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# Quantifying the importance of ontogeny and prey type in modeling top-down and bottom-up effects of an ectothermic predator

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Dietary decisions by predators can affect prey abundance and overall food web dynamics. Many predators do not forage on the same prey at the same frequency throughout their lives. Ontogenetic shifts in prey preference are not, however, often accounted for when modeling food web relationships, despite growing literature that suggests that stage specific dietary relationships may be an important consideration when modeling trophic interactions. We investigated the importance of considering size-structure of a predator population with ontogenetic diet shifts in evaluating relationships with prey response using a manipulative experiment with the brown treesnake (*Boiga irregularis*) in Guam. After removing ~ 40% of the snake population via toxic mammal carrion, we measured the strength of the relationship between snake density and the response of two types of prey (lizards and mammals). We evaluated these relationships based on total population size or division of the population into stage specific size categories based on diet preference predictions. We hypothesized that the density of juvenile snakes would correlate more strongly with lizard detections, while adult snakes would better correlate to rodent detections. We also measured reproductive output following changes in rates of prey detection. As expected by known ontogenetic shifts in dietary preference, explicit stage-based models better predicted shifts in rates of observed prey items than did total predator density for both lizards and mammals. Additionally, rodent detections were predictive of one reproductive pulse from snakes, while lizard detections were not predictive or correlated. Our findings support that consideration of predatory species stage-based dietary preference can be meaningful for understanding food web dynamics, particularly when a predator has a broad diet or one that changes through time.

**Keywords** Food web, Ontogenetic diet shifts, Size-structured population, Trophic linkages

Prey abundance is a major driver of predator vital rates, such that predators are predicted to have increased per capita birth rates as a function of prey availability<sup>1</sup>, while prey are anticipated to have reduced fecundity or survival in environments with greater predator abundances<sup>2</sup>. In traditional Lotka-Volterra dynamics, predators are predicted to overshoot their prey's carrying capacity, causing prey declines and subsequently predator declines after a lag period<sup>3</sup>. However, the overall stability of food webs is generally governed by the strength of the relationships between community members, with weaker links contributing to greater stability<sup>4</sup>. Stage-structured populations, in which juveniles and adults use the same resources, but allocate their time differently, can thus have stabilizing effects on food webs by decreasing linkage strength<sup>5</sup>.

Based on the effects that population stage structure can exert on food webs, the importance of ontogenetic diet shifts (ODS)—transition of diet over the lifetime of an individual—as a theoretical consideration in food web stability is of growing interest and can be a key component of how trophic communities are structured<sup>6–9</sup>. Thus, the relative importance of top down control on food webs may be dependent on the stage structure of the predator population<sup>10</sup>. Moreover, failure to account for shifts in prey resource use based on ontogeny can affect

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the accuracy of consumption rates and downstream trophic effects<sup>11</sup>. In a system in which the apex predator undergoes ODS, the stage structure of the population, and thus the relative consumption rates, may determine the relative importance and strength of top-down or bottom-up control of trophic linkages<sup>12</sup>. Failure to account for the stage structure of the population when measuring or modeling food web dynamics may therefore dampen or mask the detection of direct trophic linkages.

Despite growing attention towards the effects of ODS in regulating trophic interactions, the majority of field studies investigating them have been conducted on aquatic organisms<sup>13,14</sup>. In reptiles, ontogenetic shifts often co-occur with habitat, physiological, metabolic, or morphometric changes that occur with age or size<sup>15–18</sup>. Therefore, understanding how predator ontogeny affects trophic dynamics may be particularly important for assessing the community linkages or their strength when evaluating reptiles. Reptiles are recognized to be important ecological effectors<sup>19–21</sup>. Reptiles can perturb relationships sufficiently to cause disruptive events in food webs, including causing ecological cascades<sup>20,22</sup>. However, additional studies that evaluate the effect of ontogenetic relationships may refine understanding of trophic linkages involving reptiles. Better understanding of the strength of trophic interactions based on the stage structure of populations will likely improve overall understanding as to the influence of terrestrial ectotherms in their communities.

In the island of Guam (540 km<sup>2</sup>; also known as Guåhan in CHamoru), introduced brown treesnakes (*Boiga irregularis*) are documented in their ability to influence native and non-native prey populations<sup>17,23–25</sup>. There effects on vertebrate loss are linked to trophic cascades in subsequent decades<sup>20</sup>. Although the potential for this species and other invasive reptiles to modify or destabilize prey communities has been documented, the stage dependencies of relationships that may result from ODS have not been examined<sup>21,26,27</sup>. In Guam, brown treesnakes have a diet composition that is primarily composed of ectothermic lizards until ~1250 mm snout-vent length (SVL), although they begin to incorporate endothermic prey (small mammals and birds) at ~650–700 mm SVL<sup>17</sup>. They are also documented to be reliably attracted to rodent lures at around 900 mm SVL<sup>28,29</sup>. Based on diets in the wild and subsequent laboratory trials, the attractiveness of rodents appears to decline around 1550 mm SVL, with birds becoming an increasingly dominant or preferred food source for individuals when available<sup>17,30</sup>.

