INVASION NOTE

No survival of native larval frogs in the presence of invasive Indian bullfrog *Hoplobatrachus tigerinus* tadpoles

Nitya Prakash Mohanty ⓘ · John Measey ⓘ

Abstract Invasive amphibians have considerable negative impacts on recipient ecosystems, however, impact has been assessed for only a few species, limiting risk assessments. In particular, the impact of invasive anurans with carnivorous tadpoles have not been examined thoroughly. The Indian bullfrog (*Hoplobatrachus tigerinus*), native to the Indian subcontinent, is rapidly invading the Andaman archipelago, Bay of Bengal after its recent introduction. We aimed to evaluate the effect of carnivorous *H. tigerinus* tadpoles on two species of endemic anuran tadpoles *Microhyla chakrapanii* and *Kaloula ghoshi*, in a mesocosm experiment. Rapid predation by larval *H. tigerinus* resulted in no survival of endemic frog tadpoles. Survival of *H. tigerinus* was density-dependent. The study is timely in elucidating the impact of invasive larval *H. tigerinus* on native anurans and substantiates the need to manage invasive populations (or potential incursions) of the species on the Andaman archipelago and elsewhere.

Keywords Amphibia · Andaman Islands · Cannibalism · Impact · Invasion impact · Mesocosm experiment

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Invasive amphibians have considerable negative impacts on recipient ecosystems with the magnitude of impact being similar to that of invasive birds and fishes (Measey et al. 2016). However, amphibians remain a relatively understudied taxon in invasion science (Pyšek et al. 2008), despite the increasing number of established non-native amphibian species and populations globally (Capinha et al. 2017). Amphibian invaders, with biphasic life-histories, require assessment of their effect on native species in both terrestrial and aquatic environments, as the outcome of interactions in the aquatic stage may have carry-over effects influencing the terrestrial stage (Chelgren et al. 2006). Invasive larval anurans are known to have negative effects on survivorship or performance of native larval anurans through competition (Kupferberg 1997; Smith 2005a) and toxicity (see Shine 2010); however, impacts of carnivorous larvae have not been well studied. Although the number of studies on the impact of larval amphibians is greater than those on post-metamorphic amphibians

(Measey et al. 2016), there is considerable bias in the species assessed. Three species (the cane toad *Rhinella marina*, the American bullfrog *Lithobates catesbeianus*, and the African clawed frog *Xenopus laevis*) account for nearly 80% of published research on amphibian invasions (van Wilgen et al. 2018).

The Indian bullfrog (*Hoplobatrachus tigerinus*, Daudin 1802), native to the Indian sub-continent (Dutta 1997), is currently invading the Andaman archipelago, Bay of Bengal (Mohanty and Measey 2019). The Andaman archipelago, comprising of nearly 300 islands (ca. 6400 km^2), is situated between 10°30′N to 13°40′N and 92°10′E to 93°10′E. The Islands are a part of the Indo-Burma biodiversity hotspot with a 40% endemism level in herpetofauna. Introduced in early 2000s, the invasive range of *H. tigerinus* (Dicroglossidae) in the archipelago has expanded exponentially since 2009, resulting in established populations on six out of the eight human-inhabited islands of the Andaman archipelago (Mohanty and Measey 2019). *Hoplobatrachus tigerinus* is uncommon or absent in forested and coastal regions but occurs as a human commensal in plantations and agricultural fields (Daniels 2005). This large-bodied frog (up to 160 mm) is known to prey upon a host of small endemic vertebrates on the archipelago and its diet overlaps significantly with larger native anurans, indicating a potential for competition (Mohanty and Measey 2018). The species is also established on Madagascar and is reported from the Maldives and Laccadive Islands (see references in Mohanty and Measey 2019).

