Differential antipredator behavioral responses in tadpoles of *Duttaphrynus melanostictus* (Anura: Bufonidae): an experimental demonstration

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Abstract

Differential antipredator behavioral responses in tadpoles of *Duttaphrynus melanostictus* (Anura: Bufonidae): an experimental demonstration. In aquatic systems, many prey animals including larval anurans predominantly use chemical cues to assess predation risk. In such systems, a variety of predators (e.g., insects or their larvae, sympatric carnivorous/omnivorous tadpoles) can affect the behavioral responses and life history of prey tadpoles. Many anuran tadpoles are able to discriminate chemical cues of different predators and exhibit differential antipredator behavioral responses according to the perceived risk. The behavioral responses of tadpoles of *Duttaphrynus melanostictus* to different predators (predaceous insects, *Lethocerus* sp., omnivorous tadpoles of *Euphlyctis cyanophlyctis,* and carnivorous tadpoles of *Hoplobatrachus tigerinus*) were studied in the laboratory. The predator's diet-derived metabolites released in the excreta of the predators after consumption of conspecific (*D. melanostictus*) prey tadpoles were used to simulate predation threat. The *D. melanostictus* tadpoles showed antipredator behavioral responses i.e., reduced swimming movements and overall time spent swimming, and had a higher burst speed in response to water-borne cues released from the excreta of all predators that were fed with conspecific prey. Further, *D. melanostictus* tadpoles showed the strongest antipredator behavioral responses to cues released by carnivorous, active predatory tadpoles, *H. tigerinus,* moderate responses to the cues of the sit-and-wait carnivorous insect, *Lethocerus* sp. and low responses to those of omnivorous *E. cyanophlyctis* tadpoles. The hierarchy of antipredator behavioral responses in *D. melanostictus* tadpoles to different predators is *H. tigerinus* > *Lethocerus* sp. > *E. cyanophlyctis* > chemical blank solution. The findings of the present study thus show that tadpoles of *D. melanostictus* appear to modulate the intensity of their defense behavior in accordance with the level of threat posed by each predator.

Keywords: Anuran larvae, Chemical and visual cues, Dietary metabolites, Predator, Prey, Reduced activity, Swimming speed.

Resumo

Respostas comportamentais antipredador diferenciais em girinos de *Duttaphrynus melanostictus* (Anura: Bufonidae): uma demonstração experimental. Nos sistemas aquáticos, muitas presas, incluindo larvas de anuros, utilizam predominantemente sinais químicos para avaliar o risco de predação. Nesses sistemas, uma variedade de predadores (por exemplo, insetos ou suas larvas, girinos carnívoros/onívoros simpátricos) pode afetar as respostas comportamentais e a história de vida dos girinos. Muitos girinos são capazes de discriminar sinais químicos de diferentes predadores e apresentam respostas comportamentais antipredadores diferenciadas de acordo com o risco percebido. As respostas comportamentais dos girinos de *Duttaphrynus melanostictus* a diferentes predadores (insectos predadores, *Lethocerus* sp., girinos onívoros de *Euphlyctis cyanophlyctis* e girinos carnívoros de *Hoplobatrachus tigerinus*) foram estudadas em laboratório. Os metabólitos derivados da dieta do predador liberados nas excreções após o consumo de girinos de presas conspecíficas (*D. melanostictus*) foram utilizados para simular a ameaça de predação. Os girinos de *D. melanostictus* apresentaram respostas comportamentais antipredadoras, isto é, reduziram os movimentos e o tempo total gasto na natação e tiveram uma maior velocidade de explosão em resposta a sinais de água liberados pelos excrementos de todos os predadores que foram alimentados com presas específicas. Além disso, os girinos de *D. melanostictus* apresentaram as respostas comportamentais anti-predador mais fortes aos sinais liberados pelos girinos predadores carnívoros e ativos, *H. tigerinus*, respostas moderadas aos sinais do inseto carnívoro *Lethocerus* sp. e respostas baixas às dos girinos onívoros de *E. cyanophlyctis*. A hierarquia das respostas comportamentais antipredador nos girinos de *D. melanostictus* a diferentes predadores é *H. tigerinus* > *Lethocerus* sp. > *E. cyanophlyctis* > solução tampão. Os resultados do presente estudo mostram que os girinos de *D. melanostictus* parecem modular a intensidade do seu comportamento de defesa de acordo com o nível de ameaça representado por cada predador.