We explored the relationship between demographic stage structure of brown treesnakes (or snakes) on two prey types through a manipulative experiment involving snake removal in a large enclosure. After removing snakes that ate toxic rodent baits, we evaluated whether total density of snakes or ontogenetically segregated densities were better predictors of rat (endothermic) or lizard (ectothermic) prey detection rates, as estimated by sightings-per-unit-effort (SPUE) metrics for prey. Subsequently, we evaluated whether the abundance of rodents or lizards was driven by the abundance of particular snake stage classes to determine whether predator mediated effects were correlated to ontogenetic foraging shifts. We hypothesized that larger snakes would demonstrate tighter linkages to rats and smaller snakes to lizards, while rat detections would be the primary driver of reproductive events.

## Methods

### Study site

Guam is the largest and southernmost island in the Mariana Island archipelago, with temperatures between 25 and 32 °C. The island undergoes two rainfall seasons: dry and wet, with peak rainfall occurring from July–October and cumulative annual rainfall averaging 2133–2946 mm. Seasonal differences in rainfall can have substantive effects on plant and insect productivity<sup>31</sup>. We did not, however, monitor plants or insects during our experiment.

We completed this work in a 5-ha experimental snake enclosure located on Andersen Air Force Base, in northern Guam (13.639 N, 144.862 E). The enclosure was designed to prevent the ingress and egress of snakes. Therefore, the population could be accurately counted through mark-recapture, such that births represent the only source of new individuals and deaths the cause of disappearance<sup>29</sup>. Habitat within the enclosure was mixed native limestone forest and non-native *Leucaena leucocephala* forest, with a limestone karst substrate<sup>32</sup>. Within the enclosure, we maintained 27 forest-interior and 2 forest-edge transects that were 220 m long and spaced at ~8 m intervals (2-m width). In addition to snakes, the experimental enclosure housed several other vertebrate species, including two nocturnal geckos (*Lepidodactylus lugubris* [mourning gecko], *Hemidactylus frenatus* [common house gecko]), two diurnal skinks (*Emoia caeruleocauda* [Pacific blue-tail skink], *Carlia ailanpalai* [curious skink]), one diurnal mangrove monitor [*Varanus tsukamotoi*], and various non-native rodents<sup>24,33,34</sup>. The specific species of rodent present has not been identified. The enclosure was not closed to lizards or rats and prey immigration and emigration remained possible throughout the course of study.

Overall, rodents have occurred at substantially lower densities in Guam compared to other Mariana Islands, which is a difference thought to be caused by the snakes<sup>24</sup>. Additionally, there were no native forest birds observed at the study site during the period of this study. The only other potential predators of rats, lizards, and snakes were thus monitor lizards and cats. Although we were not directly monitoring either species, no change was detected in their abundance during nocturnal visual surveys over the period covered herein<sup>35</sup>.

### Snake population manipulation

We defined discrete snake stage classes based on the reliability with which they are attracted to ectothermic (<700 mm SVL) versus endothermic rodent (≥900 mm SVL) prey<sup>17,28,29,36</sup>. To quantify stage-based effects, we artificially manipulated the population by removing snakes that we expected to consume toxic rodent carrion laced with 80-mg of acetaminophen<sup>37</sup>. Specific baiting protocols and intervals are described more thoroughly in Nafus et al.<sup>38</sup>. Briefly, beginning on 31 March 2017, we distributed 594 toxic mouse baits in an 8 × 10 m array at 7 treatment periods. We applied baits in accordance with the Environmental Protection Agency (EPA) pesticide registration label for “Acetaminophen for Brown Treesnake Control” (80-mg uncoated tablets; Reg. No. 56228–34). Outside of exposure to toxicants we did not euthanize snakes and thus removal was anticipated to

affect snakes  $\geq 850$  mm SVL or those eating rodent carrion. Dead mice are not consumed by small lizards and mammals have much higher tolerances to acetaminophen, limiting the expected effect of baits on other species<sup>37</sup>. Monitors may consume the baits, which may be lethal for smaller individuals<sup>39</sup>, but as a large diurnal species these non-target effects should not have overly biased prey counts. Therefore, we did not expect that application of baits to have adverse effects on the other vertebrate species we monitored.

### Visual surveys for snakes, lizards, and rats

To quantify the relationship between snake abundance and prey response, we divided surveys into two discrete periods: pre-snake removal (24 October 2016–30 March 2017,  $N=67$  surveys, mean = 11 surveys/month) and post-snake removal (31 March–31 January 2018,  $N=128$  surveys, mean = 13 surveys/month). We conducted surveys continuously during the period referenced, with survey effort typically ranging from 2 to 4 per week. Each survey was completed by two teams of two trained observers and we surveyed all 29 transects on a given survey night. Each observer scanned their respective side of a given transect using powerful headlamps (Wilma 3200-lumens, Lupine Lighting Systems, Pennsylvania, USA) per methods described in Christy et al.<sup>40</sup>. During surveys, observers documented all lizards, small mammals (rats), and snakes. We counted but did not mark rats and lizards along each transect. When we detected a snake, we attempted to capture and mark the individual each time creating a known population.