Hoplobatrachus tigerinus has a high reproductive potential (up to 5750 eggs per clutch) with egg survival of ca. 40% (Dash and Hota 1980). Given the common occurrence of the frog in the Indian sub-continent, many autecological studies have described its breeding biology and the larval stage (reviewed in Saidapur 2001). Tadpoles of *H. tigerinus* are known to be carnivorous, feeding on zooplanktons, other anuran larvae and even display cannibalism (Khan 1996; Grosjean et al. 2004). On the Andaman archipelago, *H. tigerinus* co-occurs with native anurans of the genera *Microhyla*, *Kaloula*, *Duttaphrynus*, *Fejervarya* and *Limnonectes* (NPM unpublished data), which could include up to 12 species (see Harikrishnan and Vasudevan 2018). In human-modified areas, the invasive *H. tigerinus* and all the syntopic native anurans breed in ephemeral pools in waterlogged

agricultural fields and plantations. The syntopic native species likely breed at the onset of the south-west monsoon in May, along with *H. tigerinus* (Harikrishnan and Vasudevan 2013). Native anurans, however, appear to have a relatively longer breeding season compared to *H. tigerinus*, which is an explosive breeder (NPM *pers. obs.*). Given its high reproductive potential and carnivorous tadpoles, the impact of larval *H. tigerinus* on native larval anurans requires urgent evaluation.

We aim to evaluate the effect of invasive *H. tigerinus* tadpoles on two species of endemic anuran tadpoles, in a mesocosm experiment. We hypothesize that *H. tigerinus* tadpoles negatively impact endemic anuran tadpoles and predict that, (1) predation by *H. tigerinus* tadpoles, decreases the survival of both species of endemic anuran tadpoles and (2) *H. tigerinus* tadpoles have increased survival, growth rates, and metamorph size, and a reduced larval period in the presence of native anuran larvae as compared to treatments with only *H. tigerinus* conspecifics.

We conducted the study in and around the Andaman Nicobar Environment Team (ANET) field station, located in Wandoor, South Andaman Island. Following heavy rains, breeding commenced on the night of 12 May 2017. Four clutches of eggs belonging to invasive *H. tigerinus* were collected from water-logged paddy fields and plantation moats. We were able to collect an adequate number of egg clutches (n = 4) only for two endemic anurans, *Microhyla chakrapanii* and *Kaloula ghoshi* (both Microhylidae), which breed syntopically and synchronously with the invasive *H. tigerinus*. Upon emergence of tadpoles, we mixed the clutches and assigned individuals to treatments randomly, to avoid any parental bias (Dash and Hota 1980). We started the experiment on 16 May 2017 for 21 pools and on 19 May 2017 for the remaining four pools. All tadpoles had reached Gosner stage 25 at the onset of the experiment.

Our experiment comprised of seven treatments: three with single-species, three with two-species, and one with three-species. We replicated each treatment three times for single-species (3 × 3), and four times each for two-species (4 × 3) and three-species (4 × 1) treatments, with a total of 25 pools. The circular plastic pools (125 cm in diameter x 40 cm in depth) were filled with ca. 150 litres of untreated pond water. We kept the total tadpole density of pools constant across all treatments, at 30 tadpoles (two-

species pools: 15 tadpoles/species; three-species pools: 10 tadpoles/species). This density is equivalent to the 'high density' (0.213 larvae/litre) treatments for larval *Lithobates catesbeianus* mesocosm experiments (following Kiesecker et al. 2001). We provided uniform food resources (40 g of leaf litter and algae per week) collected from the ephemeral pools in the study site used for breeding by the three species. The pools were cleaned once a week, with the tadpoles (segregated by species) being held temporarily in plastic buckets filled with water. Cleaning involved draining of used water and scrubbing of the pool manually to remove any sediments, followed by restocking with fresh water (including zooplanktons) and food. A nylon net was used to cover each pool to avoid external predation, as the pools were placed outdoors to experience a natural photoperiod. Mean temperature (measured by Davis-Vantage Pro 2 weather-station) of the study area during the experiment was 27.52°C (SD = 1.95; range: 23.2–33.3°C).

