# A seasonally dependent change in the distribution and physiological condition of *Caiman crocodilus yacare* in the Paraguay River Basin

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**Abstract.** The distribution and physiological condition of 116 *Caiman crocodilus yacare* was assessed over one year in the Southern Pantanal. Body mass and intermediary plasma metabolites were measured at three different time periods, representing large differences in the abundance of surface water. During the wet season the study site was completely submerged under water and *C. c. yacare* were distributed evenly throughout. High levels of [glucose] and [triglyceride] in the plasma indicated regular feeding. As the dry season progressed *C. c. yacare* became increasingly crowded around the remaining ponds. They showed a reduction in plasma [glucose] and [triglyceride], and an increase in plasma [β-hydroxy-butyrate], signifying that they were feeding less and utilising fat reserves. At this sampling period, ~40% of the male *C. c. yacare* that were >10 years old inhabited dry grassland and did not have access to water. These animals were significantly lighter than males of a similar length that had immediate water access, and plasma [uric acid] indicated that they had not fed for a long time and were metabolising tissue proteins. Essentially, the adult male *C. c. yacare* that inhabited dry grassland were in a state of energy deficiency. This was so severe in some animals that recovery seemed unlikely. The study suggests that fluctuations in the abundance of surface ground water may influence the size and structure of the *C. c. yacare* population in the Pantanal.

## Introduction

The alligatorid *Caiman crocodilus yacare* is a large opportunist predator and scavenger of the Paraguay River basin (The Pantanal). Population surveys estimate that over 4 million *C. c. yacare* inhabit the 140 000 km$^2$ of seasonally flooded wetland (Mourao *et al.* 2000). The wet season runs from December until March, flooding 95% of the grasslands, and inundating them with river fishes and aquatic invertebrates (Swarts 2000). During the dry season, the retreating water forms isolated ponds, which results in fish being stranded in sizeable numbers. This provides a temporally and spatially isolated food supply, and *C. c. yacare* become ever more crowded around the remaining ponds (Coutinho and Campos 1996; Da Silveira *et al.* 1997). As the dry season progresses many of the isolated ponds cease to exist and, consequently, the food supply for *C. c. yacare* becomes ever more sporadic in space and time. The effect of the seasonal drought on the nutritional and physiological condition of the inhabiting *C. c. yacare* population has never been assessed, and may provide information about the carrying capacity of the *C. c. yacare* population within the Pantanal.

During periods of high food abundance many animals store surplus energy as fat. This provides an energy reserve for the animal to buffer periods of low food availability. The quantity of fat reserves and the speed of utilisation will determine the animals' ability to endure periods of unfavourable resource conditions. The nutritional and physiological condition of an animal during a period of fast may be characterised by certain plasma

intermediary metabolites circulating in the blood. Development of sensitive bioassay techniques has enabled the accurate determination of these metabolites, and such methods have been used successfully to show the physical condition of fasting birds and mammals (Jenni-Eiermann and Jenni 1998; Shmueli *et al.* 2000; Hollmen *et al.* 2001; Rodriguez *et al.* 2005; Artacho *et al.* 2007).

Three discrete metabolic phases relating to the degree of fasting have been described. These are: Phase I, plasma [glucose] is maintained, fat mobilisation increases and protein catabolism decreases; Phase II, lipids are used as the primary source of energy, resulting in decreased plasma [triglyceride] and an increase in plasma [β-hydroxy-butyrate] oxidation; Phase III, other reserves become exhausted and protein catabolism increases. This results in elevated plasma [uric acid], and is accompanied by a rapid reduction in body mass. It is typically considered 'premortal', and once this phase is reached organ damage can occur, making recovery a long complicated process (Robin *et al.* 1988; Castellini and Rea 1992; Rodriguez *et al.* 2005). Although on different time scales, the shift in metabolic phases is similar between fast-adapted and non-fast-adapted animals; with fast-adapted species being able to prolong the consumption of fat stores and minimise protein utilisation (Cherel and Le Maho 1988; Cherel *et al.* 1988). The few studies that have observed changes in plasma metabolites during fasting in reptiles generally agree with the three-phase

model (Gist 1972; Martin and Bagby 1973; McCue 2007). However, no studies have examined plasma metabolites in free-ranging reptiles, or the response of plasma metabolites to fasting in any crocodilian.

The purpose of this study was to determine how the interannual drought affected the physiological condition of the *C. c. yacare* population in the Paraguayan river basin. The hypothesis was that *C. c. yacare* would be subjected to a period of fasting at the height of the dry season, when water and subsequently food resources were scarce. However, due to the known ability of reptiles to survive long periods without food, it was uncertain whether *C. c. yacare* endured the dry season by metabolising stored fats (Phase II), or entered the latter stages of starvation (Phase III). Additionally, because of the lack of information on plasma intermediary metabolites in crocodilians, a comparative study was undertaken on a captive population of *C. c. yacare*, where the level of feeding and fasting was controlled.

