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Similar antipredator behaviour in tadpoles of two closely associated anuran species *Duttaphrynus melanostictus* and *Sphaerotheca breviceps* (Anura) to the common predator *Pantala flavescens* (Odonata)

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Predation is one of the most vital selective forces acting on prey animals. Therefore, prey animals must assess predation risk and develop necessary antipredator defence strategies in order to optimize their survival and fitness (LIMA & DILL 1990). An elemental way in which many aquatic organisms including anuran larvae assess predation risk is by using chemosensory information (KATS & DILL 1998, FERRARI et al. 2010, MOGALI et al. 2011, 2012). Earlier studies from various animal taxa including anuran larvae show that prey may detect a predator based either on the alarm cues from injured conspecific prey or kairomones of predator origin or on cues released from dietary metabolites of predators (MATHIS & SMITH 1993, WILSON & LEFCORT 1993, LAURILA et al. 1997, KATS & DILL 1998, WISENDEN 2000, MOGALI et al. 2011). Consequently, they exhibit diverse types of antipredator defensive behaviours such as predator area avoidance, decrease or increase of activity level, high burst swimming, remaining motionless for a longer time, hiding under structural refuges, or aggregation, to a perceived potential predation threat (WALDMAN & ADLER 1979, SAIDAPUR et al. 2009, MOGALI et al. 2011, 2019, 2022).

In southern India, most anurans including the Asian common toad *Duttaphrynus melanostictus* (SCHNEIDER, 1799) and Indian burrowing frog *Sphaerotheca breviceps* (SCHNEIDER, 1799) breed in ephemeral ponds following south-west (June–July months) monsoon rains. This leads to sympatric larvae of *D. melanostictus* and *S. breviceps* (SAIDAPUR 2001, SAIDAPUR et al. 2009, MOGALI et al. 2021). The ephemeral ponds in this region also harbour many potential vertebrate and invertebrate predators. The vertebrate predators are mainly carnivorous tadpoles of the Indian bullfrog *Hoplobatrachus tigerinus* (DAUDIN, 1802) and

omnivorous tadpoles of the Indian skipper frog *Euphlyctis cyanophlyctis* (SCHNEIDER, 1799). The invertebrate predators are mainly aquatic insects such as water beetles, water scorpions, and dragonfly larvae (SHARMA et al. 2008, SAIDAPUR et al. 2009, MOGALI et al. 2011, 2015). Among many sympatric anuran species in such water bodies, *D. melanostictus* and *S. breviceps* have many similarities: eggs of both species hatch within 24 h after oviposition. The keratodont formula of both larvae is 2(2)/3 throughout their development (Gosner stages 25–40; GOSNER 1960, HIRAGOND 2002). Both tadpoles are benthic dwellers, herbivores or detritivores and occur in very close association in these ponds (personal observations). Earlier studies showed that tadpoles of *D. melanostictus* and *S. breviceps* detect active predatory tadpoles of *H. tigerinus* based on chemical cues. They avoided the predator's area and reduced their activity levels when exposed to conditioned water (predator fed with prey tadpoles) of *H. tigerinus* tadpoles (SAIDAPUR et al. 2009, MOGALI et al. 2011, 2015). However, the behavioural responses of *D. melanostictus* and *S. breviceps* tadpoles to another aquatic insect predator, larvae of the dragonfly *P. flavescens* which also inhabits the same water bodies, is not known. In contrast to *H. tigerinus* tadpoles, the *P. flavescens* larvae are sit-and-wait and gape-limited predators that move slowly and do not hunt actively. Herein, we explain antipredator behavioural responses of these two closely associated tadpoles to various chemical stimuli of dragonfly larvae.

Eggs of *D. melanostictus* ($n \sim 1000$) and *S. breviceps* ($n \sim 1000$) were collected in June 2010 from temporary ponds on the Karnatak University Campus, Dharwad (latitude 15.440407° N, longitude 74.985246° E). The eggs of *D. melanostictus* are laid in strings at the pond bottom and

