DIET ANALYSIS OF THE INTRODUCED SPECTACLED CAIMAN (*CAIMAN CROCODILUS*) IN TORTUGUERO LAGOON, PUERTO RICO

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“Arriving at one goal is the starting point to another”

- John Dewey

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ABSTRACT

The spectacled caiman (*Caiman crocodilus*) was introduced to Puerto Rico over 50 years ago with the Tortuguero Lagoon Natural Reserve (TLNR) as its epicenter, where it is now established as an apex predator. Although concerns have been raised regarding the potential impact of this naturalized predator on Puerto Rico's native fauna, little was known of the caiman's diet on the island. Therefore this study was conducted to determine the diet of the spectacled caiman and its potential impact on island animals. For this study, measurements were obtained from 138 caimans across all life stages (12–94 cm snout–vent length; SVL) from October 2014 to May 2015 within the TLNR. Stomach contents were retrieved and analyzed based on prey category occurrence frequency. Caiman muscle samples were obtained to conduct a stable isotope analysis. Representative prey species were collected based on principal prey groups observed in stomach contents. Isotopic signatures of both predators and prey were analyzed using the Bayesian mixing model (SIAR) to determine nitrogen and carbon source proportions from the different life stages. Insects were the most abundant prey items encountered with 90.7% and 68.8% in hatchling (SVL < 20 cm) and juvenile (SVL = 20 – 59.9 cm) stomach respectively. In adult (SVL > 60 cm) caimans, fish remains were the most significant prey items with 38.3% frequency of occurrence. Fish, insects, and gastropods were the only categories of ten designated prey categories to show significant variation among the three caiman age classes. This study provides novel information on dietary habits of spectacled caimans in Puerto Rico relevant to the design of management strategies and further promotes the use of stable isotopes in diet studies.

CHAPTER 1

REASEARCH BACKGROUND AND CURRENT KNOWLEDGE OF *CAIMAN CROCODILUS* INTRODUCTION TO PUERTO RICO

The following chapter provides an introduction to *Caiman crocodilus* in Puerto Rico based on published literature and unpublished reports from the Department of Natural and Environmental Resources (DNER). It also includes its current status on the island and the objectives of this research study.

Introduction

The introduction and successful establishment of apex predators has been widely documented as an important cause of biodiversity loss. Invasive top predators have been found to disrupt food webs at multiple trophic levels, modify habitats, out-compete native species for resources or to serve as vectors for disease and parasite transmission (Work et al. 2002; Levy et al. 2008; Mortensen et al. 2008; Dorcas et al. 2011). The absence of evolutionary history and limited ability for species to geographically disperse render insular ecosystems particularly vulnerable to the effects of non-native predators (D'Antonio and Dudley 1998).

The spectacled caiman *Caiman crocodilus* (Linnaeus, 1758), also referred to as common caiman, is a medium-sized crocodilian considered an opportunistic omni-carnivorous species as its diet includes a wide array of invertebrates, fish, amphibians, reptiles, birds, and even mammals for larger specimens (Thorbjarnarson 1993). It earned its common name due to the bony structure located between its eyes resembling a pair of spectacles. Males reach greater size (approx. 2 meters) than females (approx. 1.5 meters) as evidence of sexual dimorphism. Among the 23 described crocodilian species in the world, it is the most abundant with an estimated population size of millions of individuals, the most widespread, and the most diversified as it includes four subspecies (*C.c. crocodilus, C.c. fuscus, C.c. apaporiensis, and C.c. chiapasius*). It is considered a well-adapted and least concerned species (IUCN, last assessed in 1996). Its native distribution includes the southern part of Mexico, most of Central America, and the Northern region of South America. It has been introduced and is considered fully established in Florida

(USA) since the 1950's and Isla de la Juventud (Cuba) since 1959 (Ellis 1980; Estrada and Ruibal 1999).

First appearance of *Caiman crocodilus* in Puerto Rico

In the 1960's, it was introduced in Puerto Rico principally associated with the legal caiman pet trade during these times (Thomas and Joglar 1996). Hatchling caimans could be purchased in stores such as Woolworths for about two dollars. A combination of deliberate and accidental release from caiman pet owners is likely to be the principal cause of introduction to the natural environment. Back then, the presence of *Caiman crocodilus* was principally associated with the northern coastal plains of the island with the Tortuguero Lagoon Natural Reserve (TLNR) as its source of proliferation.

Population control program and first data collection

During the 1970's, sightings of caimans within the TLNR and surrounding areas became increasingly more frequent (Santo Reyes, 1988). In November 1982, the DNER considered eradicating this non-native species from the island. In 1983, the first attempt to establish a population control program failed due to the lack of knowledge regarding which subspecies was present in the Tortuguero Lagoon. During these times, *C.c. apaporiensis* and *C.c. yacare* (now considered a full species) were two endangered species. In 1984, a caiman was captured from the lagoon and send to Dr. Wayne King, a herpetologist and former director of the Florida State Natural History Museum. Dr. King identified the caiman as *C.c. fuscus*, a non-endangered subspecies. In May 1985, the amendment of the Wild Life Law allowed the capture of spectacled caimans. In June 1985, over 20 years since the introduction of caimans in Puerto Rico, the Population Control Program was initiated and remains active to this date.

The DNER organized control activities during the night (from 8p.m. to 3 a.m.) due to the nocturnal habits of caimans. The Tortuguero Lagoon was divided in nine areas to obtain data on distribution, density, and movement of caimans within the areas. A 12 volts spotlight was used to detect the light reflecting eyes of caimans above the water surface. A low annual recruitment of individuals into the population was determined based on the observed structure of the caiman size diversity. In addition, the population was described as mature and well-established accordingly with obtained data.

When the Population Control was still relatively recently established, 18 stomachs from adult and subadult caimans were analyzed. The analyses indicated that the caiman's diet was highly diversified. Some of the retrieved stomach contents included small rodents, small birds, fish, amphibians, crustaceans, gastropods, and insects.

