

**PROJECT**

**Conservation Biology of Yellow Anacondas (*Eunectes notaeus*) in Argentina: Assessing habitat use and somatic growth through radio-tracking techniques**

**Summary Report**



**Fundación Biodiversidad, Argentina**

June 2017

**Project title: Conservation Biology of Yellow Anacondas (*Eunectes notaeus*) in Argentina: Assessing habitat use and somatic growth through radio-tracking techniques**

**Summary report**

**Introduction**

The yellow anaconda is one of four species of boas that inhabit South America, and is the largest found in Argentina. Yellow anacondas have harvested for decades, often illegally, for their highly valuable skins. They are currently being managed under a sustainable-use program, the Yellow Anaconda Management Program (YAMP) launched in 2002. This program promotes anaconda conservation, as well as research, while providing a genuine source of income for impoverished rural communities.

Between the years 2007 and 2011, the Office vétérinaire federal (Switzerland) funded research activities of YAMP, on an annual basis, through the support of the Project “*Harvest of Yellow anacondas (Eunectes notaeus) in Argentina: Assessing Parameters of Biological Sustainability*”. Between 2013 and 2016 the Office veterinaire federal continued its financial cooperation by funding a radio-tracking study on the yellow anaconda (*Eunectes notaeus*) in Formosa.

The uninterrupted cooperation provided by the Office vétérinaire federal during all these years, has permitted the inclusion of 2 field biologists from 2008 to 2016, with the additional benefit of gathering invaluable biological and harvest data throughout this period.

This report summarizes activities, main results and financials of the activities funded by the radio-tracking project.

**Study site**

Field work was carried out at “Bañado La Estrella”, located in the Northwest extreme of the Province of Formosa (Argentina), bordering Paraguay, and limiting west to the Province of Salta. The working site was “Fortín Soledad”, located in the geographical core of the “Bañado La Estrella”.



Fig. 1. Map of Formosa with study site (red).

### Project aim

To evaluate somatic growth, habitat use patterns, thermal preferences, and reproductive behaviour in the yellow anaconda (*Eunectes notaeus*) by using an innovative approach based on radio-tracking techniques.

### Expected results according to original project

- Somatic growth rate and trajectories during the first year of life.
- Habitat use for different life classes.
- Movement patterns throughout different seasons.
- Thermal preferences among life classes.
- Dry-season refugia
- Additional biological information

### Main activities carried out

- Design and acquisition of temperature data loggers and radio tracking equipment for implantation (2013).
- Setting of air, water and operational temperature data loggers in the field to capture environmental temperature options (2013).
- Search, capture and implantation of equipment in 13 yellow anacondas: 8 mature females + 5 mature males (2013 – 2014).
- Monthly radio tracking of implanted snakes by walk or canoe (2013 to 2016).
- Occasional radio tracking by plane or helicopter (2014 and 2016).
- Data gathering on snake status, weight, activity and position in habitat on each capture session (2013 to 2015).
- Recovery of data loggers from snakes after an 8 to 12-month period to obtain body temperature data (2014 to 2016).
- Collection of harvest and biological data from hunting sessions (2013 to 2016).
- Diet and functional biology studies in collaboration with Brazilian herpetologists.

| #  | Serial | Frequency | Logger? | Lat       | Lon       | Implant date | Name     | Sex | SVL (cm) | Weight (g) |
|----|--------|-----------|---------|-----------|-----------|--------------|----------|-----|----------|------------|
| 1  | F153   | 164.2601  | yes     | -24.10622 | -60.68893 | 23-jun-13    | Willy    | M   | 156      | 3550       |
| 2  | F154   | 164.3001  | yes     | -24.10637 | -60.68417 | 25-jul-13    | Masahiro | M   | 180      | 3750       |
| 3  | F155   | 164.3394  | yes     | -24.10536 | -60.68845 | 27-jul-13    | Chela    | F   | 200      | 4540       |
| 4  | F156   | 164.3800  | yes     | -24.10682 | -60.68279 | 16-ago-13    | Nono     | M   | 178      | 3620       |
| 5  | F157   | 164.4201  | yes     | -24.10630 | -60.68418 | 16-ago-13    | Felipe   | M   | 156      | 2640       |
| 6  | F158   | 164.4600  | yes     | -24.10630 | -60.68418 | 16-ago-13    | Max      | M   | 157      | 2400       |
| 7  | F159   | 164.5002  | yes     | -24.10694 | -60.68424 | 23-jun-13    | Misa     | F   | 194      | 4900       |
| 8  | F160   | 164.5396  | yes     | -24.10531 | -60.68972 | 23-jun-13    | Kristina | F   | 187      | 4040       |
| 9  | F161   | 164.5800  | yes     | -24.10645 | -60.68417 | 25-jul-13    | Nadia    | F   | 257      | 12010      |
| 10 | F162   | 164.6201  | yes     | -24.10574 | -60.68387 | 16-ago-13    | Julia    | F   | 196      | 4850       |
| 11 | F163   | 164.6600  | yes     | -24.10657 | -60.68228 | 16-ago-13    | Mercedes | F   | 281      | 13800      |
| 12 | F164   | 164.6995  | yes     | -24.10613 | -60.68406 | 27-sept-13   | Olivia   | F   | 204      | 7130       |
| 13 | F165   | 164.7404  | yes     | -24.10554 | -60.68846 | 27-sept-13   | Mariel   | F   | 228      | 8460       |

Table 1. Data on 13 yellow anacondas radio tagged.

### **Project results overview**

In 2013 when we started this project, the environmental conditions in the study area were extraordinary. A serious drought obliged to suspend the yellow anaconda harvest season and complicated the development of our normal activities in the area due to low water levels that impede the access to the snakes. Aimed to radio-track mostly pregnant females, the few specimens found were very thin or in bad body condition due to the drought, so in order to keep the season, we decided to implant any available specimen and to follow their fate in the following years with the hope to capture a reproductive event.

The conditions of work in La Estrella marsh were extremely difficult because of the distances involved and the physical obstacles due to wetland vegetation overgrowth. This situation limited our ability to reach every animal in every campaign. The result is that the number of captures and recaptures were low (110 in total for the 13 specimens), sometimes we missed animals for several months or even for the entire study lapse (males). To recover some missed individuals, it was necessary to hire an airplane once, and to ask the support of the local army helicopter (2016) to detect last specimens before the battery run off.

Because specimens were not reproductive due to the drought, offspring were not available and it was not possible to accomplish the somatic growth part of the study. This aspect (the growth in early life stages) continues to be an intriguing but hard to solve issue for this species. Although the preliminary information we have from size distributions obtained in several years of research suggests that the species growth in the first year is fast, doubling its size from one season to another, this still cannot be confirmed by factual data.

As a result of this, we could summarize as follows the success in the different aspects we proposed to study:

- Somatic growth rate during the first year of life: not accomplished.
- Habitat use / movement patterns: partially accomplished
- Thermal ecology: accomplished
- Dry season refugia: accomplished
- Additional information: a collaboration with researcher from Brazil resulted in two papers, one on diet ecology and another on functional biology.

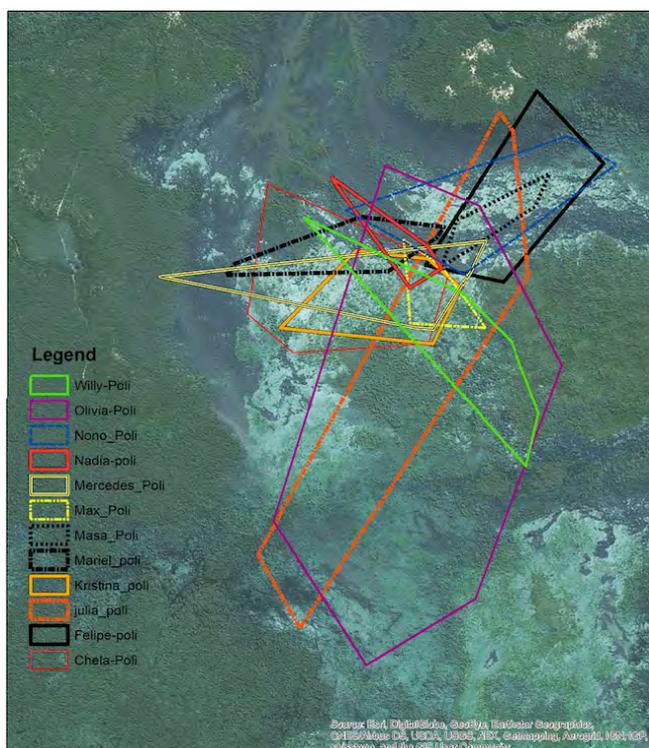
With regard to dry refugia, we confirmed that yellow anacondas do not generally use the vegetation type “totorales” (*Typha latifolia*) during the dry season. This is an important outcome because it was supposed that this type of habitat was used by the anacondas to hide during the annual droughts. That selection would imply an additional mortality source because “totorales” are burnt by local people during every dry season to reduce vegetation cover. Only 5.6% of dry season captures (N=71) involved totorales and always in the ecotone of this habitat with other vegetation types. The commonest habitat types used during the dry season by anacondas were pure palm savannas (59%) and palm-mandiyurales savannas (21%), both habitats that are mostly not affected by year fires.

The commonest specific hiding site during the dry season was concealed in dry or partially flooded “mandiyues” (*Ipomoea carnea*) bushes (39% of sightings), open vegetated water ponds (22%), and inside fallen palm trunks in the dry (12% of sightings). In one case, we found a medium sized specimen hiding inside a tree that was accessed by a 5-cm wide hole. The specimen remained for near a month inside the tree waiting for the water to come until it moves to another place.

