SUCCESSFUL PREDATION OF INVASIVE SOUTH AMERICAN CANE TOADS (*RHINELLA MARINA*) BY SOUTHERN WATER-SNAKES (*NERODIA FASCIATA*)

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ABSTRACT: South American Cane Toads (*Rhinella marina*) have been introduced into several regions outside of their native distribution. Outside of their native range, few predators have been documented preying upon *R.marina* due to their potent toxins secreted in defense. However, prevalence of a toxin resistance gene makes it possible for some snakes of the sub-family Natricinae to consume native toads. We documented successful consumption of the invasive cane toad by the Southern Watersnake (*Nerodia fasciata*) in southwest Florida, both in the wild and in the laboratory. Southern Watersnakes from populations that existed both with and without cane toads successfully consumed toad prey without obvious ill-effect. Southern Watersnakes in southwest Florida are thus resistant to, and readily consume cane toads, an otherwise relatively predator-free invasive species in Florida. More dietary field data and controlled experiments that measure resistance to multiple prey items, sizes, and frequency will serve to determine the extent to which Southern Watersnakes can impact the size and structure of sympatric populations of cane toads.

Key Words: Invasive species, Bufotoxin, Predation, Natricine, Bufonidae, Resistance

INTRODUCTION

Invasive species can negatively impact other species through competition (Ramsay et al., 2007; Perdereau et al., 2011; Kraus, 2015; Le Louarn et al., 2016), predation (Hays and Conant, 2007; Rodda and Savidge, 2007; McCleery et al., 2015), introduction of nonnative pathogens and parasites (Crowl et al., 2008; Vredenburg et al., 2013; Miller et al., 2018), and biomass that is difficult for native predators to ingest (Crossland, 1998; Lages et al., 2010; Vilcinskas et al., 2013). However, in some situations invasive species may facilitate native species by serving as prey items (Meshaka, 2011; Meshaka and Ferster, 1995; Rodriguez, 2006; Pratt and Grason, 2007; Maljković et al., 2008; Yong et al., 2014).

The diet of some snakes includes true toads of the family Bufonidae, despite chemical defenses. Toads secrete biologically active substances often referred to collectively as Bufotoxins and Bufogenins (Meyer and Linde, 1971). These substances produce a multitude of effects in potential predators, including cardiotoxic impacts, seizures, vomiting, and respiratory distress (Eubig, 2001). Consequently, many snake species avoid toads as prey, although other species such as hog-nosed snakes (*Heterodon*) are well-known exceptions for their dietary specialization on toads (Edgren, 1955; Platt, 1969; Cooper and Secor, 2007). Although they do not specialize on toads, many snakes in the sub-family Natricinae in Europe, Asia, and North America feed on toads (Arnold and Wassersug, 1978; Kephart and Arnold, 1982; Filippi et al., 1996; Griffiths et al., 1998; de Queiroz et al., 2001; Hutchinson et al., 2008). For example, several North American watersnakes in the genus *Nerodia* consume toads (Gibbons and Dorcas, 2004). Successful predation of toads by *Nerodia* is likely linked to Bufotoxin resistance genes, with related mutations found in a large percentage of the Natricinae (Mohammadi et al., 2016). However, even with such mutations present the ability to metabolize and/or sequester bufotoxins is likely dependent on the dosage, potency, and composition of the toxin variant, which is variable across the Bufonidae (Gao et al., 2010). In turn, these factors may also vary among *Nerodia* species.

The Southern Watersnake (*Nerodia fasciata*) is a common species indigenous to the southeastern United
States (Hebrard and Mushinsky, 1978; Balfour and Stitt, 2008; Reed et al., 2016). This species feeds on a wide range of invertebrates, fish, and amphibians, including several native toad species (Gibbons and Dorcas, 2004; Durso et al., 2013). Given its wide geographic range and ability to survive in a variety of natural and altered habitats (Keck, 1998; Camper and Chick, 2010), it is possible that Southern Watersnakes might encounter overlap with toad species that thrive in disturbed and/or urban environments in south Florida, such as the South American Cane Toad (Rhinella marina) (Krakauer, 1968; Savage, 2002; Meshaka et al., 2006; Ljustina and Barrett, 2018).

