What's for dinner? – Diet and trophic impact of an invasive anuran *Hoplobatrachus tigerinus* on the Andaman archipelago

Comentado [U1]: I suggest "Diet and **potential** trophic impact" since no direct measure of negative impact (e.g. demographic change, survival rate) has been performed. Predation accounts and niche overlap estimates only indicate a potential for impacts.

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ABSTRACT

Amphibian invasions have considerable detrimental impacts on recipient ecosystems. However, reliable risk analysis of invasive amphibians still requires research on more non-native amphibian species. An invasive population of the Indian bullfrog, *Hoplobatrachus tigerinus*, is currently spreading on the Andaman archipelago and may have significant trophic impacts on native anurans through competition and predation. We assessed the diet of the invasive *Hoplobatrachus tigerinus* (n = 358), and native *Limnonectes* spp. (n = 375) and *Fejervarya* spp. (n = 65) in three sites, across four habitat types and two seasons, on the Andaman archipelago. We found a significant dietary overlap of *H. tigerinus* with *Limnonectes* spp. Small vertebrates, including several endemic species, constituted the majority of *H. tigerinus* diet by volume, suggesting potential impact by predation. Diets of the three species were mostly governed by the positive relationship between predator-prey body sizes. Niche breadth analyses did not indicate a large change in diet between seasons. *Hoplobatrachus tigerinus* and *Fejervarya* spp. chose evasive prey, suggesting that these two species are mostly ambush predators; *Limnonectes* spp. elected sedentary prey; although a large portion of its diet consisted of evasive prey, such electivity indicates 'active search' as its major foraging strategy. All three species of anurans mostly consumed terrestrial prey. This intensive study on a genus of newly invasive amphibian contributes to the knowledge on impacts of amphibian invasions*,* and elucidates the feeding ecology of *H*. *tigerinus*, and species of the genera *Limnonectes* and *Fejervarya*. We stress the necessity to evaluate prey availability and volume in future studies for meaningful insights into diet of amphibians.

Key Words: diet overlap, ecological niche, resource use, predator-prey, food electivity; Dicroglossidae; invasive impact; Anura

INTRODUCTION

Accelerating rates of biological invasions (Seebens et al., 2017) and their consequent negative impacts (Simberloff et al., 2013) have led to increased efforts towards pre-invasion risk assessment and prioritization based on impacts (van Wilgen et al. *in review*; Blackburn et al., 2014). Amphibian invasions have considerable detrimental impacts on recipient ecosystems (Pitt et al., 2005; Kraus, 2015), the magnitude of impact being comparable to that of invasive freshwater fish and birds (Measey et al., 2016). Impact mechanisms of amphibian invaders remain relatively understudied (Crossland et al., 2008) and are varied. Impact via predation and competition (*sensu* Blackburn et al., 2014) in particular has been frequently examined, with documented impact on invertebrates (Greenlees et al. 2006; Choi and Beard 2012; Shine 2010), fishes (Lafferty and Page 1997), amphibians (Kats & Ferrer,

Comentado [U2]: This expression seems to contradict the previous sentence, "impact mechanisms (...) remain relatively understudied". Please rephrase sentences to provide a clearer statement.

2003; Wu et al., 2005; Measey et al., 2015; but see Greenlees et al., 2007) and birds (Boland, 2004), though other taxa may also be affected (Beard & Pitt, 2005).

However, reliable risk analysis of invasive amphibians still requires research on more non-native amphibian species (van Wilgen et al., *in review*), as the existing knowledge on impacts is mostly based on the cane toad *Rhinella marina* and the American bullfrog *Lithobates catesbianus* (Measey et al., 2016; van Wilgen et al., *in review*). Comparisons of impact across taxonomic groups for management prioritization (Blackburn et al., 2014; Kumschick et al., 2015) may also be impeded by the relatively understudied category of amphibian invasions as compared to other vertebrate invasions (Pyšek et al., 2008). This knowledge gap is further compounded by geographic biases in invasion research, with limited coverage in Asia and Africa (Pyšek et al., 2008); developing countries also have relatively less invasion research (Nunez & Pauchard 2010; Measey et al., 2016).

An invasive population of the Indian bullfrog, *Hoplobatrachus tigerinus* (Daudin, 1802), is currently spreading on the Andaman archipelago, Bay of Bengal, following its introduction in the early 2000s (Mohanty & Measey, *in review*). The bullfrog has its native range on the Indian sub-continent encompassing low to moderate elevations in Nepal, Bhutan, Myanmar, Bangladesh, India, Pakistan, and Afghanistan (Dutta, 1997). The bullfrog has previously been introduced to Madagascar (Glaw & Vences, 2007), and possibly to the Maldives (Dutta, 1997) and Laccadive Islands (Gardiner 1906). This large bodied frog (up to 160 mm) has high reproductive potential (up to 20,000 eggs per clutch, Khan & Malik 1987) and is uncommon or absent in forested and coastal regions, but occurs as a human commensal (Daniels 2005). It is considered a dietary generalist, feeding on invertebrates and even large vertebrates such as *Duttaphrynus melanostictus* (Padhye et al., 2008; Datta & Khaledin, 2017); however,

Comentado [U3]: *catesbeianus*.

