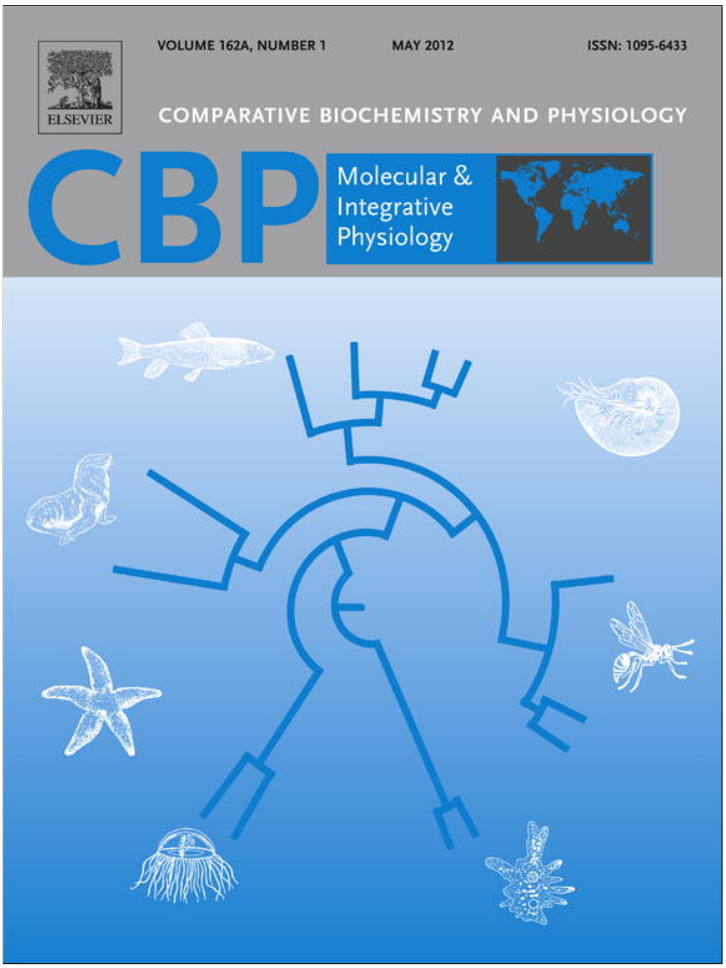

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Physiological variability in yearling alligators: Clutch differences at rest and during activity

B. Bagatto [a,*], D.A. Crossley II [c,1], J. Altimiras [d], R.M. Elsey [e], J.W. Hicks [b]

[a] Department of Biology, University of Akron, Akron, OH, 44325-3908, USA
[b] Department of Ecology and Evolutionary Biology, University of California at Irvine, Irvine, CA, 92697-2525, USA
[c] Developmental Integrative Biology Cluster, Department of Biological Sciences, University of North Texas, Denton, TX, 76203, USA
[d] Avian Behavioural Genomics and Physiology, IMF Biology, Linköpings Universitet, SE-58183 Linkoping, Sweden
[e] Rockefeller Wildlife Refuge, Louisiana Department of Wildlife and Fisheries, Grand Chenier, LA, 70643, USA

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ABSTRACT

The adult phenotype of an organism is the result of its genotype, the environment, and the interaction between the two. Assessing the relative contribution of these factors to the final adult phenotype continues to occupy researchers. Studies have shown clutch effects early in development but few have investigated the persistence of clutch effects on a longer time scale. Five clutches of American alligators were reared for 1 year in a common environment then assessed for the presence of clutch effects as they related to morphological and physiological characteristics. After 1 year, significant clutch effects were evident in all size related variables despite open access to food. Additionally, lung and liver masses remained different between clutches after animal mass was taken into account. Although clutch had no effect on resting heart rate, it significantly contributed to mean arterial pressure. During swimming and exhaustive exercise, the resulting respiratory and metabolic acidoses were strongly dependent on clutch. Therefore, while the environment can have significant influences on the American alligator from hatching to death, the measureable contribution of genetics to the morphology and physiology of the organism remains evident, even after 1 year of common rearing conditions. It behooves researchers to acknowledge and control for clutch effects when designing experiments.

