

Chemical cues of conspecific predation induce strong behavioural changes in cannibalistic Indian bullfrog tadpoles, *Hoplobatrachus tigerinus*

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Abstract

Cannibalism is an act of intraspecific predation. In cannibalistic species, conspecifics can be both predators and prey. Cannibalism in anuran tadpoles is a survival strategy driven by environmental stress, rapid population density, and scarce resources. To better know how individuals respond to the complex chemical cues, I studied cannibalistic tadpoles of the Indian bullfrog, *Hoplobatrachus tigerinus*. I exposed prey (small sized *H. tigerinus*; Gosner stage 27–28) tadpoles to chemical cues of either kairomones of predators or heterospecific or conspecific tadpole fed predators (large sized *H. tigerinus*; Gosner stage 37–38). I have not noticed any behavioural changes in prey tadpoles when exposed to chemical cues of either kairomones or heterospecific tadpole fed predators. However, I noticed a strong behavioural changes in prey tadpoles to chemical cues of conspecific tadpole fed predators. The behavioural changes includes; overall reduction in their swimming activity levels (not easy to detect) and high burst speed (fast escape from predators). In conclusion, small sized *H. tigerinus* tadpoles show strong antipredator defense behavior to its own large sized tadpoles that fed with conspecifics (own members). Hence, they clearly identify the death of their conspecific members as a serious threat.

Introduction

The most important challenge for all living organisms is to detect and respond to environmental stimuli and it is more applicable to aquatic animals (Abrahams and Dill 1989; Lima and Dill 1990; Kats and Dill 1998; Cavailles et al. 2025). Because majority of the animals are living in aquatic environments. In such systems, detection of food and predator is primarily very much important for all animals for their survival (Mogali et al. 2011). Many aquatic animals including anuran tadpoles detect both food and predator through chemical senses rather than visual. Because these animals are mainly found in turbid water or water filled with dense vegetation or leaf litter with poor visibility. In such conditions, chemical cues may reach to animals fast and from long distance and further-more anuran tadpoles are near-sighted (Hoff et al. 1999; Mogali et al. 2012, 2023).

Majority of the anuran tadpole species are found in the temporary water bodies. Such waters housed mostly herbivorous (prey) tadpoles and also sometime carnivorous tadpoles (predator). The carnivorous tadpoles are usually feeding on herbivorous tadpoles and also on other available species. If there is a scarcity of food occurs in such waters then carnivorous tadpoles for their survival, must and should attack and feed on its own species. Cannibalism, defined as is the act of killing and consuming an individual of the same species (conspecifics) either in part or as a whole. More specifically, it is predator (strong individual) and prey (weak individual) interactions between conspecifics (Fox 1975; Ferrari and Chivers 2009). It is a high-risk, high-reward interaction behaviour, the one who wins in this battle is survive and loss is lethal (Surber-Cunningham et al. 2024). Although cannibalistic behaviour may appear counterintuitive to species perseverance, it can be a firm evolutionary strategy when the advantages to individuals in times of limited food availability are greater than the disadvantages during times of food abundance (Nishimura and Isoda, 2004). The benefits of cannibalism include resource gaining, reduction in intraspecific competition and in reproductive success (Wise 2006), while the costs of cannibalism

include increased risk of injury and disease transmission and decreased inclusive fitness from cannibalism of family members (Pfennig 1997, Pfennig et al. 1991; Ward et al. 2006). Particularly in the absence of supplementary cues that can disambiguate an individual's status as predator against prey, conspecific chemical cues can simultaneously signal the extremes of predation risk against diet accessibility. Thus, individuals of cannibalistic species may exceptionally adjust their responses to conspecifics or its cues.