Palavras-chave: Atividade reduzida, Larvas de anuros, Metabólitos da dieta, Pistas químicas e visuais, Predador, Presa, Velocidade de natação.

Introduction

Anuran amphibians often breed opportunistically in ephemeral water bodies during the rainy season, and the larval anurans live in such waters until metamorphosis (Saidapur 2001, Cogălniceanu *et al.* 2012, Goldberg *et al.* 2012, Mogali *et al.* 2020). Hence, tadpoles of different anuran species that co-occur in such waters face threats from desiccation, crowding, competition for food and space, and most importantly predation (Skelly 1997, Loman 1999, Lardner 2000, Benard 2004, Mogali *et al.* 2011, 2016, 2020). Ephemeral ponds can house diverse types of predators, the most common of which are aquatic insects and their larvae, and omnivorous and carnivorous predatory tadpoles (Heyer *et al.*1975, Skelly 1997, Relyea 2001a, Saidapur 2001, Mogali *et al.* 2020). Furthermore, in the ephemeral water bodies where different species

of tadpoles reside, the water is often turbid or filled with aquatic vegetation or leaf litter, causing poor visibility (Hoff *et al.* 1999, Mogali 2018, Mogali *et al.* 2023a,b). Moreover, most species of anuran tadpoles are near sighted (Kiesecker *et al.* 1996, Hoff *et al.* 1999, Mogali 2018). In such conditions, chemical cues are more useful than visual cues to detect food or predators (Kiesecker *et al.* 1996, Nystrom and Abjornsson 2000, Hickman *et al.* 2004, Fraker 2008, Saidapur *et al.* 2009). Earlier studies have shown that the source of chemical cues emanating from predators and detected by prey tadpoles may differ (Takahara *et al.* 2008, Smith and Awan 2009, Ferrari *et al.* 2010). Chemical cues may arise from the starved predators, kairomones (Petranka *et al.* 1987, Schoeppner and Relyea 2005, Mogali 2018), dietary metabolites derived following consumption of conspecific prey and released through feces (Wilson and Lefcort 1993,

Laurila *et al.* 1997, Chivers and Mirza 2001, Kiesecker *et al.* 2002, Mogali *et al.* 2012, Scherer and Smee 2016), alarm pheromones released by injured or damaged prey (Hews and Blaustein 1985, Hews 1988, Summey and Mathis 1998, Schoeppner and Relyea 2005, Carlson *et al.* 2015), or a combination of these factors (Schoeppner and Relyea 2009a,b). Earlier studies also revealed that anuran tadpoles showed a variety of antipredator behaviors when exposed to chemical cues of predators including increased activity levels or high swimming speed to escape predators (Hews 1988, Van Buskirk and McCollum 2000, Dayton *et al.* 2005, Mogali *et al.* 2021), reduction in activity levels (Lawler 1989, Saidapur *et al.* 2009, Mogali *et al.* 2012), formation of dense aggregations or schools (Spieler and Linsenmair 1999), or increased use of refuge sites (Stauffer and Semlitsch 1993, Relyea 2003, Hossie and Murray 2010, Mogali *et al.* 2022), depending on the species.

The Asian common toad, *Duttaphrynus melanostictus* (Schneider, 1799) (Anura: Bufonidae), is widely distributed in India. In South India, during early monsoon season, it generally breeds in ephemeral water bodies along with other sympatric anuran species (Mogali *et al.* 2011, 2017, 2023a). The temporary water bodies where herbivorous tadpoles of *D. melanostictus* reside are also home to a variety of invertebrate predators such as dragonfly and damselfly larvae, giant water bugs, crabs, and beetles as well as vertebrate predators including an omnivorous [*Euphlyctis cyanophlyctis* (Schneider, 1799)], and carnivorous tadpoles [*Hoplobatrachus tigerinus* (Daudin, 1802)]. During our regular field visits, we noticed that herbivorous tadpoles of *D. melanostictus* are preyed upon by all these predators. Earlier studies suggest that different predators present different levels of predation risk to prey tadpoles (Relyea 2001a,b). Hence, in the present work, we studied the behavioral responses of *D. melanostictus* tadpoles to chemical cues (of a dietary origin) of three different types of predators. They are giant water bugs, *Lethocerus* sp. (Hemiptera: Belostomatidae) which are sit-and-wait insect predators exclusively carnivorous in nature, tadpoles of *E. cyanophlyctis* (Anura: Dicroglossidae), which are omnivorous in nature and basically feed on detritus or algae and also on other sympatric anuran tadpoles including *D. melanostictus*. On the other hand, tadpoles of *H. tigerinus* (Anura: Dicroglossidae) are primarily predators; they are carnivorous, active in nature, feeding on other sympatric anuran tadpoles.