1. Introduction

The adult phenotype of any organism is the result of its genetics, the environmental conditions in which it develops, and the interaction between the two. The role of genetics in dictating subtle yet heritable physiological characteristics during development has been explored primarily by minimizing (but not eliminating) genetic variation through the use of closely related sibling groups. Several studies in birds (Burggren et al., 1994), amphibians (Burggren et al., 2003), reptiles (Crossley et al., 1997, 1998, 2003) and mammals (Bagatto et al., 2000) have attempted to isolate the influences of environment, genetics, and their interactions by selecting species with appropriate life histories (see Burggren, 2000). Of particular relevance is the study by Bagatto et al. (2000) on the physiological variability in genetically identical quadruplets of the neonatal armadillo (*Dasypus novemcinctus*). In neonatal quadruplets, variation in mass, heart rate, ventilation rate, and metabolic rate was significantly less within sibling groups compared to non-sibling groups. This research confirmed that a sibling or litter effect was due to the genetic components determining physiological characters, since the siblings in this case were genetically identical (Bagatto et al., 2000). However, that also demonstrated that the variation in some physiological traits followed a developmental trajectory that would mask the sibling effect. In other words, the physiological variation within sibling groups significantly increased over the first week of neonatal life. Because that study focused on a very brief window of post-natal life, the degree to which the sibling effect persists into juvenile stages and into adulthood remains unknown. It is important to understand the nature of clutch effects since this source of variation could confound the comparison of physiological measurements.

To further the understanding of how genetic and environmental factors combine to shape the physiology of an animal, we studied the influence of relatedness on cardiovascular function in American alligator (*Alligator mississippiensis*). The choice of study animal was based on two factors. First, apart from the initial material investment in the egg contents, parental care plays little or no role in the maturation of the newly hatched animal. Second, the large number of available individuals produced from each group or clutch of eggs provides a sufficient sample for the assessment of clutch variability.

The goal of this study was to evaluate physiological variability for the presence or absence of a clutch effect in one-year-old juvenile

* Corresponding author. Tel.: +1 330 972 2571; fax: +1 330 972 8445.
E-mail address: bagatto@uakron.edu (B. Bagatto).
[1] Current contact information.

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alligators grown in a common environment. We hypothesize that for many of the resting variables measured, a clutch effect will be present even after 1 year of development. We further hypothesize that a physiological challenge will amplify the differences between clutches in some measured variables revealing clutch effects that may not have been measured in resting variables.

2. Materials and methods

2.1. Animals

Thirty two yearling American alligators (*A. mississippiensis* Daudin) were obtained from the Rockefeller Wildlife Refuge at Grand Chenier, LA. During the previous breeding season clutches of eggs were collected and hatched as previously described (Elsey et al., 1990). Incubation was completed at the Rockefeller Refuge in a single custom-built incubator with similar conditions described previously (Joanen and McNease, 1976). Briefly, eggs collected from field nests were placed in wire mesh containers and packed in nest materials. Eggs were placed on shelves 8 cm above water that was heated (Joanen and McNease, 1977; Joanen and McArthur, 1987). Temperature was maintained at 31 °C and eggs were monitored daily for water level and egg quality.

Upon hatching, animals were marked by clutch via tail scute clippings and placed in custom-built enclosures at Rockefeller Wildlife Refuge (2.5 wide by 5 m long × 1 m deep) (Joanen and McNease, 1976). The chamber was temperature controlled via heating the 10 cm of water, which filled approximately half of the chamber. Animals had access to equal parts dry and water-filled areas. Feeding, care, and maintenance followed a previously published protocol (Elsey et al., 1990).

After 1 year of care, the animals were collected and transported to the Department of Ecology and Evolutionary Biology at the University of California at Irvine, Irvine, California. All animals (5 clutches: 3 clutches with $n=6$ and 2 clutches with $n=7$) were maintained at 28 °C randomly distributed at animal densities of 10, 11, and 11, in three containers (1.3 m wide by 3 m long by 1 m deep) with 10 cm of water, allowing equal access to water and dry basking sites under a heat lamp. Animals were fed raw chicken twice per week and food was withheld at least 5 days prior to experimentation.

2.2. Surgery

Each animal was placed in a plastic box containing a cloth saturated with Isoflurane to induce anesthesia. Once anesthetized, the glottis was intubated with PE 90 tubing, and ventilated (SAR-830 ventilator) at 2 breaths min^{-1} (tidal volume = 10 mL/kg) with a 2% Isoflurane/room air mixture using a FluTec vaporizer until the surgical plane of anesthesia was achieved. A 1 cm cut was made in the skin at the midline of the dorsal surface of a rear limb above the femur; the skin was retracted and underlying musculature separated to expose the femoral artery and vein. Once isolated, both vessels were occlusively catheterized using heparinized saline-filled heat-pulled PE-50 tubing. Both catheters were then tunneled under the skin, externalized and fixed with a single suture to the back of the animal. A blind saline filled injection port was connected to the end of the arterial catheter and the venous catheter was heat-sealed. The skin incision was sutured closed with 4–0 suture and sealed with Vet-bond tissue adhesive. Once surgical procedures were completed, animals were placed in a 40 L glass aquarium covered with cardboard with 1 cm of water in the bottom and maintained at 28 °C. All animals were allowed 24 h to recover individually in opaque aquaria prior to experimentation. This ensured that measurements made via the catheters did not disturb the animals. All surgical procedures were approved by the University of California at Irvine IACUC committee in protocol # 1999–2123.

