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Predator-induced plasticity in tadpoles of *Polypedates cruciger* (Anura: Rhacophoridae)

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***Abstract.*—Aquatic tadpoles morphologically respond to presence of predators in various ways. Depending on the type of predator, tadpoles develop enhanced escape response abilities in acceleration, maneuverability, and speed, and these are correlated to suites of morphological characters, such as wider, longer, and robust tail related dimensions. Laying eggs away from water, such as in an arboreal foam nest from which partially developed tadpoles fall into water, could be an adaptation for predator avoidance of eggs and early tadpole stages. Since predation is of concern, even for these partially developed larvae, we sought to detect predator-induced morphological response (if any) of these forms compared to fully aquatic tadpoles. We exposed the tadpoles of foam-nesting *Polypedates cruciger* to a natural fish predator, *Belontia signata*. We show that at an early (Gosner stage 29-32) stage, tadpoles exposed to this predator develop a larger body size and increased tail-length related dimensions.**

Key words. Tadpole morphology, plasticity, foam nesting, *Polypedates cruciger*, predator-induced, morphological response, amphibian declines

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Introduction

It is well known that aquatic tadpole predators, such as some dragonfly larvae and fish, induce morphological changes in aquatic tadpoles (Anderson and Brown 2009; Buskirk 2002; Teplitsky et al. 2003). Morphological features of fully aquatic tadpoles, especially the ones that are important in swimming, such as tail dimensions, are known to change in response to predator-type, such as ambush predators and run-down predators. In the presence of ambush predators, tadpoles become acceleration/maneuver specialists, while in the presence of run-down predators, tadpoles become speed specialists. Morphological adaptations for such escape pathways include a broader tail (Lardner 1998; Laurila et al. 2006; Relyea 2002; Relyea 2003; Sosa et al. 2009; Teplitsky et al. 2003) or a longer tail, respectively (Higginson and Ruxton 2010; Moore et al. 2004; Relyea 2000). In some cases, the presence of predators causes early metamorphosis (Benard 2004; Higginson and Ruxton 2010; Relyea 2007; Werner 1986).

Morphological changes in response to predator presence occur in a diversity of amphibian taxa that are disparate both in phylogenetic and life-history traits. Frog species possessing different life-history traits show different anti-predator responses to different predators and competitors (Laurila et al. 2006; Relyea 2001a; Relyea 2001b; Relyea and Yurewicz 2002). For fully aquatic tadpoles, these morphological responses are now well known.

Laying eggs away from water in a foamy mass, in which tadpoles develop up to a pre-metamorphic stage before falling into water, is an alternative life history strategy, often known as foam nesting (Duellman and Trueb 1986). This strategy is considered to facilitate predator avoidance of eggs and early-stage tadpoles (Hodl 1992; Magnusson and Hero 1991), and to reduce the duration of the larval stage (through rapid development during the out-of-water phase).

The Hourglass treefrog (*Polypedates cruciger*), a Sri Lankan endemic, shows a derived reproductive strategy from aquatic egg deposition. These frogs make foamy nests overhanging water bodies, in which they lay their eggs. Tadpoles develop within the nest, up to Gosner stage 23 and then fall into water, where they undergo further development reaching metamorphosis. Adult *P. cruciger* are arboreal, but sometimes visit pools at night, apparently to rehydrate.