We recorded survival of tadpoles in each pool weekly and photographed five tadpoles (or fewer if unavailable due to mortality), haphazardly selected from each pool, on a gridded sheet (10 mm × 10 mm) thrice a week. The experimental setup was monitored daily to detect metamorphosing tadpoles (and dead tadpoles), which were removed at Gosner stage 42 (emergence of forelimbs). We provided a floating *Colocasia* leaf in each pool to enable metamorphosing individuals to avoid drowning. Upon completing metamorphosis, individuals were photographed on the gridded sheet; we processed the photographs in the image analysis software 'ImageJ' and obtained body length (BL) and total length (TL) for all tadpoles and snout-vent length (SVL) for metamorphs. Time to metamorphosis was recorded in days for all individuals, with the start set at the night of spawning (12 May 2017).

We obtained the final proportion of survival for each species per pool based on the initial number of allocated tadpoles. Time to metamorphosis was computed as the median value of the number of days to metamorphosis for all tadpoles in a pool. Body length and total length were measured up until the median date of metamorphosis for each pool. We fitted linear regressions to both size measurements (truncated to peak size) over time to obtain growth slopes. We removed one pool (*Microhyla-Kaloula* treatment)

from all analyses, as there was a mass die-off on the first day, probably due to contamination of the pool. One other pool of the same treatment (*Microhyla-Kaloula*) overflowed 14 days from the start of the experiment, hence no statistical tests were carried out for survival, time to metamorphosis, and metamorph size on this treatment. However, we were able to estimate growth rates for three pools of the *Microhyla-Kaloula* treatment.

A Shapiro–Wilk test was carried out to test for normality of all response variables, failing which we executed non-parametric tests. We performed a Kruskal–Wallis one-way analysis of variance on survival, growth slopes, time to metamorphosis and metamorph size, for the three species separately (i.e. four treatments per species). A Dunn's test of multiple comparisons using rank sums, was carried out as a post hoc test to determine pair-wise differences between treatments per species, using the 'dunn.test' package in the statistical software R (Dinno 2017).

Microhyla chakrapanii was the first species to reach metamorphosis (median: 21 days), followed by *Hoplobatrachus tigerinus* (32 days) and *Kaloula ghoshi* (38 days; Table 1). Growth was fastest for *H. tigerinus* and resulted in the largest metamorphs (ca. 20 mm; Table 1). Survival for both endemic anurans, *M. chakrapanii* and *K. ghoshi*, reduced to zero in the presence of invasive *H. tigerinus*, as compared to 0.89 (SE = 0.04) and 0.62 (SE = 0.11) in their respective single species treatments (Fig. 1). In the three-species treatment, all individuals of *M. chakrapanii* and *K. ghoshi* were also preyed upon (Fig. 1). Both endemic anurans were completely consumed by *H. tigerinus* within the first week, in 10 out of 12 pools (83.33%); the remaining two pools had no surviving endemic anurans by the third week.

We found no significant difference between treatments with and without endemic anurans in terms of *H. tigerinus* growth in body length (Kruskal–Wallis $\chi^2 = 1.44$; $p = 0.70$) and total length (Kruskal–Wallis $\chi^2 = 1.23$; $p = 0.75$), time to metamorphosis (Kruskal–Wallis $\chi^2 = 0.38$; $p = 0.94$), and metamorph size (Kruskal–Wallis $\chi^2 = 3.48$; $p = 0.32$). Mean number of *H. tigerinus* surviving across treatments was 2.80 tadpoles (SE = 0.48; range: 1 to 6) and did not differ significantly between treatments (Kruskal–Wallis $\chi^2 = 1.06$, $p = 0.79$). However, proportion of *H. tigerinus* surviving was significantly greater in the presence of both endemic anurans ($p = 0.012$; n = 15;

Table 1 Species-wise growth rates (mm/day), time to meta-morphosis (days), and metamorph size (snouth-vent length in mm) for larval invasive *Hoplobatrachus tigerinus* (Dicroglossidae) and the native *Microhyla chakrapanii* and *Kaloula ghoshi* (Microhylidae), in the mesocosm experiment

