

## Increasing photoperiod enhances growth in captive hatchling *Caiman latirostris*



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### ABSTRACT

Captive crocodylians reduce their intake of food during cold periods, even when kept under controlled conditions and fed *ad libitum*. These manifestations may be due to photoperiod length which is known to influence circadian rhythm. To test this hypothesis, 72 *Caiman latirostris* hatchlings from three wild-harvested nests, were reared under controlled conditions of temperature and humidity with varying hours of light exposure. Three treatments were performed: A) increasing photoperiod to 16 h light; B) decreasing photoperiod to 8 h light; C) and the control treatment with constant 12 h light / 12 h dark. Caiman growth was measured as both weight and linear measures (snout-vent length and total length). Individuals from the increased photoperiod treatment group (16 h) showed a significant increase in all growth parameters compared to other treatments (control = 12 h; reduced photoperiod = 8 h). There was no difference in stress (plasma corticosterone levels) between the treatment groups ensuring that the additional growth benefit was not antagonistic to welfare. From this, it can be postulated that photoperiod, along with availability of temperature, is another important factor which influences the growth of *C. latirostris*.

### 1. Introduction

Broad-snouted caiman (*Caiman latirostris*), like all ectotherms, rely on external heat sources, mainly from solar heat and/or radiation, to regulate metabolic rate and physiological activities (Coulson et al., 1996; Lance, 2003). Multiple studies have shown that food consumption and growth rate were higher in alligatorids maintained at 32 °C (like *Alligator mississippiensis* and *Caiman crocodylus yacare*) or 34 °C (*C. latirostris*) than individuals maintained at 28–29 °C (Staton et al., 1992; Miranda et al., 1999; Parachú Marcó et al., 2009; Bassetti et al., 2014). At temperatures below 25 °C, food intake is reduced as is metabolic rate, as well as speed and duration of digestive processes (Bonilla, 2006). Conversely, when they are held above 35 °C, they may be thermally stressed (Bonilla, 2006; Webb et al., 2013). However, within these boundaries (25–34 °C), *Caiman* kept at higher temperatures, eat more (Piña and Larriera, 2002) and have more efficient food conversion

ratios (Parachú Marcó et al., 2009).

When seasons change, temperature is not the only climatic condition that changes. Other climatic variables such as photoperiod and humidity can also have seasonality. Under natural conditions, photoperiod is shorter when seasonal temperatures decrease. For example in Argentina, there is 14 h of light in summer but only 10 h in winter (Naval Hydrography Service, 2014). Photoperiod, as defined by Ricklefs (1996), is the “length of daytime clarity” which influences circadian rhythm and is the biological clock that controls a variety of cycles, such as behavior, sleep and activity (Arreaza-Cardier and Arreaza-Padilla, 2002). Palmer and Palmer (1994) reported that the predatory behavior of *A. mississippiensis* at different times of day (morning, afternoon, evening and night) had a suggested relationship with circadian rhythm. During winter, wild adult *C. latirostris* reduce their movements and food intake, however during spring they resume feeding until the peak of activity in the breeding season during summer

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between November and March (Larriera and Imhof, 2006; Simoncini et al., 2013).

The influence of photoperiod on crocodylians, particularly in relation to juvenile growth, is poorly understood (Perry et al., 2008). Herbert et al. (2002) compared the growth of hatchling Chinese alligators (*A. sinensis*) and *A. mississippiensis* over one year using natural lighting while maintaining a constant temperature (31 °C). Despite these two species being closely related, *A. sinensis* appeared to go into a hibernation-like state (reduced food intake and growth) during autumn and winter while *A. mississippiensis* continued to eat and grow so it was hypothesized that photoperiod might be the causal effect. To test this hypothesis, in the following year, Herbert et al. (2002) imposed an 18 h photoperiod on eight *A. sinensis* and compared their growth to eight others maintained in natural lighting. To begin, it appeared that the 18 h photoperiod group was stimulated to continue growing at the faster rate but after a few months this growth slowed whereby the natural lighting group caught up to and surpassed them in growth. More so, those *A. sinensis* exposed to the increased photoperiod began to appear emaciated and took several months to regain their normal growth pattern. Similar to *A. sinensis*, *C. latirostris* growth rates have been reported to lag even in temperature-controlled environments along with declines of some metabolic and nutritional indicators (Barboza et al., 2006, 2008) so it is of interest to see if increased photoperiod could have a beneficial effect.