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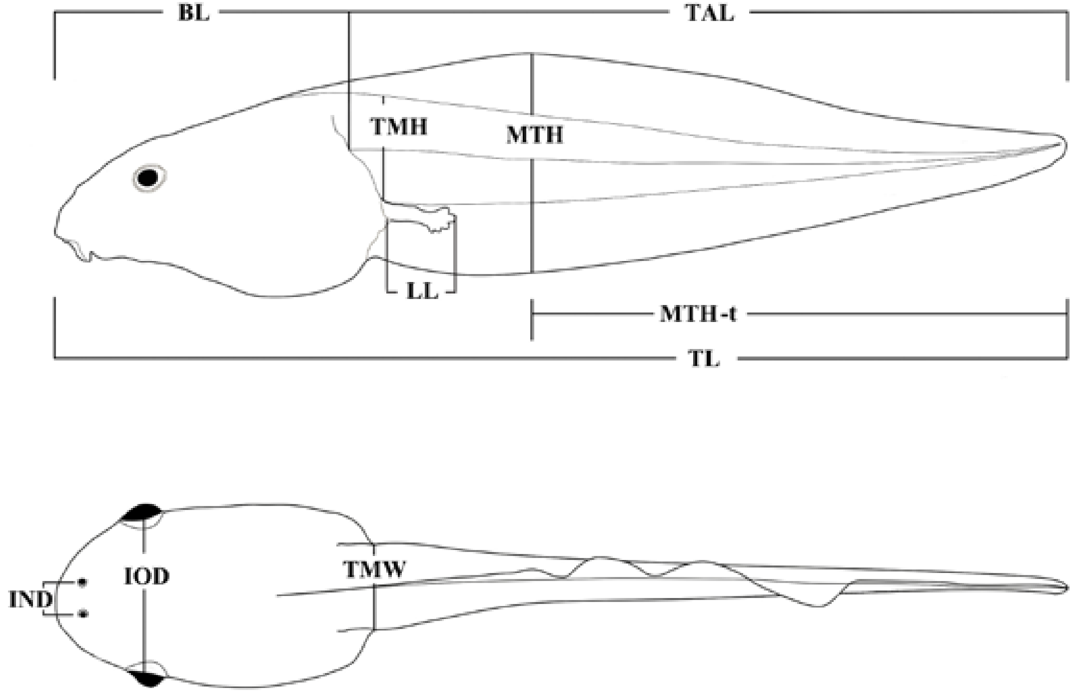

Figure 1. Outline of tadpole (lateral and dorsal views), depicting measurements that were used in this study: total length (TL), tail length (TAL), maximum tail height (MTH), maximum tail height to tip of tail (MTH-t), total muscle height (TMH), total muscle width (TMW), body length (BL), inter-orbital distance (IOD), internasal distance (IND), and limb length (LL).

Fish prey on such early-stage tadpoles that fall into water (this has been documented for other species, in which tadpoles of arboreal gel-encapsulated egg layers fall into water and are eaten by various aquatic predators; Magnusson and Hero 1991). Tadpoles of *P. cruciger* are preyed on by various fish species, including the Combtail, *Belontia signata* (Belontiidae), the Snakehead, *Channa orientalis* (Channidae), and nonnative and introduced Guppy, *Poecilia reticulata* (Poeciliidae; M. Meegaskumbura, pers. obs.). This study tests the developmental response of *P. cruciger* tadpoles to aquatic predation pressure.

Methods and materials

A single foam nest of *Polypedates cruciger* attached to a twig above a pond was observed in Peradeniya University Gardens, Sri Lanka (7°15’34.02”N, 80°35’49.71”E; 600 m asl). Tadpoles that emerged six days after the foam nest was first made (fertilization was observed) were reared in a glass aquarium for seven days, until the experiment began.

The experimental setup was as follows: eleven equally sized glass aquaria (size: 45 × 30 × 30 cm) each with 25 tadpoles was set up. Three of these were used as controls, and contained only tadpoles. Of the eight experimental aquaria, four contained tadpoles and fish, but visual contact between the tadpoles and fish was prevented by an opaque, water-permeable screen so that they shared the same water (chemicals produced by fish or tadpoles could thus be detected by any individual in the aquarium); these treatments were termed “closed” (they were established to provide tadpoles with an attenuated predator presence). The other four aquaria contained both tadpoles and fish, but allowing for visual (though not physical) contact between the predators and potential prey. They too, shared the same water, and were termed “open.”

All other experimental conditions were kept identical for all tanks. The fish and tadpoles were fed a protein-rich aquarium-fish food. Daily partial water changes were made using water from an animal-free aquarium that had a UV-C sterilizer (to remove pathogenic organisms) and an aerating power filter (to aerate water and remove traces of chlorine and ammonia that could be present in tap water).

Samples were taken 12 days after the beginning of the experiment. They were anesthetized in MS222 and measured using a vernier caliper under a stereo microscope. Six tadpoles were sampled arbitrarily from each replicate. They were measured to ±0.01 mm using a digital caliper. The following measurements were taken: total length (TL), tail length (TAL), maximum tail height (MTH), maximum tail height to tip of tail (MTH-t), total muscle height (TMH), total muscle width (TMW), body length (BL), inter-orbital width (IOD), and internasal distance (IND; Fig. 1).