During the dry season half of the recaptures took place in the dry and half in water. Against our intuition, during the extreme dry season yellow anacondas do not necessarily use remaining water bodies, and prefer to conceal under bushes or inside dead palms. Remaining water bodies are usually small, over crowded with other animals and water usually deteriorates in quality soon. Cattle trampling might also be another factor that makes this habitat type not necessarily an option during the dry season.

During the wet season yellow anacondas utilized all flooded habitat types in the marsh, being the less common habitat used the “totorales”. This could be explained by the fact it is a very close and homogeneous habitat that might impose limitations for ambushing and capturing prey. The commonest habitat type were open palm savannas with fallen logs and climbing plants where anacondas can hide, bask and eventually ambush preys. During the wet season all captures are in water situations, either exposed basking in emerging vegetation or immersed within vegetation.

Female anacondas made large movements from one month to another but always in a same territory. Some distances covered 2.5 km from one recapture to another. Distance travelled between captures was larger



in the wet season than in the dry season, and so on the territories. The scarce number of recaptures per specimens precludes us from final conclusions but data suggests that females are philopatric, keeping large territories to ambush for preys, while males are the dispersers that moves all over the range without specific territories. The largest area (Minimum Convex Polygon) was covered by a female (Olivia): 410 has, seconded by another female (Julia): 201 has. We prefer not to make conclusions on smaller areas because of the lack of enough data.

Fig. 2. Polygons built from different anaconda recaptures.

We obtained a large body temperature data set from 8 specimens: 1 male and 7 females. These datasets are comprised by 12 temperature records per day from implanted temperature data loggers during 6 to 18 months depending on specimen, accounting for thousands of records. These datasets are being analysed and will permit to understand the thermal ecology of the species in the area. Yellow anacondas in Argentina are in the southernmost limit of its range, so winter temperatures are a limiting factor for them. The thermal data base also includes two year records for air, water and operational temperatures (model that mimics anaconda coloration to measure potential absorption temperatures if basking). Here we present some examples of the information obtained.

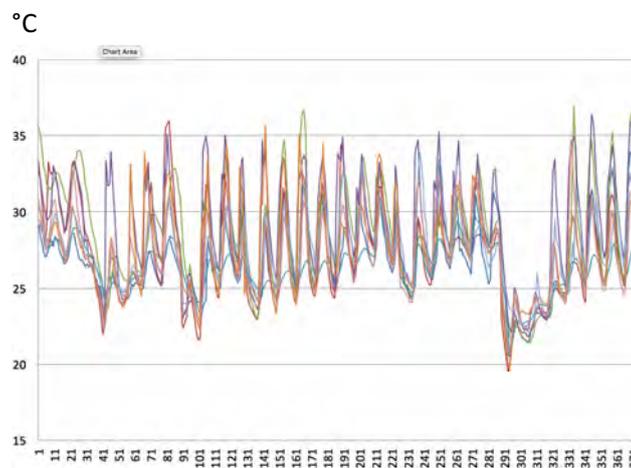


Fig. 3. January (summer) temperature curves for 8 anacondas in La Estrella. Each color represent a specimen. Each peak represents the daily temperature peak achieved. Average summer temperatures for all females pooled averaged 27 degrees Celsius. Striking temperature patterns were observed among snakes, suggesting that temperature achieved is very variable depending on habitat and physiological status of snake (fed vs not fed, gravid vs not gravid, in dry vs wet habitats, etc).

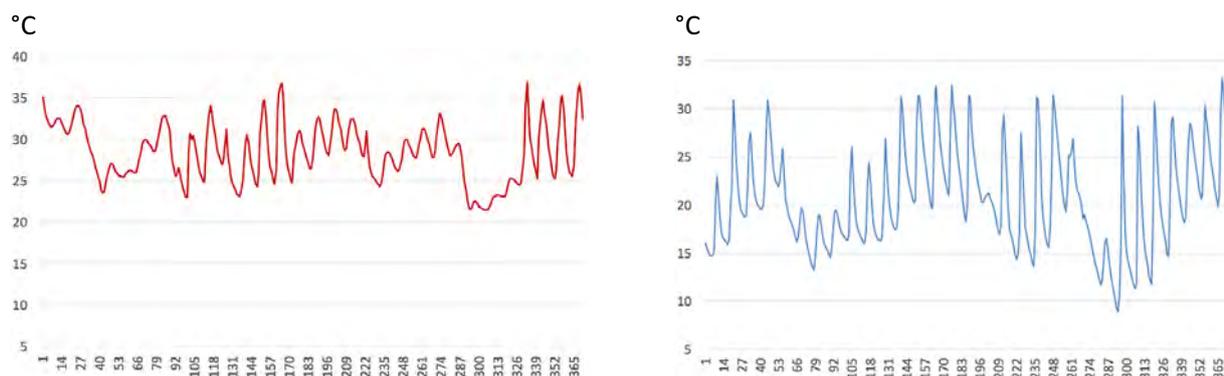


Fig. 4. Summer (January) temperature curve for a female anaconda (left in red) vs Winter (July) temperature curve (right in blue) of the same specimen. Each peak represents the daily temperature peak achieved. Summer temperature averaged 28°C and exhibited a 15°C total range of oscillation with most temperature records between 25° to 35°C. Winter temperature averaged 21°C with great ups and downs due to low temperatures exhibiting an oscillation range of up to 20°C sometimes in the same day.

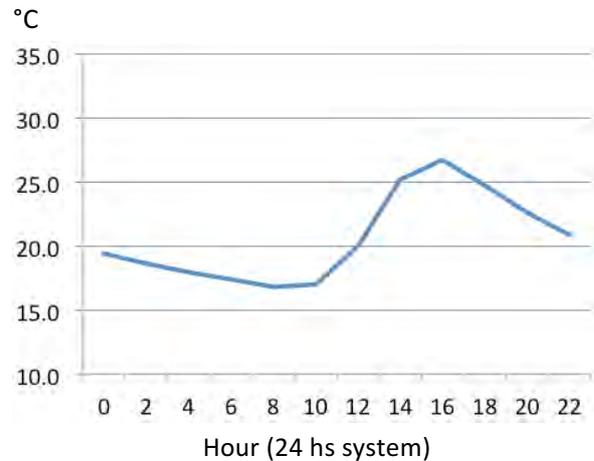
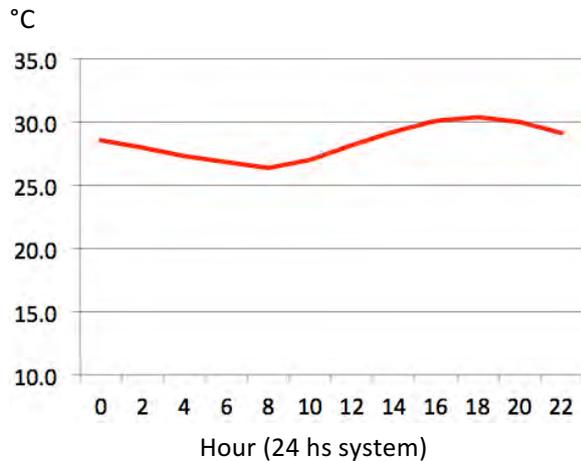


Fig. 5. Single day average temperature pattern in summer (left-red) vs winter (right-blue) for a same specimen. These graphs result from averaging the 31 temperature records obtained in each hour from January (red) and from July (blue), summer and winter, respectively.

### Additional studies carried out with provided funding

The support provided by the Office veterinaire federal permitted to have two biologists in the field for most part of the year to gather biological information during the harvest season, by accompanying hunters during their hunting sessions. This financial support has allowed us to monitor the impact of the harvest at the site level (Fortin Soledad, Formosa), the build-up of a data base of near 150 hunting sessions (days) with more than 2,000 records of snake captures, dissections and basic demographics. Additionally, during 2015 we carried out a collaborative study that was recently published and the support from the Swiss government acknowledged. A second paper has been recently approved for publication (Annex II). Both studies were done in cooperation with two biologists from the University of Mato Grosso (Brazil): 1) "Penny and penny laid up will be many: large Yellow anacondas do not disregard small prey", 2) "Adaptive regulation of digestion-related organs in wild Yellow Anacondas (*Eunectes notaeus*)"

Journal of Zoology

**Penny and penny laid up will be many: large Yellow anacondas do not disregard small prey**

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**Abstract**

The study of diet may help to predict the consequences of energy and avoid size discrimination in resource use. Although diet changes are reported in dieltempic species, integracy can be a factor in determining the degree of size variation within a species. We studied large aquatic dieltempic predators, the Yellow anacondas, *Eunectes notaeus*, to learn how influence of sex and size on diet might lead to increased adult divergence. Dieltempic feeding strategies were used to assess the consequences of sexual size dimorphism on prey size and feeding frequency. To test the consequences of integracy on night catch success, we related changes in feeding frequency and maximum prey size to increase in anaconda body size. Finally, we tested whether diet composition changed between sexes in the jaws when it could lead to reduced competition. While females did larger prey did not larger prey compared to males, the other divergent when we measured the effect of body size. Females ate more frequently than males, most with both diet effect not measured. Predation rate was not generally affected by maximum prey size, and is expected from feeding theory. All and female maximum prey size feeding frequency did not display any integracy effect. While diet composition varied between sexes, feeding is high. This indicates that variation in resource use is a product of variation of metabolic rate and not an insight in Yellow anacondas. Although females feed more frequently, this may be an effect of the general integracy of metabolic rate. Changes had a positive effect on maximum prey size, though this is a general trend, and has already been demonstrated for several other species. Finally, understanding of sex-based changes in resource use will be improved if it can be determined whether such phenomena are consequences rather than causes for sexual size dimorphism.

**Introduction**

Niche is an multi-axis hypothesis and food has been considered one of its main components (Hutchinson, 1957). Foraging theory predicts that animals should eat or maximize energetic gains from feeding, while maximizing both feeding opportunities and individual safety (Stephens & Ydenberg, 2005). One prediction of foraging theory is that body size may determine prey size. It is expected that this may affect feeding habits (Olson, 1993). The ways in which male and sexual dimorphic affect one another has been investigated recently for dieltempic (Ribeiro, 1993), and foraging theory offers a pathway to understanding the processes at work on increased dietary divergence.