## Materials and methods

### Study site

The study was undertaken at Fazenda Sao Joao in the Nhecolandia subregion of the Pantanal, Brazil. The boundary of the study area was 3 km × 200 m (19°23.62′S, 57°02.90′W), and encompassed an area of seasonally flooded grassland, interspersed with patches of woodland and small isolated freshwater ponds (baias). The study site was completely submerged under 0.5–2 m of water from December until April. After April, the water receded, and by mid-June the area comprised a mosaic of dry grassland and shallow ponds. By mid-September the site was composed mainly of dry grassland, and surface water existed in only two ponds, ~50 m in diameter and 2 m deep. The inhabiting population of *Caiman crocodilus yacare* was studied during three discrete periods throughout the year. These were carefully chosen to provide a snapshot of the study site under very different surface-water conditions. The date of each study episode was: 2–8 June 2006, 16–22 September 2006, and 5–11 March 2007.

### Survey and capture of caimans

Survey counts of *C. c. yacare* were made nightly at 2100 hours, and the study site was systematically covered with a powerful 12-V torch (Maglite, CA, U.SA). The location of each *C. c. yacare* was identified from the reflection of the torch light back from the animals' eye (eye shine). The total area covered by each spotlight survey was ~1.5 km$^2$.

*C. c. yacare* were captured between 1900 and 2400 hours because the animals were easier to locate by torchlight, and appeared more subdued than during daylight hours. Capture occurred from either a small boat or by wading into shallower water, except during September when some *C. c. yacare* were captured on dry grassland. The *C. c. yacare* were captured at random on an encounter basis, but only animals with a snout–vent length (SVL) >55 cm were kept for further study. First, a noosed 4-mm-diameter steel cable was passed over the animal's head using a 3-m length of bamboo. The cable had a stitch plate locking mechanism and, once secured, the animal could not slip backwards through the noose. Upon capture the animal would immediately struggle, but would quickly become subdued after the eyes were covered by a wet cloth. A rubber band was then tied around the snout to secure the jaws shut. The animal was immediately carried to a close suitable flat area of ground, or sampled in the boat.

A 5-mL syringe with 21-G needle was inserted into the cranial sinus and 3 mL of blood extracted. The blood was immediately put into a Vacutainer tube containing EDTA to prevent clotting. All blood samples were taken and stored on ice within 5 min of capture. The animal was then weighed using hanging scales (Tetron, UK), and the total body length and the distance from snout tip to the bottom of the cloacae was measured (SVL). Sex was determined by feeling for the presence or absence of the phallus with a sterile gloved finger. A purpose-designed nylon T-bar anchor tag with a unique ID code (FLOY, Seattle, USA) was inserted subcutaneously between the nuccal plates, using a tagging applicator with reinforced 4-cm needles (FLOY). The longitude and latitude of the capture location was determined by hand-held GPS (Marine 67, GARMIN, USA) before release. The procedure from capture to release took no more than 15 min. In the 72 h following sampling, ID-tagged *C. c. yacare* were seen swimming and basking within the study site. All animals appeared to be behaving normally and no fatalities of sampled *C. c. yacare* were ever recorded.

### Laboratory animals

A controlled feeding experiment was undertaken on 20 captive *C. c. yacare* at the Universidade Estadual Paulista, Rio Claro, São Paulo, from March 2006 until April 2007. The animals had a SVL between 55 cm and 97 cm at the start of the study, and were kept within an outdoor enclosure (20 × 15 m) that contained native grasses, foliage and a deep-water pool to simulate natural conditions. They received a diet of juvenile chicks to satiation fortnightly. In the week succeeding the field studies, the captive *C. c. yacare* underwent capture, their weight and length was determined, and a blood sample was taken. All procedures were identical to field capture and sampling techniques. The captive *C. c. yacare* were not fed 7 days before sampling to reduce any immediate effects of digestion on the measured plasma metabolites. During September and October 2007 an additional 10 captive *C. c. yacare* (SVL 70–89 cm) were weighed and blood sampled before undergoing a 53-day fast. After this period of fasting the animals were measured and blood samples were taken using the described methods.

### Condition index

The body mass index (BMI) or condition factor was determined by the residual of the regression between body mass (BM) and (SVL) from all the wild *C. c. yacare* that were measured. This produced the regression line of best fit: predicted body mass (PBM) = 0.466 – 22.24 × SVL ($r^2$ = 96, $P$ < 0.01). This equation was used to calculate the PBM for an individual related to SVL, to see if it was over- or under-weight for the group.

### Haematology

Immediately after the blood was extracted from *C. c. yacare*, it was treated with EDTA and stored on ice. Then, 250 μL of whole blood was placed into 1 mL of perchloric acid (PCA). This

preserved the plasma concentration of glucose and β-hydroxy-butyrate. The remaining blood was kept on ice for a maximum of 2 h before it was centrifuged at 5000*g* for 10 min. The separated plasma was removed and stored at –20°C. The frozen blood was transported to the Universidade Estadual Paulista, Rio Claro, São Paulo, and analysed within 8 days of collection.