When we captured unmarked snakes, we clipped ventral scales in a unique pattern using scissors and team members trained by veterinary professionals implanted a passive integrated transponder (PIT) tag into the abdominal cavity. For each captured snake, we scanned for the presence of identification marks (numerical caudal scale clips) and a PIT tag to census the population during each survey. We collected morphometric data, including SVL by gently stretching the snakes along a tape measure each night a snake was captured. We also completed a series of trapping events using a  $13 \times 13$  grid of traps (169 traps), with roughly  $16 \times 16$  m spacing (as per methods described in<sup>41</sup>) during 18 January 2017 (51) days and 04 April 2017 (9 days). Methods of estimating total abundance of individuals are described in detail in Nafus et al.<sup>38,42</sup>. Briefly, however, for new snake detections, we estimated birth date by projecting growth using size and sex specific growth rates created for this population during this period to identify the first month each individual was predicted to be  $< 400$  mm SVL, as well as size in a given month when the individual was not detected. We used the last month of detection as the estimate of when a snake was no longer present in the population, which may have underestimated the true duration of time individuals were present. At the close of the study, we thus had monthly estimates of absolute brown treesnake abundance by size and per transect SPUE (individuals sighted per km) for lizards and rats. For the purposes of this manuscript, we did not attempt to differentiate prey by species under the assumption that snakes eat all the species present in the enclosure.

### Ethics statement and data availability

We completed all work in accordance with Institutional Animal Care and Use Committee protocols U.S. Geological Survey Fort Collins Science Center #2017-03 and Colorado State University protocol #15-5892A, such that we performed all methods in accordance with the relevant guidelines and regulations. Methods are reported in accordance with ARRIVE guidelines. Experimental protocols were approved by U.S. Geological Survey. All data used are publicly available for download<sup>35,43</sup>.

### Statistical analysis

The primary purpose of the analyses described herein was to test the relationship between stage-based snake abundance and rodent or lizard SPUE. We, therefore, selected two discrete snake size classes based on evidence for preference of consuming ectotherms ( $< 700$  mm SVL) or reliable attraction to endothermic rodent prey ( $\geq 900$  mm SVL)<sup>17,29,36</sup>. Based on their highly transitory state for diet we discounted present individuals from 700 to 899 mm SVL by removing individuals within those sizes from all analyses. For each prey dataset, we calculated monthly SPUE averages by averaging the SPUE across all transects surveyed by all observers in any given month (for full description of methods, refer to Nafus<sup>43</sup>). We opted to combine data across all transects and within months to help minimize bias from spatial, temporal, or observer effects on SPUE<sup>33,44</sup>.

We used two statistical approaches to evaluate the data: negative binomial regression and time series regression. We completed the negative binomial regression using the ‘MASS’ package in R<sup>45</sup>. We completed time series regression using the ‘forecast’ package in R<sup>46</sup>. We used two approaches based on imperfect ability to account for violating specific assumptions by each modelling approach. For example, data were not normally distributed and demonstrated evidence of overdispersion, which violates linear regression assumptions for time series models<sup>46</sup>. However, given the sequential nature of our study, individual data points were temporally correlated and thus not independent, as would be ideal for a negative binomial regression. We tested model fit using a chi-square goodness of fit test with an acceptable fit determined by  $P > 0.05$ . The time series regression demonstrated generally poor predictive power and both models identified the same predictors as significant.

In our first analysis, we tested whether snake density (snakes/ha) predicted rat or lizard SPUE from 24 October 2016 to 30 September 2017 using negative binomial regression with prey as the response and snake density as a fixed effect. Because rat SPUE was very low ( $< 0.1$  rats/km), we multiplied monthly averages by 1000 to create whole numbers. We completed two additional negative binomial regressions with snake density evaluated as two independent fixed effects:  $< 700$  mm SVL or  $N_{<700}$  or  $\geq 900$  mm SVL or  $N_{\geq 900}$ . One model had rat SPUE as the response and the other lizard SPUE. We also completed time series regressions for these models, using a univariate, autoregressive integrated moving average (ARIMA) approach. We used a 1-year interval subdivided into monthly estimates to forecast time series models, maintaining  $N_{<700}$  or  $N_{\geq 900}$  as predictors. In order to test whether lizard or rats correlated with snake reproduction, we used lizard and rat SPUE as predictors in a negative

binomial regression and time series regression (univariate, ARIMA, with monthly intervals over a 12 month period), for the response of density of juvenile snakes (< 700 mm SVL) after a four month lag period. We selected four months as the lag period to account for a three-month egg gestation period, as well as a one-month lag in detection of juveniles or follicle production. We report the regression estimate ( $\hat{\beta}$ ), 95% confidence intervals (CI), and significance for negative binomial regressions, and  $\hat{\beta}$  and 95% CI for time series regression by parameter. For all models, we accepted significance at  $\alpha < 0.05$  and we report descriptive statistics as mean  $\pm$  standard error.