2.3. Blood pressure and heart rate

A saline filled section of PE 90 tubing fitted with a 21-gauge needle was connected to the catheter port. This section of PE 90 tubing was connected to a pressure transducer (DP6100, Peter von Berg) calibrated against a static column of water. The arterial catheter was attached to a pressure transducer amplified by a 4CHAMP amplifier (Somedic AB, Sweden), with the output connected to a PowerLab 8sp (ADInstruments). Data were collected on a Macintosh Computer via a PowerLab data acquisitions system (Chart 5, ADInstruments) for later analysis. The experimental zero pressure was set at the level of the heart of the animal. Heart rate and blood pressure were allowed to stabilize for 1 h to establish resting values (consistent values were typically recorded for a minimum of 30 min prior to treatments). During this period, arterial pressure was recorded and heart rate was determined from the pressure trace with an online software tachograph.

2.4. Blood measurements

Resting and post-exercise blood measurements were taken to determine the effect of exercise. A total of three 500 μL blood samples were withdrawn from the arterial catheter for a total of less than 5.0% of the total volume of the smallest animal. The post-exercise samples were withdrawn within 1 min of the cessation of the treatment. Two hundred microliters of whole blood was used for analysis of pH, Po_2, and Pco_2 simultaneously using a BMS 3 MK2 blood micro system (Radiometer, Copenhagen). Two 50 μL aliquots of blood were centrifuged at 10,000*g* for 5 min in micro-hematocrit tubes for determination of blood hematocrit. One hundred microliters of blood were mixed with 5 μL of an EGTA/glutathione solution (0.2 M/0.2 M) to prevent catecholamine oxidation and immediately centrifuged at 10,000*g*. The plasma was then separated and stored at −70 °C until analysis was carried out (within 1 month). HPLC analysis of plasma catecholamines was carried out as previously described (Fritsche and Nilsson, 1990). The remaining 100 μL of blood was centrifuged at 10,000*g* and the plasma portion was mixed with an equal amount of 8% perchloric acid and frozen for later determination of plasma lactic acid (Lowry and Passonneau, 1972). The red cell pellet was frozen in liquid nitrogen for later determination of intracellular erythrocyte pH (Zeidler and Kim, 1977).

2.5. Exercise

Two exercise protocols were used in this study to determine the physiological variability within and between clutches during activity. During the sustainable component of exercise, a constant swimming effort was designed to provide an equal minimal effort exercise challenge to all animals. In contrast, the flipping component was designed to measure the limits of the non-sustainable anaerobic capacity. For an assessment of sustainable activity, animals were placed in a custom-built swimming flume and allowed to acclimate with no flow for 30 min. Following the acclimation period, each animal was exercised for 10 min at a constant laminar 10 body lengths per second after which an arterial blood sample was taken. After the blood sample was processed, exhaustive exercise was achieved by placing the animal in a clear rectangular box (2 L) equipped with several large holes to allow for airflow. The box was then gently turned over causing the animal to roll onto its back. The animal would then attempt to right itself to a ventral side down position. This procedure was repeated until the animal no longer attempted to right itself—defined as exhaustion. This method of exercise has been reliably used in amphibians to elicit Vo_{2max} (Hillman et al., 1979). Once the animal was exhausted, a blood sample was taken for analysis as described above. All animals were then euthanized with an intravenous overdose (125 mg) of sodium pentabarbitol, after which the internal

organs were removed, trimmed of connective tissue and drained of excess blood, and weighed to the nearest 0.01 g. All tissues were then dried in a fume hood at 25 °C to a constant mass and weighed to the nearest 0.01 g.

2.6. Statistical analyses

Clutch effects in all resting variables were determined with a one-way analysis of variance (ANOVA) as assumptions of equal variance and normality were met. Since animal mass displayed a significant clutch effect, regressions were performed on mass versus all variables. Where mass significantly affected a variable, mass was then used as a covariate in the subsequent MANCOVA and least square means plotted (LSMs) from regression analyses. Clutch effects, exercise effects, and their interaction were determined with a two-way repeated measures MANOVA. Where significant effects were observed, *post-hoc* pair wise comparisons were performed using Tukey's multiple-comparisons procedure. All analyses were performed using SAS® and the level of significance was set at $P<0.05$. Data are shown as arithmetic mean or LSM ± SE.

3. Results

3.1. Animal size, liver and lung mass are clutch dependent

After 1 year of growth, there was a significant clutch effect present ($P<0.001$) on mass (Fig. 1A). Snout-vent length showed a significant clutch effect even when using mass as a covariate ($P=0.0186$) (Fig. 1B), meaning that clutches had different body shapes. The majority of the clutch differences in the organs were due to body mass (Table 1). However, despite mass effects, clutch effects persisted for both liver ($P=0.0011$) and lung wet masses ($P=0.0196$). Dry masses showed the same trends and thus we report only wet masses.