Some initial research in *C. latirostris* has showed that exposure to 12 h or 24 h light periods achieved higher growth rates than those kept in total darkness (Príncipe, 2007). To extend on Príncipe (2007), this study uses the same control photoperiod (12 h light) and compares the change in weight and length of three month old *C. latirostris* hatchlings to those exposed to an increasing photoperiod of 16 h or decreasing the photoperiod to 8 h. In addition, corticosterone was used as the predictor of “stress” to ensure that no adverse animal welfare effects resulted (Lance and Elsey, 1999; Moore and Jessop, 2003; Parachú Marcó et al., 2015).

## 2. Materials and methods

### 2.1. Experimental animals and housing

Seventy-two (72) three month old *C. latirostris* hatchlings, individually identified by scute clipping (Richardson et al., 2002) from three wild nests were used for this study. All hatchlings were housed in plastic trays (75 cm long × 40 cm wide × 29 cm deep), inclined to create land and water areas, with lids to ensure no unwanted light could enter. The trays were kept in a temperature controlled room at 31 ± 1 °C using thermostatically-controlled air and water heaters and isolated from external light sources. Hobo™ data loggers (Onset Computer Corporation, MA) were used to monitor water and dry area temperatures (average 31.4 ± 0.4 °C; range 29.5–31.9 °C). The *Caiman* were fed a mince of 60% chicken heads and 40% reptile pellets (Avigan, Agroservicios Humboldt S.A., Argentina) *ad libitum* every second day in the morning. Cleaning of the trays was performed on alternate days by removing any leftover food and changing the water which was pre-heated to 31 ± 1 °C.

### 2.2. Experimental design

Twelve hatchlings, four from each clutch, were assigned to one of six trays to dissociate any clutch effects (Garnett and Murray, 1986; Isberg et al., 2005; Montini et al., 2006). This was also the optimal stocking density for the size of the trays (Poletta et al., 2008). Two trays were assigned to each of three treatments: A) increasing photoperiod to 16 h light; B) decreasing photoperiod to 8 h light; C) and the control treatment with constant 12 h light / 12 h dark. Photoperiod was controlled using low energy (13 W; Osram) white light bulbs. Prior to the experiment, it was confirmed using the HOBO data loggers that these

light bulbs did not affect the ambient temperature and generated 800Lux (EXTECH 0–2000Lux measuring range), which is characteristic of daylight.

All animals were initially placed under the control treatment (12 h light/12 h dark) for 7 days. Thereafter, the photoperiod was decreased or increased by 15 min every four days until the treatment photoperiods (8 h and 16 h) were reached at day 64. The decision to gradually change the photoperiod was to simulate the gradual changes that occur with the natural change in seasons. The measurement at day 41 corresponds to the maximum difference of photoperiod (4 h) of *Caiman latirostris* population distribution such as in Argentina. At 71 days, when the 8 h photoperiod difference was reached, another measurement was performed. The last measurement (day 109) was made 38 days after reaching the final treatment photoperiods.

### 2.3. Animal assessments

The animals were weighed (Bwt; BOECO® balance ± 0.1 g) and measured (snout-vent length, SVL; total length, TL) before the habituation period (Day 0). Measurements were again taken at days 41 (midway through photoperiod adjustment), 71 (treatment photoperiod) and 109 (trial terminated). To quantify corticosterone (Lance et al., 2000), blood samples were taken from the spinal vein (Zippel et al., 2003) at Day 109. The blood was centrifuged at 3000 rpm for 15 min, the plasma separated and stored at –20 °C until quantification using radioimmunoassay (RIA) according to Parachú Marcó et al. (2015). Samples were analyzed in duplicate. The intra-assay variation coefficient was < 5%, while the inter-assay was below 10%. Lower detection limit of the assay was 0.1 ng/tube.

### 2.4. Statistical analysis

Statistical analysis was performed by Restricted Maximum Likelihood (REML) using the statistical package GenStat (Version 16.1; VSN International Ltd). Bwt was transformed to achieve normality using natural logarithm (*i.e.* LnBwt). For the morphometric data, fixed effects included treatment (factor; *t* = 8, 12 or 16 h), day of measurement (factor; *d* = 0, 41, 71 and 109 days) and tank (factor; *j* = 1, ..., 12). Since the data were repeated measures, Animal ID was included as a random effect to link the observations of an individual. Clutch was also included as a random effect to account for any clutch effects (*e.g.* Turton et al., 1997). A similar analysis was conducted for corticosterone but as there were no repeated measures, only treatment, tank and clutch could be modelled. All interactions were included and 5% significance level was chosen to evaluate the explanatory variable using backwards elimination. The significance of variance components were assessed using likelihood ratio tests again at the 5% level. All results are reported as back-transformed model-based means ± standard errors (SE) unless otherwise stated.