In the present study we hypothesized that *D. melanostictus* tadpoles which coexist with all these predators in the natural water bodies should show antipredatory behavioral responses to predators' chemical cues. Additionally, we hypothesized that the antipredatory responses of *D. melanostictus* tadpoles should depend on the level of threat presented by each type of predator. We expected that *D. melanostictus* tadpoles would show strong antipredator behavioral responses to *H. tigerinus* tadpoles because these tadpoles are basically carnivorous in nature, active hunters and also detect their prey by means of both visual and chemical senses (Saidapur *et al.* 2009). We expected moderate antipredator behavioral responses in *D. melanostictus* tadpoles to *Lethocerus* sp. because these are carnivorous insects but sit-and-wait predators. We expected weak antipredator responses to *E. cyanophlyctis* because these are omnivorous tadpoles mainly feeding on detritus or decayed matter or algae, and also on other sympatric tadpoles. Evaluating these hypotheses will provide novel information in the field of behavioral ecology of anuran tadpoles.

Materials and Methods

Three egg clutches of *Duttaphrynus melanostictus* were collected from an ephemeral pond in the Karnatak University Campus, Dharwad (latitude 15.440407° N, longitude 74.985246° E), Karnataka state, India in the early monsoon period and were immediately transported to the laboratory. They were placed separately in plastic tubs (32 cm diameter and 14

cm depth) containing 5 L of aged (dechlorinated) tap water. The eggs from all clutches hatched synchronously at stage 19 (Gosner 1960) the next day. The tadpoles were mixed and reared for stocking in two separate glass aquaria (75 × 45 × 15 cm) containing 20 L of aged tap water. Approximately two hundred tadpoles were stocked in each aquarium. Tadpoles of *D. melanostictus* from stage 25 (Gosner 1960) onwards were fed with sufficient amount of boiled spinach. The carnivorous predatory insect, *Lethocerus* sp. (*N* = 20; 42.50 ± 0.60 mm total length, mean ± SE), omnivorous tadpoles of *Euphlyctis cyanophlyctis* (Gosner stages 33–34; *N* = 20; 42.02 ± 0.65 mm total length) and carnivorous tadpoles of *Hoplobatrachus tigerinus* (Gosner stages 33–34; *N* = 20; 41.15 ± 0.40 mm total length) were collected with the help of net from the same temporary pond where the eggs of *D. melanostictus* were obtained. All three species of predators were reared individually to avoid cannibalism in separate plastic tubs (19 cm diameter and 7 cm depth) containing 0.5 L of aged tap water. Tadpoles of *E. cyanophlyctis* are omnivorous in nature so they were provided with boiled spinach and tadpoles of *D. melanostictus*. The tadpoles of *H. tigerinus* and the insect, *Lethocerus* sp. are carnivorous and were therefore fed exclusively with tadpoles of *D. melanostictus*. The behavioral responses of the prey (*D. melanostictus*) were studied by exposing them to stimulus solutions of dietary metabolites of predators (either *E. cyanophlyctis* or *Lethocerus* sp. or *H. tigerinus*) exclusively fed with conspecific tadpoles (*D. melanostictus*). The preparation of stimulus solution was as follows.