3.2. Blood pressure, but not heart rate, is clutch dependent

Standard cardiovascular measurements revealed a high level of variation within clutches; therefore, there were no significant differences in heart rate between clutches ($P=0.1060$) (Fig. 2A). Despite a significant mass effect on mean arterial pressure (MAP) ($P=0.0430$), a significant clutch effect ($P=0.0442$) persisted (Fig. 2B).

3.3. Blood chemistry and exercise

Blood samples were taken under resting and exercising conditions via an implanted catheter to investigate possible clutch differences in various blood parameters, and how they might respond to exercise. Resting values for blood plasma pH (pH_e) did not show a significant clutch effect ($P=0.5696$); however, resting mean intracellular blood pH (pH_i) was significantly different between clutches ($P=0.0031$) (Fig. 3). Both pH values decreased significantly ($P<0.0001$ for both) during aerobic swimming and decreased further after flipping ($P<0.0001$ for both). Most importantly, in both pH parameters there was a significant interaction between the exercise treatments and clutch (pH_e $P=0.0181$; pH_i $P=0.0005$), meaning that clutches responded differently to the exercise treatments (Fig. 3).

The measure of non-sustainable capacity was defined as the number of flips and the total length of flipping time. There was a significant mass effect in anaerobic capacity ($P=0.0326$) with larger animals flipping for longer periods of time (Fig. 4). When mass was taken into account, there was no clutch effect observed for anaerobic performance per se (Table 2).

Larger animals tended to have a higher blood Po_2 and lower blood Pco_2, and these relationships were significant (Po_2 $P=0.0046$; Pco_2 $P=0.0067$). Despite these mass effects, a significant clutch effect was observed in the resting partial pressure of oxygen but not carbon dioxide (Po_2 $P<0.001$; Pco_2 $P=0.0907$) (Fig. 5). While there was no exercise treatment effect on Po_2 and Pco_2 (Po_2 $P=0.4476$; Pco_2 $P=0.8333$), there was, however, a significant interaction between clutch and exercise for both Po_2 ($P=0.0317$) and Pco_2 ($P=0.0002$) (Fig. 5).

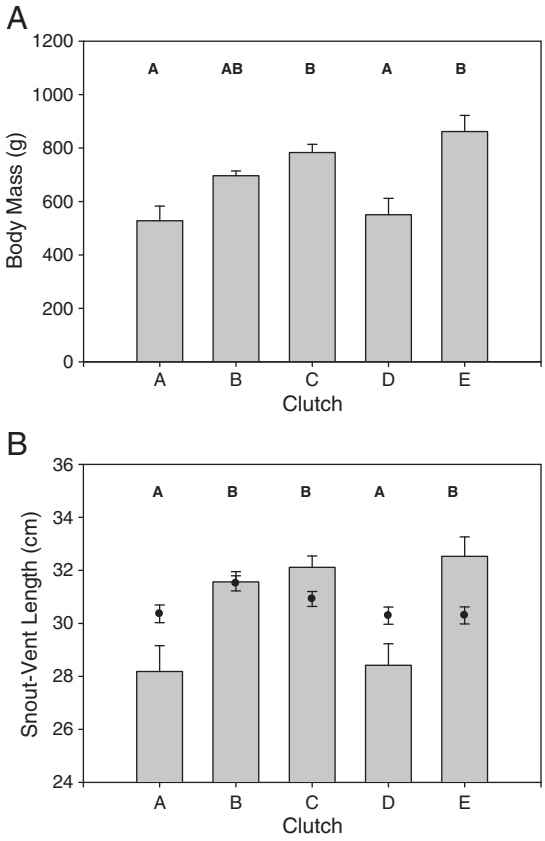

Fig. 1. (A) Mean whole body mass (g) and (B) mean snout-vent length (cm) of yearling alligators from five different clutches. Different letters denote significantly different groupings based on *post hoc* analyses. Black circles denote LSM ± S.E.

Mean plasma lactate concentrations displayed a clutch effect during rest ($P=0.0024$) and each clutch responded significantly in varying degrees to the exercise treatments ($P<0.001$ treatment; $P=0.0224$ interaction clutch x treatment) (Fig. 6A). There was no clutch effect present in mean resting plasma epinephrine; however,

Table 1
Wet tissue mass (g) from each of the 5 alligator clutches studied (A–E). $N=6$ for clutches A, B, and D; $N=7$ for clutches C and E. An asterisk denotes a significant clutch effect independent of animal mass. Data are presented as both arithmetic mean ± SEM and LSM ± SEM as labeled.