## 3. Results

### 3.1. Photoperiod effect on growth

All measures were not significantly different on days 0 and 41 (*P* > 0.05) although a trend was becoming apparent. By day 71, the linear measures of growth (SVL and TL) for the 16 h treatment group were significantly longer than the 8 h treatment group (*P* < 0.001; Fig. 1A & B). This trend continued and by day 109, the 16 h group was significantly longer than both the control (mean difference SVL 1.61 cm) and 8 h (mean difference SVL 1.83 cm) groups. Interestingly, there was no effect of treatment on Bwt between treatment groups (*P* = 0.33) although all caimans continued to gain weight throughout the study (Fig. 1C). However, when data from day 109 was subset, the 16 h treatment group was significantly heavier than both the control (by mean of 30.1 g) and 8 h (by mean of 34.8 g) groups (*P* = 0.05;

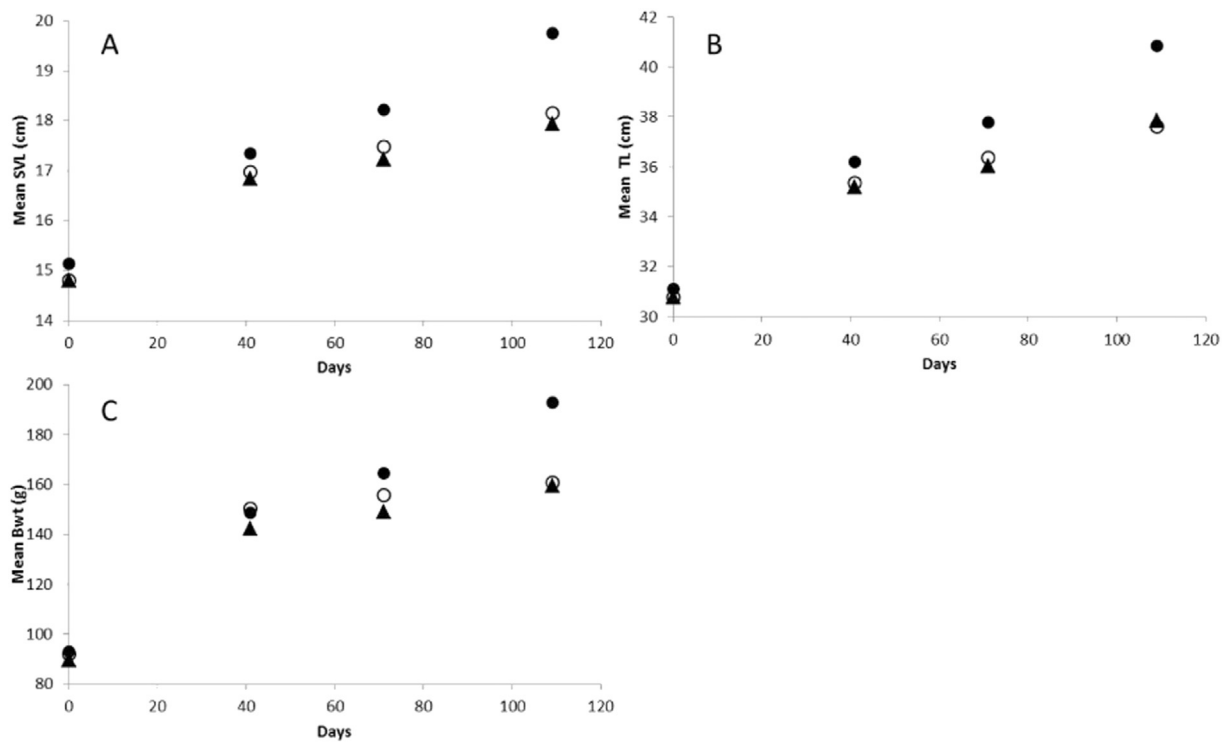


Fig. 1. Effect of different photoperiods on caiman growth: A) snout-to-vent length (SVL), B) total length (TL) and C) body weight (Bwt). Treatments were 16 h photoperiod (closed circles), 8 h (closed triangles) and a control (12 h, open circles).

Fig. 1C).

The tank that the caimans were housed in were not significant for any growth variable ( $P > 0.77$ ) but there was a highly significant Clutch effect ( $P < 0.05$ ).