Preparation of Dietary Cues of Conspecific Origin

Dietary cues of E. cyanophlyctis *fed conspecific tadpoles.*—A single *E. cyanophlyctis* tadpole was placed in a plastic tub (*N* = 10 tubs; 19 cm diameter and 7 cm depth) containing 200 mL of aged tap water along with four tadpoles of *D. melanostictus* at Gosner stages 29–30 (at about 08:30 h). The tadpoles of *E. cyanophlyctis* consumed all the tadpoles provided to them by the evening (18:30 h). On the following day between 09:30 h and 11:30 h, predators were removed and the water from the tubs was filtered using fine cheesecloth. The filtrate served as the stimulus solution containing the diet-derived excretory metabolites or substances of predators (*E. cyanophlyctis*) fed conspecific prey and are unlikely to have contained the alarm cues of prey. Prey alarm cues are known to be labile in nature (Peacor 2006, Ferrari *et al.* 2008, 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.

Dietary cues of Lethocerus sp. *fed conspecific tadpoles.*—A single *Lethocerus* sp. was placed in a plastic tub (*N* = 10 tubs; 19 cm diameter and 7 cm depth) containing 200 mL of aged tap water along with four tadpoles of *D. melanostictus* at Gosner stages 29–30 (at about 08:30 h). *Lethocerus* sp. consumed all the tadpoles provided to them by the evening (18:30 h). On the following day filtrate was obtained and served as a stimulus solution.

Dietary cues of H. tigerinus *fed conspecific tadpoles.*—A single *H. tigerinus* tadpole was placed in a plastic tub (*N* = 10 tubs; 19 cm diameter and 7 cm depth) containing 200 mL of aged tap water along with four tadpoles of *D. melanostictus* at Gosner stages 29–30 (at about 08:30 h). The tadpoles of *H. tigerinus* consumed all the tadpoles provided to them by the evening (18:30 h). On the following day filtrate was obtained and served as a stimulus solution.

Behavioral Responses of D. melanostictus Tadpoles to Dietary Cues of Different Predators

Behavioral responses of D. melanostictus *tadpoles to dietary cues of omnivorous tadpole*

predator, E. cyanophlyctis *fed with* D. melanostictus.—The behavioral responses of *D. melanostictus* tadpoles to dietary cues (water conditioned with predators fed with conspecific tadpoles) were recorded by placing a single tadpole (*D. melanostictus*; Gosner stage 29–30; mean total length 24.15 ± 0.35 mm) in a rectangular glass tank (28 × 15 × 15 cm) containing 600 mL of aged tap water. A video camera (Sony, DCR-SR300/E) was fixed above the tank such that it recorded the entire area. The video camera 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 (dietary cues) to the test tank. The Ethovision system was used to record swimming activities such as 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 tadpole of *D. melanostictus* 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 solution) was then added at the rate of ~1 mL/s to simulate the disturbance the later chemical cue would make. 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 dietary cues of the predator (*E. cyanophlyctis*) fed with *D. melanostictus* tadpoles was added as described above. Movement of the tadpole was recorded for another 5 min to determine the activity pattern after exposure to dietary cues.

Similarly, the behavioral responses of *D. melanostictus* tadpoles to dietary cues were recorded for the other predators, the carnivorous insect, *Lethocerus* sp. and carnivorous tadpoles, *H. tigerinus* fed with *D. melanostictus*. The testing procedure was exactly the same as that of the earlier one. A new test tadpole was used for each trial. Twenty-five trials were conducted for

each group (25 trials × 3 types of predators = 75 trials altogether).

Statistical Analysis

After checking for normality, initially data were analyzed using one-way MANOVAs for various swimming activities (response variables are maximum swimming speed, frequency of swimming spurts, time spent swimming and total distance moved). After getting significant MANOVAs, Further the data on the behavioral responses of *D. melanostictus* tadpoles to chemical blank solution vs. different predator type (*Euphlyctis cyanophlyctis, Lethocerus* sp., *Hoplobatrachus tigerinus*) and similarly stimulus solution vs. predator type were analyzed separately by one-way ANOVA followed by Tukey's HSD *post-hoc* test. Finally, the data on the behavioral responses of *D. melanostictus* tadpoles, before and after addition of the stimulus solution (dietary cues) of each predator were compared separately by using the paired-samples t test. All the statistical tests were performed using SPSS ver. 16.0.

