues of food, tadpoles touched the mesh cage with their snouts, presumably trying to reach the food, but no tadpoles approached or touched the glass containers.

Mechanism of Food Detection at Medium Stages of Development

In the end-bias tests, tadpoles at medium stages moved freely throughout the test tank (Z = -0.978; *p* = 0.328; Table 2). They showed no bias toward any particular zone of the tank or toward the containers (glass container or mesh cage).

In tests involving food providing only visual cues, there was no significant difference in the time spent by the tadpoles between the stimulus zones with glass containers with or without food (Z = -0.243; *p* = 0.808; Table 2). In trials involving food providing only chemical cues, the tadpoles spent significantly more time in the zone with food inside the mesh cage compared to the zone with the mesh cage without food (Z = -4.373; *p* = 0.000; Table 2). In tests involving food providing both visual and chemical cues, tadpoles spent a significantly greater amount of time in the zone with food in the mesh cage compared to the zone providing visual cues of food (Z = -4.372; *p* = 0.000; Table 2).

In the 25 trials with only visual cues of food, no tadpoles approached the glass container or touched it. In contrast, in 22 of 25 trials involving only chemical cues of food, tadpoles touched the mesh cage with their snouts, presumably trying to reach the food that could not be seen but was sensed through chemical cues. In 22 of 25 trials involving both visual and chemical cues of food, tadpoles touched the mesh cage with their snouts, presumably trying to reach the food, but no the tadpoles approached or touched the glass containers.

Discussion

In Southern India, tadpoles of *P. maculatus* occur in temporary and permanent water bodies that are generally turbid or murky with low visibility (Hiragond and Saidapur 2001, Saidapur *et al.* 2009, Mogali 2018). The ability of these tadpoles to detect food based on visual cues may be limited. It is generally believed that anuran tadpoles are near-sighted (Hoff *et al.* 1999, Mogali 2018). If so, visual detection of food or prey would be limited. In our laboratory experiments, tadpoles of *P. maculatus* at early and medium developmental stages were tested to understand the mechanism of food detection in

Table 2. The amount of time spent by tadpoles of *Polypedates maculatus* (at medium stages of development; stages 35–36) in response to visual/chemical cues of food, boiled spinach. #Wilcoxon matched-pairs signed-ranks test. *Significantly different.

Tests	Mean time spent ± SE (s)		*Z* and *p* values#
	Zone A	Zone B	
End-bias	296.66 ± 8.64	303.34 ± 8.64	Z = -0.978; p = 0.328
Blank (A) vs. Visual (B)	300.60 ± 14.80	299.40 ± 14.80	Z = -0.243; p = 0.808
Blank (A) vs. Chemical (B)	76.04 ± 5.83	523.96 ± 5.83	Z = -4.373; p = 0.000*
Visual (A) vs. Chemical (B)	71.88 ± 6.11	528.12 ± 6.11	Z = -4.372; p = 0.000*

clear water. Tadpoles in both early and medium stages did not detect the food (boiled spinach) placed in transparent glass containers, even though they were starved for 24 hours, indicating the ineffectiveness of visual cues. Blocking visual food cues did not limit detection of food that is solely based on water-borne chemical cues (see Sugur *et al.* 2008). Tadpoles in both early and medium stages moved toward chemical cues from food hidden in a mesh cage wrapped in cheesecloth. A large proportion of tadpoles of both stages (68% by early and 88% by medium stages) touched the mesh cage with their snouts, presumably trying to reach the food that could not be seen. No tadpoles touched the glass containers with visible food, indicating that tadpoles of *P. maculatus* detect food using chemical cues.

Our results conform to an earlier study on tadpoles of *Indosylvirana temporalis* (Veeranagoudar *et al.* 2004), *Sphaerotheca breviceps* (Sugur *et al.* 2008), and *Clinotarsus curtipes* (unpubl. data). The present study shows that tadpoles in medium stages spend more time (18.1%) near food than tadpoles in early stages. More tadpoles in medium stages (88%) touched the mesh cages with food than tadpoles in early stages (68%). Time spent foraging increases as tadpoles grow.

The ability to detect food based on chemical cues may have evolved under poor or low visibility conditions, such as in murky or turbid water or benthic areas covered by leaf litter and detritus. This ability may allow tadpoles to forage at night. All stages of herbivorous tadpoles like *P. maculatus* forage during the night hours (SMM personal observations). The present study shows that tadpoles of *P. maculatus* of different developmental stages detect food through chemical, not visual, senses. Failure to detect food based on visual cues by the tadpoles of *P. maculatus* supports the general view that anuran tadpoles, especially those dwelling in turbid water, have poor vision.

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