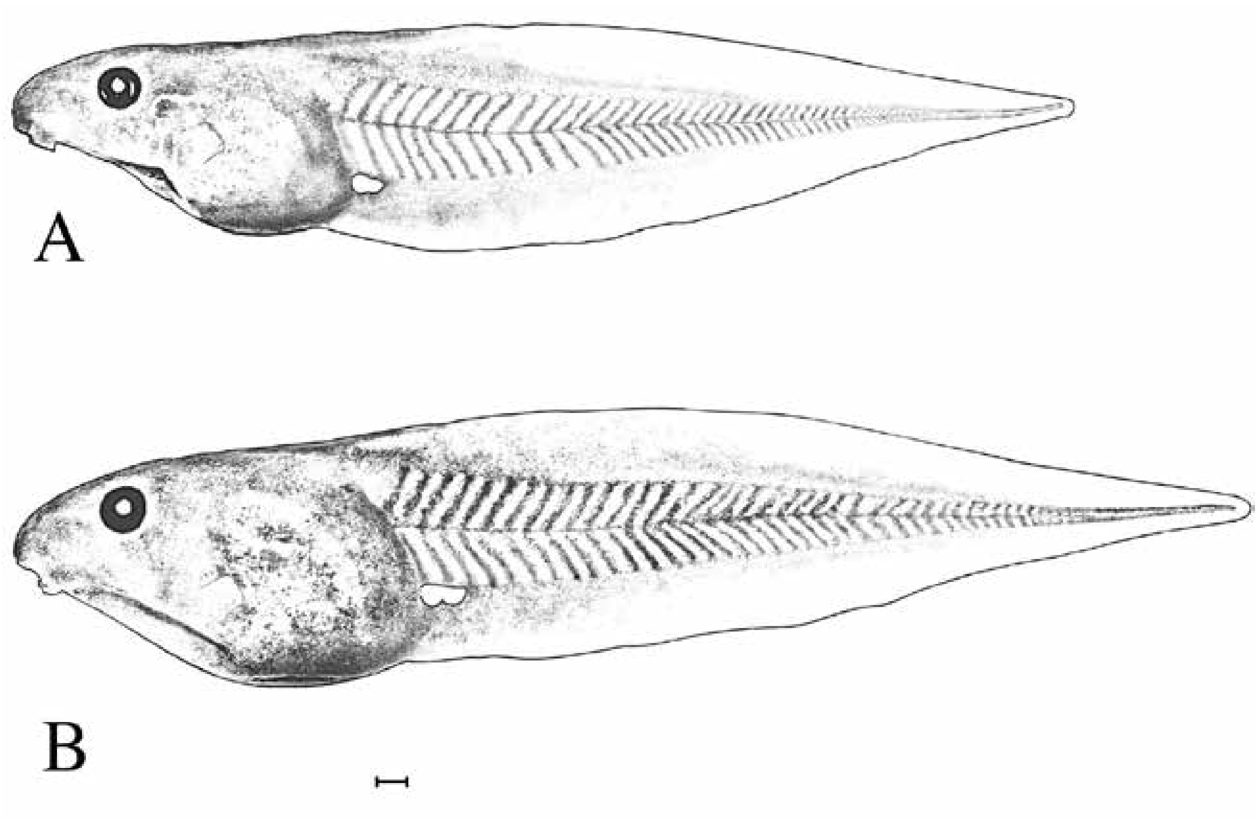

Figure 2. The morphology of early tadpole stages: A, control; B, "open." Scale bar 1 mm.

Coefficients of variation (CV scores) were determined and variables that had CV > 5%, and individuals that were outliers, were excluded from analyses. Prior to all analyses (except determination of CV scores) data were normalized through $\log_{10}$ transformation. The mean of each replicate was used in the subsequent analyses.

Systat version 11.00.01 for Windows XP was used for the statistical analysis. Principal Components Analysis (PCA) of means of character covariance matrix was used to reduce the dimensionality of morphological variables and to identify variables that may discriminate between the treatments. Different axis rotations were tested, and the one that yielded optimal interpretability of variation among variables is reported.

Discriminant Functions Analysis (DFA) was carried out to distinguish between the three experimental groups.

To visualize relationships between the variables of tadpole morphology, box plots depicting mean and standard error were made.

Results

Variables having CV scores > 5%, IND and LL, were excluded, leaving seven variables (TL, TAL, MTH, MTH-t, TMH, TMW, and BL) available for further analysis.