Two main hypotheses have been developed to explain sexual dimorphism. Under the ecological divergence hypothesis (Olson, 1993), morphology of males and females would diverge as a consequence of variation in resource utilization between sexes. The sexual selection hypothesis (Olson, 1993), on the other hand, predicts that dimorphism is a result of sexual selection that forces large males or females. Recent work on diet has been proposed to clarify the contribution of each of these processes to the evolution of sexual dimorphism. Dietary variation as a consequence of integracy has been observed in a wide range of vertebrates particularly for species with wide size variation, such as sharks (Elliott et al., 2012) and snakes (Pomati & Lopez, 2012). Differently sized individuals of the same species may vary in their morphology, physiology and life-history constraints. It is therefore plausible additional-

Adaptive regulation of digestion-related organs in wild Yellow Anacondas (*Eunectes notaeus*)

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Running headline: Postprandial physiological responses in anacondas

## Fund expenditure

|                                 | Year 1    | Year 2    | Year 3    | Year 4          |
|---------------------------------|-----------|-----------|-----------|-----------------|
| Researcher fees                 | 2,120.00  | 2,250.00  | 3,150.00  | 3,550.00        |
| Transmitters                    | 3,212.00  | 1,615.00  | -         | -               |
| Radio receptors                 | 1,718.00  | 833.00    | -         | -               |
| Antennas                        | 244.00    | 160.00    | -         | -               |
| Ibutton loggers                 | 2,952.00  | -         | -         | -               |
| Microchips                      | -         | 1,225.00  | -         | -               |
| Ultrasound scanner              | -         | -         | -         | -               |
| Surgery and veterinary supplies | 325.00    | 345.00    | 178.00    | 55.00           |
| Flight survey                   | -         | -         | 1,954.00  | -               |
| Vehicle maintenance             | 663.00    | 459.00    | 620.00    | 977.00          |
| Fuel and transportation         | 734.00    | 3,725.00  | 4,157.00  | 4,428.00        |
| Per diem                        | -         | 6,466.00  | 5,950.00  | 6,680.00        |
| Bank fees and taxes             | 450.00    | 500.00    | 450.00    | 500.00          |
| FB Coordination and Supervision | 1,150.00  | 1,380.00  | 1,350.00  | 1,400.00        |
| Expended                        | 13,568.00 | 18,958.00 | 17,809.00 | 17,590.00       |
| Budgeted                        | 15,150.00 | 18,150.00 | 17,500.00 | 18,350.00       |
| Carry on balance                | 1,582.00  | 774.00    | 465.00    | <b>1,225.00</b> |

\*although originally no major researcher fees were budgeted, due to the extra time in the field beyond the time frame of the harvest season it was necessary to allocate funding to cover for it. Most of this came out from other items in the budget that were not finally applied (ultrasonography, extra transmitters and microchips that were budgeted in year 1, 3 and 4 were not acquired due to the fact that no pregnant females were available).

There is a positive balance that we could apply as credit for a future project.

**ANNEX I – Pictures**



a) Shallow waters complicate the access to snakes.



b) Just captured thin yellow anaconda.



c) Transmitters with temp data logger attached.



d) Transmitter and logger wax/paraffin-covered.



e) Anesthetizing a yellow anaconda (isoflurane).



f) Implanting a radio transmitter into anaconda cavity.



g) Yellow anaconda basking on a fallen tree.



h) Anaconda basking on a shrub.



i) Tracking anacondas.



j) Anaconda being captured.



k) Tracking anacondas from a Cessna 182.



l) Air view of La Estrella marshes.

# Penny and penny laid up will be many: large Yellow anacondas do not disregard small prey

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## Keywords

sexual size dimorphism; Boidae; intersexual niche divergence; ontogeny; predator-prey size ratio; intraspecific competition; prey size; diet variation.

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## Abstract

The study of diet may help to predict the consequences of ontogeny and sexual size dimorphism in resource use. Although diet changes are expected in dimorphic species, ontogeny can be a factor in determining the degree of diet variation within a species. We studied large sexually dimorphic predator, the Yellow anaconda *Eumeces notaeus*, to learn how influences of sex and size on diet might lead to intersexual niche divergence, therefore avoiding intraspecific competition. We tested the consequences of sexual size dimorphism via two foraging metrics: prey size and feeding frequency. To test the consequences of ontogeny on trophic niche metrics, we related changes in feeding frequency and maximum prey size to increase in anaconda body size. Finally, we tested whether diet composition changed between sexes to the point where it could lead to reduced competition. While females (the larger sex) did eat larger prey compared to males, this effect disappeared when we removed the effect of body size. Females ate more frequently than males, even with body size effect was removed. Predator-prey size ratios were positively affected by maximum prey size, and as expected from foraging theory, did not increase minimum prey size. Feeding frequency did not display any ontogenetic effects. While diet composition varied between sexes, overlap is high. This indicates that variations in resource use as a product of sex-based differences in size are negligible in Yellow anacondas. Although females feed more frequently, this may be an effect of the greater energetic costs of reproduction. Ontogeny has a positive effect on maximum prey size, though this is a general trend, and has already been demonstrated for several other species. Finally, understanding of sex-based changes in resource use will be improved if it can be determined whether such phenomena are consequences rather than causes for sexual size dimorphism.

## Introduction

Niche is a multi-axis hypervolume and food has been considered one of its main components (Hutchinson, 1957). Foraging theory predicts that animals should act to maximize energetic gains from feeding, while maximizing both breeding opportunities and individual safety (Stephens, Brown & Ydenberg, 2007). One predication of foraging theory is that body size may determine prey size. If a species shows sexual size dimorphism (SSD), it is expected that this may affect feeding habits (Shine, 1989). The ways in which niche and sexual dimorphism affect one each other has been intriguing scientists for decades (Fisher, 1930), and foraging theory offers a pathway to understanding the processes at work on intersexual dietary divergence.

Two main hypotheses have been developed to explain sexual dimorphism. Under the *ecological divergence hypothesis* (Shine, 1989), morphology of males and females would diverge as a consequence of variation in resource utilization between sexes. The *sexual selection hypothesis* (Darwin, 1859), on the other hand, predicts that dimorphism is a result of sexual selection that favors large males or females. Knowledge on diet has the potential to clarify the contribution of each of these processes to the evolution of sexual dimorphism.

Dietary variation as a consequence of ontogeny has been observed in a wide range of vertebrates, particularly for species with wide size variation, such as sharks (Kim *et al.*, 2012) and snakes (Natusch & Lyons, 2012). Differently sized individuals of the same species vary in their morphology, physiology and life-history constraints. It is therefore plausible different-sized

conspecifics might also differ in their dietary requirements or have different selection criteria when foraging. Certainly, larger individuals have higher energetic needs, and can generally access larger prey (Carbone & Gittleman, 2002).

Prey composition of many vertebrate species have been described, including carnivorous mammals (Cavalcanti & Gese, 2010), predatory reptiles (Bhupathy, Ramesh & Bahuguna, 2014) and birds of prey (Miranda, 2015). Although several trends stand out, theoretical analysis of dietary data are still uncommon. For snakes, experiments on ontogeny effects on diet (Jayne, Voris & Heang, 1988; Shine, 1991) have been complemented by observational approaches (Natusch & Lyons, 2012). Regarding giant snakes, field observations do not record their feeding on small prey as they grow larger, contrary to what was predicted from experimental approaches or from foraging theory (Arnold, 1993).

Snakes are suitable models for studying sex-related dietary divergence and the effects of ontogeny on diet because they: (1) show a wide range of sizes (Andrews, 1982); (2) are gape-limited, making predator size a precise predictor of prey maximum size (Arnold, 1993); (3) are trophic-independent at any age; (4) have some species that are highly sexually dimorphic (Rivas, 2015). One limitation in the use of snakes in research is their low detectability in nature. This problem can be circumvented by taking samples from snake trade (Natusch & Lyons, 2012).

We investigated sex-based diet variation and potential ontogenetic shifts in prey consumption using gut contents of a giant snake species, the Yellow anaconda, *Eunectes notaeus* Cope 1862 (Fig. 1), sustainably managed in Argentina. To better understand food habits divergence between sexes and ontogenetic shifts on prey size in this species, we tested the following predictions: I – female anacondas will eat larger prey than males; II – females will eat more frequently than males; III – prey use varies between sexes; IV – body size will have a positive effect on feeding frequency; V – individual body size is positively related with maximum prey size but will have no effect on its minimum size.

## Materials and methods

### Study area

We worked at La Estrella, a marsh formed around 1960, from natural silting of a portion of the Pilcomayo River, in Formosa province, northern Argentina. La Estrella was established in Chaco vegetation, with extensive occurrence of hardwood species (Brown *et al.*, 2010). The decomposition resistance of the dead trees has helped determine the current physiognomy of La Estrella, as they have been colonized by lianas and epiphytic vegetation, forming structures locally known as 'champas', where Yellow anacondas bask during the old periods that occur during winter months. In addition to champas, there are lagoons, dense monodominant formations of Cattail (*Typha* sp.), and extensive areas of Caranday palms *Copernicia alba*. La Estrella has an abundance fish fauna that attracts aquatic birds (Brown *et al.*, 2010). Birds, together with rodents that become trapped in champas by flooding, form main prey of Yellow anacondas (Waller, Micucci & Alvarenga, 2007). Other



**Figure 1** Yellow anaconda lying in water at La Estrella marsh, Formosa province, Argentina. Reproduced with permission. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)].

large vertebrates as caimans *Caiman* spp., turtles *Phrynops hilarii*, capybaras *Hydrochoerus hydrochaeris* and nutrias *Myocastor coypus* are locally uncommon (pers. obs.). The marsh floods annually between February and May, and dries-out between October and December. During 1961–1990, the average monthly highest temperature (33.4°C) was recorded in January, and the lowest (12.1°C) in July (NOAA 2015).