The South American Cane Toad is native to Central and South America, where it thrives in a variety of habitats and is especially abundant in anthropogenically influenced ecosystems (Zug and Zug, 1979; Lever, 2001). Its toxicity, high fecundity, and generalist tendencies have enabled cane toads to become a successful invasive species, most notably across northern Australia, but also in the Caribbean, and South Florida (Riemer, 1958; Lever, 2001; Meshaka, 2011; Meshaka et al., 2004). The majority of studies in Australia indicate a negative impact of cane toads on local fauna, with reptiles suffering population-level declines (Letnic et al., 2008; Doody et al., 2009; Price-Rees et al., 2010). However, at least one species of Australian snake, the Common Keelback (Tropidonophis mairii), can consume cane toads without apparent ill-effect (Phillips et al., 2003; Llewelyn et al., 2010; 2011) and several snake species that occur sympatrically with cane toads consume this species in their native range (Eterovic et al., 2001; Oliveira et al., 2007; Kaefer and Montanarin, 2011).

Initial Observations — On 23 February 2017, an adult female Southern Watersnake (SVL = 61.6 cm) was captured at an artificial lake on the property of a local High School, in Naples, Florida during a biology class field trip. The snake regurgitated its recent meal within 30 min after having been captured and held in an 18.9 L bucket to show filed trip participants (Figure 1a). The regurgitated prey item was an adult female cane toad (Fig. 1b) measuring 8.5 cm Snout-Urostyle Length (SUL) and weighing 43 g (post-mortem) (Zug et al., 1975). The snake was kept for seven days before it was released, during which time it showed no ill-effects from ingesting the toad and readily fed on wild caught non-native Cichiliform fish from the same lake.

After these observations, we conducted feeding trials in a controlled laboratory setting to determine if Southern Watersnakes would successfully feed upon this novel food source. Although Southern Watersnakes readily feed on native toads, there are no published observations of them consuming cane toads. We tested snakes from a population in which predator and prey were sympatric (co-existing for >4 years) and a population where cane toads do not occur. Our prediction was that these snakes would successfully ingest cane toads without any ill effects, regardless of prior exposure to this potential prey source, due to their evolved resistance to toad toxins from native bufonids.

METHODS

Field sites — The first site, designated the Naples site, consisted of roadside drainage ditches and a man-made pond in a suburban neighborhood near Naples, Florida. Cane toads have been established at this site for at least four years but likely longer (J. Donini, pers. obs.). The second site, designated the Immokalee site, consisted of roadside drainage ditches in agricultural areas near Immokalee, Florida. The Immokalee site was presumed to have not been colonized by cane toads based on absence of calls or visual sightings during several road surveys in the area since 2001 (Daniel Parker, pers. comm.), including five surveys in May–June 2020 by the authors.

Snakes were captured by hand during nocturnal road surveys conducted during March–June. Snakes were placed into separate cloth holding bags for processing. Snakes were then individually marked by scale clipping as per the method of Brown and Parker (1976), and measured in cm snout-vent length (SVL), weighed, and sexed if possible, with the use of a cloacal probe (Fitch, 1960).

All toads used for this study were from the Naples site. Individuals measuring 3.0–7.0 cm snout-urostyle length (SUL) were used for trials, as larger toads were too large to be consumed by even the largest snakes found in the study. Conversely, smaller toads than the ones used might have less potent toxin than other size classes (Hayes et al., 2009) and were thus, excluded.

Laboratory Conditions and Experiments — Upon capture, snakes were taken to the lab at Florida SouthWestern State College and placed into 91 x 43 cm Sterilite® enclosures on a custom-built snake rack. Snakes were allotted 24–48 hours to acclimate to temporary enclosures before being offered food. Each enclosure was equipped with under-tank heating to ensure a temperature of 23–27°C. Snakes were kept on a substrate of newspapers or paper towels, where they were given access to water at all times. Enclosures were checked and cleaned daily as needed. Once snakes attempted to consume food items placed in the enclosure, experimental trials began.

Snakes from both sites were randomly sorted into an experimental group that was offered cane toads or a control group that was offered frozen-thawed store bought Silverside fish (Menidia sp.) to determine if there were population level differences in ingestion success. A total of nine snakes were placed in the experimental group from the Naples site, along with an additional nine snakes from the Immokalee site. Five snakes from the Naples side were placed into control groups, along with a single individual from the Immokalee site (Table 1). Snakes that refused to eat in captivity were removed from the study.