In the PC space of unrotated PC 1 and PC2 axes, the two treatments ("closed" and "open"), and the "con-

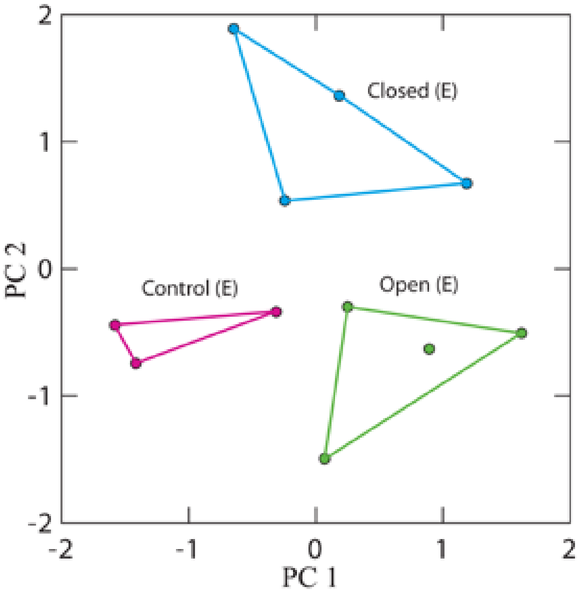

Figure 3. Principal components space of PC1 vs. PC2 (unregressed) of tadpole measurements in the two experimental conditions ("open" and "closed") and the controls of the early sampling regime. The PC1 axis, which explains 46% of the variance, is mostly represented by tail length, total length, and inter-orbital width. The PC2 axis, which explains 24% of the variance, mostly represents tail height-related variables.

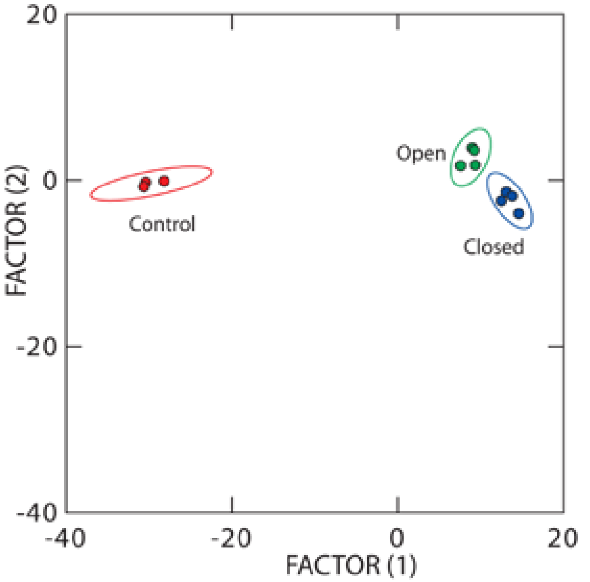

Figure 4. Canonical variables plot of discriminant function analysis (unregressed) of the two experimental conditions ("open" and "closed") and the control. Ninety-five percent confidence elipses of these three do not overlap with one another, and are centered on the centroid each group.

trol" tadpoles separate well (Figs. 3, 4). On the PC 1 axis, which explains 46% of the variance, several variables representative of tail and total lengths, and IOD load heavily (component loadings: TAL = 0.889, MTH-t = 0.871, IOD = 0.869, TL = 0.825; TMW = 0.667; Table 1). On this axis, "control" and "open" do not overlap, but "closed" overlaps with both the former cases and is placed in between these. Hence, presence of fish seems to increase total and tail-length related dimensions in tadpoles. On the PC 2 axis, which explains 24% of the variance, "closed" does not overlap with either "open" or "control." However, both "open" and "control" overlap with each other completely on this axis, which is mostly explained by tail height-related variables (component loadings TMH = 0.811, MTH = 0.624; Table 1). Considering unrotated PC 1 vs. PC 3, PC 1 vs. PC 4, PC 2 vs. PC 3, and PC 2 vs. PC 4 for these, the treatments and controls overlap with each other to various degrees on the PC 3 and PC 4 axes (not shown) but, as explained above, not on the PC 1 and PC 2 axes.

The Discriminant Functions Analysis shows that the 95% confidence ellipses do not overlap with each other (Fig. 4).

Some of the tail-length associated variables (means and standard errors) (TAL, MTH-t, TL, and TMW) show distinctions among the three groups; only the box plot of MTH-t is shown (Fig. 5).