By the end of 1985, an average of 50.1 caimans per census was recorded within the lagoon (3.55 observations per kilometer of shore). However by the end of 1986, an average of 20.8 caimans was observed per census. This finding reveals a reduction of the number of caimans by almost 2.5 less than the original population density. By February 1987, density estimates fluctuated between 1.05 to 4.77 caimans per kilometer with an average of 2.29 observations per kilometer. Caiman detections were greatest in the Small Lagoon (West part of the Tortuguero Lagoon) representing a little over one fourth of the entire lagoon. By the end of February 1987, 170 caimans had been captured in 35 hunting trips with an average of 4–5 captures per night. These 170 caimans included 56 hatchlings, 48 juveniles, and 66 adults. By the end of April 1988, a total of 226 adult caimans were removed from the Tortuguero Lagoon resulting in an estimated average of 1.48 individuals per kilometer of shore. The established Population Control Program of 1985 was determined to be effective and in compliance with its stated purpose. Meanwhile, *C*. *crocodilus* population kept on expanding in various regions of Puerto Rico.

Rapid assessment survey of *Caiman crocodilus* in the San Juan Bay Estuary of Puerto Rico

The frequency of caiman sightings and encounters increased far beyond the TLNR. Authorities and inhabitants of the San Juan Bay Estuary became concerned with the caiman's presence. As a result, a rapid assessment survey was conducted by Proyecto Coquí and Ambienta Inc. Environmental Consultants to estimate population abundance and distribution within these areas (Joglar et al. 2010). As part of this rapid assessment, 25 interviews were conducted revealing 965 sightings in Puerto Rico. In addition, nine field trips were organized, eight of which were performed within the San Juan Bay Estuary. By the end of this study, 4-14 caimans were observed per night resulting in a total of 38 observations. The population density was calculated to average 3.55 individuals per kilometer within the San José Lagoon and the Juan Méndez Creek similar to initial population estimates made in the Tortuguero Lagoon. The following study revealed a drastic range expansion since its first appearance in the TLNR. It concluded that caimans are widely distributed and abundant within the San Juan Bay Estuary watershed. In addition, it presented numerous reports of caiman sightings and captures at various other localities within Puerto Rico's coastal plain and its satellite island of Viequez.

Research outline

Caiman crocodilus has been extensively studied in its native range including population dynamics, diet, breeding, and harvesting studies (Staton and Dixon 1977; Gorzula 1978; Ayarzagüena 1983; Thorbjarnarson 1993; Allsteadt 1994; Thorbjarnarson and Velasco 1999; Moreno-Arias et al. 2012; Laverty and Dobson 2013). In Puerto Rico, two unpublished population studies have been conducted (Santos Reyes 1988; Joglar et al. 2010). However, despite having been established on the island for over 50 years, there have been no thorough ecological studies of the caiman's effects on the native fauna. Therefore, our study was initiated to address the need for new information on the introduced caiman's foraging behavior in Puerto Rico. The objectives of our study were three-fold: 1. Use both stomach content and stable isotope analyses to predict the potential effects of caiman foraging on local prey. 2. Promote the use of stable isotope analysis as an efficient tool in evaluating dietary habits. 3. Provide recommendations for caiman management, based on the findings of this study.

CHAPTER 2

STOMACH CONTENT ANALYSIS OF THE INTRODUCED SPECTACLED CAIMAN (*CAIMAN CROCODILUS*) IN TORTUGUERO LAGOON, PUERTO RICO

Introduction

Common methods to assess potential ecological impacts of introduced predators include predictive techniques through observations of documented impact in other geographical location, predator/prey population dynamic correlative studies, experimental removal, and dietary studies (Park 2004). The latter method has been widely employed by means of food extraction and stomach content analysis (SCA) across a variety of taxa (Michael Anthony et al. 2000; Pierce et al. 2004; Kidera 2008; Somaweera et al. 2011). SCA represents a standard tool used by ecologists in the study and determination of crocodilians diet (Nifong et al. 2012). Good quantitative dietary data can be obtained when collecting relatively large sample size (Pierce et al. 2004). As accurate as this approach can be in providing a snap-shot of the consumer's diet, it also presents some drawbacks. The relatively extensive sample size, labor, cost, and amount of time required to obtain dietary knowledge of a species via SCA makes it a challenging technique to use, particularly when dealing with a predator feeding on a wide variety of prey (Pethybridge et al. 2011; Bowen and Iverson 2013). There are several flushing techniques used to collect stomach contents with variable respective efficiency (Fitzgerald 1989). However, retrieving content from recently sacrificed individuals is by far the most reliable method as no prey items (e.g. crustacean parts) are subject to being caught within the stomach or esophagus as observed by Nifong et al. (2012). Sacrificing the animal is not always feasible or appropriate, especially when dealing with endangered crocodilians. For this study, it became a possible approach due to the exotic and least concern status associated with spectacled caimans in Puerto Rico (IUCN, last assessed in 1996).

Regarding the analysis of stomach content, biases in terms of prey importance may result from different rate of digestibility depending on the type of prey (Janes and Gutzke 2002). For example, arthropods chitinous exoskeleton is likely to remain in the consumer' stomach for longer periods of time than an annelid, thus, resulting in a potential overestimation of consumed arthropods. Another source of bias may occur due to secondary ingestion. For instance, invertebrates found in a crocodilian' stomach may have been previously ingested by a fish which in turn became prey to the crocodilian. However, secondary ingestions have been disregarded as a substantial source of bias in many crocodilian species (i.e. *Crocodylus niloticus*, *Alligator mississippiensis*, *Crocodylus johnstoni*, *Melanosuchus niger*, and *Caiman crocodilus*) while differences in prey items retention were a more important limitation (Cott 1961; Jackson et al. 1974; Webb et al. 1982; Laverty and Dobson 2013). In spite of discounting secondary ingestion as a substantial source of bias, it must be taken in consideration when making inferences about foraging habits of larger caimans (Jackson et al. 1974).

Several methods can be used in the analysis of stomach content. Every individual prey item may be counted to quantify the diet. However, doing so present many potential sources of error considering that prey item may be fragmented making it difficult to determine, for example, how many coleopterans were consumed out of a cluster of legs, thoraxes, abdomens, and heads. Quantifying volume and mass is another approach that can be used to analyze stomach contents. However, this type of approach is constrained by the time of the last meal and digestion rate rather than by the amount of prey consumed (Taylor 1979). Although not completely free of biases, calculating frequency of prey group occurrence may reduce some of the errors associated with digestive rate variation (Rosenberg and Cooper 1990, Baker et al. 2013).