In Southern India (Dharwad city, Karnataka State), the Indian bullfrog, *Hoplobatrachus tigerinus* are usually breed in the early monsoon season along with other sympatric anuran species in temporary ponds. The *H. tigerinus* tadpoles are generally considered as the top predators at our area because *H. tigerinus* feeding on almost all available tadpole species which are found in the temporary ponds. They are basically carnivorous in nature and also exhibit cannibalistic behavior both in wild and in laboratory if there is a scarcity of food (Saidapur 2001, 2025). Because of its cannibalistic nature, they unable to detect their kin (Rajput et al. 2011). Cannibalism in *H. tigerinus* tadpoles likely to evolved as a method of eliminating competition and as a secondary source of nutrition (Caldwell and De Araújo 1998). Development and cannibalism are linked as larger individuals are at lower risk of being eaten and are better able to eat easily smaller conspecifics (Petranka and Thomas 1995; Fouilloux et al. 2022). I also personally noticed this kind of behavior in *H. tigerinus* tadpoles both in nature and laboratory. Hence, keeping on this view, I designed the experiment by exposing small sized *H. tigerinus* (Gosner stage 27–28) tadpoles (considered as prey) to various chemical cues of large sized *H. tigerinus* (Gosner stage 37–38) tadpoles (considered as predator). The various chemical cues of the large sized predator used in this experiment are; kairomones, dietary cues of predator fed with heterospecifics (other tadpole species e.g. *Sphaerotheca breviceps*) and dietary cues of predator fed with conspecifics (own species). In the present study, I hypothesized that small sized *H. tigerinus* tadpoles do not show any antipredator behavioral responses to chemical cues of either kairomones or dietary cues of heterospecific tadpole fed predator. Because small sized *H. tigerinus* tadpoles are also basically a carnivorous tadpoles, feeding on other sympatric tadpole species. So the chemical cues either kairomones (smell from starved predator) or cues that contains falling the prey of heterospecific member may not affect the behavioural responses of small sized *H. tigerinus*. However, small sized *H. tigerinus* tadpoles should show strong antipredator behavioral responses to chemical cues of large sized *H. tigerinus* tadpoles that fed with conspecifics (own species). If all hypothesis comes true then the outcome of this study will provide a novel information in the behavior of cannibalistic tadpoles.

Materials and methods

Collection and maintenance of tadpoles

Tadpoles of *Hoplobatrachus tigerinus* of various developmental stages from 25 to 38 were collected time to time from the temporary ponds in the Karnatak University Campus, Dharwad, Karnataka State, India (15°27'N, 75°05'E, 750 m a.s.l.). Tadpoles of *H. tigerinus* are basically carnivorous in nature, feeding on all available sympatric tadpoles in the pond. They also exhibit the phenomenon of cannibalism if there is

a scarcity of food. Because of its highly predaceous/ cannibalistic nature, maintaining tadpoles of *H. tigerinus* in the laboratory is very difficult hence they were collected whenever needed but 3 days prior to the experiment. The tadpoles of *Sphaerotheca breviceps* (Gosner stage 25–26; Gosner 1960) are herbivorous in nature and were also collected from the temporary ponds. They are used to feed *H. tigerinus* tadpoles. In this experiment both prey and predator are of the same species (*H. tigerinus*). Hence, for experimental trial purpose, the tadpoles of *H. tigerinus* are divided into two groups i.e., small sized (prey; Gosner stage 27–28; 28.25 ± 0.54 mm in total length; mean $\pm$ SE; N = 20) and large sized (predator; Gosner stage 37–38; 40.58 ± 0.73 mm in total length; mean $\pm$ SE; N = 20). Prey tadpoles (for experimental trials) of *H. tigerinus* were reared individually in plastic tub (19 cm in diameter and 7 cm in depth) in 0.5 L of aged tap water and are fed with tadpoles of *S. breviceps*. In the present experiment, the behavioural responses of prey tadpoles (*H. tigerinus*; Gosner stage 27–28) was studied by exposing them to stimulus solution of either starved predator or dietary cues of predator fed with heterospecifics or conspecifics. Here various chemical cues are obtained by large sized tadpoles (Gosner stages 37–38) of *H. tigerinus*. All experimental prey tadpoles (*H. tigerinus*; Gosner stage 27–28) which are used for trials are healthy and uninjured.