Species	Growth (Body length)	Growth (Total length)	Time to metamorphosis	Metamorph size
Hoplobatrachus (n = 15)	0.56 ± 0.08	1.69 ± 0.22	32.00 ± 2.86	19.82 ± 0.48
Microhyla (n = 5*)	0.38 ± 0.04	1.18 ± 0.12	21.00 ± 1.44	6.88 ± 0.17
Kaloula (n = 5*)	0.25 ± 0.04	0.70 ± 0.11	38.00 ± 2.64	9.86 ± 0.43

Values reported as mean ± Standard Error, except for time to metamorphosis shown as the median value ± SE. 'n' denotes the number of mesocosms used for calculating values

*Growth measurements obtained from an additional mesocosm (n = 6)

Fig. 1 Proportion of survival to metamorphosis in larval invasive *Hoplobatrachus tigerinus* (HT) and native *Microhyla chakrapanii* (MC) and *Kaloula ghoshi* (KG), across seven treatments in a mesocosm experiment. Lines denote statistically significant differences in species-specific survival between pairs of treatments based on Kruskal–Wallis one-way analysis of variance tests and Dunn's test of multiple comparisons using rank sums
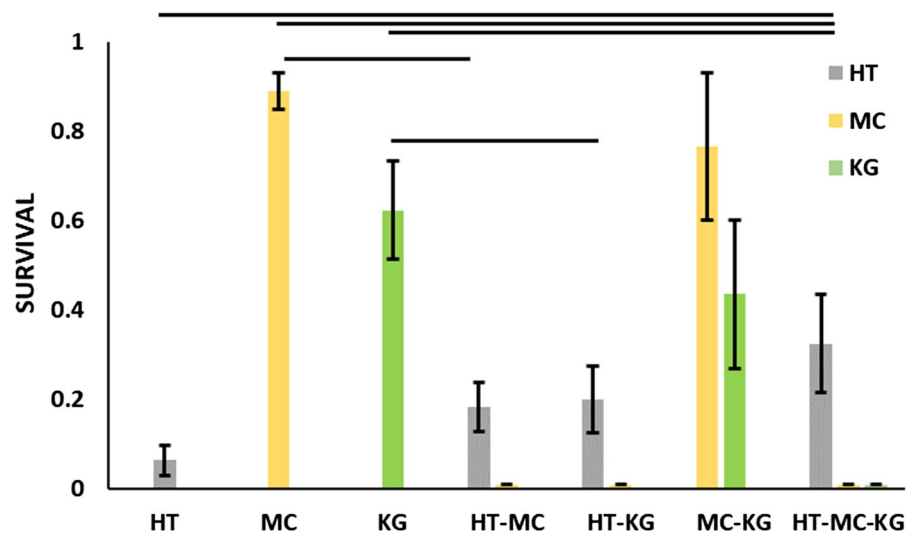
Fig. 1), as compared to the treatment with only conspecifics.

This study, elucidating the potential impact of invasive larval *H. tigerinus* on two endemic species of the Andaman archipelago, is timely as the rapidly expanding invasion is likely to affect other native anurans including the many anuran genera awaiting formal taxonomic re-assessments (Harikrishnan and Vasudevan 2018). Our findings support the prediction of reduction in survival of endemic larval anurans due to predation by invasive *H. tigerinus* tadpoles (prediction 1), to the extent of no survival of any native tadpoles. These results augment the limited existing knowledge on the impact of amphibian invaders with carnivorous larvae (Kiesecker and Blaustein 1997; Smith 2005b). However, the complete extermination of native larval anurans by *H. tigerinus* (0% survival) has not been observed either in the American bullfrog *Lithobates catesbeianus* (87.7% survival of syntopic native tadpoles; Kiesecker and Blaustein 1997) or the Cuban treefrog *Osteopilus serpentrionalis* (35% survival of syntopic native tadpoles; Smith 2005b), albeit with variation in experimental design. Apart from *M. chakrapanii* and *K. ghoshi*, considered in our mesocosm experiment, the carnivorous larvae of *H. tigerinus* are likely to impact other native anurans breeding in ephemeral pools of human-modified areas.