Comentado [U4]: I think “large anurans” would sound better here, because “large vertebrates” might be associated to larger animals such as mammals and crocodilians.

quantitative diet assessment with adequate sample size across habitats and seasons is lacking (but see Khatiwada et al., 2016 for diet of *H. tigerinus* in rice fields of Nepal).

Hoplobatrachus tigerinus on the Andaman archipelago co-occurs with native anurans of the genera *Duttaphrynus*, *Fejervarya*, *Limnonectes*, and *Microhyla* (NPM unpublished data; Harikrishnan et al., 2010). Given the large size of *H. tigerinus*, it is likely to feed on proportionately large prey, including amphibians and other vertebrates (Datta & Khaledin, 2017; Measey et al., 2015). The high volume of prey consumed by *H. tigerinus* (Padhye et al., 2008) may lead to direct competition with native anurans, especially under relatively high densities of *H. tigerinus* in human modified areas (Daniels, 2005). Although the diet of native anurans has not been assessed on the Andaman Islands, *Fejervarya limnocharis* is considered to be a generalist forager on terrestrial invertebrates (Hirai & Matsui, 2001), *Limnonectes* spp. are known to feed on vertebrates in addition to arthropods (Emerson, Greene & Charnov 1994; Das 1996), and Microhyids and Bufonids are considered to be myrmephagous. In terms of size, *H. tigerinus* is much larger than native anurans of the Andaman archipelago (Fig. 1) and may impact the native anurans through both predation and competition.

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Comentado [U6]: Microhylids.

Niche overlap, in combination with prey availability (electivity), can be used to assess trophic competition between species (e.g. Vogt et al., 2017). In addition to taxonomic evaluation and enumeration of the prey consumed, it is crucial to consider prey volume and frequency of prey occurrence to ascertain overall importance of a particular category of prey (Hirschfield & Rödel, 2011; Boelter et al., 2012; Choi and Beard 2012); classification by functional type (hardness and motility of prey) is useful in understanding predator behaviour (Toft 1980; Vanhooydonck et al., 2007; Carne & Measey 2013). Further,

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Comentado [U8]: Cited as 2015 in the list of references.

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seasonality in prey availability may influence diet in amphibians (Hodgkison & Hero 2003; de Oliveira & Haddad, 2015), therefore, there is also a need to assess diet across seasons, to fully capture the range of prey. Another important driver of prey choice may be the positive relationship between predator-prey body sizes (Werner et al., 1995; Wu et al., 2005).

We aimed to assess the trophic impact of the invasive *Hoplobatrachus tigerinus* on the native anurans of the Andaman Islands through potential competition and predation. We carried out diet analyses of the invasive *H. tigerinus* and native anurans, across four habitat types and two seasons, to ascertain the nature and magnitude of trophic impact. We hypothesized that i) small vertebrates constitute a majority of the *H. tigerinus* diet, particularly, by volume and ii) the diet of *H. tigerinus* significantly overlaps with the diet of native anurans, thereby, leading to potential competition. Additionally, we aimed to characterize the predation behaviour of these anurans in terms of electivity and predation strategy (ambush or active search).

Comentado [U10]: Why? Since the diets of native anurans have not been assessed (until this study) and *H. tigerinus* is much larger than the native anurans, why should you expect a significant overlap? I think a stronger background is necessary to support this hypothesis.

METHODS

We carried out the study in the Andaman archipelago for six months, from February to July 2017. The Andaman archipelago comprises nearly 300 islands situated between 10°30'N to 13°40'N and 92°10'E to 93°10'E (Fig. 2), which are~~is~~ part of the Indo-Burma biodiversity hotspot (Myers et al. 2000) ~~and~~ with a 40% endemism rate in herpetofauna (Harikrishnan et al., 2010). ~~This group of nearly 300 islands is situated between 10°30'N to 13°40'N and 92°10'E to 93°10'E (Fig. 2).~~ The tropical archipelago receives an annual rainfall of 3000 mm to 3500 mm (Andrews and Sankaran 2002); primary and secondary forests encompass nearly 87% of the entire archipelago (Forest Statistics 2013), whereas the remaining human modified areas comprise of settlements, agricultural fields, and plantations. Of the nine

Comentado [U11]: I made this suggestion, however the map on Fig. 2 should display a grid so that these coordinates could be removed from the text.

species of native amphibians recorded, four species (*Ingerana charelsdarwinii*, *Blythophryne beryet*, *Microhyla chakrapani*, and *Fejervarya andamanensis*) are endemic to the Andaman Islands (Das 1999; Harikrishnan et al., 2010; Chandramouli et al., 2016), however, taxonomic uncertainties still persist (Chandramouli et al, 2015; Harikrishnan Surendran, *Pers. Comm.*). The range restricted ~~*Ingerana*~~ *I. charlesdarwinii*, the semi-arboreal ~~*Blythophryne*~~ *B. beryet*, the arboreal *Kaloula baleata ghosi* and the littoral ~~*Fejervarya*~~ *F. cancrivora* are unlikely to co-occur with *H. tigerinus* at present (Das 1999; Chandramouli 2016; Chandramouli et al., 2016). Thus, we constrained our choice for comparative species to those which were strictly syntopic. As the taxonomy of the Andaman amphibians remains in flux, we limited our identifications to the genus level as the taxonomic identities of these species are pending formal re-assessments (Chandramouli et al., 2015). Hereafter, *Fejervarya* spp. and *Limnonectes* spp. are referred to as *Fejervarya* and *Limnonectes*, respectively.

