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DOES TEMPORAL VARIATION IN PREDATION RISK AFFECT ANTIPREDATOR RESPONSES OF LARVAL SKIPPER FROGS (EUPHYCTIS CYANOPHYCTIS)?

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Running title: Responses of larval skipper frogs to variable predation
**Abstract:** Predation risk varies on a moment-to-moment basis, through day and night, lunar and seasonal cycles and over evolutionary time. Hence, it is adaptive for prey animals to exhibit environment-specific behaviour, morphology and/or life-history traits. Herein, the effects of temporally varying predation risk on growth, behaviour, morphology and life-history traits of larval *Euphlyctis cyanophlyctis* (Schneider, 1799) were studied by exposing them to no risk, continuous, predictable and unpredictable risks at different time points. Our results show that larval skipper frogs could learn the temporal pattern of risk leading to weaker behavioural responses under predictable risk and stronger responses to unpredictable risk. Temporally varying predation risk had a significant impact on tadpole morphology. Tadpoles facing continuous risk had narrow tail muscles. Tadpoles facing predictable risk during the day were heavy with wide and deep tail muscles whereas those facing predictable risk at night had long tails. Tadpoles facing unpredictable risk were heavy with narrow tail muscles. Metamorphic traits of *E. cyanophlyctis* were also affected by the temporal variation in predation risk. Tadpoles facing predictable risk during the day emerged at the largest size. However, tadpoles facing predictable risk at night and unpredictable risk metamorphosed earlier while those facing continuous risk metamorphosed later.

**Key Words:** Antipredator behaviour - *Euphlyctis cyanophlyctis* - Larval morphology - Larval skipper frogs - Metamorphic traits – Temporal variation in predation risk.
Introduction

Predation is a major selection pressure acting on prey animals determining their spatiotemporal distribution and abundance in any ecosystem. Predation has both direct and indirect effects on prey populations: predators directly affect prey density by killing and reducing the population size and indirectly affect other aspects such as behaviour, morphology and life-history traits leading to compromised phenotypes (Fonner and Woodley 2015; Gazzola et al. 2015; Mitchell et al. 2017). However, predators are not always present but often come and go. Moreover, they are not always active: some forage during the dawn or the dusk while others during the day or night. Consequently, predation risk is dynamic and varies not only through seasons but also through day and night (Sih et al. 2000; Touchon and Vonesh 2016; Mitchell et al. 2017). Hence, it is adaptive for prey animals to adjust to the spatiotemporal fluctuation in predation risk by exhibiting environment-specific behaviour, morphology and/or life-history traits (Kats and Dill 1998). The risk allocation hypothesis (RAH) proposes that prey animals should adjust their antipredator responses based on the frequency and intensity of risk (Helfman 1989; Lima and Bednekoff 1999). In other words, prey animals are expected to exhibit stronger responses under high risk and weaker responses under low risk. However, with an increase in the frequency of higher-risk situations, they are expected to exhibit weaker responses to both higher and lower risks, more specifically to low-risk situations. Animals engaged in feeding during temporally varying predation risk have to strike a balance between allocating time and energy to antipredator responses across different levels of predation risk (Lima and Bednekoff 1999). Further, the RAH predicts that temporal variation in predation risk influences the antipredator behaviour of prey.
animals among more or less risky environments. Consequently, the antipredator response should be weaker under constant predation risk as prey animals have to survive the danger and forage to meet their fitness and energy demands (Lima and Bednekoff 1999). Furthermore, they should adjust their foraging time to minimise vulnerability to predation under predictable risk thus reducing the trade-off between predator avoidance and foraging (Lima and Dill 1990).