### Study species

The Yellow anaconda is commonly found along the Paraguay River Basin (Henderson *et al.*, 1995). Individuals range in mass from 0.101 kg in newborns to 27.5 kg in large females. The species shows high levels of SSD, with mean mass of 3.72 versus 7.16 kg, and 174 versus 216 cm snout-vent length for adult males and females, respectively (Waller *et al.*, 2007).

Anacondas forage widely on a variety of fish, reptiles, birds and their eggs, and mammals, occasionally including carrion in their diet (Strüssmann, 1997; Waller, Buongermini & Micucci, 2001). Like other anaconda species, they are persecuted as predators of domestic animals (Miranda, Ribeiro & Strüssmann, 2016), for their fat – traditionally used by its purported medicinal properties (Alves *et al.*, 2006), and for their valuable skin (Micucci & Waller, 2007). They are sustainably managed only in Argentina, through an initiative known as *Programa Curiyú*. The Red List status of this species has yet to be evaluated by the IUCN (International Union for Conservation of Nature), but it is included on Appendix II of the Convention on International Trade of Endangered Species – CITES.

## Management program

The Programa Curiyú works on the basis of surplus yield models in an adaptive management context, having no specific hunting quotas for a given year. The harvest is limited by the number of hunters, length of the hunting season and minimum skin length. For management plan details, see Waller *et al.* (2007).

During the hunting season (winter), biologists collect data on prey found in anaconda guts. Snakes are usually slaughtered and skinned close to the capture site, with the carcass being discarded. We offered a monetary reward for hunters to bring us live snakes for dissection, in addition to data collected in previous year by program biologists. We complied with the valid laws of Ministerio de la Producción y Ambiente de la Provincia de Formosa at the study time (permit number 11004048/2015). All the studied animals were part of the ongoing management plan and no individuals were killed specifically for this study.

## Field and laboratory protocols

After slaughtering, we waited for the spasms to subside and then dissected the snake to access the gut contents. These were identified visually when slightly digested, for all prey species excluding rodents. Highly digested prey species were identified by comparing feathers and feet for birds and scales for reptiles with material deposited at the Vertebrate Zoological Collection of Mato Grosso's Federal University, Brazil. Highly digested rodents were identified using trichology techniques (de Miranda, Rodrigues & Paglia, 2014). Body masses for avian species and their eggs were obtained from Dunning (1993) and Giacomo, (2005), with exception of Southern Screamer *Chauna torquata* eggs, which were weighed in the field. Reptile and mammal masses were obtained from Strüssmann (1992), Bonvicino & Almeida (2000), Pardiñas & Teta (2005) and Weksler & Bonvicino (2005). We used mean mass for each species unless prey remains suggested that a specimen was a sub-adult, when estimates were made visually. For rodent prey sampled previously by the Programa Curiyú (for which there were no voucher), we used the mean body mass of the largest non-Caviomorph rodent in our study area *Holochilus chacarius*. We made this conservative decision – based on the null hypothesis that prey increase in size as anacondas grow larger – considering that rodents are the smallest prey killed by Yellow anacondas, and that 80% of the rodents consumed belong to this species (Waller *et al.*, 2007).

## Statistical analyses

As multiple prey can be captured in successive short-spaced predation events, when multiple bird eggs or rodents were found, individual items were not considered independent replicates. This was done to avoid pseudoreplication. We only considered multiple items to be indicative of multiple predation events if they could not have been feasibly killed together (i.e. the egg of one bird species and an adult of another).

To test the effect of sex on prey size (prediction I), we performed an ANCOVA (as a case of generalized linear model, GLM), using sex as categories and body mass as a covariate. Prediction II, which implies that females have a higher feeding frequency, was tested with another ANCOVA (in this case a GLM with a binomial distribution), using body size as a covariate, which also allows testing the effect of body size on feeding frequency (prediction IV). An ANOSIM was used to test differential prey composition between sexes where Pianka's Niche overlap combined with a null model approach (Gotelli & Entsminger, 2006), was used to test whether different diet compositions would result in differential resource use (prediction III). A quantile regression was used to test prediction V, using two  $\tau$  values: 0.75, to test if size had a positive effect on maximum prey size, and 0.25, to test if body size had an effect on minimum prey size. We choose this method after noticing that it is the adequate model when the independent variable only controls maximum values of the dependent variable (Gotelli & Ellison, 2005). Alpha levels were established at 0.05 and statistical analyses were ran in R software, using the packages *Vegan*, *EcoSimR* and *QuantReg* (Gotelli & Entsminger, 2006; Oksanen *et al.*, 2007; Koenker, 2013).

## Results

Hunters brought us 95 anacondas – 59 females and 36 males from La Estrella during the hunting season of 2015. These ranged from 1.56 to 13.7 kg. Of these, 65 (68.43%) contained prey. The Programa Curiyú data base provided information on gut contents of another 112 anacondas – 96 females and 16 males – hunted between 2001 and 2010, with masses ranging from 1.7 to 23.2 kg. Descriptive data about Yellow anacondas and their prey is given in Table 1. Most common prey items were aquatic birds, followed by rodents, bird eggs and reptiles (Table 2; Fig. 2).

Mean mass of prey taken by male anacondas was 247 g ( $n = 30$ ), while mean mass of prey taken by females was 585 g ( $n = 142$ ), significantly larger than the males' mean prey mass (prediction I; ANOVA,  $n = 172$ ,  $P < 0.01$ ). However, when we take into consideration the effect of body size, the sex effect disappear (ANCOVA,  $P = 0.939$  for sex and  $<0.01$  for size; Fig. 3). Sex had a positive effect on feeding frequency, with 83.05% of the females having fed, compared with 44.44% of males (prediction II; ANCOVA,  $P < 0.05$ ,  $n = 95$ ). Size had no effect on feeding frequency of anacondas (prediction III;  $P = 0.723$ ). Prey composition was different between sexes (ANOSIM;  $P = 0.002$ ,  $n = 220$ ), with Pianka's

**Table 1** Descriptive data regarding Yellow anaconda and their prey mean mass, its minimum and maximum values and standard error in La Estrella, Formosa, Argentina

|               | Mean mass (g) | Min–Max (g) | sd(±) |
|---------------|---------------|-------------|-------|
| Males         | 3544          | 1560–8000   | 1363  |
| Females       | 7777          | 2280–23200  | 3948  |
| Male's prey   | 247           | 29–1890     | 474   |
| Female's prey | 585           | 14–6000     | 855   |

**Table 2** Prey composition of Yellow anacondas *Eunectes notaeus* from La Estrella marsh, northern Argentina

| Species and body mass (g)                             | Female %  |         | Male %    |         |
|---|-----------|---------|-----------|---------|
|   | Frequency | Biomass | Frequency | Biomass |
| <b>Birds</b>  |           |         |           |         |
| Cormorant <i>Phalacrocorax brasilianus</i> , 1576 g   | 9.4       | 30.5    | 5.1       | 28.0    |
| Rosy-billed pochard <i>Netta peposaca</i> , 1510 g    | 2.2       | 6.9     | 0.0       | 0.0     |
| Muskovy duck <i>Cairina moschata</i> , 2450 g         | 1.1       | 5.6     | 0.0       | 0.0     |
| Maguari stork <i>Ciconia maguari</i> , 4057 g         | 0.6       | 4.6     | 0.0       | 0.0     |
| Purple gallinule <i>Porphyrio martinicus</i> , 656 g  | 2.2       | 3.0     | 0.0       | 0.0     |
| Roseate spoonbill <i>Platalea ajaja</i> , 1490 g      | 0.6       | 1.7     | 0.0       | 0.0     |
| Whistling duck <i>Dendrocygna bicolor</i> , 710 g     | 1.1       | 1.6     | 2.6       | 6.3     |
| Anhinga <i>Anhinga anhinga</i> , 1235 g               | 0.6       | 1.4     | 0.0       | 0.0     |
| Brazilian teal <i>Amazonetta brasiliensis</i> , 595 g | 1.1       | 1.4     | 0.0       | 0.0     |
| Limpkin <i>Aramus guarana</i> , 1080 g                | 0.6       | 1.2     | 0.0       | 0.0     |
| Great egret <i>Ardea alba</i> , 882.2 g               | 0.6       | 1.0     | 0.0       | 0.0     |
| Whistling duck <i>D. autumnalis</i> , 778.3 g         | 0.6       | 0.9     | 0.0       | 0.0     |
| Silver teal <i>Anas versicolor</i> , 617 g            | 0.6       | 0.7     | 0.0       | 0.0     |
| Chachalaca <i>Ortalis canicollis</i> , 539 g          | 0.6       | 0.6     | 0.0       | 0.0     |
| Southern screamer nestling <i>C. torquata</i> , 500 g | 0.6       | 0.6     | 0.0       | 0.0     |
| Cattle egret <i>Bubulcus ibis</i> , 364 g             | 0.6       | 0.4     | 0.0       | 0.0     |
| Snail kite <i>Rostrhamus sociabilis</i> , 320 g       | 0.6       | 0.4     | 0.0       | 0.0     |
| Monk parakeet <i>Myiopsitta monachus</i> , 120 g      | 1.1       | 0.3     | 7.7       | 3.2     |
| Striated heron <i>Butorides striata</i> , 226 g       | 0.6       | 0.3     | 0.0       | 0.0     |
| Jacana <i>Jacana jacana</i> , 100.9 g                 | 1.1       | 0.2     | 0.0       | 0.0     |
| Guira cuckoo <i>Guira guira</i> , 141 g               | 0.6       | 0.2     | 0.0       | 0.0     |
| Spotted sandpiper <i>Actitis macularius</i> , 40.4 g  | 1.1       | 0.1     | 2.6       | 0.4     |
| Shiny cowbird <i>Molothrus bonariensis</i> , 40 g     | 0.6       | 0.0     | 2.6       | 0.4     |
| Brushrunner <i>Coryphistera alaudina</i> , 30 g       | 0.0       | 0.0     | 5.1       | 0.5     |
| Giant wood rail <i>Aramides ypecaha</i> , 708.2 g     | 0.0       | 0.0     | 2.6       | 6.3     |
| Southern screamer egg <i>C. torquata</i> , 164 g      | 20.2      | 4.3     | 2.6       | 1.5     |
| Limpkin egg <i>Aramus guarana</i> , 59.3 g            | 5.6       | 0.7     | 7.7       | 1.6     |
| <b>Mammals</b>  |           |         |           |         |
| Capybara <i>Hydrochoerus hydrochaeris</i> , 6000 g    | 0.6       | 6.8     | 0.0       | 0.0     |
| Marsh rat <i>Holochilus chacarius</i> , 92 g          | 22.8      | 4.3     | 25.6      | 8.2     |
| Unidentified rodent, 92 g                             | 12.8      | 2.4     | 30.8      | 9.8     |
| Guinea pig <i>Cavia aperea</i> , 637 g                | 0.6       | 0.7     | 0.0       | 0.0     |
| Vesper mouse <i>Calomys callosus</i> , 29.9 g         | 1.7       | 0.1     | 2.6       | 0.3     |
| Fornes' rice mouse <i>Oligoryzomys fornesi</i> , 14 g | 3.3       | 0.1     | 0.0       | 0.0     |
| <b>Reptiles</b>                                       |           |         |           |         |
| False water-cobra <i>Hydrodynastes gigas</i> , 1890 g | 3.3       | 12.9    | 5.1       | 33.6    |
| Yellow anaconda <i>Eunectes notaeus</i> , 1500 g      | 0.6       | 1.7     | 0.0       | 0.0     |
| Sample size   | 180       |         | 40        |         |