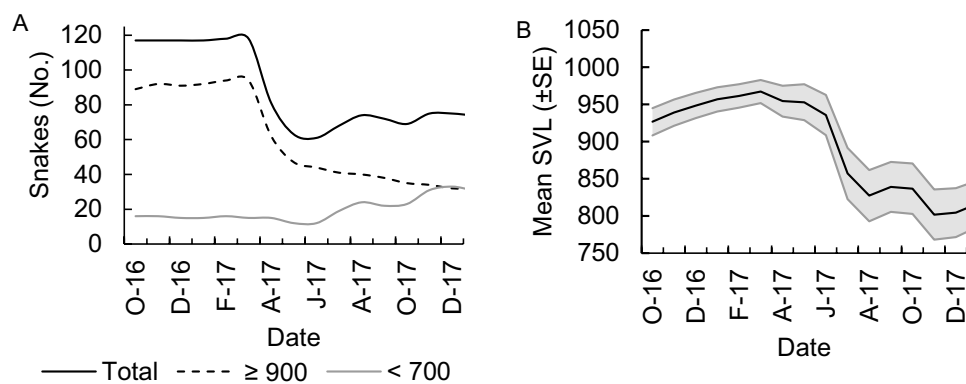
## Results

The estimated number of snakes present prior to removal was 117 individuals (23.4 snakes/ha), with  $N_{<700} = 16$  and  $N_{\geq 900} = 87$  (Fig. 1A). From 31 March to 30 September 2017, the population declined to 72 individuals (14.4 snakes/ha), with  $N_{<700} = 22$  and  $N_{\geq 900} = 37$  mm SVL. By 31 January 2018, the number of juvenile snakes (< 700 mm SVL) had roughly doubled from the starting population to  $N_{<700} = 33$ . The mean SVL of the population decreased from  $967 \pm 18$  in March 2017 to  $816 \pm 31$  mm in January 2018, supporting a shift from predominately endothermically interested snake sizes to ectothermically interested or transitioning into endothermic prey (Fig. 1B).

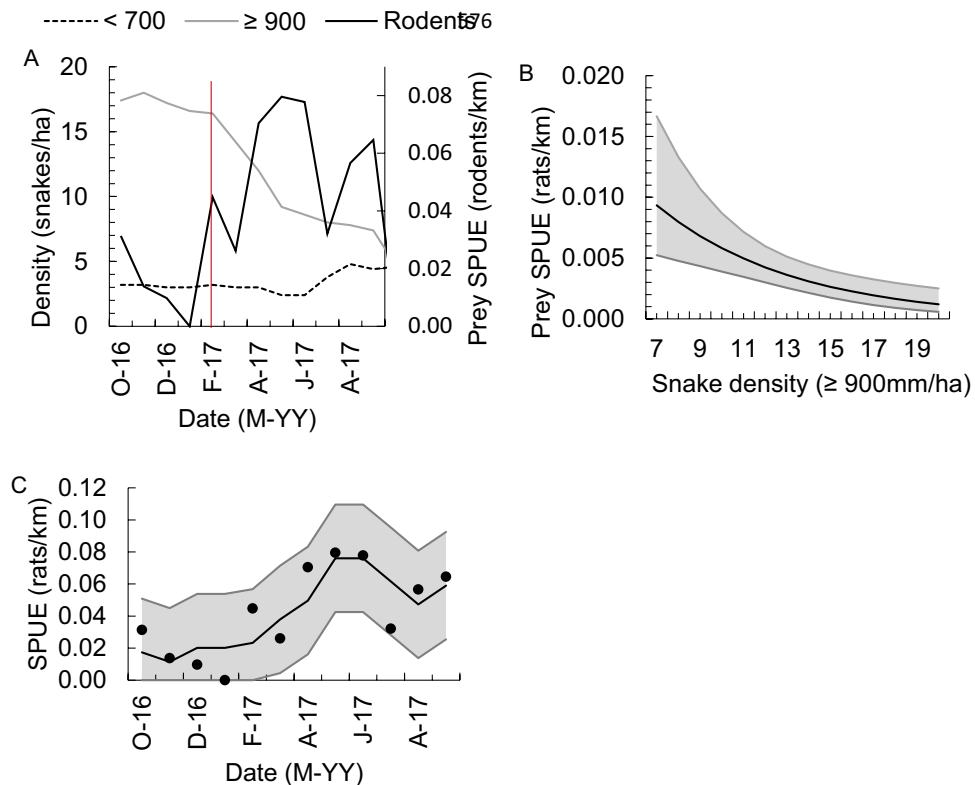
During removal of snakes susceptible to toxic mouse baits, rat SPUE increased by approximately a factor of 3, from  $0.02 \pm 0.01$  pre-snake removal to  $0.06 \pm 0.02$  rodents/km (Fig. 2A). There was a significant relationship between total snake density and rat SPUE ( $\hat{\beta} = -0.11$ , 95% CI =  $-0.17$  to  $-0.06$ ,  $t = -3.6$ ,  $P = 0.005$ ). Dividing the total population into stage-specific predictors supported that the relationship between rat SPUE and snake density was, however, stage specific. Based on negative binomial regression, the density of  $N_{\geq 900}$  was negatively correlated with rat SPUE ( $\hat{\beta} = -0.16$ , 95% CI =  $-0.23$  to  $-0.09$ ,  $t = -3.6$ ,  $P = 0.006$ ); for each decrease of  $N_{\geq 900}/ha$  corresponded to an increase in rat SPUE by a factor of 0.9 (Fig. 2B). In contrast, there was no relationship between snakes  $N_{<700}/ha$  and rat SPUE ( $\hat{\beta} = -0.26$ , 95% CI =  $-0.10$  to  $0.02$ ,  $t = -1.14$ ,  $P = 0.28$ ). Time series regression supported that  $N_{\geq 900}/ha$  was negatively predictive of rat SPUE, with a CI that did not overlap zero ( $\hat{\beta} = -0.58$ , 95% CI =  $-0.80$  to  $-0.37$ ). Time series regression indicates that  $N_{<700}/ha$  was also negatively correlated with rat SPUE ( $\hat{\beta} = -1.44$ , 95% CI =  $-0.13$  to  $-2.76$ ), with a wide 95% CI that was approaching but did not cross zero. The combined inclusion of both size classes forecasted a change in rat SPUE that encompassed actual values (Fig. 2C).