eggs are black in colour. The eggs of *S. breviceps* are laid on the water surface and appear to have embedded in a transparent sheet and eggs are brownish black in colour (HIRAGOND 2002). In the laboratory, eggs of each species were placed until hatching (at Gosner stage 19; GOSNER 1960) in separate plastic tubs (42 cm in diameter and 16 cm in height) containing 10 L of aged tap water under room temperature conditions (between 25–26°C). The eggs of both species hatched on the next day. The hatchlings were then transferred to separate glass aquaria (LWH 75 × 45 × 15 cm). Upon reaching the feeding stage (Gosner stage 25), tadpoles of both species were fed on boiled spinach *ad libitum*. Tadpoles of similar size (*D. melanostictus*; 16.35 ± 0.20 mm in total length and *S. breviceps*; 18.20 ± 1.5 mm in total length) and developmental stage (Gosner stages 27–28) were used as test prey animals in the study. The last instar larvae of the dragonfly *P. flavescens* (30.5 ± 1.2 mm in total length) were also collected from the same ponds where the tadpoles were obtained. They were reared individually to avoid cannibalism in small plastic tubs (14 cm in diameter and 7 cm in height) in 200 ml of aged tap water. They were exclusively fed with one of the tadpoles either of *D. melanostictus* or *S. breviceps* or insect larvae of *Chironomus* sp. or *Culex* sp., or remained starved (for 48 h) depending upon the experimental design or tests.

Experimental design: A rectangular glass aquarium (LWH 90 × 30 × 15 cm) as described in earlier studies (see SAIDAPUR *et al.* 2009, MOGALI 2018) served as the test tank. The test tank was equally divided into two zones (A and B). For detection of various types of chemical cues of the predator, one end of the test tank housed the predators *P. flavescens* ($n = 2$) in an open-ended mesh cage (10 cm in diameter and 15 cm high; providing chemical cues) wrapped with cloth, while the opposite end was kept empty. The test tank was cleaned prior to each trial and filled with new aged tap water to a height of 3 cm. A single test tadpole chosen arbitrarily was transferred to an open-ended mesh cage (10 cm in diameter and 15 cm high) placed at the centre of the test tank and allowed to acclimate as well as perceive various chemical cues of predators for 5 min. The tadpole was then released by gently lifting the cage with as less disturbance as possible. The time each test tadpoles (*D. melanostictus* or *S. breviceps*) spent in different zones of the test tank was recorded for 10 min using a digital stopwatch. It was assumed that when the test tadpoles detect chemical cues of predators or predation threats they would spend more time in the zone away from the one housing predators. On the other hand, failure to detect such a threat would result in random movement of test tadpoles in the test tank. A given test tadpole was used only once. No food was provided to either test tadpoles or predators during the trials. The position of predators in the test tank was reversed between trials. A set of predators was only used in three consecutive trials. All experimental trials were conducted under natural photoperiod and temperature conditions. The intensity of light during the trial periods was also recorded with the help of a lux-meter and was between 800–900 lux. All experimental trials were carried out between 0900 and 1500 h.

End-bias tests: These tests were conducted to rule out the bias of the test tadpoles towards any side of the test tank or the container used for housing predators i.e. mesh cage wrapped with cloth. These tests involved two sets of trials: (1) the stimulus zones of the test tank without any containers; (2) one stimulus zone of the test tank with a mesh cage wrapped with cloth and the other zone kept empty. For each set of tests, 25 trials were conducted using a new test tadpole each time and after cleaning the test tank before each trial. For end-bias tests, total of 100 new test tadpoles (50 *D. melanostictus* + 50 *S. breviceps*) were used.

Response to predator's various types of chemical cues: In trials to test on chemical cues of predators, *P. flavescens* larvae ($n = 2$) were placed in a mesh cage wrapped with cloth at one end of the test tank that provides various types of chemical cues to the test subjects. The opposite end zone of the test tank remained empty. In this test, five sets of trials were conducted. In the first set, starved predators (releasing kairomones), in the second set predators fed with conspecific tadpoles, in the third set predators fed with heterogeneric tadpoles, in the fourth set predators fed with insect larvae (*Chironomus* sp.) and in the fifth set predators fed with another insect larva (*Culex* sp.) were placed in a mesh cage wrapped with cloth at one end of the test tank that provided its various types of chemical cues to the test subjects whereas, the other end remained empty. Each test comprised of 25 trials with new tadpoles each time. For tests involving various types of chemical cues of predators, total of 250 new test tadpoles (125 *D. melanostictus* + 125 *S. breviceps*) were used. The test tank was washed after every trial. In all the above tests, data on the time spent by test tadpoles in stimulus zones A and B were compared by the Wilcoxon matched-pairs signed-ranks test.

In the end-bias test, *D. melanostictus* and *S. breviceps* tadpoles showed no bias towards any particular side of the test tank. The placement of the mesh cage made no difference to test tadpoles. They moved freely throughout the test tank. The data from both sets of end-bias tests were pooled and are presented in Fig. 1A (for *D. melanostictus* $Z = -0.015$, $P = 0.988$; for *S. breviceps* $Z = -0.200$, $P = 0.841$).