Discussion

Because of predation, developmental anomalies, pathogens, and unfavorable environmental conditions, not all amphibian larvae develop to metamorphosis. Often entire egg clutches are destroyed even before tadpoles become free swimming.

Predation reduction of egg and early stage tadpoles has been suggested to have driven the evolution of egg deposition out of water for many forms (Doughty 2002). This hypothesis is plausible, but predator avoidance is still important even after early-stage tadpoles of foam-nesting species fall into water. Indeed, we have observed tadpoles of *P. cruciger* being preyed upon by various fish species. Once a falling tadpole is detected by predatory fish, it lurks under the nest waiting for more tadpoles to fall (M. Meegaskumbura, pers. obs.). In such a situation, there is clearly an advantage for tadpole's ability to evacuate the impact area as soon as possible. We have observed this: tadpoles of *P. cruciger*, upon impacting the surface of the water, quickly react by swimming away rapidly, in an apparently arbitrary direction, until

Table 1. Component loadings for axes 1-4 for the Principal Component Analysis, variance explained, and percentage of total variance explained for early sample treatments and controls (unregressed: "open," "closed," and "control").

	Component Loadings			
	1	**2**	**3**	**4**
TAL	0.889	-0.341	-0.160	0.241
MTHT	0.871	-0.188	-0.297	-0.232
IOD	0.869	0.332	0.007	-0.312
TL	0.825	-0.466	0.112	0.281
TMW	0.667	0.477	0.349	0.374
TMH	-0.102	0.811	-0.242	0.464
MTH	0.407	0.624	0.514	-0.370
BL	-0.188	-0.401	0.874	0.128
Variance Explained by Components	3.642	1.914	1.335	0.796
% of Total Variance Explained	45.530	23.929	16.686	9.955

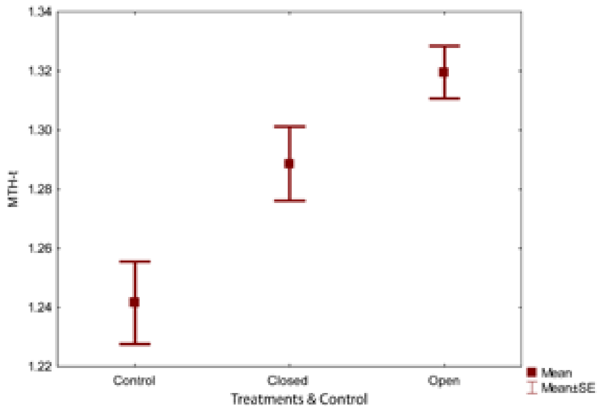

Figure 5. Boxplot depicting the means and standard errors of the two treatments ("open" and "closed") and the control.

they reach a safe submerged refuge. Furthermore, even though young tadpoles are attached by their cement glands to underwater substrates at this stage, they react quickly to any disturbance by fast and apparently random swimming (M. Meegaskumbura, pers. obs.). These observations are indications that effective swimming is an important survival attribute in tadpoles.

PCA and DFA results are complementary and show tadpoles of the "control" and "open groups" to be divergent in body morphology. It is known that a larger body confers reduced risk of predation (Buskirk and Schmidt 2000), as this enables animals to swim faster, or accelerate and maneuver better. The "open" body morphology of *P. cruciger* tadpoles matches the features of tadpoles from other unrelated taxa that respond to predation by achieving a fast-swimming body morphology e.g., longer tail, greater total length: Buskirk and Relyea (1998); Teplitsky et al. (2003).

Behavioral plasticity might be inexpensive due to absence of a need for new or altered structures to meet new challenges (Buskirk 2002). Though behavioral response of tadpoles to predators was not quantified in this study, we observed that tadpoles from "open" tanks reacted most swiftly to disturbances when compared to "closed" and "control" groups.

We have yet to study the effects of predator presence on early metamorphosis, something that several other authors have previously reported on (Gomez-Mestre et al. 2008; Lardner 1998; Vonesh and Warkentin 2006). If early metamorphosis occurs in tadpoles that develop in association with a predator, the resulting tadpoles may have a smaller body (Lardner 1998).

Although our data demonstrate that *P. cruciger* tadpoles exhibit predator-induced plasticity, they reveal little about the patterns of plasticity. For example, we do not know whether all tadpole stages show predator induced plasticity, or if the presence of predators induces early metamorphosis. Further experimentation is warranted.