Methods

Study area

Caiman crocodilus proliferation in Puerto Rico appears to have initiated principally from the Tortuguero Lagoon which was designed as a natural reserve in 1971 and currently under the management of the DNER (Schwartz and Henderson 1991; Thomas and Joglar 1996). It is located between the municipalities of Vega Baja and Manatí at the latitude-longitude coordinates of N 18.46328 and W -66.43962. The reserve contains the largest freshwater lagoon in Puerto Rico covering an approximate surface area of 2.43 km^2. The lagoon holds a water volume of approximately 2.68 million m^3 with a mean depth of 1.2 m. It is fed by subterranean, mainly fresh water sources in addition to precipitation (approx. 1,600 mm/ year) and flushed about 7.5 times per year through a canal built in 1940 leading to the sea on the north-east side (Quiñones-

Márquez and Fusté 1978). Other losses include an estimated evapotranspiration rate of 1,274 mm per year. Temperatures oscillate from 24 ºC to 31 ºC in accord with seasonal changes with the absence of vertical temperature gradients. The wet and dry season occur from May to November and from December to April respectively.

The lagoon's surrounding habitat composition includes swamps, marshes, coastal shrubs, and hills. A total of 717 plants species have been recorded including 144 endemic and/or endangered species. Consequently it is considered the fourth most important flora in Puerto Rico. Among the endemic plants, the insectivorous sundew (*Drosera capillaris*) and bladderwort (*Utricularia sp*.) are unique to this reserve. As one of the only two natural reservoirs of the island, the Tortuguero Lagoon is of great ecological importance due to its inhabiting and surrounding abundant wildlife including an observed 83 bird species, 23 fish species, native reptiles, and amphibians. It represents a rich and diversified food source for opportunistic foraging *Caiman crocodilus*. Quiñones-Márquez and Fusté (1978) suggested that due to the fragility of the lagoon as an ecosystem, disturbances should be minimized in order to maintain its stability.

Sample Collection

Between October 2014 and May 2015, 138 recently sacrificed caimans were obtained from the DRNA as part of routine night hunts conducted within the lagoon since the established population management protocol in 1985. Total Lengths (TL) and Snout-to-Vent Lengths (SVL) were measured. To account for sexual dimorphism in *Caiman crocodilus* size, each carcass was categorized into its respective size class according to its Snout-to-Vent Length (SVL, cm) following the guidelines of Ayarzagüena (1983). Measured individuals were classified as hatchlings (Size Class I < 20 cm), juveniles (Size Class II = 20–59.9 cm), sub-adults/ adult females (Size Class III = 60–89.9 cm), and adult males (Size Class IV ≥ 90 cm). Spring scales of 5 kg, 10 kg, and 20 kg were used to record the weight of larger individuals. A Denver Instrument XL-3100D electric scale was used to measure the weight of smaller caimans, mainly hatchlings. Sufficiently large individuals were sexed by finger probing through the cloacal opening. All caimans were dissected, their stomach content collected and stored in a 70% ethanol solution.

Stomach Content Analysis

Collected stomach contents were placed in a fine sieve and rinsed out with water to remove mucous substances and gastric juices following Thorbjarnarson (1993). Retrieved prey remains were identified at the lowest taxonomic level possible using a dissecting microscope when necessary and placed into one of ten designated prey categories (i.e. birds, mammals, reptiles, amphibians, fish, gastropods, crustaceans, insects, myriapods, arachnids). As mentioned before, some prey parts may remain in the stomach of crocodilians for much longer period of time than others (e.g. chitinous exoskeleton of insects, keratinized scutes of reptiles) (Janes and Gutzke 2002). To reduce some of the bias associated with different rates of digestibility for different prey items, only prey categories presenting substantial evidences (e.g. recently ingested or partially digested) were recorded. To further minimize this bias, the analysis was performed based on prey category frequency of occurrence (%) rather than quantifying stomach contents' volume or weight. This represents a convenient technique and the most robust and interpretable approach when quantification of individual prey items present considerable limitations (Rosenberg and Cooper 1990, Baker et al. 2013).

A Mann-Whitney U test and a Kruskal−Wallis analysis of variance by ranks were used to determine any possible difference of prey category utilization between sexes and across ontogenetic stages respectively, based on nonnormally distributed dietary data. A Spearman Correlation test was used to evaluate potential correlation between prey category utilization and caiman size (SVL). These statistical analyses were conducted using the R Studio software (R Core Team 2014, version 3.1.2).

Results

A total of 138 caimans from the four size classes were captured. However, only two belonging to size class IV (adult males ≥ 90 cm SVL) were encountered. This class was then merged with size class III, now including all adults regardless of their sex or size. The sex of 81 (29 females and 52 males) individuals was successfully determined. Although the presence of birds, mammals, and amphibians were only recorded in male caiman stomachs, the Chi-Square test revealed no significant difference in the consumption of any of the established prey categories between the two sexes ($P > 0.05$).

Prey Category Occurrence Frequency

Aquatic insects were the most frequently recovered prey from the stomach (Table 1). Members of the genus Belostoma made up the greatest percentage of consumed insects followed by larva of the family Stratiomyidae (Table 2). Fish were another commonly found prey item, especially in adults. Yet only two were successfully identified as *Eleotris sp*. and *Oreochromis sp*. Unlike the fish category, aquatic gastropods were found across all caiman life stages with a higher occurrence frequency of *Melanoides tuberculata* and *Tarebia granifera*. Members of the genus Macrobrachium comprised the majority of crustaceans successfully identified although this prey category was not as commonly found as those previously mentioned. 81.8% of arachnids encountered belonged to the family Pisauridae. Prey from other categories did not represent a substantial component of stomach contents. Gastroliths were recovered from seven stomachs, but were absent in hatchlings. Plant material, mainly seeds and grass, were found in 39.1% of all stomachs. Ingested plant material was found in hatchling stomachs (14.0%), juvenile stomachs (45.8%), and in the majority of adult stomachs (55.3%). Human refuse was found in juveniles and adult stomachs and included glass, rubber, metal, and plastic materials. Empty stomachs were encountered in 14 individuals, with occurrence higher in adults (17.0%) than juveniles (6.3%) and hatchlings (7.0%).

