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RESEARCH ARTICLE

Developmental oxygen preadapts ventricular function of juvenile American alligators, *Alligator mississippiensis*

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Abstract

Developmental oxygen is a powerful stressor that can induce morphological and functional changes in the cardiovascular systems of embryonic and juvenile vertebrates. This plasticity has been ascribed, at least in part, to the unique status of the developing cardiovascular system, which undergoes organogenesis while meeting the tissue oxygen demands of the embryo. We have previously reported an array of functional and morphological changes in embryonic American alligators that persist into juvenile life. Most notably, cardiac enlargement as well as functional parameters of anesthetized juvenile alligators remains after embryonic hypoxic exposure. Because the effects of developmental oxygen in crocodilians have only been investigated in anesthetized animals, we explored the pressure dynamics of both ventricles as well as systemic pressure in response to stressors of acute hypoxia and swimming. Our current findings demonstrate that developmental programming of cardiac function (intraventricular pressure and heart rate) does persist into juvenile life, but it is chamber-specific and depends on the experimental manipulation. Acute hypoxic exposure revealed that juvenile alligators that had experienced 10% O_2 as embryos maintain right ventricle function and increase left ventricle function during exposure. Finally, the data indicate blood flow in the left aorta must originate from the left ventricle during acute hypoxia and swimming.

activity; cardiovascular; crocodilian; hypoxia; programming

INTRODUCTION

The phenotype of a developing animal is amendable in response to environmental modifications, a phenomenon called "developmental phenotypic plasticity" (1, 2). This plasticity enables novel phenotypes to appear in response to environmental cues and may increase organismal fitness in a given condition later in life. The ability of environmental factors, such as oxygen (O_2) availability, to alter developmental trajectories is not surprising given the sensitivity and plasticity of the developmental process (3). As such, oxygen can be a useful tool to assess the capacity of the environment to alter structural and functional phenotypes at all phases of an animal's life history. Given the great number of vertebrates that experience either brief or long-lasting differences in oxygen during development, low oxygen likely represents the norm rather than the exception for many vertebrate taxa. During the past 20 years, several studies have been conducted on egg-laying reptiles, because these embryos naturally develop in a subterranean or mound nest, where they can be subjected to periodic or possibly sustained hypoxia (4).

The embryonic cardiorespiratory system of egg-laying reptiles has been shown to exhibit plasticity in response to low oxygen (5–13). These studies report an increase in relative heart mass, which has been used as an index of cardiac stroke volume (5–8, 10, 11, 14, 15). In two species, common snapping turtles (*Chelydra serpentina*) and American alligators (*Alligator mississippiensis*), the relatively larger heart can persist into juvenile life (16–18). We recently studied the functional traits of the right and left ventricles in anesthetized juvenile American alligators. We found that these animals retained the enlarged heart phenotype, and they maintained left ventricle contractility during acute hypoxic exposures (19). However, although the anesthetized preparation enables invasive hemodynamic measures, it does not address the impact that developmental plasticity has on cardiac performance during relevant physical activities, such as swimming. In the current investigation, we studied the influence of developmental hypoxia on cardiac function (measures of intraventricular pressures, heart rate, and systemic pressures) of recovered juvenile alligators, 5–6 years after hatching. We specifically investigated whether developmental exposure to hypoxia affects intraventricular pressures

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during acute hypoxic exposure and swimming. Both situations are relevant to the natural history of crocodilians and could shed light on whether embryonic exposure to hypoxia provides programming for improved cardiac performance. Secondarily, given that changes in ventricular pressure can directly influence central blood flows, including the right-to-left (pulmonary bypass) shunt that develops when right ventricular pressure exceeds systemic blood pressure, we investigated if this shunt occurs in surgically recovered animals (20–22). We predicted that indices of ventricular function would be unchanged in hypoxic incubated juveniles in response to acute hypoxia. We further predicted that these indices would decrease in normoxic incubated animals during acute hypoxia, as previously documented in anesthetized alligators. Furthermore, we predicted that left ventricle contractility would increase during swimming in juveniles that had experienced hypoxia during incubation.