In contrast to rodents, there was no change in average monthly lizard SPUE in the six months after snake removal ( $6 \pm 1$  lizard per km), as compared to the six months during toxic mouse baiting ( $5 \pm 1$  lizard per km), although 3 months after snake removal began there was a temporary increase (Fig. 3A). There was no relationship between total snake density and lizard SPUE as estimated by binomial regression ( $\hat{\beta} = -0.01$ , 95% CI =  $-0.03$  to  $0.01$ ,  $t = -0.65$ ,  $P = 0.53$ ). Dividing the total population into stage-specific predictors, illuminated a significant relationship between lizard SPUE and snake density that was undetectable without stage specific predictors. Based on negative binomial regression density of  $N_{<700}$  was negatively correlated with lizard SPUE ( $\hat{\beta} = -0.23$ , 95% CI =  $-0.34$  to  $-0.13$ ,  $t = -2.80$ ,  $P = 0.02$ ), such that for each decrease of  $N_{<700}/ha$  corresponded to an increase in lizard SPUE by a factor of 0.8 (Fig. 3B). In contrast, there was no relationship between snakes  $N_{\geq 900}/ha$  and lizard SPUE ( $\hat{\beta} = -0.01$ , 95% CI =  $-0.04$  to  $0.01$ ,  $t = -1.01$ ,  $P = 0.33$ ). Time series regression suggest that  $N_{<700}/ha$  was also negatively correlated with lizard SPUE ( $\hat{\beta} = -1.47$ , 95% CI =  $-2.38$  to  $-0.56$ ), with a wide 95% CI that was approaching but did not cross zero. Time series regression supported that  $N_{\geq 900}/ha$  was not correlated to lizard CPUE, with a CI that overlapped zero ( $\hat{\beta} = -0.13$ , 95% CI =  $-0.29$  to  $0.02$ ). The time series regression forecasted a change in lizard SPUE that encompassed actual values (Fig. 2C).

After accounting for a four-month lag, the number of juvenile snakes (< 700 mm SVL) in the population increased to 33 individuals from the 16 originally present (Fig. 4A). Based on negative binomial regression, rat



**Figure 1.** The change in the number (A) and mean population snout-vent length (SVL, B) of brown treesnakes (*Boiga irregularis*, snakes) in a 5-ha enclosure in Guam proceeding and during experimental removal of snakes willing to eat mouse baits. The number (No.) of snakes is the total individuals in the population, as well as individuals < 700 or  $\geq 900$  mm SVL. The mean size is the average SVL of snakes present in the population  $\pm$  the standard error (SE). The red line indicates when removal of snakes susceptible to toxic mouse baits ( $\geq 850$  mm SVL) began.



**Figure 2.** The density of brown treesnakes (*Boiga irregularis*) in a 5-ha enclosure in Guam proceeding and during experimental removal, and the monthly mean sightings of rats (prey sightings per unit effort [SPUE]) based on visual surveys. Snakes are divided into two size classes based on ontogenetic interest in rodent ( $\geq 900$  mm snout-vent length [SVL]) or lizard ( $\leq 700$  mm SVL) prey (A). The red line indicates when snake removal began. The negative binomial regression and 95% confidence interval (CI) between rat SPUE relative and snakes interested in rodent prey ( $\geq 900$  mm) was negative (B). The predicted sightings and 95% CI of rats during each time step based on the density of snakes in each size class using time series regression forecasted an increase as snakes  $\geq 900$  mm declined (C). Actual rat SPUE is represented by black circles.