We conducted the study in two sites (Webi and Karmatang) on Middle Andaman Island and one site (Wandoor) on South Andaman Island (Fig. 2). We chose sites with moderately old invasions of ~~*Hoplobatrachus*~~ *H. tigerinus* (more than 3 years since establishment; Mohanty & Measey *in review*), assuming that a relatively longer time since establishment would indicate an adequate population to sample from. In each site, we established four 1 ha plots with varying land use-land cover types: agriculture, plantations (Areca nut and Banana), disturbed (logged) and undisturbed forest (minimal use). To capture the variation in diet with respect to seasons, we carried out the sampling in both dry (January to April) and wet (May to July) seasons, the latter coinciding with the south-westerly monsoon.

Our protocol was approved by the Research Ethics Committee: Animal Care and Use, Stellenbosch University (#1260) and permission to capture anurans, was granted under the

Comentado [U12]: Although there is taxonomic uncertainty, I think it is interesting to inform the current available names for these populations. You cited a species of *Fejervarya* but suggested that this species is unlikely to co-occur with the invasive frog, so which is the *Fejervarya* species you examined? The same question for *Limnonectes* spp., which I suppose represents more than one species. We can refer only to the genus names, but should firstly present the current names by which the frogs have been associated in the literature.

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permit of the Department of Environment and Forests, Andaman and Nicobar Islands (#CWLW/WL/134/350). Diet of anurans was determined using stomach flushing, a standard and low-risk technique to determine prey consumed (Patto, 1998; Solé et al., 2005). Anurans were hand-captured between 1800 to 2200 hrs; stomach flushing was carried out within 3 h of capture. We consciously avoided capture bias towards any particular size class, by actively searching for anurans of all size classes. As our sampling focussed on sub-adult and adult ~~*Hoplobatrachus*~~ H. *tigerinus* and was completed in July (presumably before breeding and emergence of metamorphs) we did not examine the diet of metamorphs. In order to avoid mortality, we did not stomach flush individuals below 20 mm SVL and hence, individuals of co-occurring *Microhyla chakrapanii* (ca. 10-30 mm SVL; Pillai, 1977) were not sampled. After excluding native anurans which did not co-occur with *H. tigerinus*, we sampled *Duttaphrynus melanostictus* (although its taxonomic and geographic status is uncertain, Das 1999), *Limnonectes* spp., and *Fejervarya* spp. (hereafter, *Limnonectes* and *Fejervarya*). We conducted stomach flushing using a syringe (3 ml to 10 ml for anurans of 20 mm-50 mm SVL and 60 ml for anurans >60 mm SVL), soft infusion tube, and water from site of capture. In addition to SVL, we noted the sex and measured head width (HW) and lower jaw length length (LJL) of the anurans. The stomach flushed individuals were toe-clipped (following Hero, 1989; Grafe et al., 2011) to ensure that sampling bias, if any, was recorded. Individuals were released back to the capture site post completion of the procedure.

We collected the expelled prey items in a transparent beaker and sieved the contents using a mesh of 0.5 mm. Prey items from each individual were classified up to a minimum of order level, and further characterized by functional traits (hardness and motility, following Vanhooydock et al., 2007). Length and width of intact prey were measured under an 8x

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Comentado [U14]: Using a calliper?

Comentado [U15]: How did you cope with recaptures? Did you considered recaptured frogs as independent samples to calculate prey frequencies?

magnifying lens to the nearest 0.01 mm using a Vernier calliper and recorded along with the prey's life stage (adult/larvae). We preserved all prey items in 70% ethanol.

We also determined electivity of prey, based on prey consumption as compared to prey availability. Terrestrial prey were measured using five pitfall traps in each 1 ha plot, which were visited twice daily for a duration of three days (total of 30 trap occasions). Within each 1 ha plot, the pitfalls were arranged in the four corners and one in the centre of the plot. We used plastic traps, 80 mm in diameter and 300 mm high. A wet cloth was kept at the bottom to provide refuge to trapped animals, so as to prevent any predation before sample collection. We used chloroform soaked cotton balls to euthanize the invertebrate prey, prior to collection. These prey items were also identified up to the order level and measured for length and width. Our approach of estimating prey availability excludes flying evasive orders (e.g. adult lepidopterans) and vertebrate prey.

Data analyses

Comentado [U16]: I suggest the inclusion of a correlative analysis to assess how prey size (volume) is related to frog size and to frog species. See my comment below at line 248.

We did not obtain adequate numbers of ~~*Duttaphrynus*~~ *D. melanostictus* (n = 4) individuals and hence they were not included in the analyses. We pooled samples from the three sites to examine the diet of the three species of anurans, as our aim was to make inferences at the species level. We assessed the number, volume, and frequency (number of individuals with a given prey item in their stomach) of consumed prey under each taxonomic category~~, for the three anuran species~~. Volume was calculated using the formula of an ellipsoid, following Colli and Zamboni (1999),

Comentado [U17]: It is implicit.

$$\text{volume} = \frac{4}{3}\pi\left(\frac{l}{2}\right)\left(\frac{w}{2}\right)^2 ,$$

where, l is prey length and w is prey width. Prey items for which volume could not be calculated due to lack of measurement data (i.e. fragmented prey) were assigned the median prey volume for that order.