Prey animals inhabiting diverse ecosystems have evolved novel strategies to counter predation risk. A general antipredator response of the majority of prey is the behavioural alteration such as subtle changes in habitat choice and changes in the timing of foraging and/or reproduction (Lima 1998). However, some prey species exhibit morphological defences such as development of protective spines, armour or cryptic/ aposematic colouration (Appleton and Palmer 1988; Ferrari et al. 2010) whereas others exhibit changes in growth, timing of metamorphosis, reproductive patterns or life-history traits (Crowl and Covich 1990; Chivers et al. 1999). Interestingly, some of these alterations (behaviour and morphology) are plastic and can be induced at multiple ontogenetic stages (Puttlitz et al. 2001; Relyea 2003; Hossie and Murray 2012). Despite the majority of larval forms exhibiting high adaptive plasticity, many have a low probability of completing metamorphosis to reach adulthood, which is attributed to intra and inter-specific competition for resources and predation, both of which can be highly variable and unpredictable through space and time (McDiarmid and Altig 1999).

Amphibians have complex life cycles that involve an aquatic larval stage meant for the accumulation of energy and a terrestrial adult stage meant for dispersal (Werner 1986). Hence, effective foraging is critical for larval amphibians to increase chances of
metamorphosis and to reach the adult stage (Alford 1999). However, predation risk acts as a strong selective force that affects optimal foraging as there exists a trade-off between foraging and developing antipredator responses. Hence, it is crucial for amphibian larvae to accurately assess and gauge predation risk to match their antipredator responses based on the patterns of risk. This makes sensory information about predators invaluable for prey to reduce the energetic costs associated with mounting antipredator responses and increase their fitness (Lima and Dill 1990). Larval amphibians are ideal to study the effects of fluctuating predation risk on behaviour, morphology and metamorphic traits as they exhibit predator-induced plastic responses and are quite amenable to experimental manipulation (Benard 2004). Moreover, they respond to chemical cues of predators allowing researchers to simulate predictable and unpredictable predation risk using caged predators without actual predation (Wisenden 2003; Ferrari et al. 2010). The Indian skipper frog, *Euphlyctis cyanophlyctis* (Schneider, 1799) is widely distributed throughout Asia and inhabits all types of aquatic ecosystems that include both lentic and lotic as well as ephemeral and permanent water bodies. In such diverse ecosystems, larval skipper frogs have to coexist with a wide variety of vertebrate and invertebrate predators that vary in their occurrence, abundance and activity pattern. Wide distribution coupled with the abundance of *E. cyanophlyctis* throughout its range suggests that its larval forms may have novel strategies to counteract predation risk thus metamorphosing in large numbers. Interestingly, larval *E. cyanophlyctis* lack innate ability to recognize kairomones of dragonfly nymphs but can recognize conspecific alarm cues innately. However, they can learn to recognize the kairomones of dragonfly nymphs through associative learning (Supekar and
Hence, the objectives of the study were twofold: to determine whether or not larval skipper frogs recognise temporally fluctuating predation risk by associating predator kairomones with the alarm cues of conspecifics and to assess their responses to predictable and unpredictable predation risk. If larval *E. cyanophlyctis* can assess the temporal variation in predation risk, then 1. Tadpoles facing unpredictable predation risk exhibit stronger responses in comparison to those facing predictable risk. 2. Tadpoles facing predictable predation risk during the day exhibit stronger response during the day rather than at night or vice-versa. 3. Tadpoles facing continuous risk exhibit weaker responses compared to those facing predictable or unpredictable risk. To test these predictions, we designed an experiment to assess the effect of temporal variation in predation risk on larval growth, antipredator behaviour, morphology and metamorphic traits of *E. cyanophlyctis*.

**Methods**

Four pairs of *E. cyanophlyctis* in amplexus were collected from a semi-permanent pond located on the Savitribai Phule Pune University campus (18° 55’ N and 73° 82’ E), Pune, Maharashtra, India on 20th July 2013 around 11 pm. The pairs were quickly transported to the laboratory where each pair was kept separately in a glass aquarium (60 cm x 40 cm x 45 cm) for spawning. Next morning, the embryos were transferred to another glass aquarium (45 cm x 30 cm x 10 cm) with aged tap water until hatching while the adults were returned to nature. Hatchlings obtained from all the spawns were mixed thoroughly and used for experimentation. Developmental stages were identified according to Gosner’s (1960) standard table for staging anuran tadpoles. The experiment was initiated when the tadpoles reached Gosner stage 25.
Water was renewed completely every third day and the tadpoles were fed partially boiled spinach *ad libitum*. Fourth instar nymphs of dragonfly (*Bradinopyga geminate* (Rambur, 1842) collected from the same pond (Mean ± SE = 22.7 ± 0.61 mm; *n* = 120) were used as predators. The effect of temporal variation in predation risk was studied by exposing tadpoles to predictable or unpredictable risks at different periods of the day/night. The experimental set up was as follows:

**Group I (Control)** – Rearing of tadpoles without any predator.

**Group II (Predictable risk during the day, PRD)** – Rearing of tadpoles with a caged predator for 2 h every day between 7.30 – 9.30 AM.

