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

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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.

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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

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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

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