Comentado [U18]: I think this method should be referenced. Is there any reference to this procedure?

In order to assess the overall importance of a prey category, based on the percentage of number, frequency and volume, we used the Index of Relative Importance (IRI, Pinkas et al., 1971). We characterized the niche breadth of each anuran species with the Shannon-Weaver's measure of evenness (*J'*), which is a modified from the Shannon-Weaver index (*H',* Shannon and Weaver 1964). For the niche breadth analyses, we only included habitat types where the ~~*Hoplobatrachus*~~ *H. tigerinus* and the native anurans co-occurred (plantation and agriculture); we did not find *H. tigerinus* in undisturbed and disturbed forest plots, although there have been observations of a few individuals along forest streams (Harikrishnan & Vasudevan, 2013). As sampling was carried out over two seasons only in 2017 and we lacked temporal replicates, we did not statistically test for seasonal differences in niche breadth (Fig. 3).

To test for diet overlap between the three species, we employed the MacArthur and Levins' index *Ojk* (MacArthur and Levins 1967) in the pgirmess package (Giraudoux 2017); we built null models using the 'niche_null_model' function of the EcoSimR package (Gotelli et al. 2015) to test for statistical significance of *Ojk*. We also assessed prey availability for each site across both dry and wet seasons, using the Simpson's diversity index (Supplemental Information 1). We determined electivity of terrestrial invertebrate prey by the three species of anurans, using the Relativized Electivity Index (Vanderploeg & Scavia 1979). Following Measey (1998), we computed electivity for only those prey taxa with n ≥ 10 prey items for ~~*Hoplobatrahus*~~ *H. tigerinus* and *Limnonectes*; given the low sample size for

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Comentado [U20]: Please add "in three sites in the Andaman Archipelago" in the caption of the supplemental table after the word "seasons".

Fejervarya (Table 1), we fixed the cut-off at n ≥ 5. Further, electivity for *H. tigerinus* was calculated only for agriculture and plantations; electivity for *Fejervarya* was considered only for one site with adequate sample size: Wandoor (Table 1). All analyses were carried out in the statistical software R 3.4.1 (R Core Team 2017).

RESULTS

Overall, we sampled 798 individuals of the two native anurans and the invasive *Hoplobatrachus tigerinus* (Table 1). We obtained 1478 prey items belonging to 35 taxonomic categories in the stomach of 688 anurans (Table 2, Supplemental Information 2). Vacuity index (i.e. proportion of empty stomachs) was higher in the dry season (19.68%) as compared to the wet season (8.67%). Less than 4% of prey items remained unidentified, mostly due to advanced levels of digestion. *Hoplobatrachus tigerinus* consumed prey items under the most numbers of taxonomic categories (29), followed by *Limnonectes* (25), and *Fejervarya* (14). Vertebrates were consumed by both *H. tigerinus* and *Limnonectes*, although the numeric and volumetric percentage of vertebrates consumed was higher in the case of *H. tigerinus* (2.62%, 58.03%) as compared to *Limnonectes* (0.48%, 5.16%; Table 2). Based on IRI, coleopterans and orthopterans constituted the major prey of *H. tigerinus* and *Limnonectes*, whereas, formicids and coleopterans formed the majority in the diet of *Fejervarya* (Table 2).

Niche breadth (*J'*) varied only slightly between dry and wet seasons in all three anurans (Fig. 3). It was highest for *Limnonectes*, followed by ~~*Hoplobatrachus*~~ *H. tigerinus*, and *Fejervarya* (Fig. 3). The diet of *H. tigerinus* overlapped significantly with that of *Limnonectes* (*Ojk* = 0.87, lower-tail *p* > 0.999, upper-tail *p* < 0.001) but there was no significant overlap with

Comentado [U21]: How many for each frog species?

Comentado [U22]: Please add to the caption of Table 2 the names and sample sizes for each frog species. The totals of prey items should also be included after the name of each frog species in the head line of the table. Additionally, refer only to prey taxonomic names (I found “crab”, “rodent” and “slug” in the prey list).

Fejervarya (*Ojk* = 0.35, lower-tail p = 0.919, upper-tail p = 0.08). The diet of the two native anurans overlapped significantly (*Ojk* = 0.58, lower-tail p = 0.967, upper-tail p = 0.03).

Based on availability of terrestrial invertebrates, prey electivity of all three species appeared to be driven by the relationship between predator-prey body sizes (Fig. 4). While the largest species, ~~*Hoplobatrachus*~~ *H.* *tigerinus*, strongly selected for moderately large to large prey (≥ 100 mm^3), the smallest anuran, *Fejervarya*, selected for prey items smaller than <10 mm^3; the medium sized *Limnonectes* chose small and moderately large prey items (10 mm^3 – 500 mm^3), although the magnitude of electivity (positive or negative) was lowest for this species (Fig. 1; Fig. 4). Most of the prey consumed by the three species was terrestrial, hard, and evasive; diet of *Limnonectes* included a relatively high proportion of soft and sedentary prey.

Comentado [U23]: I suggest changing the colors of the bars in the graph; replace for more vivid colors, because the current faded tonalities might not appear in a printed version of the paper.