In trials providing chemical cues of kairomones of predators or insect-fed predators i.e. *Chironomus* sp. or *Culex* sp. the prey tadpoles moved randomly throughout the test arena. There was no significant difference in the time spent in the zone either with starved ($Z = -0.632$, $P = 0.527$ for *D. melanostictus*; $Z = -0.443$, $P = 0.658$ for *S. breviceps*, Fig. 1B) or *Chironomus* sp. ($Z = -1.480$, $P = 0.882$ for *D. melanostictus*; $Z = -1.372$, $P = 0.170$ for *S. breviceps*, Fig. 1E) or *Culex* sp. fed predators ($Z = -0.161$, $P = 0.872$ for *D. melanostictus*; $Z = -0.915$, $P = 0.360$ for *S. breviceps*, Fig. 1F) or in the opposite predator-free zone (Fig. 1). In contrast, in trials with chemical cues of conspecific tadpole-fed predators ($Z = -3.565$, $P = 0.000$ for *D. melanostictus*; $Z = -4.373$, $P = 0.000$ for *S. breviceps*, Fig. 1C) and heterogeneric tadpole-fed predators ($Z = -3.175$, $P = 0.000$ for *D. melanostictus*; $Z = -4.029$, $P = 0.000$ for *S. breviceps*, Fig. 1D), the prey tadpoles spent significantly greater amount of time in the zone away from predator zone (Fig. 1).

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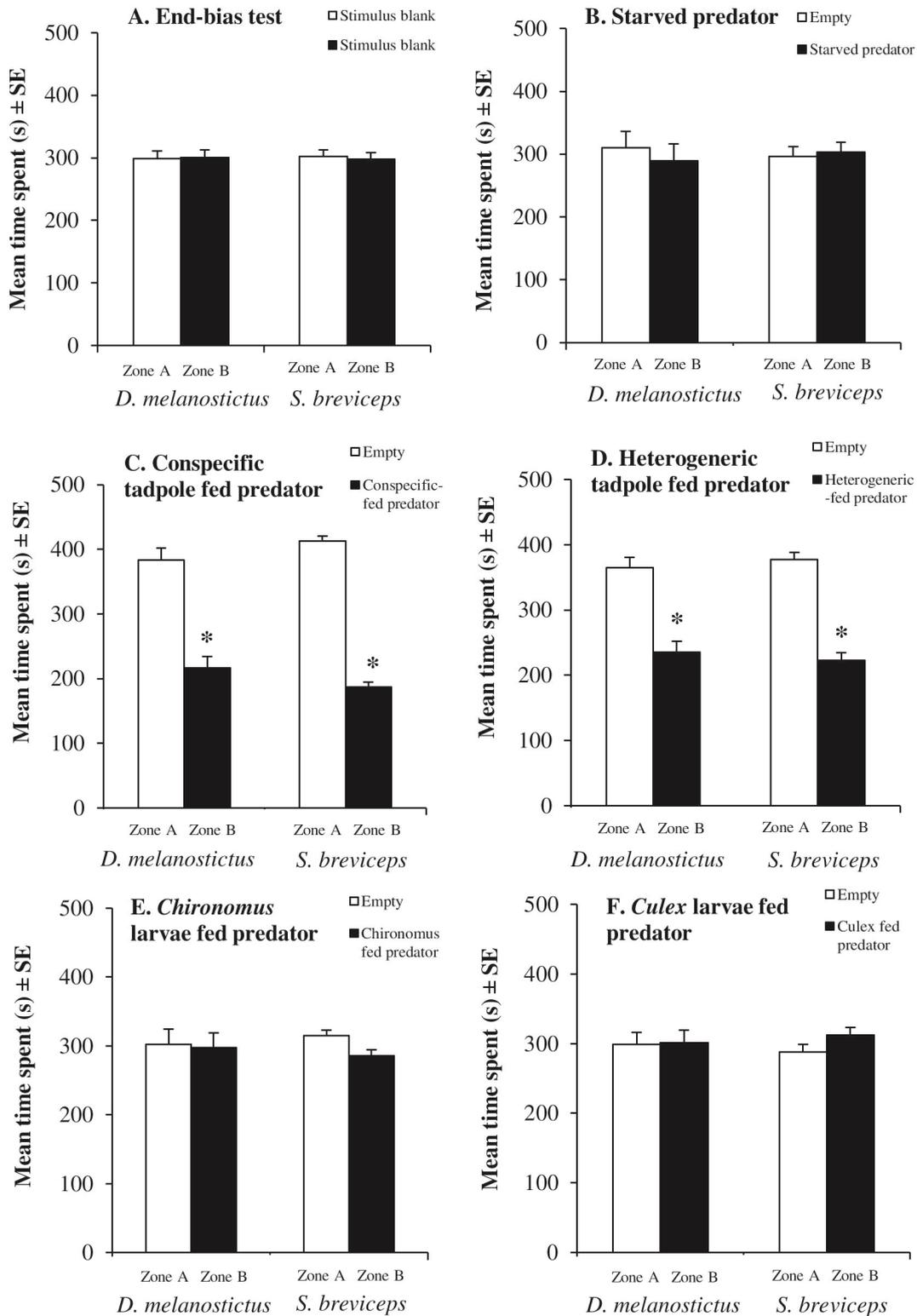