Experimental animals were offered a live cane toad in their enclosure, and ingestion was photographed and recorded on video. Snakes were initially observed for the first hour after ingestion and then again six hours

Fig. 1a. Wild adult female Southern Watersnake with recently-regurgitated female Cane Toad. 1b. same cane toad next to ruler for scale.
Table 1. Results of cane toad ingestion trials in Southern Watersnakes. Naples indicates snakes from a site with known cane toad populations, Immokalee indicates a site with cane toads absent. No snakes suffered any fatal effects and were released post trials. Snakes that refused food were removed from study and not included in this table. * indicated individuals that attempted to feed, but released toads prior to ingestion.

<table>
<thead>
<tr>
<th>Snake ID No.</th>
<th>Sample Location</th>
<th>Experimental (E) or Control (C)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>6</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>7</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>8</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>9</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>10</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>11</td>
<td>Naples</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>13</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>14</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>15</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>16</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>17</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad Swallowed completely, but regurgitated 48 hours post consumption. No detectable effects.</td>
</tr>
<tr>
<td>18</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>19</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>21</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>22</td>
<td>Immokalee</td>
<td>E</td>
<td>Toad swallowed completely. No detectable effects.</td>
</tr>
<tr>
<td>2</td>
<td>Naples</td>
<td>E*</td>
<td>Toad swallowed past paratoid glands, but released after 3 min. No Detectable effects</td>
</tr>
<tr>
<td>3</td>
<td>Naples</td>
<td>E*</td>
<td>Toad Swallowed past paratoid glands, but released after 20 min. No Detectable effects</td>
</tr>
<tr>
<td>1</td>
<td>Naples</td>
<td>C</td>
<td>Fish consumed. No detectable effects.</td>
</tr>
<tr>
<td>4</td>
<td>Naples</td>
<td>C</td>
<td>Fish consumed. No detectable effects.</td>
</tr>
<tr>
<td>20</td>
<td>Naples</td>
<td>C</td>
<td>Fish consumed. No detectable effects.</td>
</tr>
<tr>
<td>25</td>
<td>Naples</td>
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<td>Fish consumed. No detectable effects.</td>
</tr>
<tr>
<td>26</td>
<td>Naples</td>
<td>C</td>
<td>Fish consumed. No detectable effects.</td>
</tr>
<tr>
<td>12</td>
<td>Immokalee</td>
<td>C</td>
<td>Fish consumed. No detectable effects.</td>
</tr>
</tbody>
</table>
post-ingestion for any mortality or sublethal effects, such as motor function loss and mouth drooping or movements, as seen in experiments with gartersnakes (Thamnophis sp.) and Tropical Salamanders (Bolitoglossa sp.) (Brodie et al. 1991). Snakes were then observed every 24 hrs post-ingestion and were tested by pinching the snakes’ tails to test reflexes and basic motor function (Licht and Low, 1968; Churchill and Storey, 1992). To avoid inducing regurgitation, additional rigorous physical tests were not performed. Defecation was regularly observed by every individual throughout trials. After seven days, animals were assessed one final time and released at their respective capture sites.

RESULTS

Feeding trials — Fifteen individual snakes (9 females, 2 males, 4 unknown sex due to tail loss/damage to post cloacal tail region) were tested from the Naples site (cane toads confirmed present). Mean SVL was 61.1 ± 1.13 cm (range = 52–65), and mean mass was 256.5 ± 18.72 g (range = 135–350 g). Eleven snakes (8 females, 3 males) were tested from the Immokalee site; mean SVL was 46.6 ± 1.04 cm (range = 33.0–69.9 cm) and mean mass was 136.6 ± 2.93 g (range = 52–280 g).

No lethal or sublethal effects were observed in either the experimental or control snakes from either site during any of the subsequent days in captivity (Table 1). Snakes all responded to tail-pinning by tightly coiling, striking, or attempting to flee. There were no obvious signs of impairment after ingestion of toads. A single individual from the Immokalee site regurgitated its meal ~48 hrs post-consumption for unknown reasons but showed no other negative effects.