Ontogenetic Variation of Prey Category Use

The frequency of the three most abundant prey categories (i.e. Insects, fish, and gastropods) significantly varied across life stages (Fig.1). The consumption of insects decreased as caimans increased in size (Spearman $r = -0.626$, $df = 2$, $P < 0.001$). In contrast, the consumption of fish increased with caiman size (Spearman $r = 0.448$, $df = 2$, $P < 0.001$). Likewise, gastropods were found more commonly in the larger caiman sizes although they occurred slightly more frequently in juveniles (Spearman $r = 0.448$, $df = 2$, $P < 0.001$).

Discussion

The findings of this study based on stomach content analysis were, to some extent, similar to those reported from native *Caiman crocodilus* in regions of the Amazon (Magnusson et al. 1987) and in the Central Venezuelan Llanos (Thorbjarnarson 1993). For instance, the increase in consumption of fish and decrease in that of insects with size were consistent in both native and exotic caimans. Such patterns have been previously reported in many dietary studies of crocodilians as a means to reduce intraspecific competition among life stages (Horna et al. 2001). Also, the most abundant invertebrates in stomachs of both native and exotic caimans were aquatic Coleopterans and Hemipterans (Belostomatidae). However in caimans from the central Venezuelan Llanos, no Dipterans were reported in their diet whereas in the exotic caiman, larva from the families Stratiomyidae, Syrphidae, and Tabanidae combined represented major component (54.4%) of ingested insects. In some of the smaller caiman stomachs, as much as 6 to 8 individual dipteran larvae were observed although counting as a single entry for the presence of insects based on the occurrence frequency analysis. Also, Thorbjarnarson identified all consumed crustaceans as the freshwater crab *Dilocarinus dentatus*. In this study, freshwater prawns of the genus Macrobrachium and shrimps made up for the majority of identified crustaceans. In spite of high dietary similarities, it is not surprising to find some differences in composition of ingested prey considering the opportunistic and generalist nature of the spectacled caiman's foraging behavior. Its diet is likely to reflect the prey composition of the environment it inhabits (Magnusson et al. 1987), thus, explaining the variation in the frequency of occurrence of some taxa. Similar to other crocodilian species, my results indicate a much greater consumption of aquatic prey in contrast to terrestrial prey. The two most commonly encountered terrestrial prey species were members of the genus Phyllophaga (Scarabaeidae) and mice (*Mus musculus*). The former has been observed (pers. obs.) numerous times clinging onto surrounding aquatic vegetation (mainly *Typha domingensis*). These nocturnal beetles are known to be attracted to light and are considered to be poor flyers compared to other flying insects. It is possible that their attraction to the reflection of the moon in the lagoon or other light sources may be responsible for their accidental presence in the water (pers. com.). In regards with *Mus* *musculus*, it is known to be an able swimmer particularly in still water bodies (Hiadlovská et al. 2012) and they may have been consumed while swimming at the water surface.

In this study, size class IV caimans were largely lacking likely due to the project location. Caimans from the Tortuguero Lagoon are occasionally hunted for their meat by inhabitants of the surrounding municipalities in addition to the DNER's active population control. The potential occurrence of some of the largest vertebrates of the area (e.g. domestic mammals, iguanas, aquatic birds) in stomachs may therefore have been underestimated. The only evidence of bird consumption included parts of chicken (*Gallus gallus domesticus*) found in two adult males, one of them containing a curled up metal wire used for restraining live baits.

CHAPTER 3

STABLE ISOTOPE ANALYSIS OF THE INTRODUCED SPECTACLED CAIMAN (*CAIMAN CROCODILUS*) IN TORTUGUERO LAGOON, PUERTO RICO

Introduction

Dietary study methods vary depending on the specific aim of the study. Some are more informative than others. Nonetheless, the disadvantages that one technique presents can be compensated by the advantages of another to enhance the resolution of the diet composition (Tierney et al. 2008). Advances in the field of stable isotopes analysis (SIA) have proven to be advantageous in the prediction of foraging impacts in invasive top predators (Bodey et al. 2011). While SCA provides a snapshot observation of a consumer's diet, combining SIA methods becomes very useful to obtain a better understanding and a more complete dietary history of the predator by presenting both recent and average long-term intake (Meckstroth 2007). The temporal scale of the diet revealed by stable isotopes depends on the type of tissue used (e.g. scutes, teeth, muscles, blood). Moreover, biases associated with different rates of digestion present in SCA are less relevant when using SIA since food is assimilated rather than ingested (Vander Zanden et al. 1997). According to Meckstroth (2007), combining these two methods of analysis allows a more reliable assessment of diet composition, which can be used by managers to plan and evaluate their predator-removal strategies while also improving our understanding of the impacts of exotic or native predators on prey populations.

A study combining SCA and SIA on the gray snapper (Lutjanus griseus) indicates that using a single element (e.g. carbon) may not be sufficient to make a distinction between isotopically similar food sources or diets incorporating more than two food sources (Harrigan et al. 1989). However the study in question suggests that such limitation can be reduced by the use of a second element (e.g. nitrogen). These stable isotopes are present in the tissues of consumers and reflect the isotopic composition of their food sources assimilated during the synthesis of these tissues. Stable carbon isotopes ($\delta 13C$) can determine different sources of primary productivity (i.e. the predator food source ecosystem or biome) (Bodey et al. 2011) whereas stable nitrogen isotopes (δ15N) can be used to identify the trophic positions of both predators and prey revealing a 3-5 ‰ stepwise enrichment with each rise in trophic level (Hobson and Clark 1992; Pierce et al. 2004). The delta (δ) notation represents the isotope composition as the ratio of the heavy to the light isotopes (i.e. 13C:12C and 15N:14N) relative to a standard, typically the reference materials from Viena PeeDee Belemite (VPDB) and the ambient air nitrogen. Isotopic values are expressed in parts per thousand (‰) such that:

δ X = [(Rsample/Rstandard)-1] x 103 where,

X = 13C or 15N
R = 13C:12C or 15N:14N

In ecological studies, SIA can be put to many uses including but not limited to examining specific predator-prey interactions in food webs (Inger and Bearhop 2008), tracing animal migratory patterns (Hobson 2005; Zimmo et al. 2012), or evaluating intra-specific isotopic niche variation (Marques et al. 2013). However some limitations associated with SIA include an expansive procedure depending on the sample size and the possibilities of erroneous isotopes value due to inadequate storage, sample processing, and fractionation, thus, rendering the interpretation of isotopic signatures challenging. Other complications arise as the predator's diet variability increases (Philips and Gregg 2003). Still such limitation can be overcome by grouping some of the items with respect to similar functional significance (e.g. herbivorous fish, insectivorous fish, insects, aquatic plants). In fact, such aggregation of food sources is strongly recommended when the prey items are highly diversified and when their isotopic values are not significantly different (Philips et al. 2005).