Prior to analysis, plasma samples were given 2 h to thaw on the bench at room temperature. The concentration of the various metabolites in the plasma was determined by colourimetric assay, using Randox (Antrim, Northern Ireland, UK) bioassay kits. The whole blood that had been stored in PCA was first neutralised by adding 4M NaOH. The glucose in the plasma underwent enzymatic oxidation in the presence of glucose oxidase (GL 2623, Randox), and total content determined by reading the absorbance at a light wavelength of 500 nm on a UV/Visible spectrometer (Ultraspec 2000, Biotech, Austin, TX). β-hydroxybutarate content was determined by the kinetic enzyme method on the basis of its oxidation to acetoacetate (RB 1007, Randox), and read at 340 nm. The plasma samples that had been frozen at –20°C were analysed for triglyceride content by the enzymatic hydrolysis of lipases (TR210, Randox), and read at 500 nm. Uric acid content was also determined in the frozen plasma samples, by converting uricase to allantoin and hydrogen peroxidase, and reading at 520 nm (UA 230, Randox).

A pilot study was undertaken to assess whether the preparation and storage procedures preserved plasma metabolite concentration in the time between sampling and analysis. Results found that no degradation of plasma metabolites occurred using the described methods.

### Pilot study to determine whether capture of C.  c. yacare affected plasma metabolite concentration

It was vital for the study to determine whether the initial capture of *C. c. yacare* resulted in a change in any of the measured plasma metabolites. Thus, it was required to take a blood sample from an animal both before and then after it was captured. This was achieved by first catheterising the femoral artery in five *C. c. yacare* (3.2 ± 0.4 kg), then allowing them a period of rest before the experiment.

To catheterise *C. c. yacare*, the animals were first restrained, and received a 2-mL injection of local anaesthetic (Lignocaine, São Paulo, Brazil) into the muscle of the left hind leg. After the anaesthetic took effect (5 min) a vertical incision of 3 cm was made on the lateral surface of the thigh, and the femoral artery was freed of connective tissue by blunt dissection. The femoral artery was raised with forceps and a small hole made with a scalpel. A polyurethane cannulae (40 cm long × 0.8 mm in diameter), rinsed with sodium heparin (100 IU mL$^{-1}$ heparin in 0.9% NaCl solution), was inserted into the artery and advanced 0.5 cm upstream. The cannulae was externalised through a hole in the skin at the base of the limb, and fixed firmly into position using four single silk sutras. Finally, the wound was closed using three single silk sutras, and the area was cleaned with 70% ethanol. The catheterisation had no visible effect on limb movement. The procedure took 20–30 min.

The animals were kept in a darkened box with water and care was taken to not disturb the animals for 48 h. During this period the cannulae tube remained outside the box, and was flushed periodically with heparin to prevent it from being blocked by clotted blood. After the 48-h rest period 0.2 mL of blood was extracted via the cannulae without disturbing the animal. The box was then opened and the *C. c. yacare* captured using the same methods as described for the wild *C. c. yacare*. The animal was then restrained and the blood extracted (0.2 mL) via cannulae at 2-min intervals for 18 min. The blood samples were processed as described previously, stored at –20°C, and then plasma [glucose], [triglyceride], [β-hydroxy-butyrate], and [uric acid] determined using previously described methods.

The study showed that capturing and restraining *C. c. yacare* by these methods did not significantly change plasma [triglyceride], [β-hydroxy-butyrate] or [uric acid] up to 18 min after capture. Plasma [glucose] increased by 12–15% in three of the five *C. c. yacare* 12 min after initial capture. As a consequence of this result, only blood samples that were taken from *C. c. yacare* within 8 min of initial capture were used for biochemical analysis.

### Statistics

The relationship between sex and the various plasma metabolites were determined by ANOVA. This revealed no significant difference between male and female *C. c. yacare* for any of the metabolites measured, and therefore the sexes were pooled into a single group when analysing for significant temporal differences. Multiple regressions were used to test for any significant relationship between the plasma metabolites and their association with body mass. The Tukey HSD test modified for use with unequal groups (Smith 1971) was employed to examine temporal difference in plasma metabolites. Additionally, the Bartletts test was employed to test for significant differences in the within-group variability. The captive *C. c. yacare* were serial sampled and therefore a Students *t*-test for unequal variances was used to determine temporal effects, and the effects of the 53-day fast. Results that produced a $P < 0.05$ were considered to be significantly different.

## Results

### Distribution

The seasonal decline in abundance of surface ground water greatly affected the spatial distribution of *C. c. yacare* (Fig. 1). In March, the grasslands were completely submerged under water, and 82% of the total number (117 ± 8.2, *n* = 3) of *C. c. yacare* inhabiting the study site inhabited the flooded grasslands. In June ~40% of the grassland was exposed, interspersed with many shallow ponds and ditches. The number of *C. c. yacare* within the study site had not significantly changed, but a 2-fold larger proportion of the inhabiting *C. c. yacare* were now found within the permanent ponds. By late September the only surface water within the 1.5-km$^2$ study site existed in two shallow ponds (50 m diameter, 2 m depth). The total number of *C. c. yacare* in the study site had risen 46% since June, and nearly all the *C. c. yacare* now inhabited the permanent ponds, with only 11% inhabiting dry grassland.