**Group III (Predictable risk at night, PRN)** – Rearing of tadpoles with a caged predator for 2 h every day between 7.30 – 9.30 PM.

**Group IV (Unpredictable risk, UPR)** – Rearing of tadpoles with a caged predator for 2 h at any time of the day or night.

**Group V (Continuous risk, CR)** – Rearing of tadpoles continuously with a caged predator.

Each experimental unit consisted of a glass aquarium containing sixteen tadpoles with 5 L aged tap water housing a transparent, perforated plastic cage (8 cm dia x 11 cm h) either empty or with a predator. In the predator exposure groups, a dragonfly nymph was placed in the cage and allowed to feed on a conspecific tadpole. Subsequently, the predators were maintained in separate containers for at least 72 h before using them for subsequent exposure. Tadpoles belonging to groups II-IV were exposed to a caged predator for 2 h followed by the complete water change. To mimic
the effects of water changes, aquarium water of groups I and V was also changed once a day (during the day or at night). Each group was replicated five times.

2.1 Growth rate

To assess the effect of temporal variation in predation risk on growth, body masses of tadpoles were recorded at the initiation of the experiment and subsequently, at a 15-day interval until stage 42 (metamorphic climax). Body mass was recorded to the nearest 1.0 mg using an electronic balance after quickly blotting tadpoles using a paper towel.

2.2 Antipredator response using a behavioural assay

Antipredator response of the tadpole was assessed using a behavioural assay following an established protocol (Batabyal et al. 2014; Supekar and Gramapurohit 2017, 2018). The behavioural assay was carried out ~25 days after the initiation of the experiment. The tests were conducted during the day or night (testing time) in response to kairomones (predator odour) of dragonfly nymphs. We specifically used kairomones rather than water as a neutral cue in our study design because larval skipper frogs lack the inherent capacity to recognise kairomones of dragonfly nymphs as a threat and hence exhibit normal activity. Further, reduction in their activity towards kairomones after the exposure to predator feeding on conspecific tadpoles would indicate and confirm associative learning. Kairomones (K) were prepared following the protocol established earlier (Batabyal et al. 2014; Supekar and Gramapurohit 2017). Briefly, six starved dragonfly nymphs were kept in a plastic container with 400 ml aged tap water without food for 24 h. This conditioned water was filtered and used as a stimulus cue. Thirty tadpoles/group chosen randomly at stage 29-33 (6 tadpoles/tank)
were used for testing the antipredator response to kairomones. Each tadpole was tested separately and only once.

A specially designed glass aquarium (50 cm x 6 cm x 6 cm) was used as the testing chamber. The chamber was marked from outside at the bottom with lines at an interval of 5 cm. At the beginning of each trial, the test chamber was cleaned and filled with one litre aged tap water to allow free movement of tadpoles. A test tadpole was introduced at one end of the chamber and allowed to acclimatise for 10 min. Subsequently, the number of lines crossed by the test tadpole before and after the addition of stimulus cue was recorded as a measure of activity. The test tadpole was considered to have crossed the line when the whole of its body was on the other side of a line. If the tadpole did not show any activity in the test chamber up to 2 min during the acclimatisation period, it was discarded (< 3%). The total time of each trial was 10.5 min (4 min pre-stimulus activity recording followed by 2.5 min during which the added cue diffused throughout the tank and 4 min of post-stimulus activity recording). An equal volume of the stimulus cue (2.5 mL) was added at either end of the testing chamber.