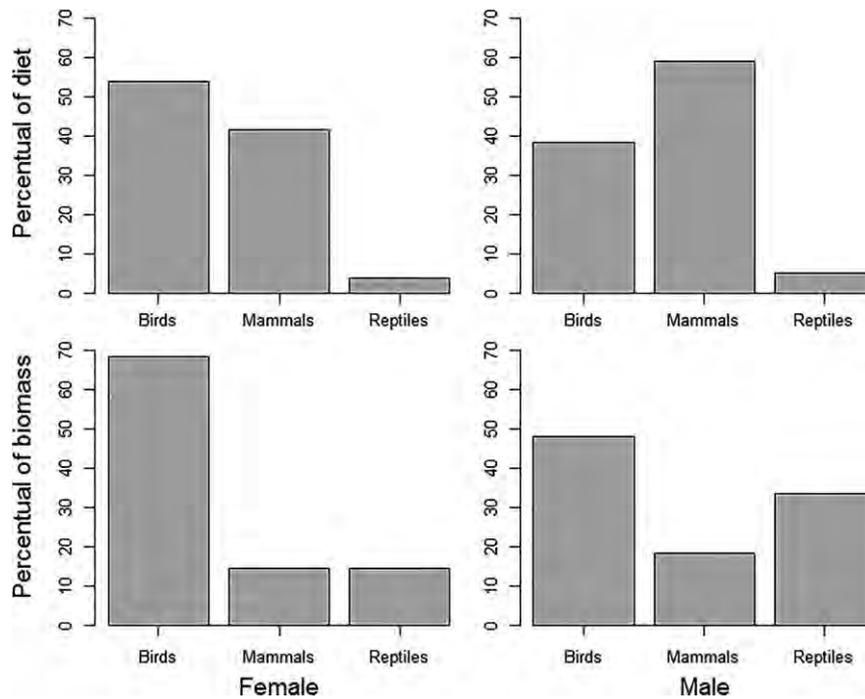
For each prey species, frequency and biomass (calculated as contribution of each species to total biomass consumed) are shown by sex.

niche overlap is 0.68, higher than expected by chance (prediction IV;  $P < 0.01$ ). Anaconda body size had a positive effect on maximum prey size (prediction V; quantile regression,  $P > 0.001$ ,  $n = 173$ ,  $\tau = 0.75$ ), but no effect on minimum size ( $P = 1.0$ ,  $\tau = 0.25$ ) (Fig. 4). Thus, the best fitting equation was prey size =  $-66.44 + 0.14 \times$  anaconda size, for  $\tau = 0.75$ .

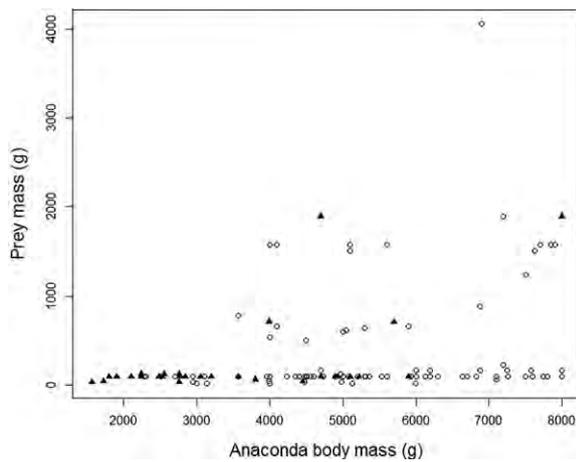
## Discussion

Here, we show that sex play a small role in diet divergence between sexes in anacondas. If intersexual niche divergence has a parallel function to reproductive success in evolution of large sized females, it may be small. As diet divergence as a

product of SSD has been demonstrated for a range of taxa (Weise, Harvey & Costa, 2010; for mammals; Natusch & Lyons, 2012 for reptiles; Cook *et al.*, 2013 for birds), this may be a secondary consequence of increases in fitness generated by larger body size in males and/or females. Although female anacondas prey is both larger and different from that of males, they feed more frequently, which may be related to fat accumulation associated with the higher costs of reproduction (Lourdais, Lorigou & DeNardo, 2013). This notion is supported by the fact that, while diverging in composition, males and females diet overlaps extensively, excluding the possibility of SSD being a way to reduce competition. Ontogenetic changes in diet increase maximum size of prey that anacondas



**Figure 2** Prey composition of Yellow anacondas *Eunectes notaeus* from La Estrella marsh, northern Argentina. For each prey Class, bars represent frequency or biomass percentiles for each sex.

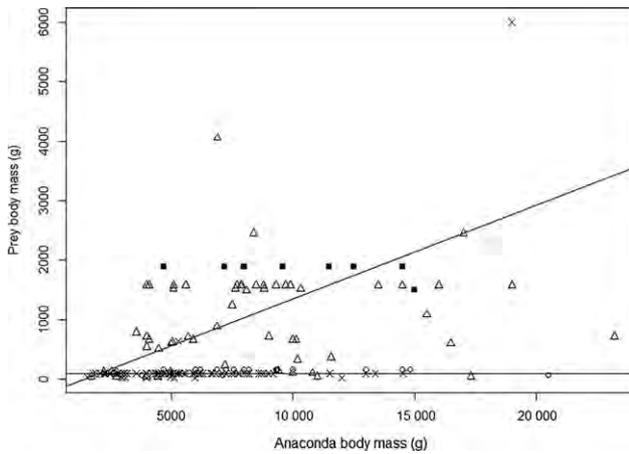


**Figure 3** Effect of Yellow anaconda sex on prey body mass. Prey size is plotted against anaconda size; males (black triangles) do not eat smaller prey than females (open circles) ( $P > 0.05$ ). Anaconda's size has been truncated by the largest male in order to afford a better view of data.

may catch, without affecting minimum prey size. This is predicted on both physiological and foraging theoretical grounds because energetic demands increase with size, despite greater efficiency in energy use by larger organisms (Makarieva, Gorshkov & Li, 2005; Stephens *et al.*, 2007).

The diet composition of Yellow anacondas from northern Argentina shows a high proportion of aquatic birds (Table 2). This is followed by small mammals and reptiles in frequency for females, while for males, small mammals are the most common prey. When we take biomass into consideration, the general trend of great avian importance is maintained for females, while for males, birds and reptiles become more important than mammals. The absence of amphibians is probably because La Estrella is mostly formed of open waters where amphibians are uncommon. Fish prey have been rarely recorded by us and other authors because the anacondas lacks the head morphology needed not reduce drag when striking at prey underwater (Vincent *et al.*, 2009). Therefore, predation on fish may be restricted to dry season, when they can be founded stranded or dying in shallow pools. Head morphology is considered a variable that can cause intersexual divergence on diet (Vincent, Herrel & Irschick, 2004). However, contrary to predictions of previous research (Vincent & Herrel, 2007), head size and shape does not show changes between sexes in Yellow anacondas (Waller *et al.*, 2007).

High predation of birds has been shown elsewhere for Yellow and Green anacondas (Strüssmann, 1997; Rivas, 2015). However, our records of rodents and other small prey were higher than recorded in other studies, which we believe may be due to the methodological constraint imposed by other studies use only of forced regurgitation to obtain stomach contents. This method may underestimate small-sized prey present in the snake's intestines because anacondas – like pythons – are large and muscular snakes and small prey may go unnoticed during



**Figure 4** Yellow anaconda's size plotted against prey size, showing that anacondas feed on larger prey as they grow in size while continuing to eat small prey. Prey types: reptiles – closed squares; avian eggs – circles; birds – triangles; mammals – crosses. The quantile regression of prey size against predator size was significant for  $\tau = 0.75$  (upper line,  $P < 0.05$ ) but not for  $\tau = 0.25$  (lower line,  $P = 0.10$ ).

stomach palpation. If studying large snakes, we recommend using feces collection as a complement to forced regurgitation. Nevertheless, since large prey will make snakes produce more feces, some statistical correction would need to be undertaken (Ackerman, Lindzey & Hemker, 1984), otherwise strong pseudoreplication will occur (see Bhupathy *et al.*, 2014 for an example).