### Morphometrics

In total, 116 *C. c. yacare* that inhabited the study site were studied. The details of these *C.  c. yacare* are shown in Table 1. The sex ratio of *C.  c. yacare* was male-biased at all three sam-
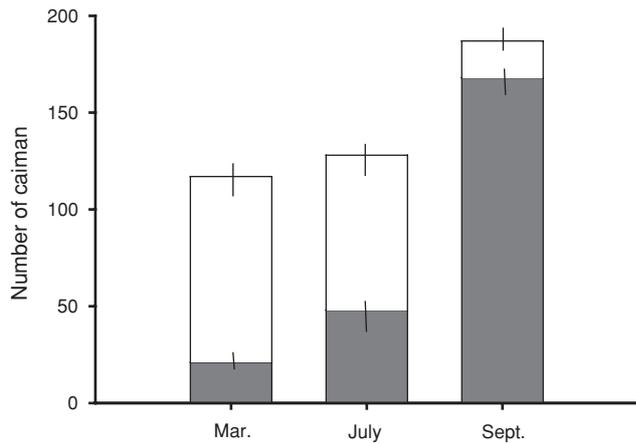
**Fig. 1.** Total number of *C. c. yacare* (mean ± s.e., number of observations = 3) counted within the study site (3 km × 200 m) during respective months. To illustrate the distribution of *C. c. yacare*, each bar is divided into the number of animals that inhabited the permanent pond (black) and those that inhabited dry grassland (white).

pling episodes. Male *C. c. yacare* had a significantly greater body mass ($F_{2,116} = 12.32$, $P < 0.01$) and snout–vent length ($F_{2,116} = 8.37$, $P < 0.01$) than the females studied. All *C. c. yacare* with a body mass >23 kg and a SVL >91 cm were male. Calculation of BMI for all the *C. c. yacare* studied showed that there was no significant difference ($F_{2,232} = 0.39$, $P = 0.54$) between the body mass of males and females for a given SVL. The captive *C. c. yacare* showed similar results to wild animals, with males being significantly greater in body mass and SVL than the females ($F_{2,18} = 7.8$, $P < 0.01$) (Table 1), but the sexes showed a similar body mass for the same SVL. Comparison of a calculated BMI between wild and captive *C. c. yacare* showed that the captive animals were significantly heavier than their wild counterparts ($F_{1,176} = 16.4$, $P = 0.02$).

Over the duration of the study the captive *C. c. yacare* increased body mass by 5.2 ± 0.8 kg year$^{-1}$ and SVL by 7.3 cm year$^{-1}$. The captive *C. c. yacare* decreased body mass by 17 ± 1.2% after a 53-day period of fasting (Table 2).

Nine *C. c. yacare* were found inhabiting dry grassland during September (Table 3). These *C. c. yacare* were all males and they had a body mass that was 31% lighter ($F_{2,9} = 9.6$, $P < 0.05$) than males of similar SVL that inhabited permanent ponds. Prediction of body mass from SVL, using the calculated BMI, showed the *C. c. yacare* from the dry grassland to have a body mass that was 24.8% less than predicted ($F_{1,17} = 13.6$, $P < 0.01$), whereas the *C. c. yacare* inhabiting ponds had a body mass that was 14.6% greater than that predicted ($F_{1,19} = 12.1$, $P < 0.01$).

*Plasma intermediary metabolites*

There was no association between sex and any of the measured plasma metabolites (ANCOVA, $F_{1,125} = 2.6$, $P = 0.86$). There was also no significant relationship between body mass and SVL, with any of the plasma metabolites (Multifactor ANOVA, $F = 0.59$, $P = 0.64$). There was a significant logarithmic relationship between plasma [glucose] and [triglyceride] ($r^2 = 0.67$, $n = 146$, $P < 0.01$), and an inverse exponential relationship between plasma [triglyceride] and [β-hydroxy-butyrate] ($r^2 = 24$, $n = 146$, $P < 0.05$) (Fig. 2). A positive linear correlation was also observed between plasma [β-hydroxy-butyrate] and [uric acid], $y = 0.91x + 0.128$ ($r^2 = 38$, $n = 146$, $P < 0.05$).

In wild *C. c. yacare*, plasma [glucose] significantly decreased between March and September ($F = 9.2$, $P < 0.05$) (Fig. 3). However, the captive *C. c. yacare* showed no seasonal variation ($F = 3.2$, $P = 0.9$) and had significantly more plasma [glucose] than the wild *C. c. yacare* for all 3 sampling episodes ($F = 20.6$, $P < 0.01$). A similar pattern was observed for plasma [triglyceride], which decreased ($F = 8.7$, $P < 0.05$) in the wild *C. c. yacare* between March and September. The captive *C. c. yacare* exhibited no seasonal variation ($F = 2.2$ $P = 0.81$),