2.3 Morphological traits

The effect of varying predation risk on morphology was determined by recording body and tail parameters of thirty tadpoles chosen randomly from each group. Morphology was recorded between stages 37–39 using a digital calliper after quickly anaesthetizing the tadpoles using MS - 222. Body mass (BM), tail length (TL), tail muscle width (TMW), tail depth/tail fin height (TD) and tail muscle depth (MD) were
measured to the nearest 0.01 mm using a digital calliper. Body mass was recorded to
the nearest 1.0 mg using an electronic balance.

2.4 Metamorphic traits

At metamorphosis, snout-vent length (SVL), body mass (BM) and larval
duration (LD) were recorded. The length of larval duration was determined as the
days elapsed from hatching to reach metamorphic climax.

Statistical Analysis

All the data were confirmed for normality using probability plots and Anderson-
Darling test before subjecting to statistical analyses. A few outliers (<1%) from some
groups were excluded from the analyses. In all mixed-model ANOVAs, rearing tanks
were included as random effect while treatment as fixed effect. Behavioural (activity),
morphological (BM, TL, TW, TD, MD) and life-historical (SVL, BM and LD at
metamorphosis) responses were included as response variables. All the tests were two-
tailed and the significance was set at 0.05. The statistical tests were performed using
PAST 2.17c and SPPS ver. 19.

3.1 Growth rate

Differences in the body masses of tadpoles among groups were analysed using
nested analysis of variance (ANOVA) with intervals nested under the treatments,
followed by univariate tests for each time interval and Tukey’s test for multiple pair-wise
comparisons.

3.2 Antipredator response

The number of lines crossed by test tadpoles in pre and post-stimulus period was
converted into a proportional change in activity using the formula: (number of lines
crossed in the post-stimulus period - number of lines crossed in the pre-stimulus period) /number of lines crossed in the pre-stimulus period. To determine if there is an interaction between the temporal pattern of predation risk and the testing time, the responses were analysed using two-way ANOVA with temporal pattern of risk and testing time as independent variables and antipredator response as a dependent variable (after confirming that individual tanks within each group did not influence the antipredator behaviour of tadpoles using mixed model ANOVA’s) followed by univariate tests and Tukey’s *post-hoc* test.

### 3.3 Morphological traits

Differences in larval morphology (BM, TL, TW, TD and MD) were analysed using Mixed model ANOVAs followed by Tukey’s *post-hoc* test. For mixed model ANOVA’s, mass-independent estimates of tadpole shape were used. To produce mass-independent estimates of tadpole shape (for each tadpole) estimated marginal means were added to the saved residuals (McCoy et al. 2006; Schoeppner and Relyea 2008, 2009). Estimated marginal means were generated using multivariate analysis of covariance (MANCOVA), using the cube root of body mass as a covariate.

### 3.4 Metamorphic traits

Differences in the metamorphic traits were analysed using Mixed model ANOVAs followed by Tukey’s *post-hoc* test.

## Results

### 4.1 Growth rate
There was no mortality in any of the experimental groups and tadpoles metamorphosed within 2-3 months. The overall growth of larval *E. cyanophlyctis* was comparable among groups (*F*<sub>16, 100</sub> = 1.476, *p* = 0.124; Fig. 1).

### 4.2 Antipredator response

Tadpoles of *E. cyanophlyctis* do not recognise dragonfly nymphs as a threat inherently but can do so through associative learning. Hence, reduction in their activity towards kairomones during behavioural assay confirms the associative learning. In this context, the antipredator response of tadpoles was significantly different among groups (*F*<sub>9, 290</sub> = 68.758; *p* < 0.001). Temporal pattern of predation risk had a significant effect on the antipredator responses of tadpoles (*F*<sub>4, 290</sub> = 153.345; *p* < 0.001; Fig. 2) while testing time did not have any effect (*F*<sub>1, 290</sub> = 0.173; *p* = 0.678). Further, the interaction between the temporal pattern of predation risk and testing time was also not significant (*F*<sub>4, 290</sub> = 1.318; *p* = 0.263). When tested during the day, tadpoles facing predictable or unpredictable risks reduced activity significantly compared to those facing no risk (control; *p* < 0.001). Further, when tested during the day, the response of tadpoles facing predictable risk at night (PRN) and continuous risk (CR) was significantly weaker than those facing unpredictable risk (UPR; *p* = 0.005). Similarly, when tested at night, tadpoles facing predictable or unpredictable risks reduced activity significantly compared to those facing no risk (*p* < 0.001). However, reduction in the activity of tadpoles facing predictable risk during the day (PRD) and continuous risk (CR) was significantly weaker than those facing unpredictable risk, when tested at night (UPR; *p* = 0.033).