Comentado [U24]: This information should be followed by a table or a bar graph to summarize the data. You mention "most of" and "relatively high proportion" but the amounts of each category (e.g. terrestrial, aquatic, hard, soft) are not available for the reader.

We observed several endemic vertebrate species in the diet of *H. tigerinus*, including the Andaman emerald gecko *Phelsuma andamanensis*, Chakrapani's narrow mouthed frog *Microhyla chakrapani*, the Andaman skink *Eutropis andamanensis*, and Oates's blind snake *Typhlophs oatesii*. We also found *Limnonectes*, one unidentified rodent, and the invasive *Calotes versicolor* in the diet of *H. tigerinus*.

Comentado [U25]: I think a call for the supplemental material 2 would be good here, since all this information can be found therein.

DISCUSSION

We expected the diet of *H. tigerinus* to overlap significantly with the diet of both species of native anurans. However, we found a significant overlap only with *Limnonectes*, such that when prey is limited competition may arise. As expected, small vertebrates constituted a majority of *H. tigerinus* diet by volume, suggesting potential impact by predation on a large proportion of the endemic island fauna. Diets of the three species were mostly governed by

Comentado [U26]: Again, the reasons why you expected this strong overlap should be informed here.

a strong positive relationship between predator-prey body sizes. Niche breadth analyses did not indicate substantial changes in diet between seasons.

We observed 86% niche overlap between ~~*Hoplobatrachus*~~ *H. tigerinus* and *Limnonectes*, which was statistically significant in comparison to the constructed null model; whereas, niche overlap of *H. tigerinus* with *Fejervarya* was not significant. On the other hand, prey electivity (based on prey availability) suggests that *H. tigerinus* strongly elected for moderately large to large prey whereas small and moderately large prey were elected by *Limnonectes* (Fig. 4). This may result in competition for prey ranging from 10 – 500 mm^3 between the two species, under the conditions of limited prey. Although there was a clear positive relationship between predator-prey body sizes at the species level (Fig. 4), we did not observe increased dietary overlap (in terms of prey taxa) for relatively large *Limnonectes* and relatively small *H. tigerinus*. Trophic competition in amphibians may lead to a decrease in fitness (e.g. growth rate) and affect population level processes (Benard & Maher, 2011). Impact of invasive amphibians (post-metamorphic) via trophic competition has been documented in fewer studies as compared to predation (Measey et al., 2016), but this mechanism may affect taxa at various trophic levels (Smith et al., 2016). Metamorphs of *H. tigerinus* may also compete with both *Fejervarya and Limnonectes* as they would fall under the same size class (20 mm-60mm; Daniels, 2005). Although our sampling did not evaluate the diet of *H. tigerinus* metamorphs, we think this may be relevant as competition between juvenile *Lithobates catesbianus* and small native anurans has been previously documented in Daishan Island, China (Wu et al., 2005).

Evaluating dietary overlap is a pre-cursor to determining trophic competition due to invasive populations, which do not have shared evolutionary history with native species. Dietary

Comentado [U27]: Even though you provided a visual comparison of the sizes of the species in Fig.1 and their electivities according to prey volume categories in Fig. 4, I expected to see in the results a correlative analysis between frog size and prey volume to support this conclusion. An analysis of covariance (ANCOVA) with frog SVL as independent variable, frog species as the covariate and prey volume as response variable is a suggestion. A GLM with this same variable arrangement is an alternative approach.

Comentado [U28]: Did you estimate diet overlaps for frogs of different size classes, i.e., different intraspecific groups? I found mention to overlap at the species level only (i.e., including data from specimens in all size classes). If you did so, then show it in the results.

Comentado [U29]: *catebeianus.*

overlap in co-occurring species may be independently influenced by prey availability (Kuzmin, 1995), prey taxa (Lima, 1998), prey size (Toft, 1981; Vignoli et al., 2009; Crnobrnja-Isailović, 2012) and a combination of these factors. Therefore, it is essential to design studies and interpret diet patterns with reference to all three factors, in order to arrive at meaningful inferences on prey consumed, dietary overlap, and probable subsequent competition (Kuzmin, 1990; but see Kuzmin, 1995 regarding criteria for competition). Further, prey size should ideally be measured in terms of volume, as it is known to be a better dietary descriptor (Vignoli & Luiselli 2012).

Hoplobatrachus tigerinus preyed upon three classes of vertebrates (Amphibia, Reptilia, and Mammalia), which accounted for a significant proportion of its diet by volume, although vertebrate prey was numerically inferior to invertebrates in the diet. Such major contribution to the volume of prey by vertebrates (despite numerical inferiority) has been observed for *Lithobates catesbianus* and *Xenopus laevis* (Boelter et al., 2012; Vogt et al., 2017); anurophagy may also contribute significantly to the diet of many amphibians (Measey et al., 2015; Courant et al., 2017). We observed several endemic species in the diet of *H. tigerinus*, which may be vulnerable if frequently preyed upon. *Limnonectes* was also consumed by *H. tigerinus*, thereby, indicating a potential two-pronged impact through predation and competition. However, demographic change (if any) in *Limnonectes*, due to predation and competition by *H. tigerinus*, was not evaluated in this study. The invasive *H. tigerinus* on the Andaman Islands reportedly consume poultry (Manish Chandi pers comm., Mohanty & Measey, *in review*) and stream fish (NPM unpublished data). Despite the presence of a large portion of vertebrates in the diet of *H. tigerinus*, its trophic position (consistency of vertebrate prey consumption) can only be ascertained with stable isotope

Comentado [U30]: *catesbeianus.*

Comentado [U31]: This justifies the inclusion of the word "potential" in the title of the study.

analyses (Huckembeck et al., 2014). Although, diet analysis of invasive species can identify vulnerable taxa and confirm at least ‘minimal’ to ‘minor’ levels of impact through predation and competition (*sensu* Blackburn et al., 2014; Hawkins et al. 2015), such analysis must be complimented with evidence of trophic level effects to evaluate the degree of impact (Smith et al., 2016).