Most snakes fed within minutes of introducing the food item (Fig. 2); however, two individuals did not, and were left with a toad for up to twelve hours before their lack of ingestion led to removal from the study. Two snakes in the experimental group began to ingest toads and swallowed toads past the parotid glands before struggling with the toad’s bloating defenses and releasing them. Parotid secretions were obvious upon the toads’ release. These snakes were still monitored for seven days post attempt to document any potential negative impacts but were not documented as successfully ingesting toads.

DISCUSSION

Our study is the first to describe successful predation of cane toads by a native North American snake in the wild, and in laboratory feeding trials. Ingestion was successful by Southern Watersnakes from sites with and without cane toad prey, likely due to evolved resistance to bufotoxins that are also produced by native toad species. Other snakes of the genus Nerodia naturally overlap with bufonids through much of their ranges in North America (Gibbons and Dorcas, 2004) and have also evolved resistance to toad toxins (Ujvari et al., 2015). However, it is unknown if other known toad-consuming species such as the Saltmarsh Watersnake, (Nerodia clarkii) and the Diamond-backed Watersnake, (Nerodia rhombifer) could successfully feed on cane toads in the same manner observed here.

In the only other study to investigate resistance of snakes to Cane Toad toxins in North America, Licht and Low (1968) orally administered varying concentrations of toad toxin to Common Gartersnakes (Thamnophis sirtalis), which eat toads, and to Striped Whipsnakes (Coluber taeniatus), Eastern Coachwhips (Coluber flagellum) and Eastern Patch-nosed Snakes (Salvadora grahamiae), which do not regularly consume toads. Their study found that the non-toad-eating species suffered from cardiac and muscular irregularities and even death at much lower doses (3 mg/g of body weight) than toad-eating species. Most of toad-eating individuals did not show appreciable symptoms until dosage was increased between 10-20 mg/g of body weight (some then died at these higher doses). It should be noted that the authors conceded that doses approaching these concentrations are unlikely to be ingested from preying upon a toad in nature.

Despite our general results, two snakes did release cane toads during the trials possibly due to toxin effects or the bloating defense of the toads; one snake held the toad for ~20 minutes prior to releasing it. However, both of these snakes showed no obvious conspicuous ill-effects for the seven day period. Additionally, apparent mortality of an individual Southern Watersnake due to ingestion of a cane toad has been documented (Asplund, Instagram, 2018.) In this instance, an adult Southern Watersnake died while attempting to prey upon a large adult cane toad on the Florida east coast. Variation in the feeding outcomes within a species could reflect population-level or individual-level differences in toxin strength in toads, toxin resistance in the snakes, or some other factor such as health of the snake or relative size of predator vs. prey. Even the largest Nerodia may struggle to consume a fully-grown adult cane toad given constraints of head and gape size along with the potential physiological implications for higher volumes of toxins from larger individuals (Forsman and Lindell, 1993; Vincent et al., 2006; Kowalski et al., 2020). For instance, In the Japanese Yaeyema Islands, where cane toads have also been introduced, the native Red-Banded Snake (Dinodon rufozonatum) is known to successfully ingest single juvenile/sub-adult sized toads similar in size to those used in the present study without ill-effect; however, they may suffer mortality in attempts to consume larger adult toads and/or multiple toads (Kidera and Ota, 2008).

Overall, our experimental trials and observations indicate that cane toads are likely included in the diet of Southern Watersnakes in south Florida, at least during the small to medium-sized post-metamorphic life stages.
Given the ability of Southern Watersnakes to successfully ingest toads, there is potential for the species to serve a role in regulating size and structure of cane toad populations. However, dietary frequency of native toads in previous studies was relatively low for Southern Watersnakes and congeners (5.0-12.1 %; Mushinsky and Hebrard, 1977; Camp et al., 1980; Vincent et al., 2007). More dietary data from wild Southern Watersnakes from southern Florida and experimental testing of toxin resistance of Southern Watersnakes exposed to consecutive meals and toads of varying sizes are needed to determine both the importance of toads in its diet and the extent to which this species may actually impact the relatively predator free cane toad.

ACKNOWLEDGEMENTS
We thank Daniel Parker and Chris Lechowicz for their assistance in sampling snakes. Animals were collected and released under FWC Permit LSCC-17-0022A. Animals were handled and cared for in a manner consistent with the Herpetological Animal Care and Use Committee of the American Society of Ichthyologists and Herpetologists.

LITERATURE CITED