### 4.3 Morphological traits

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Exposure to temporal variation in predation risk had a significant impact on the body (BM) and tail parameters (TL, TW, TD and MD) of larval *E. cyanophlyctis* (Table 1). Overall body masses of tadpoles facing predictable or unpredictable risks at different times were significantly different (Table 1). Tadpoles facing predictable risk during the day (PRD; 6% change) or unpredictable risk (UPR; 5% change) were significantly heavier than those facing no risk (Fig. 3A; \( p < 0.001 \)). However, body masses of tadpoles facing no risk, continuous risk (CR) or predictable risk at night (PRN) were comparable (\( p \geq 0.244 \)). The overall tail length of tadpoles facing temporal variation in predation risk was significantly different (Table 1). Tadpoles facing predictable risk at night had longer tails than those facing no risk (2%) or continuous risk (2%; Fig. 3B; \( p \leq 0.012 \)) whereas tail length of the tadpoles facing continuous risk, predictable risk (day) or unpredictable risk were comparable to that of no risk group (Fig. 3B; \( p \geq 0.121 \)). Tail muscle width of tadpoles was also significantly different among treatments (Table 1). Tadpoles facing predictable risk (day) had significantly wider tail muscles than those facing no risk (4% change), continuous risk (7% change), the predictable risk at night (4% change) or unpredictable (8% change) risk (Fig. 3C; \( p \leq 0.001 \)). Interestingly, tadpoles facing continuous risk had narrower tail muscles than those facing no risk (3% change) or predictable risk at night (3% change; Fig. 3C; \( p \leq 0.012 \)). Further, tadpoles facing unpredictable risk had narrower tail muscles than those facing no risk (4% change) or predictable risk at night (4% change; Fig. 3C; \( p \leq 0.001 \)). Tail fin depth of tadpoles belonging to different treatments was comparable (Table 1; Fig. 3D; \( p \geq 0.165 \)). However, the overall tail muscle depth of tadpoles belonging to different groups was significantly different (Table 1). Tadpoles facing predictable risk during the day had
significantly deeper tail muscles than those facing no risk (3% change), continuous risk (3% change) or predictable risk at night (3% change; Fig. 3E; \( p \leq 0.009 \)). Further, tadpoles facing unpredictable risk had significantly deeper tail muscles than those facing continuous risk (3% change) or predictable risk at night (3% change; Fig. 3E; \( p \leq 0.014 \)). However, tail muscle depths of tadpoles facing no risk, continuous risk and predictable risk during the night were comparable (Fig. 3E; \( p \geq 0.977 \)).

4.4 Metamorphic traits

Temporally varying predation risk had a significant impact on the metamorphic traits of *E. cyanophlyctis* (Table 2). Tadpoles facing predictable or unpredictable risks at different times emerged as larger froglets (Table 2; Fig. 4A). Froglets emerging from the group facing predictable risk during the day were significantly larger than those facing no risk (3%) and continuous risk (3%; Fig. 4A; \( p \leq 0.008 \)) whereas those from the groups facing no risk, continuous risk, predictable risk at night or unpredictable risk were comparable in their SVL (Fig. 4A; \( p \geq 0.178 \)). Body masses of froglets emerging from different groups were also significantly different (Table 2; Fig. 4B). Tadpoles facing predictable risk during the day emerged as the heaviest froglets compared to those facing no risk (5%), continuous risk (4%), the predictable risk at night (7%) or unpredictable risk (4%; Fig. 4B; \( p \leq 0.035 \)). However, body masses of froglets emerging from groups facing no risk, continuous risk, the predictable risk at night or unpredictable risk were comparable (Fig. 4B; \( p \geq 0.499 \)). A significant difference was observed in the larval duration among groups (Table 2; Fig. 4C). Tadpoles facing continuous risk metamorphosed later than those facing predictable risk during the day (5 days), the predictable risk at night (8 days) or unpredictable risk (7 days; Fig. 4C; \( p \leq 0.009 \)). On
the other hand, tadpoles facing predictable risk at night were the first to metamorphose followed by those facing unpredictable risk, predictable risk during the day, no risk or continuous risk.