The large proportion of ants in the diet of *Fejervarya* does not necessarily prove specialization for myrmecophagy. Hirai and Matsui (2000) inferred relatively weaker avoidance of ants by *Glandirana rugosa* as compared to other anurans. Although we found the same pattern for *Fejervarya* based on prey electivity (*E*= -0.02), it does not prove weak avoidance either. As social insects, ants may be disproportionately captured in the pitfall traps, it is necessary to compliment diet studies on potentially myrmephagous predators with additional evidence (e. g. cafeteria experiments). *Hoplobatrachus tigerinus* and *Fejervarya* chose evasive prey, suggesting that these two species are mostly ambush (‘sit and wait’) predators; *Limnonectes* elected sedentary prey along with other prey types, indicating a combination of ‘active search’ and ‘sit and wait’ foraging (Huey & Pianka, 1981; Vanhooydonck et al., 2007). Generally, soft bodied prey are considered to provide more nutrition by size as compared to hard prey and therefore, it is hypothesized that species will select soft prey more often than hard prey, which in turn is dependent on prey availability by season (Measey et al., 2011; Carne & Measey 2013). However, we find that diet does not appear to vary considerably across the seasons (Fig. 3) and is governed more by size than hardness of prey (Fig. 4; Werner et al., 1995).

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Comentado [U33]: 2015 in the list of references.

Although our sampling for diet analysis by stomach flushing was adequate (Table 1), our assessment of prey availability did not include flying invertebrates and vertebrates, which prevented~~s~~ us from carrying out electivity analyses on these taxa.

CONCLUSION

Diet analyses of *Hoplobatrachus tigerinus* revealed significant predation on endemic vertebrates and a high diet overlap with large-bodied native anurans, indicating direct predation. Given the observed high density of *H. tigerinus* in human modified habitats on the Andaman archipelago (NPM unpublished data), trophic competition and predation by *H. tigerinus* may have a significant impact on native anuran populations in these habitats. In addition to quantifying the trophic niche of anurans belonging to three genera, we stress the necessity to evaluate prey availability and volume in future studies for meaningful insights into diet of amphibians.

Comentado [U34]: No conclusive sentence was made about predation behaviour, even though describing it is part of your goals in this study.

Comentado [U35]: Connect this to the first hypothesis; did endemic vertebrates represent most of prey volume? Yes, they did!

Comentado [U36]: This was not clear for me; is there "indirect predation"? I wonder if you intended to say "indirect competition" or a similar expression here. Please, rephrase this sentence in order to provide a clearer conclusion.

Comentado [U37]: This also justifies the inclusion of the word "potential" in the title of the study.

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REFERENCES

Andrews HV, Sankaran V. 2002. Sustainable management of protected areas in the Andaman and Nicobar Islands. Port Blair: Andaman and Nicobar Environment Team.

Beard KH, Pitt WC. 2005. Potential consequences of the coqui frog invasion in Hawaii. *Diversity and Distributions*, 11: 427-433.

Blackburn TM, Essl F, Evans T, et al. 2014. A unified classification of alien species based on the magnitude of their environmental impacts. *PLoSBiol*, 12(5), e1001850.

Boelter RA, Kaefer IL, Both C, Cechin S. 2012. Invasive bullfrogs as predators in a Neotropical assemblage: What frog species do they eat? *Animal Biology*, 62(4): 397-408.

Boland CRJ. 2004. Introduced cane toads *Bufo marinus* are active nest predators and competitors of rainbow bee-eaters *Meropsornatus*: observational and experimental evidence. *Biological conservation*, 120(1): 53-62.

Carne L and Measey J. 2015. Chameleons on the cruise: seasonal differences in prey choice of two dwarf chameleons. *Herpetological Journal*. 23:221-227.

Comentado [U38]: Appeared as 2013 in the citations within the text.

Chandramouli SR, Khan T, Yathiraj R, Deshpande N, Yadav S, Tejpal C, de Groot S, Lammes I. 2015. Diversity of amphibians in Wandoor, South Andaman, Andaman and Nicobar Islands, India. *Alytes*, *32*.

Chandramouli SR. 2016. Rediscovery and redescription of a little known, insular endemic frog, *Ingerana charlesdarwini* (Das, 1998)(Amphibia: Anura: Dicroglossidae) from the Andaman Islands, Bay of Bengal. *Alytes*, *33*.