**Table 1. Morphometric data for wild and captive *C. c. yacare***

Sampling was undertaken at three separate time intervals throughout the year, when *C. c. yacare* were subjected to very different surface water conditions. SVL = snout–vent length. PBM is the predicted body mass from the equation: PBM = 0.466 – 22.24 × SVL. Data were tested for significant differences between group means using the the Tukey HSD procedure modified for unequal numbers when required. Superscripted letters denote columns that are significantly different from each other ($P < 0.05$)

| | Sex | Total | BM | SVL | PBM |
|---|---|---|---|---|---|
| Wild *C. c. yacare* | | | | | |
| March (wet season) | M | 22 | 15.6 ± 1.6[a] | 83.5 ± 3.8[a] | 16.4 ± 1.2 |
| | F | 16 | 9.3 ± 2.1[b] | 67 ± 5.6[b] | 8.7 ± 1.6 |
| June | M | 22 | 19.3 ± 3.7 [a] | 88.9 ± 4.1[a] | 18.9 ± 2.1 |
| | F | 15 | 9.6 ± 1.5[b] | 69.8 ± 4.7[b] | 10 ± 1.1 |
| September (dry season) | M | 26 | 17.6 ± 2.3[a] | 83.5 ± 3.8[a] | 16.4 ± 1.8 |
| | F | 17 | 10.8 ± 2.4[b] | 70.2 ± 5.8[b] | 10.2 ± 1.1 |
| Captive *C. c. yacare* | | | | | |
| March (wet season) | M | 10 | 19.3 ± 1.4[a] | 88.9 ± 4.3[a] | 18.9 ± 1.8 |
| | F | 10 | 9.6 ± 1.5[b] | 69.8 ± 4.2[b] | 10.1 ± 2.1 |
| June | M | 10 | 17.6 ± 2.1[a] | 83.5 ± 2.4[a] | 16.4 ± 2.1 |
| | F | 10 | 10.8 ± 1.0[b] | 70.2 ± 4.2[b] | 10.2 ± 2.2 |
| September (dry season) | M | 10 | 15.6 ± 1.4[a] | 83.5 ± 3.8[a] | 16.4 ± 1.21 |
| | F | 10 | 9.4 ± 1.4[b] | 67 ± 4.2[b] | 8.8 ± 0.21 |

**Table 2. Morphometrics and plasma metabolites of captive *C. c. yacare* after a 7-day and then a further 53-day fast**

SVL = snout–vent length. PBM is the predicted body mass from the equation: PBM = 0.466 – 22.24 × SVL. Superscripted letters denote a significant a difference between columns as tested by Students *t*-test assuming unequal variances ($P < 0.05$)

|  | 7-day fast | 53-day fast |
|---|---|---|
| Number | 10 | 10 |
| Males : females | 0.25 | 0.25 |
| Body mass (g) | 18.4 ± 1.2[a] | 15.8 ± 1.25[b] |
| SVL (cm) | 80.4 ± 0.6[a] | 80.4 ± 0.6[b] |
| Predicted BM | 14.9 ± 0.8 | 14.9 ± 0.8 |
| Glucose (mmol L$^{-1}$) | 6.5 ± 0.71[a] | 1.44 ± 0.09[b] |
| Triglycerides (mmol L$^{-1}$) | 4.03 ± 0.81[a] | 1.3 ± 0.73[b] |
| β-HB (mmol L$^{-1}$) | 0.36 ± 0.12[a] | 6.4 ± 1.31[b] |
| Uric acid (mmol L$^{-1}$) | 0.15 ± 0.08[a] | 0.91 ± 0.08[b] |

and had [triglyceride] 10–15-fold greater than the wild *C. c. yacare* ($F = 48.2$, $P < 0.01$). The plasma [β-HB] and [uric acid] were similar between wild and captive *C. c. yacare* in March and June ($P > 0.53$), but by September wild *C. c. yacare* had significantly greater plasma [β-HB] ($F = 36.6$, $P < 0.01$)and [uric acid] ($F = 12.3$, $P < 0.01$) than the captive animals. After captive *C. c. yacare* were subjected to a fast for 53 days, plasma [glucose] and [triglyceride] were reduced by 93% and 67%, respectively, and plasma [β-HB] and [uric acid] had increased by 20-fold and 6-fold, respectively. All changes were significant to 95% (Table 2).

By the September study period, there was a large interindividual difference in the concentration of plasma metabolites between *C. c. yacare* that inhabited dry grassland and those that inhabited the permanent ponds (Table 3). Plasma [glucose] and [triglyceride] were 50% and 87% less ($F = 14.4$ and 23.2, $P < 0.05$), respectively, and plasma [β-HB] and [uric acid] were ~3-fold and ~10-fold greater ($F = 12.2$ and 9.6, $P < 0.01$), respectively. The plasma metabolites from captive *C. c. yacare* that had not been fed for 53 days (Table 2) were compared with

**Table 3. Morphometrics and plasma metabolites in *C. c. yacare* inhabiting either a permanent pond or dry grassland in the month of September**