Although SIA is deficient in providing high taxonomic resolutions of consumed prey species, it reveals the relative diet source proportion with a priori knowledge of the prey species inhabiting the novel environment. Especially in the context of a generalist predator, where prey categories rather than specific prey species are to be assessed, SIA provides relevant information to predict direct dietary impacts. Time is of the essence when dealing with recent introductions. In spite of failure to prevent the spread of an exotic, an early detection of the potential impacts can play a major role in the careful design of management strategies and establishing priorities in mitigating the threat.

Methods

Sample Collection

For this study, 19 hind leg muscle samples were collected from caiman of all life stages and both sexes. In addition, samples from 3 distinct individuals of selected food sources were obtained representing the following groups: aquatic plants, gastropods, crustaceans, insects, insectivorous fish, and herbivorous fish. The different selected prey groups were those most commonly found in preliminary caiman stomach content collections, as well as potential prey species observed in the lagoon. All predator and prey samples were obtained from randomly selected location of the lagoon. Depending on the size of the organism, muscle samples or the whole organism was processed. Samples were dried at 60 ºC (Shellab oven) for 72 hours. They were then reduced to a fine powder processed through the Retsch MM200 grinder for one minute at 27 revolutions per second. Between 0.9 mg and 1.2 mg for each animal samples and 4.5 mg to 4.8 mg for each plant samples were weighed using an electronic scale (Metller Toledo Classic Plus; last calibrated on October 7, 2013) and placed into tin capsules pressed for micro analysis. Capsules were sent to the Stable Isotope Laboratory of Miami University, Florida to determine nitrogen and carbon isotopic compositions. δ13C values were lipid-normalized prior to analysis based on the non-linear model provided by Kiljunen et al. (2006).

Stable Isotope Analysis

Potential difference of isotopic composition (δ13C and δ15N) between sexes and life stages were evaluated using MANOVA. When significant differences were found, ANOVA was then used to enhance the resolution on the contribution of each isotope to the results obtained with MANOVA. Spearman’s correlation analysis was used to determine the relationship of stable isotope compositions and size (SVL). The relative contribution of each prey group or food source to hatchling and adult caimans was determined using the Bayesian stable isotope mixing model in the SIAR (Stable Isotope Analysis in R) statistical package following guidelines of Parnell et al. (2010). The model was based on 5 x105 iterations with burnin set to 5 x 104 and thinning set to 15. Trophic enrichment or discrimination factors are the differences in isotopic values between an organism's tissue and its diet. Stable isotope mixing models rely on these factors for dietary reconstructions and trophic-level estimations (Vander Zanden et al. 2012). For this study, we used trophic enrichment factors of 3.49 ± 0.23 and 0.8 ± 0.2 for Δ15N and Δ13C respectively. These values were based on average values obtained from a compilation of aquatic food web studies (Vander Zanden and Rasmussen 2001). Statistical analyses were conducted using the R Studio software (R Core Team 2014, version 3.1.2).

Results

Carbon and Nitrogen Isotopic Values

A total of 19 caimans from different life stages, 15 prey, and 3 plants were used to obtain carbon and nitrogen isotopic values (Table 3). Selected prey reflected an aquatic diet enriched in δ15N (Fig.2), especially observed in crustaceans (16.65 ± 1.33 ‰). Stable isotopes composition did not significantly differ between caiman sexes. However, they did show significant variation across ontogenetic stages (MANOVA: $F_{2,16} = 5.35$; $P = 0.002$). A separate analysis revealed that δ13C values did not differ significantly (ANOVA: $F_{2,16} = 51.62$; $P = 0.228$) between caiman life stages whereas δ15N differed significantly (ANOVA: $F_{2,16} = 13.71$; $P < 0.001$) between life stages. The Tukey's test revealed a significant difference in δ15N values between all stages except between hatchlings and juveniles. A Spearman's correlation analysis indicated a significant positive relationship (Spearman $r = 0.539$, $df = 13$, $P < 0.047$) between δ15N and SVL when excluding hatchling (Fig. 3).

Isotopic Source Proportions

In both adults and juveniles, herbivorous fish and insects, on average constituted the most important prey sources (Fig. 4). Nevertheless herbivorous fish (33%) rather than insects (29%) were slightly more important in adult diets. The opposite was true for juvenile diets (26% from herbivorous fish and 29% from insects). Insectivorous fish, aquatic snails, and crustaceans were slightly (2–3%) more important in juvenile than adult diets.

Discussion

Stable isotope analyses in addition to my previous stomach content analysis provided a greater resolution of the caiman diet in Puerto Rico. Surprisingly, higher nitrogen isotopic values were found in prey relative to the predators. This is likely due to the nitrogen rich ecosystem that the TLNR represents. Fertilizers used for pineapples cultivation is a primary source of ground water nitrate concentrations in these areas (Conde-Costas and Gómez-Gómez 1999). Nonetheless, high nitrogen values were not reflected in the caimans suggesting that their food source may be obtained from additional environments outside the reserve. Adult male caimans can be territorial (especially during the breeding season; Staton and Dixon 1977), however, heavy hunting activities in these areas may be responsible for a constant flow of immigrants from nearby areas as unattended suitable territories become available. Moreover, every night of sample collection at the lagoon, 20-30 caiman sightings were reported regardless of the number of caimans previously removed. Their seemingly rapid dispersal behavior and ability to fill in empty territories may have contributed to their successful establishment throughout Puerto Rico. The lack of statistical significance in carbon isotopic values between life stages suggested that the assimilated food source belonged to a similar ecosystem. In contrast, nitrogen isotopic values significantly varied across ontogenetic stages representing food assimilated from different trophic level except for values between hatchlings and juveniles (Table 3). In fact, the mean nitrogen values were found to be higher in hatchlings than juveniles. Such results seem contradictory to predictions that nitrogen isotope composition increases with diet trophic level as caimans develop. However, hatchlings may assimilate the higher nitrogen values from their yolk sacs, which are representative of the adult female's tissues before the hatchlings begin to feed independently. A subsequent decrease in nitrogen values is then expected as recently hatched caimans forage on primary and secondary consumers of lower nitrogen compositions. Consequently, hatchlings were excluded from the assessment of source proportion across life stages. Food source relative proportions determined between juveniles and adults generally agreed with the results obtained from stomach content analyses (SCA) in terms of prey group importance and diet variation. For instance, fish, particularly herbivorous ones, and insects made up the greater portion of their diet (Fig. 4). Another agreement between the two analyses was observed where gastropods became less important in adults compared to juveniles. However SIA results suggested that crustaceans were a more important diet source than aquatic gastropods in the diet of both life stages in contrast to SCA findings. The over representation of gastropods in the SCA relative to SIA results likely is attributable to their lower digestibility in caiman stomachs. With that exception in mind, the dietary patterns obtained from SIA remained consistent with those of SCA. This study demonstrates how SIA can provide a reasonably good quantitative estimate of various food source contributions given prior knowledge of the diet of a predator.