	A	B	C	D	E
Arithmetic mean ± SEM					
Atrium	0.43 ± 0.07	0.55 ± 0.05	0.60 ± 0.10	0.41 ± 0.08	0.65 ± 0.06
Ventricle	1.20 ± 0.20	1.30 ± 0.10	1.37 ± 0.25	1.26 ± 0.18	2.12 ± 0.24
Liver*	9.61 ± 1.10	8.98 ± 0.44	12.07 ± 1.03	8.90 ± 1.05	13.17 ± 1.31
Lung*	5.30 ± 0.71	5.90 ± 0.30	5.80 ± 0.49	3.63 ± 0.65	7.14 ± 0.67
Spleen	0.81 ± 0.18	1.07 ± 0.11	1.10 ± 0.14	0.74 ± 0.10	1.18 ± 0.27
Kidney	2.41 ± 0.33	2.74 ± 0.14	3.40 ± 0.19	2.44 ± 0.29	3.94 ± 0.44
LSM ± SEM					
Atrium	0.57 ± 0.07	0.55 ± 0.07	0.53 ± 0.06	0.53 ± 0.07	0.50 ± 0.07
Ventricle	1.48 ± 0.22	1.30 ± 0.18	1.22 ± 0.18	1.51 ± 0.21	1.83 ± 0.21
Liver*	12.51 ± 0.55	8.91 ± 0.47	10.48 ± 0.46	11.40 ± 0.53	10.20 ± 0.53
Lung*	6.62 ± 0.46	5.86 ± 0.39	5.06 ± 0.39	4.77 ± 0.44	5.79 ± 0.44
Spleen	1.02 ± 0.19	1.06 ± 0.16	0.99 ± 0.16	0.93 ± 0.18	0.96 ± 0.18
Kidney	3.16 ± 0.21	2.72 ± 0.18	2.99 ± 0.17	3.09 ± 0.20	3.16 ± 0.20

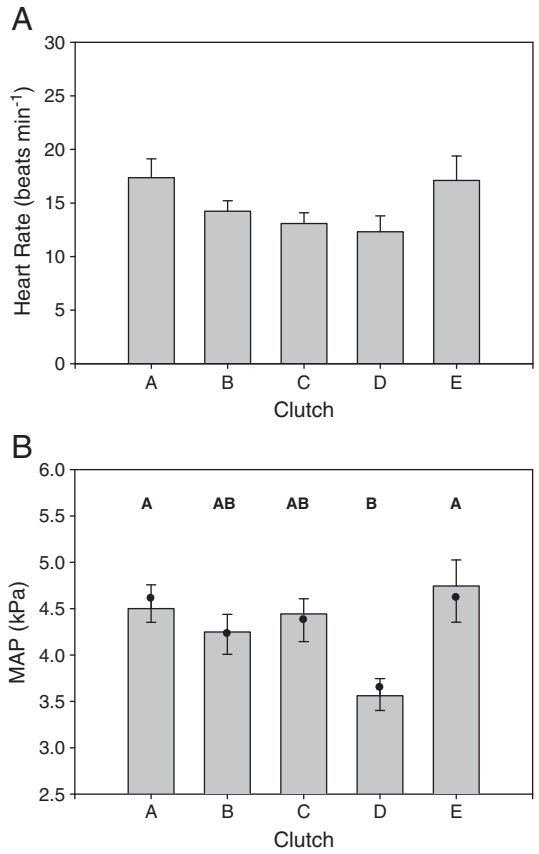

Fig. 2. (A) Mean heart rate (beats min^{-1}) and (B) mean arterial pressure (kPa) of yearling alligators from five different clutches. Different letters denote significantly different groupings based on *post hoc* analyses. Black circles denote LSM ± S.E.

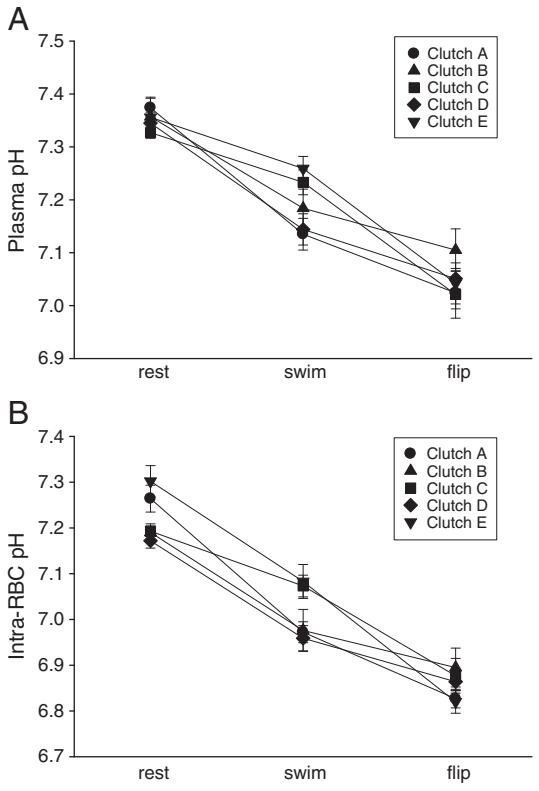

Fig. 3. (A) Mean plasma pH and (B) mean intracellular pH of yearling alligators at rest, following 10 min of swimming at 10 body lengths per second, and following exhaustive flipping.