SVL = snout–vent length. PBM is the predicted body mass from the equation: PBM = 0.466 – 22.24 SVL. Superscripted letters denote a significant difference between column means as tested by Tukey HSD procedure modified for unequal numbers ($P < 0.05$)

|  | Water-inhabiting caiman | Water-inhabiting adult males | Dry land-inhabiting adult males |
|---|---|---|---|
| Number | 34 | 10 | 9 |
| Males : females | 1.25 | 10 | 9 |
| Body mass (g) | 13.8 ± 2.3[a] | 25 ± 4.6[b] | 17.2 ± 2.9[c] |
| SVL (cm) | 72.9 ± 4.0[a] | 95.2 ± 4.2[b] | 97.5 ± 4.2[b] |
| PBM | 11.5 ± 2.8[a] | 21.8 ± 4.1[a] | 22.9 ± 3.2[b] |
| Glucose (mmol L$^{-1}$) | 6.81 ± 0.40[a] | 6.62 ± 0.51[a] | 1.11 ± 1.20[b] |
| Triglycerides mmol L$^{-1}$) | 0.91 ± 0.04[a] | 1.2 ± 0.2[a] | 0.11 ± 0.03[b] |
| β-HB (mmol L$^{-1}$) | 1.91 ± 0.11[a] | 1.80 ± 0.21[a] | 7.3 ± 0.64[b] |
| Uric acid (mmol L$^{-1}$) | 0.19 ± 0.07[a] | 0.18 ± 0.06[a] | 2.3 ± 0.08[b] |
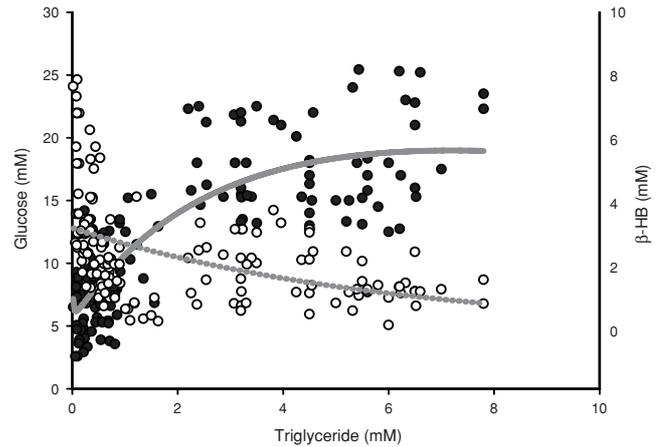


**Fig. 2.** The relationship between plasma [triglyceride] and [glucose] in *C. c. yacare* is shown with black circles, and the grey solid non-linear regression line ($y = (5.3 \times X)/(-0.005 + X) + (37.3 \times X)/(4.69 + X)$, $r^2 = 0.67$, $n = 146$, $P < 0.01$). The relationship between plasma [triglyceride] and [β-HB] in *C. c. yacare* is shown with white circles and the grey dotted non-linear regression line ($y = 3.24 \times \exp(-0.167 \times X)$, $r^2 = 0.20$, $n = 146$, $P < 0.05$).

those of wild *C. c. yacare* that inhabited dry grassland (Table 3). The results showed the two groups to have similar plasma [glucose], [triglyceride] and [β-HB] ($P < 0.05$); however, the wild *C. c. yacare* had significantly higher levels of uric acid ($F = 19.6$, $P > 0.01$) than the captive *C. c. yacare*.

## Discussion

The abundance of surface water varied greatly over the duration of this study. As the dry season progressed, *C. c. yacare* became ever more crowded around the remaining water bodies. The diet of *C. c. yacare* is mainly composed of aquatic vertebrates and invertebrates (Schaller and Crawshaw 1982; Santos *et al.* 1996), and thus, access to food was probably the main determinate of the species' distribution. However, other factors such as mating, thermoregulation, energy preservation and predator evasion cannot be discounted. The determination of plasma intermediary metabolites from free-ranging individuals throughout the year showed a seasonally dependant shift in the fasting state. Only a few laboratory studies have previously determined plasma intermediary metabolites in fasting reptiles (Gist 1972; Martin and Bagby 1973; McCue 2007), and we could not find information on any crocodilian. Therefore, conclusions about the physical condition of free-ranging *C. c. yacare* are drawn from these reptilian studies, avian and mammalian literature, and from results presented here on captive *C. c. yacare* that were fasted for 53 days.