**Discussion**

In the present study, we assessed the antipredator responses of larval *E. cyanophlyctis* to temporally varying predation risk. Unlike previous studies, larvae were exposed to daily changes in predation risk as well as the time of day during which predation risk varied. In contrast to many other studies, not only were larval behaviour and morphology assessed but also growth and metamorphic traits because behavioural and morphological responses could directly or indirectly affect larval growth and life-history traits.

Larval *E. cyanophlyctis* lack the innate ability to recognise dragonfly nymphs as a threat but can do so through associative learning (Supekar and Gramapurohit 2017, 2018). Hence, they were expected to respond to the temporal pattern of predation risk by pairing kairomones with conspecific alarm cues. The results show that larval skipper frogs facing temporally varying predation risk reduced activity in response to kairomones of dragonfly nymphs thus reinforcing our earlier findings that these tadpoles respond to nymph odour through associative learning. Moreover, they could learn to assess the temporal pattern of predation risk and adjust their activity depending on the predictability of risk. For instance, activity reduction of larval skipper frogs was weaker in response to predictable risk whereas stronger in response to unpredictable risk. Similarly, tadpoles of *Lithobates sylvaticus* (LeConte, 1825) and *Hyla versicolor* (LeConte, 1825) could learn to match the intensity of their antipredator response based
on the temporal pattern of predation risk (Ferrari and Chivers 2009b; Ferrari et al. 2008b). In some systems, predictable risk allows prey to adjust their behaviour and habitat choice to decrease their vulnerability to predators while optimizing trade-offs between predator avoidance and foraging (Lima and Dill 1990; Ferrari and Chivers 2009b). Such behavioural adjustments are learnt by associating alarm cues with predator odour (Ferrari et al. 2008c; Ferrari and Chivers 2009b). The ability of prey to exhibit more intense antipredator responses during riskier periods should allow them to maximize trade-offs between predator avoidance and other activities such as foraging (Ferrari et al. 2008c). Moreover, unpredictable risk requires a quick and immediate response. Hence, behavioural alteration appears to be the best strategy for larval skipper frogs under unpredictable risk.

The RAH predicts that prey animals should allocate their foraging efforts (hence their exposure to predators) adaptively across high and low-risk situations (Lima and Bednekoff 1999). Prey animals facing long periods of frequent risk are expected to forage actively during brief periods of safety than those facing infrequent risk. The behavioural responses of larval *E. cyanophlyctis* were weaker under predictable risk in comparison to unpredictable risk. Further, the response of larval *E. cyanophlyctis* facing continuous risk was comparable with that of predictable and unpredictable risk. Larval anurans facing temporally varying predation risk are known to exhibit species-specific variation in their antipredator responses. For instance, behavioural responses of larval *L. pipiens* (Schreber, 1782) under no risk, weekly-risk, half-risk and continuous-risk were comparable (Hossie and Murray 2012). In contrast, behavioural responses of *R.*
*temporaria* were strongest in tadpoles facing continuous risk whereas weaker in those facing infrequent or frequent risk (Laurila et al. 2004).

Results of the present study indicate that growth of larval *E. cyanophlyctis* was not affected by temporally variable predation risk as evidenced by similar growth rates among tadpoles facing no risk, continuous risk, predictable or unpredictable risks. Similarly, larval *L. pipiens* exposed to temporally varying predation risk grew at a similar rate than those facing no risk (Hossie and Murray 2012). The growth responses of larval skipper frogs to temporally fluctuating predation risk were similar to no risk group probably due to the shorter exposure period. Hence, we assume that larvae could balance their foraging activity during periods of safety to vigilance during periods of risk and attain similar growth to that of no risk group. Moreover, they might not be energy deficient as the period of risk was short (2 h) and plenty of food was available readily all the time. If repeated exposures to the same regimen enable larval *E. cyanophlyctis* to learn and become habituated to the particular pattern, they can minimise foraging efforts during short periods of risk and compensate the loss by foraging actively during long safety periods to achieve growth similar to those facing no risk (Ferrari et al. 2009; Hossie and Murray 2012). Consequently, the growth of larval skipper frogs could be less responsive to temporally varying levels of predation risk.