Figure 1. Time spent (s) by prey tadpoles (*Duttaphrynus melanostictus* and *Sphaerotherca breviceps*) in different zones (A or B) of the test tank in response to various types of chemical stimuli of a predator (*Pantala flavescens*). End-bias test (A), Chemical cues of starved predator (B), Chemical cues of conspecific tadpole fed predator (C), Chemical cues of heterogeneous tadpole fed predator (D), Chemical cues of *Chironomus* sp. larvae fed predator (E), and Chemical cues of *Culex* sp. larvae fed predator (F). Data represented as mean \pm SE and analyzed by Wilcoxon matched-pairs signed-ranks test ($n = 25$ trials per test; asterisks over the bar indicate significantly different from other).

In the present study, both prey species showed anti-predator behaviour meaning that they avoided the predator area and spent most of the time away from the predator zone, when the predator consumed conspecific tadpoles. Interestingly, both species also showed antipredator behaviour to predators when these consumed heterogeneric tadpoles. Both prey species in contrast did not show any anti-predator behaviour (no avoidance of the predator area and almost an equal amount of time was spent in the predator and empty zone) to the predator when that was fed with insect larvae or when it was starved. The result of the present study indicates that the behavioural responses of both prey species to their common insect predator are almost the same. Earlier studies have also reported that tadpoles preyed-on respond more strongly to predators which fed conspecific tadpoles and the behavioural response goes on weak or nil as and when predator consumes other than the species (LAURILA et al. 1997, CHIVERS & MIRZA 2001, SCHOEPPNER & RELYEA 2009a, b, MOGALI et al. 2012). In the present study predator detection by *D. melanostictus* and *S. breviceps* tadpoles were judged by their behaviour like avoiding the zone with only diet-derived chemical cues of conspecific/ heterogeneric prey consumed predator.

In the present study, both prey species do not respond to predator-derived kairomones or diet comprises *Chironomus* sp. or *Culex* sp. larvae. Perhaps they do not perceive such cues as a predation risk. Interestingly, kairomones of *H. tigerinus* tadpoles elicit strong antipredator responses in both prey species (SAIDAPUR et al. 2009, MOGALI et al. 2011). This differential response in the behaviour of both prey species is perhaps related to the behaviour of the predators used. The tadpoles of *H. tigerinus* are active hunters and visually oriented (SAIDAPUR et al. 2009). In contrast, larvae of dragonflies are sit-and-wait and gape-limited predators and move at a low pace (MOGALI et al. 2016). Possibly, both prey species treat the cues of dragonfly larvae as less risky and continue to remain active when compared to those exposed to cues of *H. tigerinus* tadpoles. These findings are in conformity with earlier studies suggesting that the magnitude of antipredator response is related to the magnitude of risk posed by the predator (LIMA & DILL 1990, RELYEA 2001, FRAKER 2010). On the other hand, dietary cues of predators preying upon conspecific or heterogeneric prey rather than the starved predators (releasing kairomones) or insect-fed (*Chironomus* sp. or *Culex* sp. larvae) predators seem to provide more reliable information on the vulnerability to predation. Hence, both prey species exhibit antipredator defence strategies only when they perceive a real predation threat (i.e. their conspecific, heterogeneric or closely-associated prey species being attacked). These findings support the threat-sensitive hypothesis according to which prey species assess and adjust their behaviour in accordance with the predation risk (HELPMAN 1989, CHIVERS et al. 2001, FERRARI et al. 2008).

In summary, the present study shows that chemical cues of starved or insect-fed predators do not evoke antipredator behaviour in *D. melanostictus* and *S. breviceps* tadpoles. However, they exhibit antipredator behaviour when pred-

ators fed on conspecific or heterogeneric tadpoles. Both species at larval stage live in the same habitat and live in very close-association so it is very important for them to be aware of their closely-associated species at larval stage being attacked by predators. The present study clearly shows that *D. melanostictus* and *S. breviceps* tadpoles discriminated among different chemical cues of predators and their defensive behaviour in accordance with the perceived threat.

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