Plasma [glucose] and [triglyceride] were significantly lower in the wild, compared with the captive, *C. c. yacare*. Circulating plasma [glucose] reflects carbohydrate metabolism (Phase I) and will originate from the diet, or in the fasting state from stored glycogen or from glucaneogenesis (Jenni-Eiermann and Jenni 1998). Plasma [triglyceride] reflects the amount of fat eaten in foods or utilised from carbohydrate stores. Essentially, the results suggest that the 5–7 chicks (~1.5 kg) ingested fortnightly by the captive *C. c. yacare* were a significantly larger
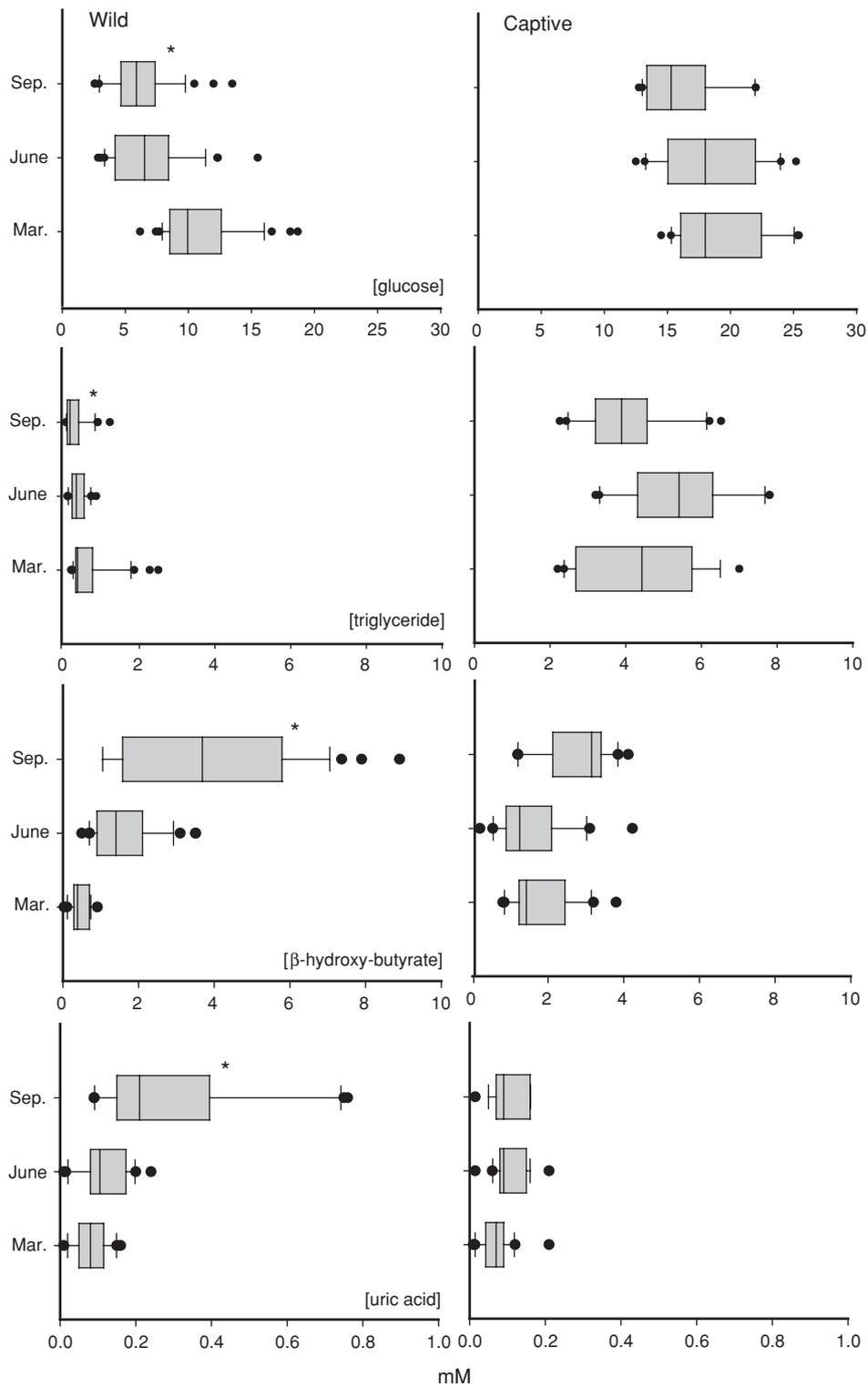
**Fig. 3.** Plasma [metabolite] in wild and captive *C. c. yacare* at three periods during the year, each exhibiting very different surface water conditions. The various metabolites are displayed vertically and labelled for each graph. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. In addition, the outlying data points are shown. An asterisk indicates where the group in September is significantly different from the group in March ($P < 0.05$).

ration of carbohydrates and fats than was ingested by wild *C. c. yacare*. Wild *C. c. yacare* that inhabited permanent water bodies during the dry season had lower plasma [glucose] and [triglyceride] than recorded during the wet season, indicating that they had surpassed the Phase I criteria of the fast and were utilising fat reserves. At the same time *C. c. yacare* that did not have access to water had far lower plasma [glucose] and [triglyceride] than *C. c. yacare* that inhabited ponds, and their plasma [glucose] and [triglyceride] was comparable with captive *C. c. yacare* that had not fed for 53 days. Glucose is important to most tissues and, additionally, for the integrity of the nervous system (Jenni-Eiermann *et al.* 2002). A fasting animal will try to maintain plasma [glucose] as long as physiologically possible, and therefore very low plasma [glucose] indicates an energy-deficient physiological state.