Results

Intensity of Behaviors in D. melanostictus *Tadpoles to Different Predators*

The results of the one-way MANOVAs clearly showed that each swimming activities (V_{max}, no. of swimming spurts, time spent swimming and total distance moved) of *D. melanostictus* tadpoles are statistically significant difference by predator type (*Euphlyctis cyanophlyctis, Lethocerus* sp., *Hoplobatrachus tigerinus*) and treatment type (chemical blank solution, stimulus solution) (Table 1). Further, results of one-way ANOVA with Tukey's HSD *post-hoc* test revealed that there was no significant difference in the various swimming activities (V_{max}, no. of swimming spurts, time spent swimming and total distance moved) of *D. melanostictus* tadpoles between chemical blank

solution vs. different predator type (*Euphlyctis cyanophlyctis*, *Lethocerus* sp., *Hoplobatrachus tigerinus*) (Table 2). However, there was a significant difference in the V_{max}, swimming spurts, time spent swimming and total distance travelled by *D. melanostictus* tadpoles between stimulus solution vs. different predator type

(Table 2). The test tadpoles exposed to the stimulus solution of *H. tigerinus* exhibited significantly higher V_{max} ($p < 0.01$) but overall significantly reduced their activity i.e., spent less time swimming ($p < 0.01$) with reduced number of swimming spurts ($p < 0.01$) and moved a shorter distance ($p < 0.01$) compared to any

Table 1. Results of one-way MANOVAs for various swimming activities of *Duttaphrynus melanostictus* tadpoles (the response variables are maximum swimming speed, frequency of swimming spurts, time spent in swimming and total distance moved) to chemical cues of predator type (*Euphlyctis cyanophlyctis*, *Lethocerus* sp., *Hoplobatrachus tigerinus*) and treatment type (chemical blank solution, stimulus solution). Asterisks indicate significant difference.

Source	Wilks' Lambda	F	p
Swimming speed ($V_{max,}$ cm/s)	0.087	770.60	$< 0.01^*$
Number of swimming spurts	0.079	855.60	$< 0.01^*$
Time spent swimming (s)	0.081	836.92	$< 0.01^*$
Distance moved (cm)	0.119	544.30	$< 0.01^*$

Table 2. Results of one-way ANOVAs with Tukey's HSD *post hoc* tests for various swimming activities of *Duttaphrynus melanostictus* tadpoles (the response variables are maximum swimming speed, frequency of swimming spurts, time spent in swimming and total distance moved) to chemical blank vs. predator type (*Euphlyctis cyanophlyctis*, *Lethocerus* sp., *Hoplobatrachus tigerinus*) and stimulus solution vs. predator type. Dissimilar letters along the same column indicate significant difference between different treatment groups.

One-way ANOVA for chemical blank vs. predator type				
Predator type	Swimming speed (Vmax, cm/s)	No. swimming spurts	Time spent swimming (s)	Distance moved (cm)
Euphlyctis cyanophlyctis	11.80 ± 0.08a	65.92 ± 1.43a	63.10 ± 1.31a	399.11 ± 8.81a
Lethocerus sp.	11.75 ± 0.07a	65.60 ± 1.46a	63.59 ± 1.45a	399.63 ± 8.52a
Hoplobatrachus tigerinus	11.82 ± 0.07a	67.00 ± 1.35a	65.43 ± 1.57a	394.48 ± 5.22a
F value	$F_{2,72} = 0.226$	$F_{2,72} = 0.267$	$F_{2,72} = 0.795$	$F_{2,72} = 0.112$
p value	$p = 0.798$	$p = 0.766$	$p = 0.456$	$p = 0.894$
One-way ANOVA for stimulus solution vs. predator type				
Predator type	Swimming speed (Vmax, cm/s)	No. swimming spurts	Time spent swimming (s)	Distance moved (cm)
Euphlyctis cyanophlyctis	18.48 ± 0.70a	31.00 ± 0.56a	29.36 ± 0.55a	256.48 ± 3.89a
Lethocerus sp.	21.15 ± 0.22b	22.36 ± 0.70b	21.05 ± 0.68b	195.97 ± 3.51b
Hoplobatrachus tigerinus	24.55 ± 0.30c	14.52 ± 0.53c	13.47 ± 0.49c	148.51 ± 3.83c
F value	$F_{2,72} = 151.768$	$F_{2,72} = 183.101$	$F_{2,72} = 187.64$	$F_{2,72} = 197.846$
p value	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$