Preparation of stimulus solutions

Kairomones: Tadpoles of *H. tigerinus* (Gosner stage 37–38; Gosner 1960) were placed individually in separate plastic tubs (N = 5 tubs; 19 cm in diameter and 7 cm in depth) containing 200 mL of aged tap water without food for 96 h. This procedure resulted in a solution with only kairomones. After 96 h of starvation period, the predators were removed from the tubs, and the stimulus solution was filtered thrice to remove any small quantities of faecal matter. The filtered solution, rich in kairomones was used immediately for the experimental trials.

Dietary cues of predator fed with heterospecifics: Tadpole of *H. tigerinus* (Gosner stage 37–38; n = 1) was placed in a plastic tubs (N = 5 tubs; 19 cm diameter and 7 cm deep) containing 200 mL of aged tap water along with four tadpoles of *S. breviceps* (Gosner stages 25–26; at 8:30 h). The *H. tigerinus* tadpoles consumed all the tadpoles provided to them by the evening (18:30 h). On the next day between 9:30 h and 11.30 h, predators were removed and the water from the tubs was filtered using fine cheese cloth thrice. The filtrate served as the stimulus solution containing the diet-derived excretory metabolites or substances of predators fed heterospecific prey (*S. breviceps*) + kairomones and are unlikely to have contained the alarm cues of prey.

Dietary cues of predator fed with conspecifics: Tadpole of *H. tigerinus* (Gosner stage 37–38; n = 1) was placed in a plastic tubs (N = 5 tubs; 19 cm diameter and 7 cm deep) containing 200 mL of aged tap water along with four tadpoles of *H. tigerinus* (Gosner stages 25–26; at 8:30 h). The *H. tigerinus* tadpoles consumed all the conspecific tadpoles provided to them by the evening (18:30 h). On the next day between 9:30 h and 11.30 h, predators were removed and the water from the tubs was filtered using fine cheese cloth thrice. The filtrate served as the stimulus solution containing the diet-derived excretory metabolites or substances of predators fed conspecifics (*H. tigerinus*) + kairomones and are unlikely to

have contained the alarm cues of prey. Earlier studies suggest that prey alarm cues are known to labile in nature (Ferrari et al. 2008, Wisenden et al. 2009, Chivers et al. 2013). Thus, it is unlikely that prey alarm cues were present in the stimulus solution because all prey were consumed more than 15 h before the solution was collected (Mogali et al. 2020). The water temperature of the testing test tank fluctuated between 24-24.2 °C. All experimental trials were conducted in a room with uniform light. The experiments were conducted in the absence of wind and human-induced noise. The experimental trials were started in the morning at 8:00 h and continued until afternoon (15:00 h). Since the experimental trials were carried out in the rainy season, the climatic conditions remained almost the same during entire trial period.

Behavioral responses of *H. tigerinus* (prey; small sized) tadpoles to kairomones of cannibalistic predatory tadpoles, *H. tigerinus* (large sized)

The behavioral responses of *H. tigerinus* tadpoles to kairomones of *H. tigerinus* (water conditioned with starved predators) were recorded by placing single *H. tigerinus* tadpole in a rectangular glass tank (28 L × 15 W × 15 H cm) containing 600 mL of aged tap water. A handycam (Sony, DCR-SR300/E, Japan) was fixed above the tank such that it recorded the entire tank. The handycam was connected to a computer with the Ethovision Video Tracking System (Noldus Information Technology, The Netherlands) to track movements of the tadpole before and after addition of stimulus solution to the test tank. The Ethovision system was used to record maximum swimming speed ($V_{\max}$), distance traversed by the tadpole, number of swimming spurts and time spent swimming during an entire trial. For each trial, a new *H. tigerinus* tadpole was first introduced into the tank and left undisturbed for 5 min. A burette was placed ~ 1 cm above the water level and 50 mL of aged tap water (chemical blank water) was then added at the rate of ~ 1 mL/s. The burette was then removed gently. Movement of the tadpole was then recorded for 5 min using Ethovision to record its baseline activity in the absence of any cues. After tracking baseline activity, 50 mL of stimulus solution containing kairomones of *H. tigerinus* was added as described above. Movement of the tadpole was recorded for another 5 min to determine the activity pattern after exposure to kairomones. Twenty trials were conducted.