Phase II of the fast is characterised by the breakdown of stored triglyceride, which results in the liberation of free fatty acids that are then oxidised by the liver to form β-hydroxybutyrate (β-HB) (Jenni-Eiermann *et al.* 2002; Artacho *et al.* 2007). The inverse relationship between [triglyceride] and [β-HB] in *C. c. yacare* suggests that crocodilians possess β-HB dehydrogenase enzyme activities that are comparable to those reported in birds (Jenni-Eiermann *et al.* 2002; Artacho *et al.* 2007), and similar to fast-tolerant migratory birds (Cherel and Le Maho 1988; Cherel *et al.* 1988). Crocodiles may prolong fasting by reducing protein oxidation and utilising lipid reserves. The ability of an animal to sustain Phase II of the fast, will be dependant on both the amount of fat reserves and the rate of expenditure. *C. c. yacare* that had inhabited dry grassland had elevated plasma [β-HB] over those that inhabited water, suggesting that the utilisation of stored fats occurred at a greater rate in *C. c. yacare* that did not have access to water. The reason for this could be the increased cost of locomotion for *C. c. yacare* inhabiting dry grassland; although not quantified energetically for this species, the morphology of crocodilians results in their being far more efficient at aquatic, compared with terrestrial, locomotion. Additionally, crocodilians have a limited ability to physiologically control their body temperature and, instead, will keep their daily body temperature within the preferred thermal range by using the thermal latency of water (O'Connor 1999). During the dry season the daily air temperature in the Pantanal averages 30°C, but can reach 37°C, and night-time temperature averages 18°C, but can be as low as 7°C (Swarts 2000; Campos *et al.* 2006). This large shift in diurnal air temperature probably results in large fluctuations in body temperature outside of the animals' preferred thermal range. Therefore, the energy required for the maintenance of basic metabolic processes may be increased in *C. c. yacare* that do not have access to water for thermal buffering. The energetic cost may be quite significant; for example, the standard metabolic rate of the salt water crocodile (*C. porosus*) was increased 3-fold when body temperature was elevated from 20°C and 30°C (Grigg 1978).

Once all the stored fats have been utilised by a fasting animal, only tissue proteins remain. The catabolisation process will generate urea as a by-product and the animal converts this to uric acid for excretion. Most of the *C. c. yacare* sampled in this study had very low or negligible plasma [uric acid], illustrating the crocodilian's ability to prolong the utilisation of stored fats (Phase II). This was evident even at the height of the dry season, when *C. c. yacare* were crowded within the ponds under high stocking densities. In contrast, the *C. c. yacare* that did not have water access exhibited a 10-fold increase in plasma [uric acid], indicating that these *C. c. yacare* had exhausted fat reserves and were required to catabolise proteins to maintain basic physiological function. Captive *C. c. yacare* that had not eaten for 53 days did not exhibit the high plasma [uric acid] recorded in the dry grassland *C. c. yacare*. The reason for the disparity is probably due to a combination of greater fat reserves before the fast and a reduced rate of energy utilisation. The fasting captive *C. c. yacare* were often submerged in water, which supports the theory that wild *C. c. yacare* that have access to water have the capacity to reduce the energetic cost of living over those *C. c. yacare* that do not.

### Ecological significance

The ability of *C. c. yacare* to access a large body of water during the dry season was a major determining factor in maintaining good physical condition. *C. c. yacare* that did not have access to water had used up fat reserves and were undergoing protein catabolism, which may have resulted in irreversible tissue damage (Castellini and Rea 1992; Rodriguez *et al.* 2005). Therefore, the reason some *C. c. yacare* remained starving in open grassland, when permanent water was nearby, appears paradoxical. All the *C. c. yacare* in the dry grassland were males >10 years of age (Coutinho 2000; Campos *et al.* 2006), and we hypothesise that these *C. c. yacare* were excluded by more dominant males. Social hierarchy has been documented in other crocodilians (Seebacher and Grigg 1997), and this theory is supported here by the bite scars, missing limbs and tails that were observed on the *C. c. yacare* in the dry grassland. If inter-specific aggression deprives adult males from access to water, then this may be a common mortality factor for *C. c. yacare* in the Pantanal. Other studies have also documented solitary adult males inhabiting dry grassland and recorded some of these males to be 20% lighter than similar-sized females (Schaller and Crawshaw 1982; Campos *et al.* 2003, 2006). In the present study it was calculated that the number of males that inhabited dry grassland were ~15% of the total number of male *C. c. yacare* within the study area and this proportion was increased to ~40% if only males >10 years of age were considered. At present, it is unsubstantiated how many adult males die through starvation during the seasonal drought. In the Pantanal, adult dead *C. c. yacare* have been reported towards the end of the drought although sex, size, cause of death and exact numbers have not been recorded. This is an important area of future investigation. Essentially, results presented here suggest that the social exclusion of adult male *C. c. yacare* from water holes does lead to starvation, and thus the population ecology of the species in the Pantanal is intrinsically linked to the temporal and spatial abundance of surface water.

### Management implications

The Paraguayan river basin population of *C. c. yacare* was severely over-exploited in the 1970s, 1980s and early 1990s. In recent years, the numbers have recovered substantially, due to a drop in the price of skins on the international market (Mourao *et al.* 2000). However, the present abundance of *C. c. yacare*

has been held responsible for declining fish stocks and sustainable harvesting of the species remains a hot topic. Results presented here show that, in fact, *C. c. yacare* feed mainly during the wet season, when they inhabit the flooded grasslands and fast when their numbers become crowded within ponds. It is tempting to speculate, however, that the harvesting of large adult male *C. c. yacare* may be an option, as many are effectively redundant from the breeding population and potentially will die through starvation. Essentially, before management decisions can be made, greater insight is needed into the size of the *C. c. yacare* population that a discrete area of Pantanal wetland can sustain, and how this is influenced by interannual and multi-annual fluctuations in surface water.

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