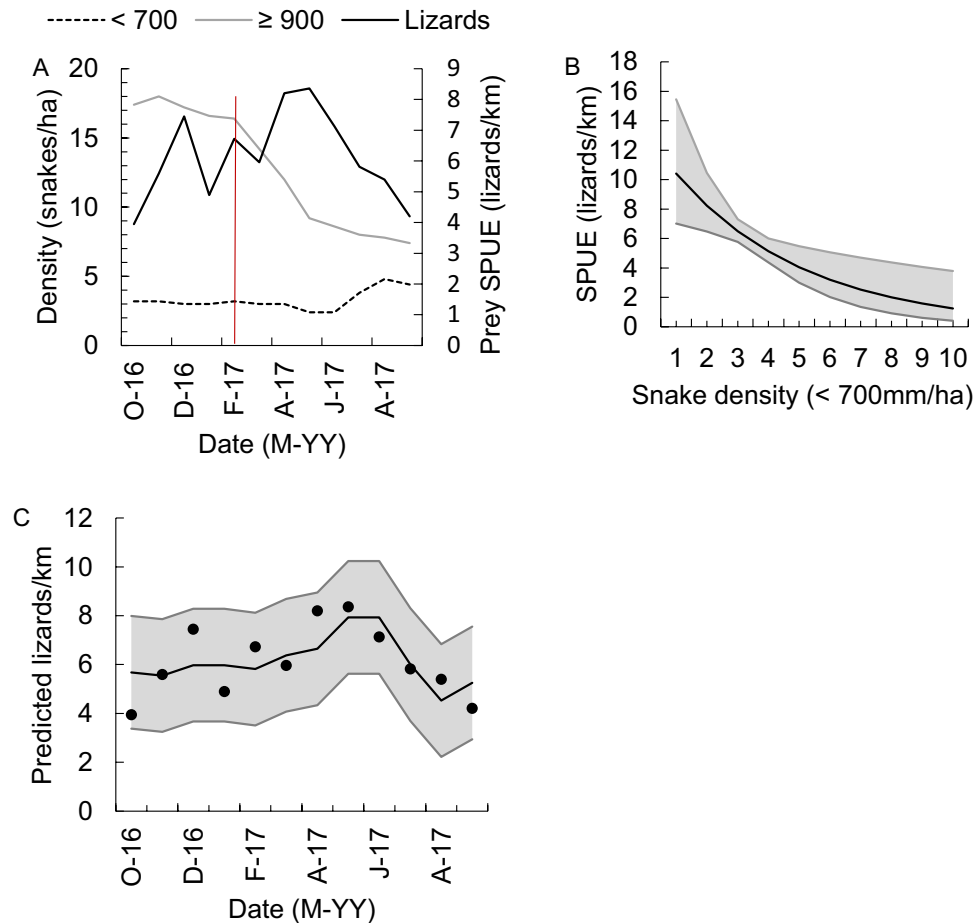
SPUE was a significant positive predictor of juvenile abundance ( $\hat{\beta} = 0.10$ , 95% CI = 0.06–0.14,  $t = 2.73$ ,  $P = 0.02$ , Fig. 4B), while lizard SPUE was not correlated ( $\hat{\beta} = -0.09$ , 95% CI = -0.16 to 0.00,  $t = -1.27$ ,  $P = 0.26$ ). The time series regression poorly fit the data and neither rat ( $\hat{\beta} = -0.19$ , 95% CI = -0.93 to 0.54) nor lizard SPUE ( $\hat{\beta} = -0.17$ , 95% CI = -1.39 to 1.05) was correlated with juvenile abundance 4 months later (Fig. 4C).

## Discussion

In this study, models that accounted for ODS in a top predator illuminated stage specific predator–prey relationships within food web dynamics involving reptilian predators. Our findings, therefore, provide limited field support for the hypothesis that stage structure of predator populations with ODS is an important consideration in accurately assessing terrestrial trophic linkages. Of note, however, are quantitative factors limiting the interpretation of these data, including how we measured prey response, the lack of spatial replication, and temporal dependence between the time series data.

Based on the scale of the study and the resources required to effectively study cryptic species at the intensity required, replication of our study was not possible. These data thus represent a single event, in a single location confounded by environmental or temporal factors for which we did not control. Temporal autocorrelation is documented to have substantive effects on populations when modeling stochastic population growth<sup>47</sup>. Although the effects of temporal autocorrelation should be lower in our study, they are likely not entirely negligible based on the slightly different statistical relationships between the snake density and prey SPUE within the time series and negative binomial regressions. Thus, although quantitative limitations in interpretation should be considered as limiting the strength of support, the data add to broader theoretical ideas on ODS as a component of trophic relationships.