As per our knowledge, very few studies have evaluated the morphological responses of anuran larvae to temporally varying predation risk (Laurila et al. 2004; Hossie and Murray 2012). Morphological responses of larval *L. pipiens* facing weekly-risk were intermediate between those facing continuous risk and no risk (Hossie and Murray 2012). Further, tadpoles facing predation risk only in the last 3 weeks did not
respond morphologically to perceived risk (Hossie and Murray 2012). The results on larval morphology of *L. pipiens* are consistent with the hypothesis of Gabriel et al. (2005), according to which prey responses should be intermediate when the frequency of environmental change is high relative to the response lag. In contrast, body length, tail length and tail muscle depth of *R. temporaria* (Linnaeus, 1758) larvae facing frequent and infrequent risks were often intermediate between those facing no risk and continuous risk (Laurila et al. 2004). Results of the present study indicate that some morphological traits of tadpoles facing predictable risk varied depending on the time of the risk. For instance, tadpoles facing predictable risk during the day were heavier and had wider and deeper tail muscles. Further, tadpoles facing unpredictable risk were heavier and had narrower and deeper tail muscles. Furthermore, tadpoles facing continuous risk had a narrower tail muscle. Possibly, larval skipper frogs initially divert their energy towards growing bigger bodies rather than investing in specific tail parameters such as length and width. In nature, larval *E. cyanophlyctis* grow to a large size, making it difficult for even the largest nymphs to attack and feed on them. Moreover, the pattern of locomotion of *E. cyanophlyctis* could be responsible for failing to exhibit tail-specific parameters in response to temporally varying predation risk. In nature, larval skipper frogs exhibit rapid startle response and change their directions swiftly while navigating. Moreover, they move rapidly from one place to another making it difficult for sit-and-wait predators such as dragonfly nymphs to attack them. Interestingly, larval skipper frogs facing predictable risk during the day and unpredictable risk had deeper and wider tail muscles. Deeper and wider tail muscles are under strong selection by dragonfly nymphs (Buskirk et al. 2002). Strong muscular
tails with heightened fins are known to aid tadpoles to exhibit rapid startle response. Moreover, stronger tail muscles are believed to aid in changing directions rapidly (Arendt 2010; Mikolajewski et al. 2016). Taken together, strong tail muscles with deep tails may help larval skipper frogs to exhibit rapid startle response and change directions while navigating and thus avoiding predators.

Information on the effects of temporal variation in predation risk on the metamorphic traits of anuran tadpoles is very scarce (Laurila et al. 2004). Only a single study has tested the effects of temporal variation in predation risk on the metamorphic traits of larval anurans (Laurila et al. 2004). In the present study, temporally varying predation risk had a strong impact on the metamorphic traits of E. cyanophlyctis. Larval skipper frogs facing predictable risk emerged with the largest size. They are more active at night and hence, tadpoles facing predictable risk during day-time might forage freely at night in the absence of risk and thus could attain a larger size. Interestingly, tadpoles facing no risk and continuous risk emerged at similar sizes. In contrast, larval R. temporaria and Hylarana indica (Biju et al., 2014) facing continuous risk metamorphosed at a larger size compared to those facing variable risk and no risk respectively (Laurila et al. 2004; Joshi et al. 2017). Probably, exposure to continuous risk enables E. cyanophlyctis tadpoles to become habituated thus ignoring the predatory cues. Alternatively, the larval skipper frogs might sense the unchanged ratio of conspecific density to the amount of predatory cue and hence forage freely to emerge at a similar size (Buskirk et al. 2011; Gazzola et al. 2018). Predictability of short-term risk during day-time may enable these tadpoles to forage freely during periods of no risk to grow and develop faster and metamorphose at the largest size. By
metamorphosing at a larger size, *E. cyanophlyctis* tadpoles could derive higher fitness as the larger metamorphic size is associated with increased juvenile survival increased foraging efficiency and higher fecundity (Buskirk and Saxer 2001; Altwegg and Reyer 2003).