Presently, the population of *H. tigerinus* is abundant in human-modified landscapes and has only been reported from forest streams based on a few observations (Harikrishnan and Vasudevan 2013). However, its recent exponential range expansion in the Andaman archipelago (Mohanty and Measey 2019) can result in substantial mortality to native anurans in their larval stage, across a large geographical area. Artefacts of landscape modification by humans, such as artificial ponds for aquaculture, facilitate the invasion of *H. tigerinus* in the archipelago (Mohanty and Measey 2019). Further, the presence of moats in Areca nut and banana plantations serve as suitable habitat for larval *H. tigerinus* (NPM *pers. obs.*). Such facilitation of breeding populations of invasive anurans and their larvae by landscape modification could prove disadvantageous for native anurans.

The invasive population of *H. tigerinus* on the Andaman archipelago bred synchronously with the native anurans, thereby not limiting *H. tigerinus* larval predation by prey size (Babbitt and Tanner 1998). The remarkable rapidity of *H. tigerinus* predation on endemic larval anurans in the experiment precluded the possibility of any inter-specific competitive effects or interactions. Similarly, any reverse competitive effects on the invasive anuran due to native anurans were not observed.

Our prediction that *H. tigerinus* benefits from preying on endemic anurans in terms of growth rate, time to metamorphosis and metamorph size (prediction 2), does not find statistical support; number of *H. tigerinus* tadpoles surviving does not vary between treatments. The increased survival proportion of *H. tigerinus* larvae in the presence of the two endemic larval anurans is probably a result of strong density-dependent survival rather than a treatment effect. This is reflected in the similar numbers of *H. tigerinus* surviving across treatments. Such density-dependence of tadpole survival has been observed in invasive populations of the American bullfrog *Lithobates catesbeianus* (Govindarajulu et al. 2005) and has important implications for management of invasive *H. tigerinus* and similar anuran invaders (Vimercati et al. 2017). Govindarajulu and colleagues (2005) found removal of tadpoles for management to be detrimental to population control as it increased larval survival; instead, they recommended the targeted removal of post-metamorphic anurans (see also Vimercati et al. 2017).

However, inferences from mesocosm experiments have limitations, as the results may not be completely transferable to natural systems (Cabrera-Guzmán et al. 2013). In natural breeding sites of *H. tigerinus* in the Andaman archipelago, a range of additional effects can alter the magnitude of the impact via larval predation. Availability of other prey in the breeding sites (e.g. mosquito larvae; NPM *pers. obs.*) could reduce predation on only larval anurans; conversely, moderate predation can increase survival of native species driven by density-dependence. Further, the presence of cover or refuge (e.g. emergent vegetation) due to structural complexity of the natural breeding site could reduce predation rates (Saidapur et al. 2009; Babbitt and Tanner 1998). Breeding asymmetry, given the likely prolonged breeding by some native anurans as compared to the explosive breeder *H. tigerinus*, may further reduce population-level impacts. Finally, other aquatic predators (e.g. odonates) can add further complexity to the interactions between larval *H. tigerinus* and native larval anurans (Smith 2006). Despite these potential offsets to *H. tigerinus* impact, the severity of predation observed in our experiment demonstrates a plausible mechanism by which native anuran populations on the Andaman archipelago may be significantly impacted.

Elucidation of potential impacts of larval invasive *H. tigerinus* has implications for other invasive anurans with carnivorous larvae, as well as other invasive populations of this species. This study adds to the existing knowledge on the impact of its post-metamorphic stage—through consumption of small endemic vertebrates and potential competition with larger native anurans for food (Mohanty and Measey 2018). Our findings substantiate the need to manage invasive populations (or potential incursions) of *H. tigerinus* on Andaman archipelago and elsewhere.

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