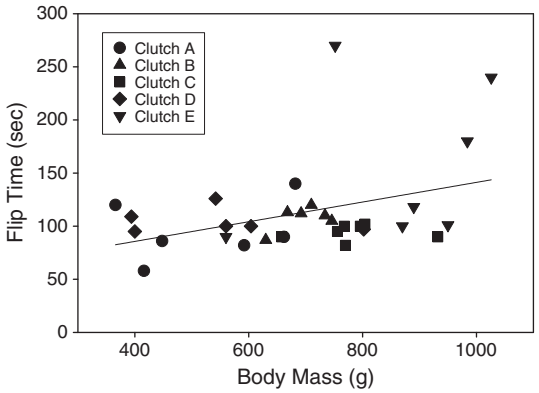

Fig. 4. Regression showing the relationship between body mass (g) and flip time (s).

significant treatment ($P<0.001$) and interaction effects ($P=0.0373$) were present (Fig. 6B). Mean plasma norepinephrine values were significantly different ($P<0.0001$) as a result of exercise treatment; however a clutch effect and its interaction with treatment were absent (Fig. 6C).

4. Discussion

Prior to hatching, the genetic makeup, maternal investment, and nest environment all contribute to hatchling phenotype (Arnold, 1987). As alligators in this study began their feeding regimes post-hatch, there were already significant morphological clutch effects present, such as mass and SVL (observation, R. Elsey). Quantifying this initial observation would have provided further insight; however, clutch effects persisting after 1 year are compelling and strongly supportive of initial clutch effects. Since egg size and/or yolk allocation have been shown to be a common denominator in hatchling size in many bird and reptile species (Sinervo et al., 1992; Radder et al., 2004; Storm and Angilletta, 2007; Wolanski et al., 2007; Mortola and Al Awam, 2010), it is likely that gross morphological similarities within a clutch (i.e. smaller hatchling size) resulted from female alligators investing comparatively less yolk per egg. While the implications of maternal allocation on hatchling size have been previously addressed (Packard and Packard, 1993; Burggren et al., 1994, 2003; Burggren, 1999; Bagatto et al., 2000), this study focused on whether previously documented physiological and morphological clutch differences in embryonic/neonatal vertebrates persist after 1 year of captive rearing in a common environment.

4.1. The clutch dependence of morphological and cardiovascular features

Juvenile alligators exhibited persistent differences in morphology following 1 year of growth, showing greater variability between clutches than within clutches. Thus, the clutch effects previously documented in hatchling American alligators (Allsteadt and Lang, 1995) do persist 1 year into juvenile life and likely longer. Alligators can grow extremely fast or relatively slowly depending on a number

Table 2
Mean number of flips (flip number) and length of time (s) until exhaustion for the 5 clutches of alligators tested. $N=6$ for clutches A, B, and D; $N=7$ for clutches C and E. Data are presented as a mean ± SE for flip number and as both arithmetic and LSM ± SE for flip time.

Clutch	Flip number	Mean Time (s)	LSM time (s)
A	35.5 ± 4.3	96.0 ± 13.1	105.7 ± 18.2
B	40.7 ± 3.3	107.8 ± 5.1	107.6 ± 15.4
C	36.0 ± 4.0	94.8 ± 3.4	88.8 ± 15.3
D	47.0 ± 4.3	104.5 ± 5.2	112.9 ± 17.6
E	44.0 ± 6.5	166.5 ± 31.0	147.1 ± 17.4

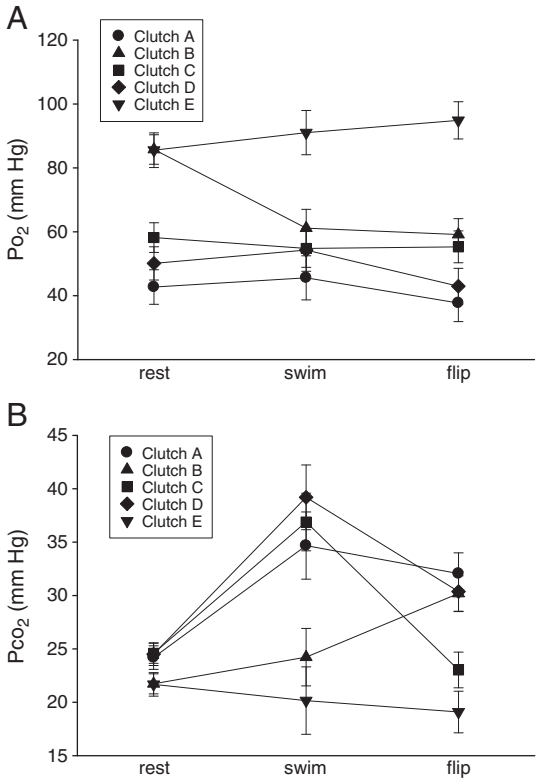

Fig. 5. (A) Least square mean whole blood Po_2 (mm Hg) and (B) whole blood Pco_2 (mm Hg) of yearling alligators at rest, following 10 min of swimming at 10 body lengths per second, and following exhaustive flipping.