Multiple layers of protection, initially through harboring of the vulnerable early developmental forms in a foam nest, and later, after partially developed tadpoles fall into water, in the accelerated development responses to aquatic predator presence, seem like adaptations to help survive in a predator high environment. If foam nesting evolved as a response to predator avoidance of early tadpole stages, it can be argued that there was a heavy predation cost for the aquatic larvae, at least historically. Then even the partially developed tadpoles would have to face some form of predation, from the very predators that would have eaten them as early-stage larvae, had the eggs been laid in water, even though at a reduced intensity. These adaptations could be a reason for the wide distribution of this species across several habitat types in the wet and the intermediate zone of Sri Lanka. It will be interesting to determine whether adaptations observed in *P. cruciger* are seen also in tadpoles of *Taruga*, its sister genus (Meegaskumbura et al. 2010).

Introduced predatory fishes may have various feeding mechanisms, which tadpoles living in these waters may not be adapted to. For instance, to avoid predation from an ambush predator, an accelerating or maneuvering tadpole body form may be needed. If this is not present, an introduced form may destroy whole populations of tadpoles.

Hence, when causes for decline of amphibians are considered in the context of to introductory predatory fishes or aquatic predators, study of tadpole morphological adaptability may be important to determine the actual mechanisms of decline.

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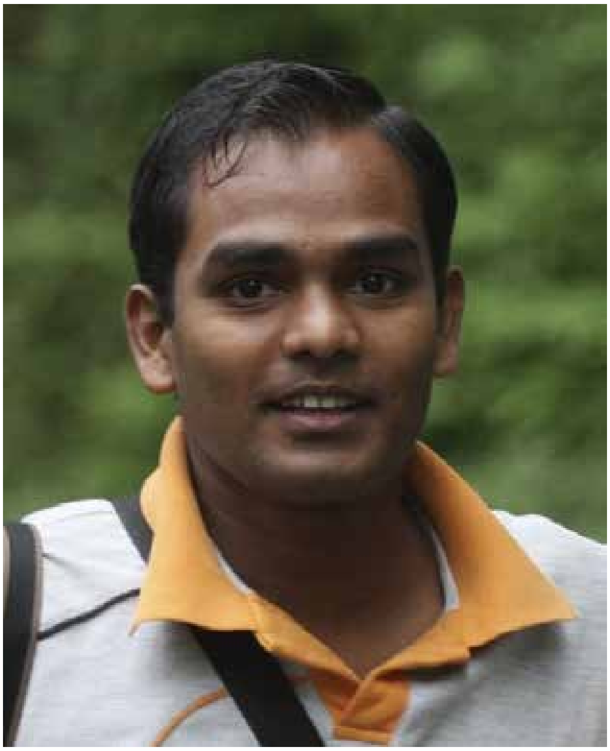

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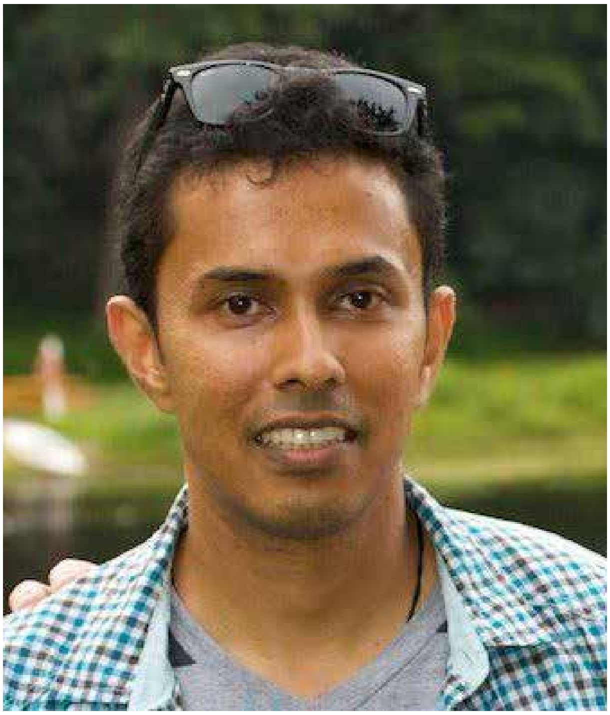

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