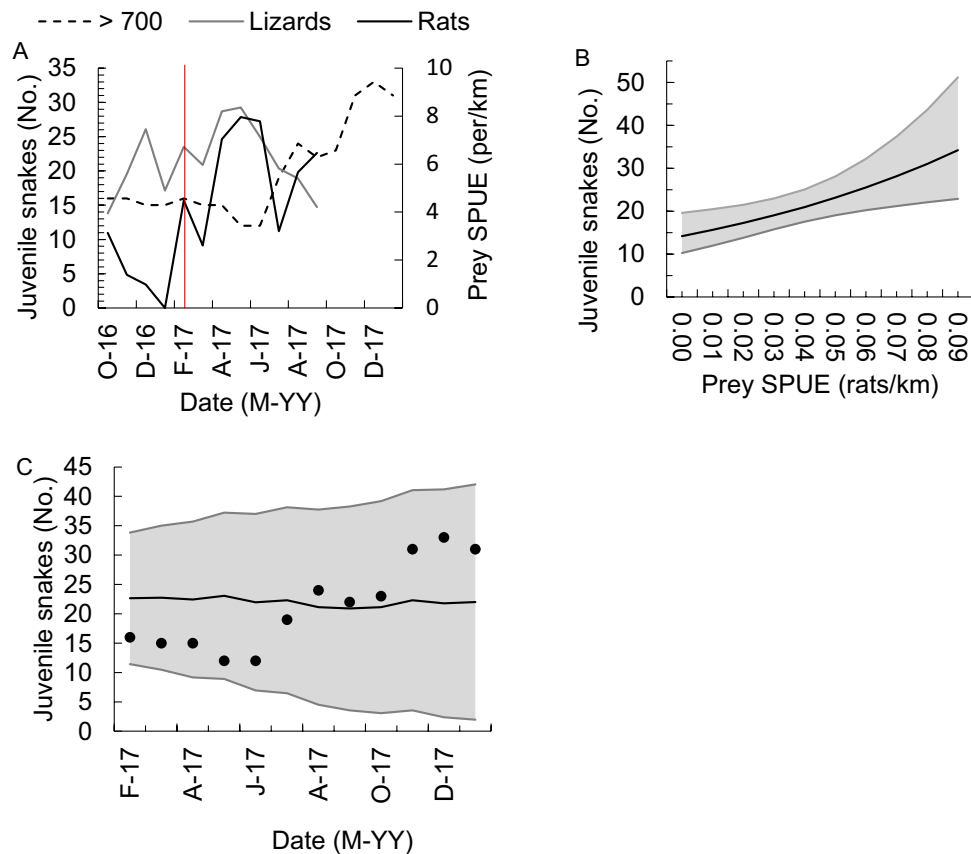
Additionally, because the study site described was closed only to snakes, rats and lizards could immigrate or emigrate freely. Thus, prey SPUE may have been a function of movement, activity levels, and reproduction. Linkages between predator and prey may be tighter in closed populations where recruitment or mortality is the only mechanism for population changes for both predator and prey. Likewise, our results also rely on the assumption that SPUE is a relatively accurate index of true abundance. In general, capture per unit effort (CPUE), as a



**Figure 3.** The number of brown treesnakes (*Boiga irregularis*) in a 5 ha enclosure in Guam proceeding and during experimental removal, and the mean monthly sightings of lizards (prey sightings per unit effort [SPUE]), based on visual surveys. Snakes are divided into two size classes based on ontogenetic preferences for rodents ( $\geq 900$  mm snout-vent length [SVL]) or lizards ( $\leq 700$  mm SVL) (A). The negative binomial regression and 95% confidence interval (CI) for the relationship between lizard SPUE relative to snakes interested in only lizard prey ( $< 700$  mm) was negative (B). The predicted and 95% CI of lizard sightings during each time step based on the density of snakes in each size class using time series regression forecasted a decrease as snakes  $< 700$  mm increased (C). Actual lizard SPUE is represented by black circles.

measure of abundance, is coarse and may be problematic without prior species-specific validation<sup>48</sup>. There are many known and unknown sources of error in using CPUE to index population abundance for lizards in Guam forests<sup>33,34</sup>. The relationship between nocturnal visual sightings of rodents and their abundance in tropical forests is untested. Known concerns with visual mammal survey effectiveness include having well-worn paths and rested surveyors, which should not be hindrances in our study<sup>49</sup>. Regardless, visual detections may be driven by both direct effects (true abundance) and indirect effects, such as reduced activity or area avoidance or attraction, which hinder our ability to determine whether decreases in SPUE reflected fewer individuals or sightings of individuals that were present.