of exogenous factors, the most obvious being temperature and caloric intake (Elsey et al., 1990). Under controlled conditions at the Rockefeller Wildlife Refuge (temperature controlled chambers with animals fed daily to satiation), the animals used in this study grew to a mean mass of 693 ± 31 g and mean snout-vent length of 30.7 ± 0.4 cm. This growth rate is much higher than the growth rates of wild alligators; however, it is much lower than the maximal rate previously reported for American alligators fed daily and maintained at warmer temperatures (31 ± 2 °C) than those animals raised in this study (28 °C). In 1 year, masses as high as 8 kg and snout-vent lengths of over 120 cm were documented (Herbert et al., 2002). So within this large growth rate range, it is interesting that smaller clutches did not catch up in body mass and length from what appeared to be a fairly small deficit upon hatching (personal communication, Ruth Elsey). After 1 year of *ad libitum* feeding (once per daily), small clutches remained significantly smaller (both in mass and length) than their larger counterparts (Fig. 1). Although it is possible that smaller alligators had trouble competing for food, a recent study with a similar feeding regime demonstrated that initial hatching mass dictated final animal size after approximately 1.5 years (Eme et al., 2010). Furthermore, genetic variation has been tied strongly to morphological differences (Bennett, 1987; Mousseau and Roff, 1987; Roff and Mousseau, 1987); therefore, as previously suggested in a study of *Eleutherodactylus cooki*, the most likely explanation for this clutch effect is genetic (Burggren et al., 2003).

Given the persistence of between clutch gross body size differences after 1 year, it is not surprising that clutch effects of internal organs were due to the strong correlation to animal mass (Table 1). However, despite strong body mass effects, clutch effects on both liver and lung mass remained when body mass effects were removed. This suggests that liver and lung mass are dictated in part by persistent genetic effects that are independent of animal size. Since the lungs and liver are organs involved in performance metabolism, it was interesting that heart size did not show the same mass independent clutch effect. Perhaps these effects, if present, were too subtle to detect. In an effort to quantify the translation of clutch effects on morphology into physiological function, we focused on cardiovascular function and blood chemistry.

To our knowledge, this is the first study to document clutch effect on physiological variables in reptiles beyond a few weeks of age. In this study, we identified a mass independent clutch effect that dictated MAP in yearling American alligators (Fig. 2B), suggesting a clutch specific cardiovascular homeostatic set point. Rigorous studies on the influence of genetics on heart rate and blood pressure in non-mammalian organisms do not exist; however clinical studies of human twins suggest that the cardiovascular response to stress and resting MAP are heritable traits (Tank et al., 2001; Vinck et al., 2001; Cerutti et al., 2009; Wang et al., 2009; Zhang et al., 2010). The influence of clutch on MAP in American alligators indicates that arterial pressure as a whole, or any of the factors contributing to arterial