By finding 68% of anacondas fed we question a common notion that giant snakes feed infrequently, a premise for a number of research fields (Whelan & Schmidt, 2007; Andrew *et al.*, 2015). In other large species, as *Python reticulatus*, 37–45% individuals had prey in their guts (Shine, Harlow & Keogh, 1998). We stress that these are very high rates considering that animals were collected with methods allowed defecation and regurgitation before dissection. In the smaller sized scrub python *Morelia spilota*, 45% of the individuals had prey in their stomachs or intestines (Slip & Shine, 1988). Since digestion takes no more than 2 weeks for extremely large prey (Secor & Diamond, 1997a; Toledo, Abe & Andrade, 2003), we argue that giant snakes feed rather more frequently than is commonly supposed. In addition, research on the metabolic response to digestion shows a hypoallometric (Secor & Diamond, 1997b) or absent (Bedford & Christian, 2001) effect of large sized prey, strengthening this idea.

Another common assumption regarding giant snakes on which the data casts doubt is the widespread use of a sit-and-wait or ambush foraging strategy (Whelan & Schmidt, 2007; Secor, 2008), as this could not explain the high incidence of egg predation found in this study, suggesting that sit-and-wait strategy is just one part of a broader suite of foraging behaviors. It is possible that this is an idiosyncrasy caused by Yellow anacondas being an aquatic species. Locomotion costs are smaller in water, and this could permit individuals to forage

more actively. However, a similar pattern of egg predation is found in the more terrestrial *Python molurus* in Everglades (Dove *et al.*, 2012), and in the terrestrial *Python natalensis* in South Africa (Alexander, 2012). Based on evidence regarding: (1) high feeding frequency; (2) presence of non-motile prey; and (3) presence of small-sized prey; we consider that the idea of the giant snake which sit-and wait to feed on enormous prey and later fast for months may have to be reassessed.

While sex has a strong effect on feeding frequency, its effect in prey size disappears when we make body size into account. Consequently, dietary divergence may result from SSD as well as from the ontogenetic relationship between body size and feeding frequency. Meanwhile, fitness-based differences between male and female body size are more parsimonious as an explanation for large body size in females (Shine, 1989). One could suggest that the strong differences in feeding frequency could be a misconception induced by our data on this being collected only during the winter months. But summer is the dry season in our study area; this is when anacondas reproduce (Waller *et al.*, 2007; Rivas, 2015), and individuals of neither sex feed often when reproductively active. Breeding season fasting has been shown to occur in several other snake taxa, including colubrids (Shine, 2003), pythonids (Madsen & Shine, 2000) and viperids (Madsen & Shine, 1993). Nevertheless, since La Estrella is a recently formed environment, we believe that data collection in seasons other than winter or in other study areas may reveal different diet composition.

Increases in maximum prey size along with increases in anaconda's body size while continuing to consume small prey reflect optimal choices under foraging theory. This theme has been the subject of a long discussion since some snakes are shown to consume small-sized prey in laboratory experiments (Shine, 1991). This, however, contrasted with field studies where relationship between snake size and prey size could be described as an 'ontogenetic shift', with an increase in the minimum prey size in larger snakes (Arnold, 1993). We believe that this is a misconception caused by: (1) increased performance of the digestive system in larger snakes (Secor & Diamond, 1997b); and (2) small sample sizes of truly large snakes (Shine *et al.*, 1998; Rivas, 2015). Both can act reducing detection of small prey, the first decreasing passing time and therefore reducing time to detection through dissection or regurgitation and the second reducing general detection probability for any prey species.

In anacondas, feeding data supports the predictions of the 'ontogenetic telescope' hypothesis, with size-based traits allowing larger animals to eat larger prey (Beerman *et al.*, 2015). Although it is difficult to accurately predict the evolutionary drivers for any morphological trait (Young & Brodie, 2004 and comments therein), our results are consistent with the hypothesis that SSD is an effect of increases in fitness resulting from larger body size in females (Rivas, 2015), while the moderate dietary divergence may be a side-effect of it. Fitness differences resulting from differential optimum size between males and females seem to be a sufficient proximal cause to explain SSD.

In summary, our study shows that there is little change in Yellow anacondas feeding habits resulting from SSD as size increases, while the ontogenetic relationship between body size and diet produces increases in maximum prey size. Our results indicate that the pattern of prey size shift by anacondas can therefore be considered general in indeterminate-growth species. Since these shifts might have evolved because of conserved physiological constraints related with increase in energetic demand by larger organisms, they may be worthy of consideration for research on foraging by indeterminate growth species. Further research on the effects of SSD on resource use will be relevant for development of our understanding of these changes as causes or consequences of differently sized males and females.

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# Adaptative regulation of digestion-related organs in wild

## Yellow Anacondas (*Eunectes notaeus*)

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Running headline: Postprandial physiological responses in anacondas

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39

## 40 **ABSTRACT**

41 The modulation of digestion-related organs in snakes is related to feeding frequency and  
42 reproduction energetic demands . It allows snakes to minimize costs and maximize the  
43 energetic gains from predation. We performed in wild Yellow Anacondas (*Eunectes*  
44 *notaeus*, Boidae) killed through a sustainable management plan directed to skin trade.  
45 This provided, for the first time, an opportunity to study the foraging-digestion  
46 continuum in this specie and to know the post-prandial hypertrophy of stomach, liver,  
47 kidney and heart in wild individuals for the first time. We also investigated intersexual  
48 differences in organs allometry . Here we show that young specimens of both sexes have  
49 similar organ mass. However, adult females have proportionally larger stomachs and

50 livers than adult males, that on the other hand have larger kidneys than adult females.  
51 Heart does not show intersexual allometric difference. We also show for the first time  
52 the postprandial hypertrophy of digestion-related organs in a wild snake. Increases in  
53 mass were significant to stomach and liver, with hypertrophy of 28% on stomach's  
54 and 57% on liver's, and were not significant to kidney and heart, with increase of  
55 1,89% and 29,98% respectively. These are of lesser magnitude than the ones observed  
56 in laboratory experiments. Since physiological variation related to costs associated with  
57 reproduction is different among sexes, this paper helps to understand how these  
58 differences affect internal morphology and how these differences may contribute to  
59 minimize energy investments with postprandial hypertrophy.

60

61 **KEYWORDS:** Boidae, allometry, digestive system, postprandial physiology,  
62 physiological plasticity, foraging-digestion continuum.

63

## 64 **INTRODUCTION**

65

66 The Foraging theory predicts that predators have adaptations in behavior,  
67 morphology and physiology that allow them to minimize the costs of food acquisition  
68 and processing and maximize the predation events, while reducing their own predation  
69 risk and affording best chances of mating (Stephen et al. 2007). Those adaptations are  
70 under variable degrees of plasticity. Some are strongly fixed by the evolutionary process  
71 (e.g. regulation of digestive performance; Secor 2008) and some are very variable (e.g.  
72 behavior; Ballen et al. 2015).

73 The digestive system can be highly variable too (Stephen et al. 2007). Digestive  
74 system modulation has direct influence on ecological traits of any animal because it

75 allows the predator to meet its metabolic needs in the face of changes — sometimes  
76 unpredictable — in ontogenetic development and resources availability (Whelan et al.  
77 2000). Digestory system modulation is also related with relative meal size (e.g. Toledo  
78 et al. 2003), food composition (proteic, fat, etc; e.g. Wang et al. 2012) and predator's  
79 foraging frequency (e.g. Andrade et al. 2005).

80 In snakes, different postprandial responses as changes on morphology and  
81 function in organs are related to different food habits. In laboratory, frequent foraging  
82 snakes show low or even none postprandial responses in digestive organs (Secor and  
83 Diamond 2000; Großmann and Starck 2006). On the other hand, infrequent foraging  
84 snakes—sit-and-wait hunters like large constrictor snakes—show widely postprandial  
85 changes in morphology and function in digestive organs (Secor et al. 1994). Some of  
86 these species shows 50-150% postprandial hipertrophy in the masses of small intestine,  
87 liver and stomach after meal (Secor e Diamond 2000).

88 Reproductive costs between the sexes may also determine different pathways to  
89 selectives pressures. The reproductive success in males and females is associated to  
90 different body sizes and visceral components (Devine 1977). Males produce  
91 energetically cheap gametes and are under strong selection for the improvement of  
92 structures and strategies to ensure greater success in inseminating the female. Females,  
93 in their turn, produce relatively onerous gametes and are under strong selection for the  
94 improvement of organs related to obtention and processing of nutrients, essential to  
95 reproduction (Bonnet et al. 1998). Despite of that fact, internal anatomic difference is  
96 still understudied, especially in snakes. Thus, the Foraging Theory predictions can help  
97 us to understand patterns that determine energetic investments in adaptive regulation in  
98 digestion-related organs, because intersexual physiological variation results in different  
99 food habits and visceral anatomic traits between the sexes.

100 In Neotropic region, boids of *Eunectes* genus are good models to investigate the  
101 foraging-digestion *contunuum* in snakes. The Yellow Anaconda (*Eunectes notaeus*  
102 Cope 1862) is a non-venomous constrictor snake. The yellow anaconda occurs in a wide  
103 geographical area among the Paraguay and Paraná river basins, where it can be found in  
104 aquatic environments. Showing strong sexual dimorphism, adult females can reach 3.78  
105 meters in length and 29 kilos in mass (Waller et al. 2007). This snake is a generalist  
106 predator and employ a mixed foraging to catch little and big meals—such as  
107 invertebrates, eggs, rats and capibaras (Waller et al. 2007; Miranda et al. 2016).