The mechanistic explanations for changes in relative prey SPUE in response to changing predator populations may, however, be less relevant when a biological response (i.e., changes in activity or abundance) was still observed. Specifically, the relationship between rat SPUE and the production of juvenile snakes after a suitable lag period supports the idea that the mechanism that caused changes in rat SPUE also affected their availability as prey for snakes. As measured by mass, rodents are a larger prey reward than most lizards that occur in Guam. Reproduction tends to be metabolically costly, with a 30% increase in metabolic activity to support egg yolk deposition in viviparous snakes and measured rates of energy assimilation from meals at upwards of 90% in pythons<sup>50,51</sup>. When each brown treesnake egg is roughly 8 g<sup>52</sup>, a clutch of 3 or more eggs would require consuming quite a few  $< 5$  g lizards to support normal metabolic costs as well as reproduction, while one 300 g rat may support both functions easily. Lizards may, therefore, primarily be intermediate meals that support survival, while larger prey items are needed to reproduce, but additional data would be necessary to corroborate this hypothesis. Our data support that a measurable increase in rat SPUE potentially predicted a reproductive pulse for BTS, but that changes in lizard SPUE did not.



**Figure 4.** The number of immature brown treesnakes (*Boiga irregularis*, < 700 mm snout-vent length [SVL]) in a 5 ha enclosure on Guam preceding and during experimental removal of adults. The monthly mean sightings of lizards and rats (prey sightings per km surveyed [SPUE]) observed during nocturnal visual surveys are depicted relative to the abundance of immature snakes (A). After a 4-month lag, the negative binomial regression relationship for the number of juvenile brown treesnakes (< 700 mm snout-vent length [SVL]) was positively correlated to rodents sighted per km surveyed (rat SPUE) but not lizards (B). The predicted and 95% CI of juvenile snakes during each time step was not related to either prey type (C). Actual juvenile snake count is represented by black circles.

Although brown treesnakes are reliably interested in endothermic prey around 900 mm SVL, lizards remain a major part of their diet into sizes well over 1100 mm SVL<sup>17,28,29</sup>. The maximum size of a snake in this population was 1299 mm SVL, indicating almost all individuals in the studied population likely consumed lizards. The long-term persistence of smaller prey items in the diet of species with ontogenetic prey changes is documented in several fish and reptile species<sup>53–56</sup>. In the forests of Guam, very few endothermic prey remain, with the few remnant native birds mostly isolated to small areas in urban landscapes<sup>23,57</sup>. Thus, daily energetic and metabolic needs may require that snakes maintain lizards in their diet. Snakes collected from forested areas on Guam, where the primary prey are anticipated to be various lizard species, maintained lizards as a higher proportion of their diet into larger size classes than snakes in urban landscapes<sup>17</sup>. Therefore, lizard populations are likely also affected by larger size classes and prior work has demonstrated lizard counts increase with brown treesnake eradication<sup>58</sup>. Trophic linkages between larger snakes and lizards may, however, be weaker as compared to those between juvenile snakes that are dependent on lizards as their primary food source. Thus, the ability for brown treesnakes to exert top-down control on ectothermic prey species is reasonable and documented. The importance, however, of considering the ontogeny in populations brown treesnake or other reptile populations in exerting top-down effects has not, to our knowledge, been previously reported.

Although the total population was reduced by ~ 40% after treatment, we did not detect a direct relationship between snake density and lizard response based on the total population density of snakes. The stronger response of rodents to the reduction of snakes, as compared to lizards, may have been biased by the use of rodents as the snake removal tool. This approach should favor the removal of snakes foraging on rodents. The separation of the population into different stages was, however, effective in unmasking a relationship between snakes and lizards that was otherwise not detectable based on the methods we used for removal. Thus, failing to consider ODS in trophic interactions and population stage structure may miss trophic linkages in community food web patterns<sup>13,59,60</sup>. For herpetofauna, this may contribute bias in understanding their overall ecological impacts or relative importance, as diet shifts in prey are relatively common in predatory herpetofauna.

## Management implications

The stage-specific and ontogenetic effects of predators on prey may be especially important to consider for invasive snake management. Native prey species may have altered risk of depredation depending on the stage structure of the invasive predator population. Regarding brown treesnakes, the primary removal tools are based on food attractants that target a subset of the population<sup>29,61,62</sup>. This removal approach may have implications for broader management goals, if conservation or recovery goals are targeting different species than those used for control<sup>42</sup>. In general, reptiles have important roles in communities and injurious invasive species, with several examples of major perturbations following the introduction of snakes<sup>21,63</sup>. ODS are common among reptiles and may also co-occur with other types of niche shifts<sup>64</sup>. In addition to many-fold ontogenetic changes in size, which can affect decisions such as toxicant dosing, individuals may display stage-based preferences for prey states<sup>36,61,65</sup>. In systems in which an invasive predator affects many trophic levels and ecological recovery is desired, consideration of population structure may be meaningful for both planning species recovery and to achieve removal targets.

## Data availability

The datasets generated and/or analyzed during the current study are available for download (Nafus et al.<sup>35</sup>, <https://doi.org/10.5066/P9QRWKQB>, Nafus<sup>43</sup>, <https://doi.org/10.5066/P9JQ9HG0>).

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## Author contributions

MGN conceived and designed experiments and statistical analysis. MGN and LNG wrote the manuscript. Both authors contributed to revisions.

### Competing interests

The authors declare no competing interests.

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