Chandramouli SR, Vasudevan K, Harikrishnan S, Dutta SK, Janani SJ, Sharma R, Das I., Aggarwal RK. 2016. A new genus and species of arboreal toad with phytotelmonous larvae, from the Andaman Islands, India (Lissamphibia, Anura, Bufonidae). *ZooKeys*, (555), p.57.

Choi RT, Beard KH. 2012. Coqui frog invasions change invertebrate communities in Hawaii. *Biological Invasions*, 14(5): 939-948.

Colli GR, Zamboni DS. 1999. Ecology of the worm-lizard *Amphisbaena alba* in the Cerrado of Central Brazil. *Copeia* 1999:733-742

Courant J, Vogt S, Marques R et al. 2017. Are invasive populations characterized by a broader diet than native populations? *PeerJ*. DOI 10.7717/peerj.3250.

Crossland MR, Brown GP, Anstis M, Shilton CM, and Shine R. 2008. Mass mortality of native anuran tadpoles in tropical Australia due to the invasive cane toad (*Bufo marinus*). *Biological Conservation*, 141(9): 2387-2394.

Daniels RR. 2005. Amphibians of peninsular India. – Universities Press, India.

Das I. 1999. Biogeography of the amphibians and reptiles of the Andaman and Nicobar Islands. Tropical Island Herpetofauna-Origin, *Current Diversity and Conservation*, 43-77.

de Oliveira EG, Haddad CF. 2015. Diet Seasonality and Feeding Preferences of *Brachycephalus pitanga* (Anura: Brachycephalidae). *Journal of Herpetology*, *49*(2): 252-256.

Datta AK, Khaledin S. 2017.Observations on an Indian bullfrog swallowing an Asian common toad, and a Checkered keelback on a skipper frog. *Zoo's Print*¸32:28-29

Dutta SK. 1997. Amphibians of India and Sri Lanka: checklist and bibliography. Odyssey Publishing House.

Forest Statistics 2013, accessed from http://forest.and.nic.in/ on 15th Aug 2017.

Comentado [U39]: There is a citation indicating 1996 in line 80.

Gardiner JS. 1906. Notes on the distribution of the land and marine animals, with a list of the land plants and some remarks on the coral reefs. – In Gardiner, J.S. (ed.), The fauna and geography of the Maldive and Laccadive archipelagoes. Cambridge University Press, Cambridge. pp. 1046–1057.

Giraudoux P. 2016. pgirmess: data analysis in ecology. R package version 1.6.4. Available at http://CRAN.R-project.org/ package=pgirmess.

Comentado [U40]: Cited as 2017 at line 196.

Glaw F, Vences M. 2007. A field guide to the amphibians and reptiles of Madagascar. ISBN-13: 9783929449037.

Gotelli NJ, Hart EM, Ellison AM. 2015. EcoSimR: Null model analysis for ecological data. R package version 0.1.0. Available at http:// github.com/ gotellilab/EcoSimR.

Grafe TU, Stewart MM, Lampert KP, Rödel MO. 2011. Putting toe clipping into perspective: a viable method for marking anurans. *Journal of Herpetology*, *45*(1): 28-35.

Greenlees MJ, Brown GP, Webb JK, Phillips BL, Shine R. 2006. Effects of an invasive anuran [the cane toad (*Bufomarinus*)] on the invertebrate fauna of a tropical Australian floodplain. *Animal Conservation*, 9(4): 431-438.

Greenlees, M. J., Brown, G. P., Webb, J. K., Phillips, B. L., and Shine, R. (2007). Do invasive cane toads (*Chaunus marinus*) compete with Australian frogs (*Cyclorana australis*)?. Austral *Ecology*, 32(8): 900-907.

Harikrishnan S, Vasudevan K. 2013. Recent introduction and spread of Indian bullfrog *Hoplobatrachus tigerinus* (Daudin, 1802) into the Andaman Islands. *Aliens*, 33:42–43.

Harikrishnan S, Vasudevan K, Choudhury BC. 2010. A review of herpetofaunal descriptions and studies from Andaman and Nicobar islands, with an updated checklist. In: Ramakrishna, Raghunathan C, Sivaperuman C (eds) Recent trends in biodiversity of Andaman and Nicobar Islands. Zoological Survey of India, Kolkata, pp 387–398.

Hawkins, CL, Bacher, S, Essl F et al. 2015. Framework and guidelines for implementing the proposed IUCN Environmental Impact Classification for Alien Taxa (EICAT). *Diversity and Distributions*. 21:1360-1363.

Hero JM. 1989. A simple code for toe clipping anurans. *Herpetological Review*, 20: 66-67.

Herrel A, Joachim R, Vanhooydonck B, Irschick DJ. 2006. Ecological consequences of ontogenetic changes in head shape and bite performance in the Jamaican lizard *Anolis lineatopus. Biological Journal of the Linnean Society,* 89: 443–454.

Hirschfeld M, Rödel MO. 2011. The diet of the African Tiger Frog, *Hoplobatrachus occipitalis*, in northern Benin. *Salamandra*, 47(3): 125-132.

Hodgkison S, Hero JM. 2003. Seasonal, sexual and ontogenetic variations in the diet of the 'declining' frogs Litoria nannotis, Litoria rheocola and Nyctimystes dayi. *Wildlife Research*, *30*(4): 345-354.

Huey RB and Pianka ER. 1981. Ecological Consequences of Foraging Mode. *Ecology*, 62:991-999.