Larval amphibians are known to exhibit predator-induced changes in behaviour and morphology that together cause slower growth and development (Buskirk 2000; Relyea 2002). Consequently, amphibian tadpoles facing predation risk constantly tend to metamorphose later. In our study, *E. cyanophlyctis* tadpoles facing predictable and unpredictable risks metamorphosed earlier than those facing continuous risk. Similarly, tadpoles of *R. temporaria, R. aurora* (Baird and Girard, 1852) and *Bufo bufo* (Linnaeus, 1758) facing continuous risk took more time to metamorphose (Kiesecker et al. 2002; Laurila et al. 2004). However, continuous predation risk did not evoke significant morphological responses in larval *E. cyanophlyctis* but induced longer larval duration. By contrast, continuous predation risk induced larval *Hylarana indica* to metamorphose early (Joshi et al. 2017). Metamorphosing earlier at a larger size has many advantages, as metamorphic size and timing of metamorphosis are known to affect juvenile survival and growth leading to increased adult fitness (Smith 1987; Berven 1990; Scott 1994; Altwegg and Reyer 2003). Metamorphosing earlier at a larger size under short-term predation risk could be the best strategy for larval *E. cyanophlyctis* to derive fitness benefits during juvenile and adult life.

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Ethical Note:
This study was carried out following the guidelines of Departmental committee for animal ethics (No. 538/CPCSEA).

Conflict of Interest: The authors declare no conflict of interest.

References


Figure legends

**Figure 1** Effect of temporal variation in predation risk on growth of larval *E. cyanophlyctis*.

PR(D)- predictable risk during the day; PR(N)- predictable risk at night; UPR- unpredictable risk; CR- continuous risk.

**Figure 2** Effect of temporal variation in predation risk on proportional change in activity of larval *E. cyanophlyctis* towards kairomones when tested during the day and night. Dissimilar letters over the bars indicate significant difference.

PR(D)- predictable risk during the day; PR(N)- predictable risk at night; UPR- unpredictable risk; CR- continuous risk.

**Figure 3** Morphology (Mean ± SE) of larval *E. cyanophlyctis* facing temporally variable predation risk, A) Body mass, B) Tail length, C) Tail muscle width, D) Tail depth and E) Muscle depth.

Parameters were calculated relative to the body mass. Dissimilar letters over the bars indicate significant difference.

PR(D)- predictable risk during the day; PR(N)- predictable risk at night; UPR- unpredictable risk; CR- continuous risk.

**Figure 4** Effect of temporal variation in predation risk on metamorphic traits (Mean ± SE) of *E. cyanophlyctis* A) Snout-vent length, B) body mass and C) larval duration. Dissimilar letters over the bars indicate significant difference.

PR(D)- predictable risk during the day; PR(N)- predictable risk at night; UPR- unpredictable risk; CR- continuous risk.
Table 1: Results of mixed model ANOVA’s showing effect of temporal variation in predation risk on each of morphological parameter of larval skipper frogs (*E. cyanophlyctis*). All the parameters except body mass were calculated relative to the body mass.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
<th>$F$</th>
<th>$Df$</th>
<th>$P$</th>
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<td>4, 16</td>
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<td>16, 124</td>
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<td>Tank ID</td>
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<td>4, 16</td>
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Table 2: Results of mixed model ANOVA’s showing effect of temporal variation in predation risk on each of metamorphic traits of skipper frogs (*E. cyanophlyctis*).

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<thead>
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<td>Treatment</td>
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</table>
Figure 1

Body mass (gm) vs. Age (days)

- Control
- PR(D)
- PR(N)
- UPR
- CR
--- Temporal pattern of predation risk ---
Figure 4

**A**

Snout-vent length (mm)

**B**

Body mass (mg)

**C**

Larval duration (days)

---Temporal pattern of predation risk---