Behavioral responses of *H. tigerinus* (prey; small sized) tadpoles to kairomones + dietary cues of large sized *H. tigerinus* fed with heterospecifics

In this experiment, the stimulus solution contained the chemical cues from excretory metabolites or substances of *H. tigerinus* tadpoles fed *S. breviceps* tadpoles (i.e., kairomones + dietary cues of heterospecifics) instead of kairomones alone of the predator. The behavioural responses of *H. tigerinus* tadpoles were recorded as described above, before and after the addition of stimulus solution (kairomones + dietary cues of *H. tigerinus* fed with heterospecifics). Twenty trials were conducted.

Behavioral responses of *H. tigerinus* (prey; small sized) tadpoles to kairomones + dietary cues of large sized *H. tigerinus* fed with conspecifics

In this experiment, the stimulus solution contained the chemical cues from excretory metabolites or substances of *H. tigerinus* tadpoles fed *H. tigerinus* tadpoles (i.e., kairomones + dietary cues of conspecifics) instead of kairomones alone of the predator. The behavioural responses of *H. tigerinus* tadpoles were recorded as described above, before and after the addition of stimulus solution (kairomones + dietary cues of *H. tigerinus* fed with conspecifics). Twenty trials were conducted. All experimental trials were run using a new *H. tigerinus* tadpole each time. The test tank was cleaned and replenished with aged tap water between trials. The data is given in the annexure 1.

Statistical analysis

After checking for normality, the data on the behavioral responses of *H. tigerinus* tadpoles before and after the addition of the stimulus solution containing kairomones or conspecific tadpole fed predator were compared separately by using Paired-Samples t-test. The data on the behavioral responses between different treatments were analyzed by a one-way ANOVA followed by a Tukey's HSD post-hoc test. All these statistical tests were performed using SPSS ver. 22.0.

Results

There was no changes in the behavioural responses (i.e., various swimming activity such as maximum swimming burst speed, swimming spurts, time spent swimming and distance travelled) of prey tadpoles before and after addition of chemical blank solution (control; Table 1a). Similarly, there was also no changes in the behavioural responses of prey tadpoles before and even after the addition of stimulus solution (i.e., either kairomones or dietary cues of predators fed with heterospecific tadpoles) (Table 1b and 1c). However, when prey tadpoles exposed to dietary cues of predator fed with conspecifics (own species), prey tadpoles showed a significant increase in the swimming burst speed ($V_{\max}$) but a significant decline in the number of swimming spurts, swimming time, and total distance moved when compared to their baseline activity (Table 1d).

Results of ANOVA revealed a significant difference in the $V_{\max}$ ($F_{4, 155} = 305.921$, $P = 0.000$), number of swimming spurts ($F_{4, 155} = 400.100$, $P = 0.000$), time spent swimming ($F_{4, 155} = 387.009$, $P = 0.000$), and total distance moved ($F_{4, 155} = 686.162$, $P = 0.000$) among different treatment groups (Table 2). Prey tadpoles only when exposed to dietary cues of predator fed with conspecifics (own species), exhibited the strong behavioural responses i.e., displaying significantly higher $V_{\max}$ ($P = 0.000$), but significantly reduced time in swimming ($P = 0.000$), number of swimming spurts ($P = 0.000$), and moved for a shorter distance ($P = 0.000$) compared to rest of the other treatment groups i.e., control or kairomones or dietary cues of predator fed with heterospecific tadpoles (Table 2). There was no significant difference between other treatment groups (control vs kairomones vs dietary cues of predator fed with heterospecifics; Table 2).