### 3.2. Effect of photoperiod on stress

Plasma corticosterone levels were similar between treatments ( $P = 0.21$ ; Fig. 2) and there was no effect of clutch ( $P = 0.92$ ). The average corticosterone value for all caiman was  $22.86 \pm 1.47$  ng/ml.

## 4. Discussion

Photoperiod had a significant effect on *C. latirostris* growth over the study period. The group with the longest photoperiod (16 h) showed the fastest growth in both linear and weight measures. However, the difference in growth rates was not proportional whereby the control (12h) and shortest (8 h) photoperiod groups never significantly

deviated from each other. This could have important implications for producers who have long understood the importance of artificial heating on caiman growth but have been missing the advantages of maximizing this potential through photoperiod manipulation.

These results are in contrast to a previous study by Príncipe (2007) who found hatchling *C. latirostris* exposed to continuous lighting grew slower than the same control (12 h) used in this study. There were obvious differences in study length (60 days in Príncipe, 2007 compared to 109 days in this study) but the more obvious explanation for the poor result in Príncipe (2007) is the extreme treatment (continuous lighting) chosen. Of interest would have been to observe if there were any difference in corticosterone values in Príncipe (2007), which were not observed in this study.

Palmer and Palmer (1994) noted a change in predatory behavior in *A. mississippiensis* under different photoperiods. A similar rate of predation was observed for over a week until the treatment groups were applied after which there was a gradual transition from the predation pattern (number of prey fish consumed wholly or partially during each test session) in response to manipulation of the light-dark cycle upon which the circadian rhythm depends. Coincidentally, in the present research, there was a gradual transition with variations in weight and length.

All vertebrate classes, including reptiles, behaviorally respond to photoperiod and temperature changes, which in turn are related to circadian rhythm, regulated by plasma melatonin levels primarily secreted by the pineal gland (Lance and Lauren, 1984; Firth et al., 2010). Studies in the Order Crocodylia have not detected the presence of this gland so it is assumed that the source of melatonin is extrapineal (Firth et al., 2010). Measurable amounts of melatonin have been detected in *A. mississippiensis* although its rate of production was not significant (Roth et al., 1980). These authors suggest that a lack of seasonal variation of photoperiod and temperature in tropical regions may explain this finding. However, for species of crocodylians that live further from the tropics, where the variation of photoperiod and temperature are substantially different between seasons, like *C. latirostris*, melatonin rhythms could be significantly different but this requires validation.

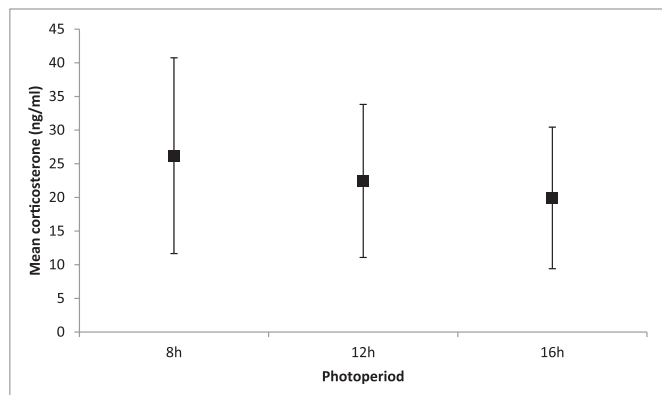


Fig. 2. Mean plasma corticosterone levels (ng/ml;  $\pm$  standard deviation) on day 109 across photoperiod treatments ( $P > 0.05$ ).

We are currently implementing these results on farms. If we are able to reproduce the outcomes on a large scale, they will benefit the producer by reducing production costs by obtaining the required caiman size faster. Also, since the caiman management program requires the re-release of a certain percentage of caiman back into the wild after one year (Larriera and Imhof, 2006), the animals released will be larger, thus increasing their chance of survival (Elsey et al., 1992) further ensuring the non-detriment of egg collection on wild populations. Whether these results are transferrable to other species and different photoperiods needs to be explored.

## 5. Conclusions

The 16 h photoperiod used in this study significantly increased all growth parameters of juvenile *C. latirostris*. Further work needs to be done using a combination of different photoperiods and different controlled temperatures to further dissociate these effects. Irrespective, manipulating photoperiod at this temperature had no effect on caiman plasma stress showing no negative welfare implications. Thus, these results not only provide relevant data on caiman biology but also provide practical information to ensure appropriate rearing conditions for sustainable use programs.

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