other groups (Table 2B). The test tadpoles exposed to the stimulus solution of *Lethocerus* sp. exhibited significantly higher V_{max} ($p < 0.01$) and overall reduced their swimming activities ($p < 0.01$) compared to the stimulus solution of *E. cyanophlyctis* and stimulus free solution (Table 2B). The test tadpoles exposed to the stimulus solution of *E. cyanophlyctis* exhibited significantly higher V_{max} ($p < 0.01$) and overall reduced their swimming activities ($p < 0.01$) compared to the chemical blank solution or stimulus-free solution (Table 2B). The degree of antipredator behavioral responses of test tadpoles (*D. melanostictus*) was greatest when exposed to dietary cues of carnivorous *H. tigerinus*, followed by carnivorous *Lethocerus* sp. (intermediate) and least to omnivorous *E. cyanophlyctis* (Table 2B).

Behavioral Responses of D. melanostictus Tadpoles to Dietary Cues of Different Predators

Behavioral Responses of D. melanostictus *tadpoles to dietary cues of omnivorous tadpole predator,* E. cyanophlyctis *fed with* D. melanostictus.—Upon exposure to dietary cues of omnivorous tadpole predator, *E. cyanophlyctis* fed with conspecific prey (*D. melanostictus*), test tadpoles (*D. melanostictus*) showed a significant increase in V_{max} ($t = -39.540$, $df = 24$, $p < 0.01$; Figure 1A), and a significant decrease in the number of swimming spurts ($t = 11.864$, $df = 24$, $p < 0.01$; Figure 1B), time spent swimming($t = 11.613$, $df = 24$, $p < 0.01$; Figure 1C) and total distance moved($t = 9.261$, $df = 24$, $p < 0.01$; Figure 1D) when compared to their baseline activities in stimulus-free water (Figure 1).

Behavioral responses of D. melanostictus *tadpoles to dietary cues of carnivorous insect predator,* Lethocerus sp. *fed with* D. melanostictus.—Upon exposure to dietary cues of the carnivorous insect predator, *Lethocerus* sp. fed with conspecific prey (*D. melanostictus*), test tadpoles (*D. melanostictus*) showed a significant increase in V_{max} ($t = -24.771$,
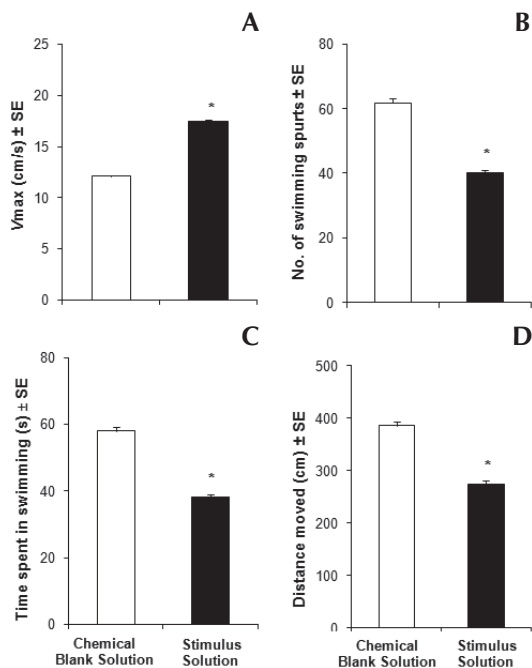

Figure 1. Maximum swimming speed (V_{max}) (**A**), Swimming spurts (**B**), Time spent in swimming (**C**), and Distance moved (**D**) by tadpoles of *Duttaphrynus melanostictus* exposed to chemical blank solution (aged tap water) or stimulus solution (dietary cues) of predator, *Euphlyctis cyanophlyctis* fed with conspecific tadpoles. Data are represented as mean ± SE; $N = 25$ trials; data analyzed by paired-samples *t-test*. Asterisks over the bars indicate significant difference between the treatment groups.

$df = 24$, $p < 0.01$; Figure 2A), and a significant decrease in the number of swimming spurts ($t = 15.906$, $df = 24$, $p < 0.01$; Figure 2B), time spent swimming ($t = 14.968$, $df = 24$, $p < 0.01$; Figure 2C) and total distance moved ($t = 14.079$, $df = 24$, $p < 0.01$; Figure 2D) when compared to their baseline activities in stimulus-free water (Figure 2).