A determination of the foraging habits of spectacled caimans in Puerto Rico was best performed using a combination of SCA and SIA rather than any one method alone. This study represents the baseline, the first study conducted on dietary habits of this exotic predator since its introduction over 50 years ago. Appropriate introduced population management is a delicate task and one that must be carefully carried out, especially dealing with established exotic predators, as their removal can have undesired repercussions on ecosystems (Prugh et al. 2009, Courchamp et al. 2011). Meckstroth et al. (2007) advise resource managers to obtain comprehensive knowledge of an introduced predator's diet before implementing removal protocol. In this study, documented predation on native species was scarce including two hatchling Puerto Rican sliders (*Trachemys stejnegeri stejnegeri*). We found no other evidence of predation on endangered or threatened species. In contrast, a much greater percentage of exotic including some considered invasive prey were found in stomachs. To this date, at least for the size classes sampled in this study, there is no substantial evidence that the spectacled caiman have a significant direct impact at multiple trophic levels or are the cause of major food web disruptions on the island. Its diversified diet as opposed to a selective diet may represent a minimizing factor in terms of predation pressure it may have on some prey population. Likewise, it could be responsible for mesopredator population control providing some predation release on lower trophic levels. In order to further our understanding on its foraging behavior, similar dietary studies should be performed in areas where caimans pertaining to size class IV are more common as their diet may include unrecorded larger vertebrate species. Despite an active population management program, changes among prey population dynamics in or outside the natural reserve remain virtually unmonitored, an essential but too often omitted step in order to effectively assess impact on the ecosystem. Furthermore, exotic caiman population estimates, geographic distribution, and growth rates are currently unknown from Puerto Rico. Thorough predator and prey population monitoring is the next measure to take to adequately assess potential exotic caiman effects on the environment.

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Table 1. Spectacled caiman prey category, plant material, gastroliths, human refuse, and empty stomachs based on occurrence frequency in stomachs sampled from the Tortuguero Lagoon Natural Reserve, Puerto Rico. n = number of caiman stomachs analyzed for each size class. No. = number of stomachs containing substantial evidence of the indicated category.

	Size Class (n)					
	I (43)		II (48)		III (47)	
Caiman Prey category	No.	%	No.	%	No.	%
Insects	39	90.7	33	68.8	7	14.9
Fish	0	0.0	9	18.8	18	38.3
Gastropods	1	2.3	12	25.0	10	21.3
Crustaceans	5	11.6	5	10.4	4	8.5
Birds	0	0.0	0	0.0	2	4.3
Mammals	0	0.0	3	6.3	2	4.3
Reptiles	0	0.0	5	10.4	3	6.4
Amphibians	0	0.0	2	4.2	0	0.0
Myriapods	2	4.7	2	4.2	2	4.3
Arachnids	5	11.6	5	10.4	1	2.1
Plant Material	6	14.0	22	45.8	26	55.3
Gastroliths	0	0.0	4	8.3	3	6.4
Human Refuse	0	0.0	2	4.2	6	12.8
Empty	3	7.0	3	6.3	8	17.0

Table 2. Spectacled caiman prey composition from caiman stomach samples obtained from the Tortuguero Lagoon Natural Reserve, Puerto Rico. Percentage composition of each prey taxa is determined separately for each major prey category (i.e., insects, fish, gastropods, birds, mammals, reptiles, amphibians, crustaceans, myriapods, arachnids) and represents the percentage of stomachs with presence of the specified prey taxa. All caiman size classes are included in this analysis and sample sizes of stomachs with each major prey category are shown in Table 1.

Prey Item	Composition (%)
Insects	
Diptera (Larva)	54.4
Belostoma sp.	43.0
Phyllophaga sp.	27.8
Dytiscidae	27.8
Odonata (Larva)	10.2
Unid. Coleoptera	6.4
Gerridae	6.3
Ephemeroptera	3.8
Hymenoptera	3.8
Hydrophilidae	1.3
Fish	
Eleotris sp.	3.7
Oreochromis sp.	3.7
Unidentified	96.3
Gastropods	
Melanoides tuberculata	47.8
Tarebia granifera	39.1
Marisa cornuarietis	13.0
Neritina sp.	8.6
Bulimulus guadalupensis	4.3
Birds	
Gallus gallus domesticus	100.0
Mammals	
Mus musculus	60.0
Unidentified	40.0
Reptiles	
Trachemys scripta elegans	50.0
Trachemys stejnegeri stejnegeri	25.0
Caiman crocodilus	25.0
Amphibians	
Lithobates catesbeianus	50.0
Unidentified Anuran	50.0
Crustaceans	
Macrobrachium sp.	28.5
Palaemon pandaliformis	14.3
Unidentified	64.3
Myriapods	
Diplopoda	100.0
Arachnids	
Pisauridae	90.9
Hydrachnidiae	9.1

Table 3. Mean (±SD) $\delta^{15}N$ and $\delta^{13}C$ values for collected caiman, prey, and aquatic plant samples obtained from the Tortuguero Lagoon Natural Reserve, Puerto Rico. Tissue type, sample size (n) and snout-vent length (SVL) are also displayed when applicable.