Kats LB, Ferrer RP. 2003. Alien predators and amphibian declines: review of two decades of science and the transition to conservation. *Diversity and Distributions*, 9: 99-110.

Comentado [U41]: I did not find citation for this study within the text.

Comentado [U42]: Hirschfield?

Khan MS, Malik SA (1987). Reproductive strategies in a sub-tropical anuran population in arid Punjab, Pakistan. *Biologia,* 33: *279-298*.

Khatiwada JR, Ghimire S, Khatiwada SP, et al. 2016. Frogs as potential biological control agents in the rice fields of Chitwan, Nepal. Agriculture, Ecosystems and Environment

Kraus F. 2015. Impacts from Invasive Reptiles and Amphibians. *Annual Review of Ecology, Evolution, and Systematics*, *46*, 75-97.

Lafferty KD, Page CJ 1997. Predation on the endangered tidewater goby, *Eucyclogobius newberryi*, by the introduced African clawed frog, *Xenopus laevis*, with notes on the frog's parasites. *Copeia*, 1997(3), 589-592.

MacArthur R, Levins R (1967). The limiting similarity, convergence, and divergence of coexisting species. *American naturalist*, 377-385.

Measey, GJ. 1998. Diet of feral *Xenopus laevis* (Daudin) in South Wales, UK. *Journal of Zoology*, 246(03), 287-298.

Measey GJ, Rebelo AD, Herrel A, Vanhooydonck B, Tolley KA. 2011. Diet, morphology and performance in two chameleon morphs: do harder bites equate with harder prey? *Journal of Zoology*, 285: 247-255.

Measey GJ, Vimercati G, De Villiers FA, et al. 2015. Frog eat frog: exploring variables influencing anurophagy. *PeerJ*, *3*, e1204.

Measey GJ, et al. 2016. A global assessment of alien amphibian impacts in a formal framework. *Diversity and Distributions*, 22: 970-981.

Mohanty NP, Measey J. *in review*. Reconstructing biological invasions using public surveys: a new approach to retrospectively assess spatio-temporal changes in invasive spread. *Biological Invasions*.

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J. 2000. Biodiversity hotspots for conservation priorities. *Nature*, 403(6772): p.853.

Nunez MA, Pauchard A. 2010. Biological invasions in developing and developed countries: does one model fit all? *Biological invasions*, 12:707-714.

Padhye A, Manamendra-Arachchi K, de Silva A, et al. 2008. *Hoplobatrachus tigerinus*. The IUCN Red List of Threatened Species.

Pinkas L. 1971. Food habits study. *Fish bulletin*, 152(5): 10.

Pitt W, Vice D, Pitzler M. 2005. Challenges of invasive reptiles and amphibians. Proceedings of Wildlife Damage Management Conference.

Pyšek, P, Richardson DM, Pergl J. et al. 2008. Geographical and taxonomic biases in invasion ecology. *Trends in Ecology & Evolution*, 23: 237-244.

R Core Team. 2017. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. Available at https://www.R-project.org/.

Seebens H, Blackburn TM, Dyer EE et al. 2017. No saturation in the accumulation of alien species worldwide. *Nature communications*, 8: p.14435.

Shannon CE, Weaver W. 1964. The mathematical theory of communication. Urbana: University of Illinois Press.

Comentado [U43]: Correct reference: “Pinkas, L., M. S. Oliphant and Z. L. Iverson 1971. Food habits of albacore bluefin, tuna and bonito in California waters. *California Department of Fish and Game Bulletin*, 152:1-350.”

Shine R. (2010). The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology*, 85(3), 253-291.

Simberloff D. et al. 2013. Impacts of biological invasions: what's what and the way forward. Trends in Ecology & Evolution 28: 58–66.

Solé M, Beckmann O, Pelz B, Kwet A, Engels W. 2005. Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. Studies on Neotropical Fauna and Environment 40:23-28.

van Wilgen N, Gilespie M, Richardson D, Measey J *in review Risk analysis for reptiles and amphibians needs directed research on more non-native species: a systematic review*. *Journal of Applied Ecology*.

Vanderploeg HA, Scavia D. 1979. Calculation and use of selectivity coefficients of feeding: zooplankton grazing. *Ecological Modelling* 7:135-149.

Vanhooydonck B, Herrel A, Van Damme R. et al. 2007. Interactions between habitat use, behavior, and the trophic niche of lacertid lizards. In: Lizard Ecology: The Evolutionary Consequences of Foraging Mode, ed. S. M. Reilly, L. D. McBrayer and D. B. Miles. Cambridge University Press.

Vogt S, De Villiers FA, Ihlow F, Rödder D, Measey GJ. 2017. Competition and feeding ecology in two sympatric *Xenopus* species (Anura: Pipidae). *PeerJ*.

Werner EE, Wellborn GA, McPeek MA. 1995. Diet composition in postmetamorphic bullfrogs and green frogs: implications for interspecific predation and competition. *Journal of Herpetology*, 600-607.

Formatado: Fonte: Itálico

Wu Z, Li Y, Wang Y, and Adams MJ. 2005. Diet of introduced bullfrogs (*Rana catesbeiana*): predation on and diet overlap with native frogs on Daishan Island, China. *Journal of Herpetology*, 39(4), 668-674.