Behavioral responses of D. melanostictus *tadpoles to dietary cues of carnivorous tadpole predator,* H. tigerinus *fed with* D. melanostictus.—
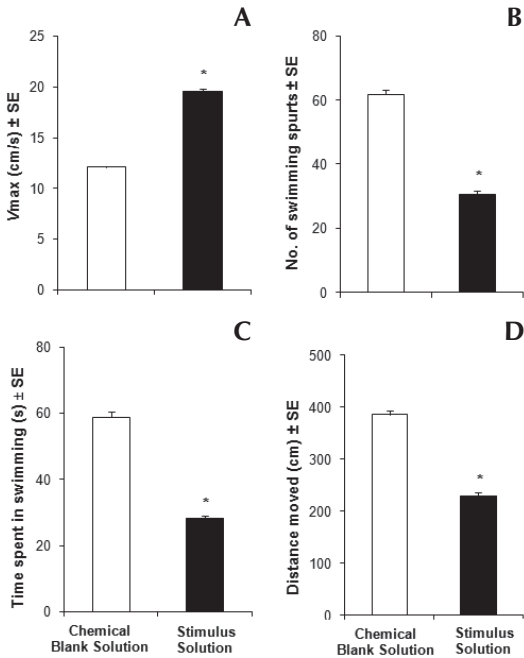
Figure 2. Maximum swimming speed (V_{max}) (**A**), Swimming spurts (**B**), Time spent in swimming (**C**), and Distance moved (**D**) by tadpoles of *Duttaphrynus melanostictus* exposed to chemical blank solution (aged tap water) or stimulus solution (dietary cues) of predator, *Lethocerus* sp. fed with conspecific tadpoles. Data are represented as mean ± SE; N = 25 trials; data analyzed by paired-samples *t-test*. Asterisks over the bars indicate significant difference between the treatment groups.
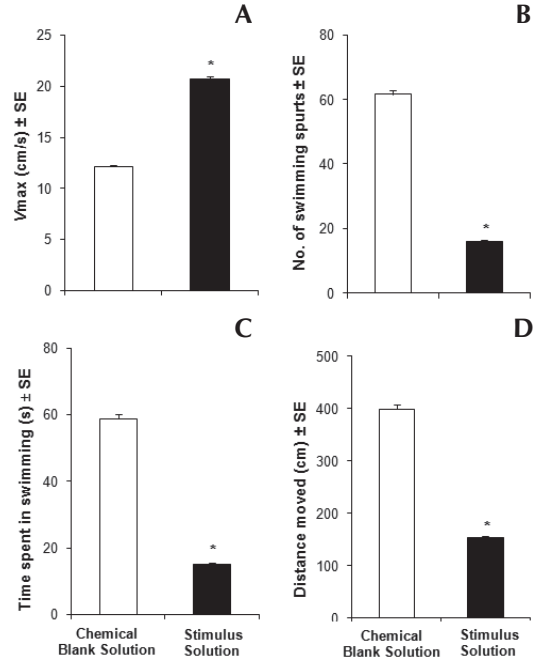
Figure 3. Maximum swimming speed (V_{max}) (**A**), Swimming spurts (**B**), Time spent in swimming (**C**), and Distance moved (**D**) by tadpoles of *Duttaphrynus melanostictus* exposed to chemical blank solution (aged tap water) or stimulus solution (dietary cues) of predator, *Hoplobatrachus tigerinus* fed with conspecific tadpoles. Data are represented as mean ± SE; N = 25 trials; data analyzed by paired-samples *t-test*. Asterisks over the bars indicate significant difference between the treatment groups.

Upon exposure to dietary cues of the carnivorous tadpole predator, *H. tigerinus* fed with conspecific prey (*D. melanostictus*), test tadpoles (*D. melanostictus*) showed a significant increase in V_{max} (t = -31.555, df = 24, p < 0.01; Figure 3A), and a significant decrease in the number of swimming spurts (t = 28.541, df = 24, p < 0.01; Figure 3B), time spent swimming (t = 27.237, df = 24, p < 0.01; Figure 3C) and total distance moved (t = 24.302, df = 24, p < 0.01; Figure 3D) when compared to their baseline activities in stimulus-free water (Figure 3).