Discussion

The previous extensive research work (across different continents) on anuran tadpoles belongs to various genera, species, and families have shown that; majority of the tadpole species can have the great ability to recognize (e.g., innate, learning etc.), detect (e.g., visual, chemical cues), distinguish type of predators (e.g., sit-and-wait, active and chase, vertebrate/ invertebrate predators etc.) and also its cues for e.g., kairomones, alarm, dietary cues, disturbance cues etc. and shown various types of plastic responses such as behavioural, morphological, physiological and life-history (Lawler 1989; Relyea 2001, 2007; Schoeppner and Relyea 2005, 2009a,b; Kishida and Nishimura 2005; Fraker et al. 2009; Mogali et al. 2011, 2016; Saidapur 2025). The majority of anuran tadpole species exhibits various types of antipredator behavioral responses to predators or its cues. The important antipredator behavioral responses to perceived predation threat include; overall decrease in its activity levels, hiding under refuge sites, aggregation, sudden increase in swimming burst speed, freezing etc. (Spieler 2003; Spieler and Linsenmair 1999; Mogali et al. 2011, 2019; Saidapur 2025).

In most of the anuran larval prey-predator interaction studies, prey is invariably the tadpole species and predator is either insects or its larvae or any other vertebrate. But in the present study, both prey (small size *H. tigerinus*) and predator (large size *H. tigerinus*) species is the same. Such kind of conspecific predation or interactions usually seen in natural waters, if there is scarcity of food resources or over density. Hence, the behavioral response of small size *H. tigerinus* to large size *H. tigerinus* is quite interesting.

The results of the present study showed that the prey tadpoles (small sized *H. tigerinus*) does not show any antipredator behavioral responses to either chemical cues of large sized starved predator (i.e., kairomones) or to chemical cues of large sized predator fed with heterospecifics. The behavioural response of prey tadpoles before (tap water) and after the addition of stimulus solution (kairomones or chemical cues of heterospecific tadpole fed predator) was same, this is a clear evidence. However, prey tadpoles identify only the dietary cues of predator fed with conspecifics (its own species) as a significant threat and decreased their activity levels during the trial period. When prey tadpoles (small sized *H. tigerinus*) moved in the stimulus solution (dietary cues of predator fed with conspecifics), their spurt speed ($V_{\max}$) was higher than in the stimulus blank solution or kairomones or heterospecific tadpole fed predator, indicating their effort to escape from the perceived predator's dietary cues that contains conspecifics. This is the unique kind of behavioral study reported only in cannibalistic *H. tigerinus* tadpoles.

The question may arises, why prey tadpoles does not responded to cues of heterospecific tadpole fed predator or kairomones of predator. The answer is clear in the natural water bodies, the prey tadpoles (small sized *H. tigerinus*) are also basically potential carnivorous, active and chase predators, and are capable of feeding on all available tadpole species and also on other aquatic organisms. That's why they have not shown any kind of antipredator behavior. Whereas, when conspecific members are being eaten by predator, the prey tadpoles really consider this as a serious threat and responded immediately; decreased their activity levels (not easily detected by the predator), and sometimes suddenly increased its burst speed (rapid escape from predation).

I rule out the influence of alarm cues released by conspecific *H. tigerinus* tadpoles as there were neither surviving injured individuals nor dead remains of tadpoles in the tubs for 15 h prior to harvesting the stimulus solution, and alarm cues are highly labile in nature (Ferrari et al. 2008; Wisenden et al. 2009; Chivers et al. 2013). Hence, the antipredator defense behavior of small size *H. tigerinus* tadpoles in the present is specifically in response to diet-derived excretory metabolites or substances released by large size *H. tigerinus* tadpoles that have consumed conspecifics.

In conclusion, the chemical cues of conspecific member predation induces strong behavioural changes (overall decrease in its swimming activity and high burst speed) in cannibalistic Indian bullfrog tadpoles.

Declarations

Compliance with Ethical Standards

This research was conducted according to ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, under Registration No. 639/02/a/CPCSEA

Conflict of Interest

The author declares no competing interests

Ethical approval

This research was conducted according to ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, under Registration No. 639/02/a/CPCSEA

Informed consent

CPCSEA, New Delhi

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None

Author Contribution

Santosh Mogali designed the experiment, conducted the experiment, analyze the data wrote the paper

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Data availability

All original data is included as supplementary material.

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Tables

Tables 1 and 2 are available in the Supplementary Files section.

Supplementary Files

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