108 In the present study we investigate the foraging-digestion continuum in wild  
109 yellow-anacondas, based in mass changes in digestion organs related and its intersexual  
110 allometric variation. Thus, we aim to answer the questions : I – Is there intersexual  
111 difference in those organs mass, given that the sexes have different reproductive  
112 investment? and, II - Will wild yellow anacondas with prey on the gut show  
113 postprandial hypertrophic response to feed in digestion-related organs?

114

## 115 **MATERIAL E METHODS**

### 116 **Management program and study area**

117 The yellow anaconda' skin is explored in Argentina through the initiative  
118 "Programa para la conservación y el aprovechamiento sustentable de la Boa Curiyú (*E.*  
119 *notaeus*) en la Argentina", or yet, "Curiyú Program" (see Micucci et al. 2006 to more  
120 information). The program is a great opportunity to research about yellow anacondas  
121 because it allows access to a large number of wild individuals legally slaughtered to  
122 skinning.

123 Implemented in 2002 in a seasonal flooded area on Chaco domain (Formosa,  
124 Argentina), around 3800 wild yellow anacondas are captured every year during the  
125 hunting season. The skins are directed for export under the supervision of Ministerio de  
126 la Producción y Ambiente de la Provincia de Formosa (Waller et al. 2011), while  
127 biologists collect data for improving management, producing data about the specie's  
128 biology.

129 La Estrella marshland (24°08'S, 60°35'W), northeast of Formosa city, in  
130 Formosa Province (Figure 1) is a subtropical region, the annual climate cycle is  
131 characterized by extreme variations between the flooding period (January-May) and dry  
132 period (October-December). The temperatures range from a minimum of 7 °C in winter  
133 to a maximum of 45 °C in summer and the rain presents an gradient from east (1200  
134 mm) to west (600 mm; Burgos 1970). Yellow anacondas bask on the sun above  
135 vegetation during the winter, when they are hunted. We paid hunters to bring us  
136 anacondas before slaughtering, in order to allow dissection before and their carcasses be  
137 discarded. Considering Natusch and Lyons (2014) killed the snakes crushing the head  
138 after spine break. We emphasize none specimens analyzed here was killed for the  
139 specific purpose of this study, but have been obtained inside the Curiyú Program, legal  
140 part of the ongoing snake trade.

#### 141 **Specie of study**

142 Yellow anacondas are active over all the year (Waller et al. 2007) but among the  
143 summer in La Estrella, yellow anacondas hide out in aquatic vegetation on small ponds  
144 and it makes them hard to detect. On the other hand, during the winter the snakes bask  
145 on the sun and are easily detected. It shows rapid growth and sexual dimorphism in size.  
146 Females are 20% longer and twice heavier (2.16 m in snout-vent length, 7.166 kg) than

147 males (1.74 m, 3.720 kg). The reproductive activity begins in spring (October and  
148 December) and the birth of the offspring in early autumn (Waller et al. 2007).

149         Anacondas are typical generalist predators. Hunting actively or ambushing its  
150 preys (Reed and Rodda 2009), its food includes invertebrates, fish, reptiles, mammals,  
151 birds and eggs (Strüssmann 1997; Waller et al. 2001, Miranda et al. 2016). Ontogenetic  
152 changes and intersexual differences in the trophic ecology were observed  
153 and discussed by Miranda et al. (2016). The anaconda has not been evaluated yet by  
154 IUCN (International Union for Conservation of Nature) but it's listed in Appendix II on  
155 the Convention on International Trade in Endangered Species of Wild Fauna and Flora  
156 – CITES.

#### 157 **Data collection**

158         We carry out the data collection in the hunting season between June and July  
159 2015. After killed, the anacondas remained stored for 12 hours until the *post-mortem*  
160 spasms stopped. We recorded the snout-vent length, weight, sex and made a  
161 longitudinal ventral incision to remove the stomach, liver, kidneys and heart. We clean  
162 organs of membranes, vessels and other tissues, and we measured the fresh mass of  
163 each organ using hand dynamometers of different scales to minimize the error.

164         We observed if the specimen contained any prey in its gut. Observations on the  
165 presence / absence of prey were grouped into three categories: a) empty gut; b) prey(s)  
166 in the stomach; and c) prey(s) in the intestine.

#### 167 **Data analysis**

168         To answer the question I (Is there intersexual difference in those organs mass,  
169 given that the sexes have different reproductive investment?) we used a Analysis of  
170 Covariance (ANCOVA) to all specimens including sex and size as variables-predictors

171 and mass of organ such as the response-variable. To answer the question II (Will wild  
172 yellow anacondas with prey in digestive system show postprandial hypertrophic  
173 response to feed in digestion organs?) we used a logistic regression analysis as a case of  
174 generalized linear model (GLM) with binomial distribution including sex, prey  
175 presence/absence in stomach or intestine and body mass as predictor variables and  
176 organ mass as response-variable. We used Vegan Package (Oksanen et al. 2007) of R  
177 Program and  $p \leq 0.05$ .

178

## 179 **RESULTS**

180

181 We analyzed 95 yellow-anacondas specimens caught in La Estrella marshland  
182 under the hunting season in 2015. The body mass ranged between 1.560 kg and 13.710  
183 kg, with a mean weight of 4.691 kg. From these, 66 individuals (69,47%) had prey on  
184 the digestory system, thus 24 females had prey on stomach, 41 had prey on intestine and  
185 10 was empty, while 7 males had prey on stomach, 15 had prey on intestine and 19 was  
186 empty.

187 We found intersexual difference on mass in some organs. While small-sized  
188 individuals showed a similar organs mass between sexes, stomach ( $p=0.04$ ; Figure 2a),  
189 and liver ( $p=0.02$ , Fig 2b) showed hiperalometric growth in females. On the other hand,  
190 the kidneys showed hiperalometric growth in males ( $p=0.01$ , Fig 2c) and the heart did  
191 not have difference between sexes ( $p<0.01$ ).

192 In general, there is no positive effect of body size, sex and presence of prey in  
193 the stomach or intestine on the postprandial response of organs ( $p>0.20$ ), except to  
194 stomach and liver, for which these relationships were significative. The presence of prey  
195 on stomach did not have significative effect in stomach ( $p>0.2$ ) or liver mass ( $p>0.1$ ),

196 while the presence of prey on intestine was related to an hypertrophy of 28% in stomach  
197 ( $p=0.046$ ; Fig 3a) and 57% in liver ( $p=0.0064$ ; Fig 3b). The masses of the kidneys and  
198 heart were not affected by the presence of prey on the gut in yellow-anacondas ( $p>0.5$ ).

199

## 200 **DISCUSSION**

201

202 Here we demonstrated the existence of intersexual allometric differences in mass  
203 of the stomach, liver (larger in females) and kidneys (larger in males) and, for the first  
204 time, postprandial responses were measured in wild snakes. In natural conditions the  
205 yellow anaconda had meager postprandial responses. In laboratory conditions, some  
206 boids and pythonids show extreme postprandial responses to feeding trials (Secor 2003;  
207 Andersen et al. 2005). By keeping snakes in experimental conditions, where the time  
208 between meals is maximized could induce more pronounced postprandial responses  
209 than that under field conditions. Furthermore, extreme meal size of prey offered in  
210 captivity (up to 65% of snake body mass in experiments; Secor and Diamond 1997), is  
211 uncommon in the wild for most or all species (Shine et al. 1998; Snow et al. 2007; Dove  
212 et al. 2012).

213 The limited postprandial response on stomach hypertrophy in yellow anacondas  
214 can be related on their behavior feeding habits. In nature, those snakes hunt frequently  
215 for small prey (Waller et al. 2007; Miranda et al. 2016). Other studies about other boids  
216 and and pythons' food habits have shown that giant snakes hunt frequently for small  
217 preys—such as rats and eggs (Shine et al. 1998; Snow et al. 2007; Dove et al. 2011,  
218 2012; Miranda et al. 2016). Small prey are easily digested and will demand modest  
219 postprandial response. Frequent feeder snakes show modest or no hypertrophic  
220 postprandial response in stomach (Bedford et al. 2001d 2000). Studies using the similar

221 models — as the Burmese Python, *Python molurus* (Linnaeus, 1758) — have  
222 demonstrated that the stomach may experiment postprandial hypertrophy (Secor and  
223 Diamond 1995; Cox and Secor 2008). It shows that the understanding about the factors  
224 that act on the structural and functional remodeling of this organ demands more  
225 research in snakes, if the hypertrophy occurs by synthesis of the gastric tissue, by the  
226 enzyme synthesis or other factor.

227         Intraspecific differences in food habits between sexes can be explained partially  
228 by morphological differences (Manicom et al. 2014). The sexual dimorphism in size  
229 implies different energy requirements, which by their time and are reflected in  
230 physiological differences. Whereas females have higher energy requirements, both to  
231 body maintenance and reproduction (Lourdais et al. 2013), they eat bigger prey  
232 compared to males (Waller et al. 2007; Miranda 2016). The larger stomach in females  
233 can be a reflex of this trait.

234         As in the stomach, the larger mass and the postprandial response in the liver is  
235 related to prey size and feeding frequency. Morphophysiological changes in the liver are  
236 related of the enzyme synthesis, metabolic reaction with the digesta and the mass  
237 increase is a result of the incorporation of lipid from the food breakdown and increased  
238 of the blood flow (Großmann and Starck 2006). Throughout their life, snakes upregulate  
239 their digestive performance (Secor 2008) and in yellow anacondas we show that females  
240 have larger stomach and liver than male making possible a digestive process that is  
241 more constant and has a higher performance, different from snakes with high  
242 postprandial responses.