Discussion

In natural aquatic environments, many prey organisms including larval anurans are at threat of predation, but the level of their threat is dependent on their defenses, which have evolved to promote their escape from predators and promote survival (Lima and Dill 1990, Kats and Dill 1998, Relyea 2001b, Schmidt and Amézquita 2001, Jara and Perotti 2010, Schalk 2016). In aquatic environments, a variety of chemical cues (e.g., kairomones of predators, alarm cues of damaged conspecifics, disturbance cues and

dietary metabolites of predators fed with conspecific prey items) affect the behavioral responses of prey (Wilson and Lefcort 1993, Wisenden 2000, Van Buskirk and Arioli 2002, Schoeppner and Relyea 2005, Ferrari *et al.* 2010, Mogali *et al.* 2012, Scherer and Smee 2016, Rivera-Harnãndez *et al.* 2022). The dietary cues of predators, especially those from predators fed with conspecific prey items, elicit strong antipredator behavior in many prey animals including anuran tadpoles (Kats and Dill 1998, Ferrari *et al.* 2010, Mogali *et al.* 2011, 2012, Scherer and Smee 2016). The results of the present study showed that tadpoles of *D. melanostictus* sensed dietary cues of all predators (*Lethocerus* sp., tadpoles of *E. cyanophlyctis* and *H. tigerinus*) when predators were fed with conspecific prey tadpoles and test tadpoles quickly decreased their activity levels during the trial period (i.e., less time spent in swimming, fewer swimming spurts and less distance travelled). Furthermore, it is interesting to note that whenever the *D. melanostictus* tadpole moved in the stimulus solution, their burst speed (V_{max}) was higher than in the stimulus blank solution, indicating their efforts to escape from the perceived risk upon exposure to dietary cues of predator. Our results are in conformity with earlier studies on tadpoles of *Rana clamitans* (Latreille, 1801) (Fraker 2009), *Rana temporalis* (Mogali *et al.* 2012) and *Clinotarsus curtipes* (Jerdon, 1853) (Mogali *et al.* 2023c). Thus, *D. melanostictus* tadpoles primarily appear to perceive *Lethocerus* sp. and tadpoles of *E. cyanophlyctis* and *H. tigerinus* as potential predators. This may be because long ecological co-existence of *D. melanostictus* tadpoles with sympatric *Lethocerus* sp. and omnivorous/carnivorous tadpoles may have led to the evolution of antipredator defense strategies in response to dietary cues of these predators.

The results of the present study also clearly showed that the antipredator behavioral responses of *D. melanostictus* tadpoles to all predators are not the same. The tadpoles of *D. melanostictus* clearly discriminated among the predators, and as a consequence they exhibited differential antipredator behavioral responses to perceived predator risk. They showed the strongest antipredator behavioral responses to dietary cues of carnivorous tadpoles, *H. tigerinus,* intermediate antipredator behavioral responses to dietary cues of *Lethocerus* sp., and the weakest antipredator behavioral responses to dietary cues of omnivorous tadpoles, *E. cyanophlyctis*. It is clear that, among these three predators; *D. melanostictus* tadpoles react to *H. tigerinus* tadpoles as the most dangerous predators; this is probably because *H. tigerinus* are basically carnivorous and also active hunters. More importantly, they detect their prey items by means of both visual and chemical cues (Saidapur 2001, Saidapur *et al.* 2009). The tadpoles of *D. melanostictus* also react to *Lethocerus* sp. as dangerous predators. They are basically carnivorous insects, sit-and-wait predators but they detect their prey through their strong vision and mechanoreceptor movements. The tadpoles of *D. melanostictus* probably show weak antipredator responses to *E. cyanophlyctis* tadpoles because these potential predators are omnivorous in nature feeding primarily on detritus matter or algae and also on other sympatric herbivorous tadpoles and detect their prey or food items only through chemical senses but not by visual senses (Mogali *et al.* 2023d).

In summary, tadpoles of *D. melanostictus* show antipredator behavioral responses to all their naturally co-existing predators and the strength of these responses depends upon likely predation risk. The findings of our study reinforce the idea that the antipredator behaviors of anuran tadpoles incorporate complex tradeoffs between risk and benefit.

Acknowledgments

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