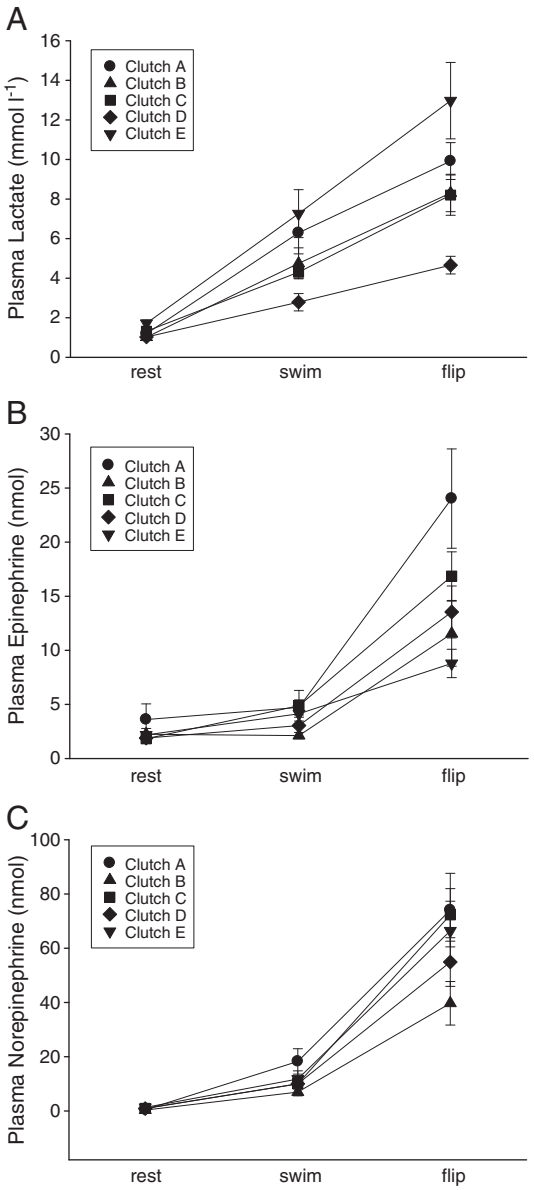

Fig. 6. (A) Mean plasma lactate (mmol L^{-1}), (B) mean plasma epinephrine (nmol), and (C) mean plasma norepinephrine (nmol) of yearling alligators at rest, following 10 min of swimming at 10 body lengths per second, and following exhaustive flipping.

pressure, has a strong genetic component. While an assessment of cardiovascular regulation was not conducted in this study, a prior study in human twins identified this in relation to the genetic influences on baroreflex function (Tank et al., 2001). This assertion is further supported by the absence of clutch influences on resting heart rate in this study regardless of the differing MAP (Fig. 2A). Previous literature has identified heart rate a useful physiological indicator between clutches (Burggren et al., 1994, 2003); however studies have been restricted to early developmental time periods. In neonatal armadillos, variation in heart rate significantly increases over 8 days of development (Bagatto et al., 2000). Therefore, it was predictable that between sibling variation in heart rate was high enough such that clutch effects were not discernable after 1 year (Fig. 2A). Taken together, we have provided strong evidence that the set point for arterial pressure regulation is dictated by a long lasting genetic influence in American alligators.

4.2. Blood chemistry and exercise

The blood chemistry values measured in American alligators at rest and following each of two different exercise treatments strongly indicated that aerobic and anaerobic capacity is more variable between clutches than within clutches (Fig. 3A and B). This genetic component to exercise capacity has been previously documented in clinical studies of monozygotic twins (Bielen et al., 1990; Hopkins et al., 2010), supporting that exercise capacity has a genetic basis in the American alligator. In humans, end-diastolic left ventricular volume is suggested as the functional manifestation of this genetic effect (Bielen et al., 1991). While further cardiovascular parameters were not measured in this study, heart rate and stroke volume may well be the key contributors to the exercise capacity and the subsequent clutch effects we see in our study. Even though there was not a mass independent clutch effect for ventricular mass, there were strong mass-independent clutch effects in both the respiratory and metabolic acidoses. It was also apparent that the aerobic swimming treatment in the flume caused a significant blood acidosis in all clutches, signifying that this treatment was not, in fact, purely aerobic. In addition, clutches that responded with a less severe respiratory acidosis to aerobic swimming did not necessarily respond with the same severity to anaerobic flipping. These interactions also take into account that the exercise treatments were not independent of one another. Plasma lactate, however, indicates that regardless of the form of exercise, there is a strong clutch effect on the amount of lactate each individual produces (Fig. 6A). This is significant because this is the first study to show a clutch effect on two different performance variables and relate this to blood acid-base status.

Clutches possessed differing liver and lung sizes that were independent of clutch mass differences. While liver size did not correlate with any performance variable, lung size correlated significantly and positively to lactate values ($P=0.0261$). Even though larger lungs meant larger lactate values, this was also true for lungs that were corrected for the mass of the animal. This means that smaller clutches could have comparatively larger lungs and be able to produce more lactate compared to equally sized alligators with smaller lungs.

Mass is an important predictor for anaerobic metabolism in crocodilians. In this study, however, mass was only a predictor of flip time (Fig. 4). Baldwin et al. (1995) show this as well as mass scaling with blood lactate and H^+. A likely explanation was that our range (600 g) of masses was quite small compared to the Baldwin study (180 kg). It is interesting that we show no scaling effect on blood lactate; however a significant amount of lactate variation is due to clutch. Although further experiments are necessary to elucidate possible mechanisms for these findings, there seems to be very interesting clutch effects on performance that are not necessarily related to the mass of the animal.

5. Summary

It is clear that both physiological and morphological clutch effects persist due to extensive genetic contribution, and last at least 1 year into post hatch life. While the majority of these effects are due to the mass of the animal, there are a few variables, such as resting MAP and lactate, which showed a significant clutch effect independent of animal mass. Clearly, the environment significantly influences the American alligator from hatching to death, however, the measureable contribution of genetics to the morphology and physiology of the organism remains evident, even after 1 year of common garden rearing. These data reinforce the importance of addressing instead of ignoring genetic (clutch) effects on physiological variables. When possible, experimental designs should either test for clutch effects independently or employ split-clutch designs. Using comparative animal models to understand the details of genetic×environment interactions provides a rich area for future studies and will be required to fully understand how organisms adapt to environments.

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