Sampling Source	Tissue Type	n	SVL (cm)	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Adult Males	Muscle	5	72.60 ± 6.43	12.38 ± 0.28	-24.64 ± 1.53
Adult Females	Muscle	4	66.50 ± 3.87	13.60 ± 1.45	-24.29 ± 2.40
Juveniles	Muscle	5	50.00 ± 3.94	9.10 ± 1.86	-24.75 ± 1.46
Hatchlings	Muscle	5	14.38 ± 0.70	10.98 ± 1.05	-22.89 ± 2.09
Herbivorous Fish	Muscle	3		14.86 ± 1.27	-25.49 ± 0.46
Omnivorous Fish	Muscle	3		14.91 ± 0.11	-21.14 ± 0.21
Insects	Whole body	3		10.34 ± 0.70	-22.3 ± 2.18
Aquatic Snails	Soft tissue	3		14.13 ± 0.35	-17.89 ± 1.44
Crustaceans	Soft tissue	3		16.65 ± 1.33	-21.95 ± 1.47
Aquatic Plants	Leaf fragments	3		2.19 ± 3.84	-28.62 ± 2.04

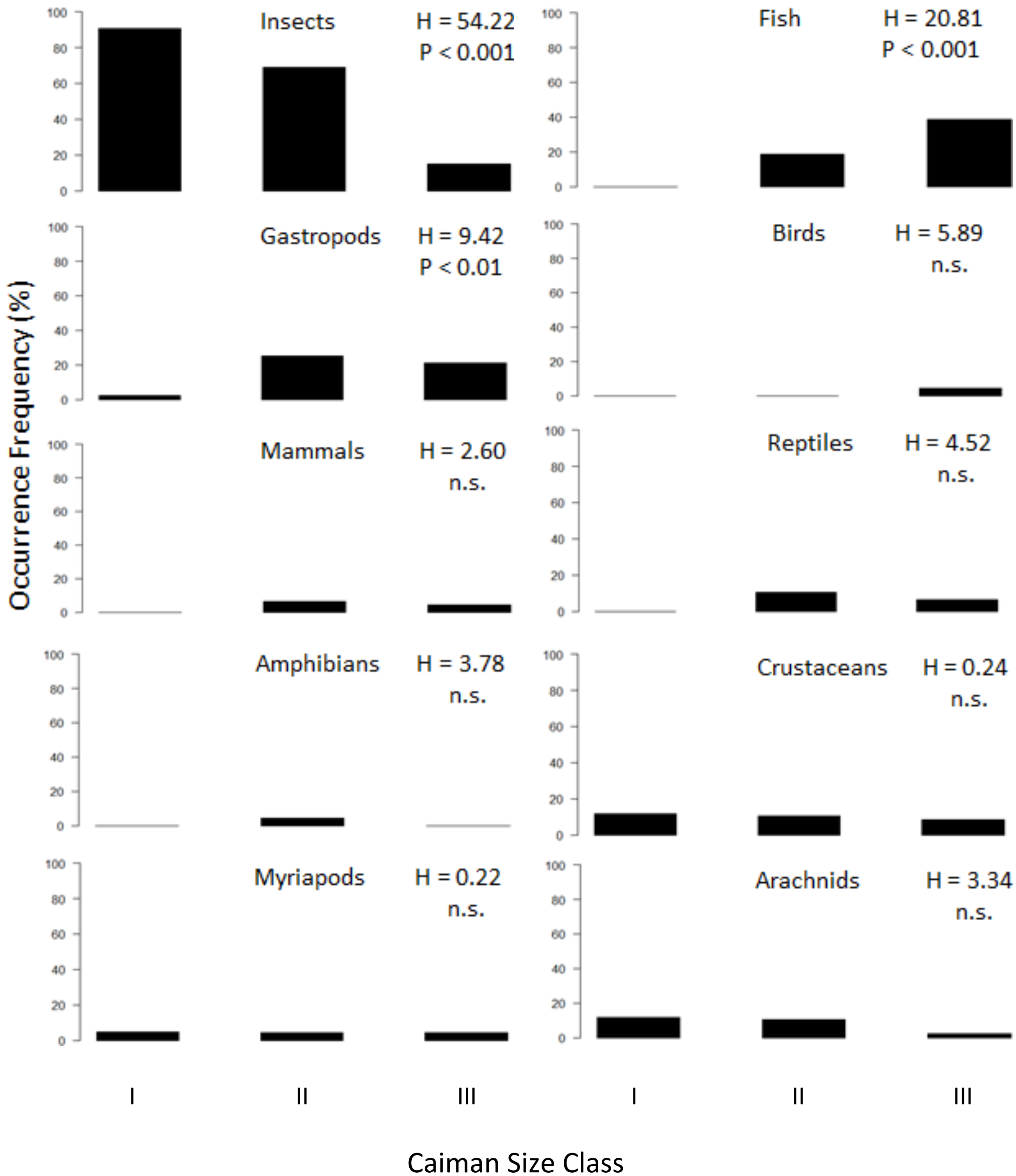

Figure 1. Variation in spectacled caiman prey category occurrence frequency across size classes I, II, and III (i.e. hatchlings, juveniles, and adults) with respective level of significance (*P*-value) as sampled from the Tortuguero Lagoon Natural Reserve, Puerto Rico. The Kruskal-Wallis statistic (H) shows the degree of discrepancy among rank sums (i.e. a higher H value represents a higher discrepancy among size classes).