243         Internal anatomical differences between the sexes have been little investigated  
244 (Aldridge and Brown 1995; Bonnet et al. 1998). Females produce high energy gametes  
245 and therefore invest in the improvement of structures related to the processing of

246 nutrients to reproductive events (Bonnet et al. 1998). Thus, it is expected that yellow  
247 anaconda females' have bigger stomach and livers compared to males to process larger  
248 meals frequently. Males, by their time, invest more energy in structures related to sex  
249 (Aldridge and Brown 1995; Bonnet et al. 1998; Shine et al. 1999). Anacondas have a  
250 reproductive behaviour known as breeding balls (Rivas 2016), where a fertile female is  
251 the center of males ready to inseminate her. In anacondas, the male can mate a few  
252 times during the breeding season (Rivas 2015). Before leaving the female, the male  
253 physically hold her for a period of time and when he can not maintain its position  
254 leaving in her a "plug" sperm, covering the cloaca of the female and preventing other  
255 males from inseminating her (Rivas 2015).

256         While female kidneys performs functions related to excretion and homeostasis  
257 regulation, in males of reptiles Squamata (lizards, anfisbaenians and skanes) a  
258 secondary sexual function is assigned to the kidneys (specifically to the sexual segment  
259 of the kidney: Devine 1977; Ross and Crews 1977). Before the breeding season, an  
260 increase in testosterone levels (Bishop 1959) prepare the sexual segment of the kidney,  
261 through hypertrophy, to produce nutrient-rich liquid. These liquids composes the semen,  
262 keeping live the spermatozoa during storage, and will form the "plug" (Aldridge and  
263 Brown 1995; Bonnet et al. 1998). Thus, the sexual selection favors larger kidneys in  
264 Squamata males, like in yellow anaconda.

265         It is also possible that the postprandial kidney hypertrophy has not been strong  
266 enough to detect it in field conditions. The meal size has a direct influence on the  
267 magnitude of postprandial responses (Secor and Diamond 1997, Toledo et al. 2003) and  
268 the anacondas analyzed showed only small preys in their gut. In addition, hypertrophy  
269 in the sexual segment of the kidney in males may also have had some influence on our  
270 results. Males of yellow anacondas have annual reproductive cycle with increased

271 gonadal—which can be related to the increase in testosterone levels—between May and  
272 September (Waller et al. 2007). But the understanding about the relationship between  
273 endocrine factors and dynamics of sexual segment of the kidneys, especially in field  
274 conditions remains limited to a few taxa (Hoss et al. 2011). Given that the kidney was  
275 already in hypertrophy as a result of gonadal cycle, a new increase in the mass of this  
276 organ, caused by the ingestion of prey, may be impracticable. New research may help to  
277 clarify if the gonadal cycle interferes or not in the occurrence of postprandial  
278 hypertrophic responses in kidney of male anacondas.

279         In this study we did not detect post-prandial hypertrophy to heart, but changes in  
280 ventricular volume, in coronary area and heart rate have been reported for the species  
281 (Zerbe et al. 2011). Research shows that postprandial cardiac hypertrophy in snakes is  
282 facultative and may occur through functional changes, but not in the mass of the heart  
283 (Jensen et al. 2011). During the digestion process there is an increase on the metabolic  
284 rate, oxygen demand and blood viscosity (Slay et al. 2014). To support a higher blood  
285 flow, supplying the organs involved in the digestive process, the ventricular cells can  
286 undergo hypertrophy by heavy chain myosin synthesis (Andersen et al. 2005) or just  
287 change the cardiac functions (Jensen et al. 2011; Zerbe et al. 2011; Slay et al. 2014). By  
288 this way, changes on cardiac function is enough to support the digestion process in  
289 yellow anacondas, without energetic expenditure in cardiac hypertrophy.

290         In conclusion, our study shows the existence of sexual dimorphism in organs  
291 related to digestion in yellow anacondas and, for the first time, the occurrence of  
292 postprandial responses in a wild snake. Since differences in the costs associated with the  
293 reproduction contribute to the establishment of physiological changes, our study helps  
294 to understand some of the proximate causes of the feeding behavior in yellow  
295 anacondas. Here we enlighten the understanding how different reproductive costs between the

296 sexes can influence the morphology and physiology of males and females and have  
297 shown that allometric differences in digestion-related organs may contribute to  
298 minimize energy investments with postprandial hypertrophy.

299

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438 Figures

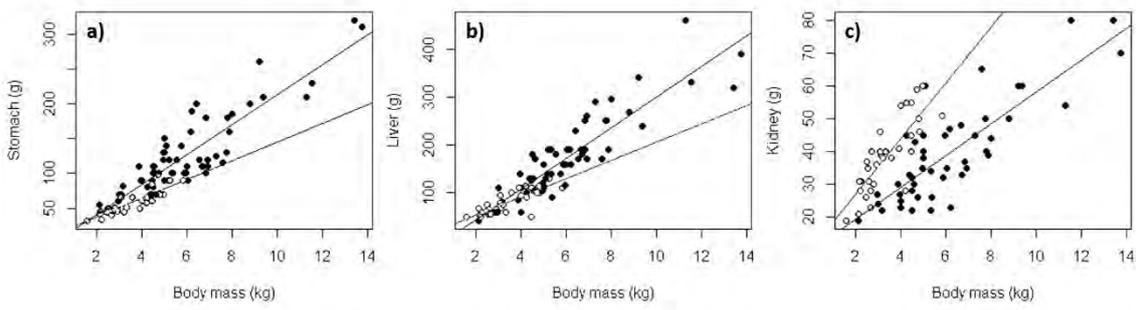


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440 **Fig. 1** Study area: La Estrella mashland location, Formosa Province, North Argentina.

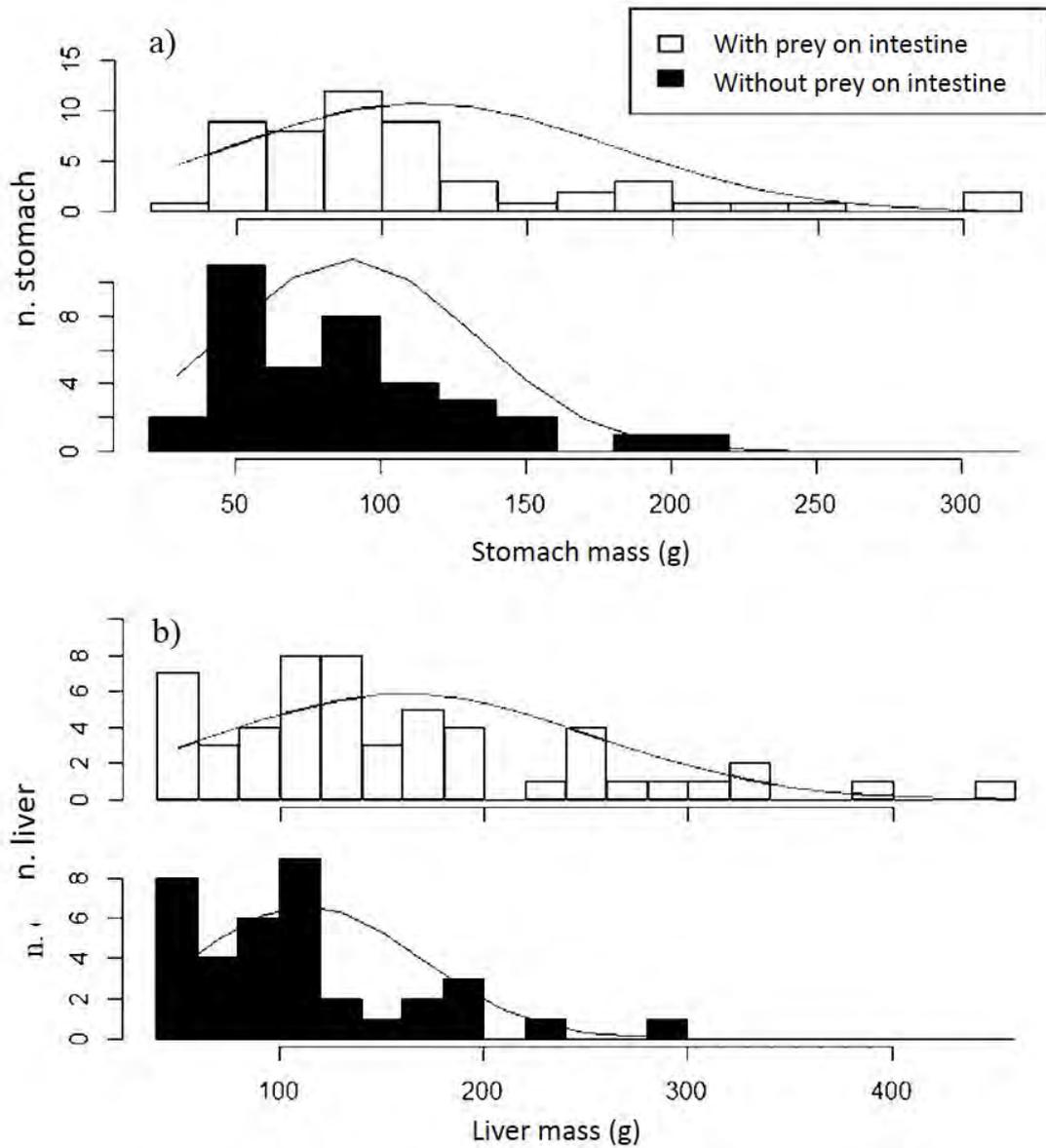
441

Source: Waller et al. (2007)



442

443 **Fig. 2** Covariant Analysis between sex and body mass under the masses of stomach (a),  
444 liver (b) and kidney (c), based in 95 yellow-anacondas. Females are represented in black  
445 circles and males in grey circles



446

447

448 **Fig 3** Masses of the stomach (a), liver (b) and its frequency in fasted yellow-anacondas

449 and in feed yellow-anacondas, based in 93 specimens (a) and 91 specimens (b)