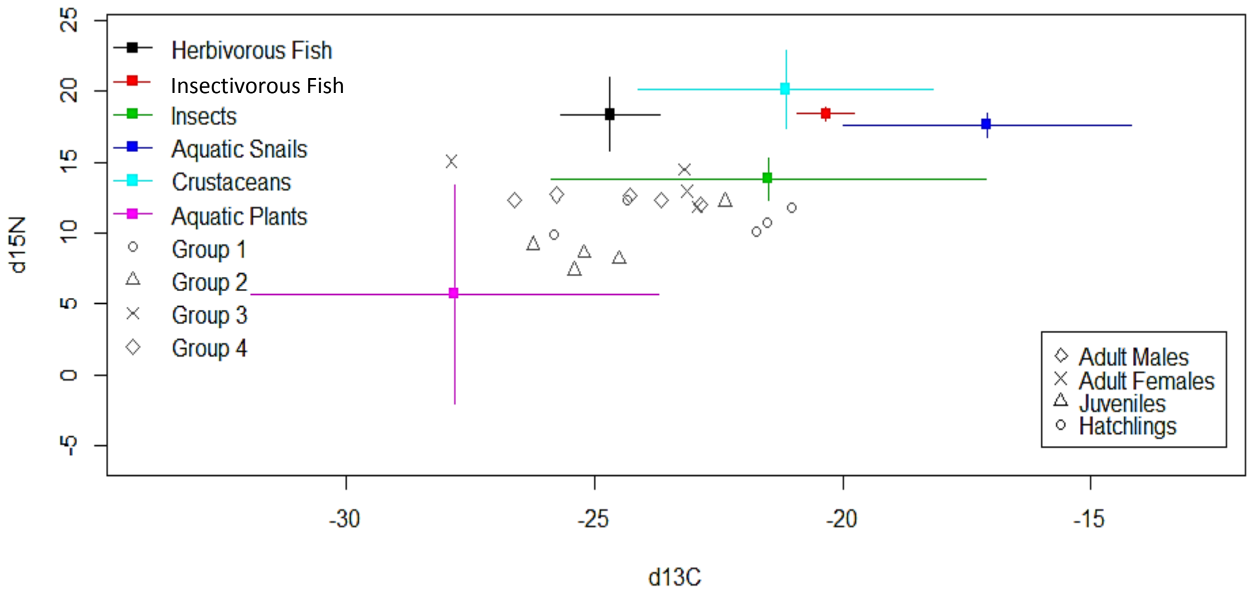

Figure 2. Carbon and nitrogen isotopic composition (‰) of spectacled caiman (adult males, adult females, juveniles, and hatchlings), prey, and aquatic plant tissues collected at random location within the Tortuguero Lagoon Natural Reserve, Puerto Rico.

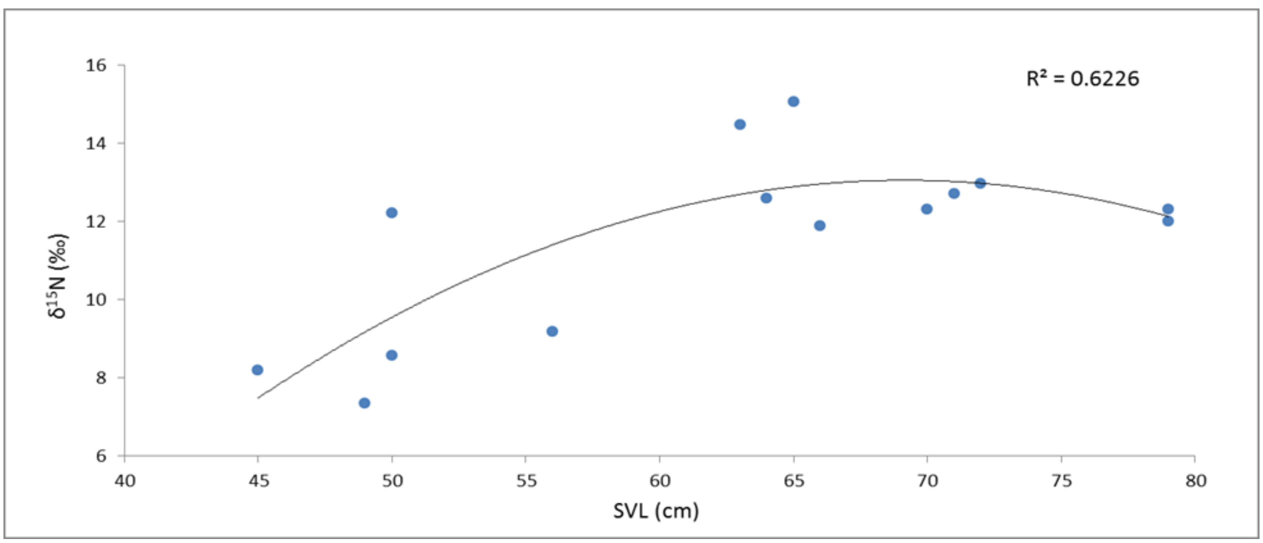

Figure 3. Relationship between stable isotope compositions of $\delta^{15}N$ and SVL of sampled spectacled caimans in the Tortuguero Lagoon Natural Reserve, Puerto Rico. The trend line represents a significant positive relationship between $\delta^{15}N$ and SVL when excluding hatchling from the analysis.

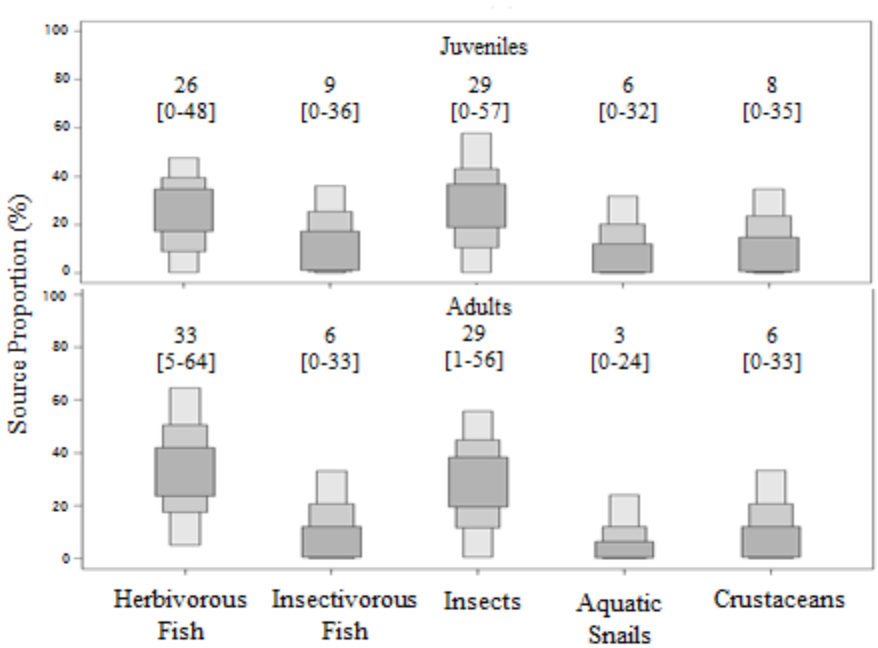

Figure 4. Diet source proportion average percentage estimated for juvenile and adult spectacled caimans using the Bayesian mixing model SIAR (version 4.2). Values [low-high] based on a 95% confidence interval are also displayed. Tissues were obtained from caimans collected in the Tortuguero Lagoon Natural Reserve, Puerto Rico.