MATERIALS AND METHODS

In the summer of 2015 and 2016, 20 clutches of American alligator eggs (average clutch number of 40 eggs) were collected from wild nests on the Rockefeller Wildlife Refuge in Grand Chenier, LA and transported to the Department of Biological Sciences at the University of North Texas. Eggs were then handled as previously described (19). Briefly, initial embryonic age was established according to Ferguson (23). All eggs were weighed, numbered, and then randomly assigned to 1-L Ziplock boxes containing a vermiculite water mixture at a ratio of 1:1. The lidless box mass was recorded for each egg box. Water content of the egg boxes was maintained by adding water three times weekly to bring the box to its original mass. Embryos were incubated in a walk-in room (Percival Scientific, Perry, IA), at 30°C, ensuring that all embryos developed as females. At ~20% of incubation (total incubation is 72 days at 30°C), 50% of all eggs were randomly assigned to either a 21% oxygen group (N21) or a 10% oxygen group (H10). These O_2 levels were based on our previous studies on the embryonic development of alligators (9, 11, 24, 25), and the similarity to previous measures from a crocodilian nest (4). To maintain the oxygen level for each treatment, the boxes were placed in 76-L Ziploc bags that were connected to a gas supply of either normoxia or 10% O_2. The normoxic gas was supplied using an air pump (LT 11 Whitewater, Pentair Aquatic Eco-Systems, Cary, NC) passed through a rotameter flow controller. Hypoxic gas was generated using rotameters (Sho Rate, Brooks instruments division, Hatfield, PA) supplied with compressed N_2 and air supplied by an air pump (Whisper AP 300, Tetra products, Blacksburg, VA). Normoxic and hypoxic air was humidified using bubbling chambers and delivered to the bags at a rate of 2–4 $L \cdot min^{-1}$. Gas composition was monitored continuously using an oxygen analyzer (S-3AI, AEI Technologies). Hatchlings were marked by tail scute clipping and photographed to identify the incubation condition and clutch of origin. All animals were maintained for 4 to 5 years in 378- and 567-L plastic containers, with free access to water, at an ambient temperature that ranged from 24°C to 28°C. The animals were fed commercial alligator food (Crocodilian Diet, Mazuri Exotic Animal Nutrition, St. Louis, MO) three times weekly and maintained under a 12:12-light:dark cycle. All experiments were approved by the University of North Texas Institutional Animal Care and Use Committee (IACUC 20009).

Surgery and Instrumentation

Thirty juvenile alligators were used in the study; pressure measurements were successfully gathered in 28 animals (N21: n = 15 and H10: n = 13). All animals were fasted for at least 10 days before instrumentation. On the day of surgery, animals were isolated and a plastic bag containing cotton gauze saturated with isoflurane (Isothesia, Henry Schein Animal Health, Dublin, OH) was held over the head of the animal. Once the righting reflex was absent, the animals were weighed, moved to a stainless-steel surgical table, and then intubated with a 15- to 20-cm section of Tygon tubing connected to a ventilator (Harvard Apparatus 665 ventilator, Harvard Apparatus, Holliston, MA), which drew room air through an Isoflurane vaporizer (FluTec vaporizer, FluTec, Ohmeda, OH) set at 2% mixed with 98% room air. Animals were ventilated at a rate of 5–7 $breaths \cdot min^{-1}$ at a volume of 20 $mL \cdot kg^{-1}$. Once the animal had reached a surgical plane of anesthesia, as determined by the lack of a pedal reflex, the vaporizer was reduced to 1% or 1.5% isoflurane delivery. In preparation for surgery, the skin of the left rear limb and the left lateral body wall were cleaned with Betadine solution and rinsed with 75% ethanol. Once cleaned, subcutaneous injections of a general antibiotic (0.1 $mL \cdot kg^{-1}$, 2.7% Baytril, Bayer Healthcare, LLC, Shawnee Mission, KS) and a general analgesic 0.025 $mL \cdot kg^{-1}$ [Flunixiject (50 $mg \cdot mL^{-1}$), Henry Schein Animal Health, Dublin, OH] were administered in the right rear limb. One milliliter of a 2% lidocaine solution (Lidoject, Henry Schein Animal Health, Dublin, OH) was then injected subdermal to the dorsal surface of the left thigh. A 2-cm incision was made in the skin to expose the femoral artery, which was isolated by separating the Iliotibialis muscles (26). Once isolated, the vessel was catheterized with PE50 tubing filled with heparinized (50 $U \cdot mL^{-1}$, Sagent Pharmaceuticals, Schaumburg, IL) saline (0.9% NaCl) that was advanced 10 to 12 cm into the dorsal aorta. After catheterization, the incision was sutured closed (Surgical Silk, size 0 USP, Medikrebs, Corp., FL). The catheter was then fitted with a pin port (Instech Laboratories, Inc., Plymouth Meeting, PA) and anchored with 2-0 silk suture (Surgical Silk, Medikrebs, Corp., FL), at the junction of the limb and the body wall, as well as on the middorsal surface line. The animal was then placed ventral surface up, and the ventral skin was cleaned as outlined earlier. Two 1-mL injections of a 2% lidocaine solution were injected subcutaneously above the sternum and abdomen. An 8- to 10-cm incision was then made in the skin starting ~5 cm below the sternum and extending up one-fourth the length of the sternum. Skin was then blunt-dissected away from the underlying sternum and an incision was made in the muscle 2 cm caudal to the sternum. Forceps were inserted under the sternum to act as a guide for the sternal and abdominal cut as the body cavity was widened to expose the underlying heart. The pericardium was cut along the long axis of the animal above the ventricles to expose the heart. Once the heart was exposed, a location in the wall of each ventricle that did not contain branches of the coronary circulation was identified.

At those locational points, a 6-0 silk suture (Surgical Silk, Medikrebs, Corp.) was used to create a purse stitch in the epicardium. Once completed, an 18-gauge needle was inserted in the middle of the purse through the ventricle wall and withdrawn. A catheter (PE 90) with holes in the side 1 cm from the tip, containing heparinized saline, was gently inserted into the chamber. The purse stitch was then tightened to close the epicardium around the catheter. This procedure was repeated in the other ventricle (Fig. 1). Once catheters were in place, the pericardium was closed with 4-0 silk suture (Surgical Silk, Medikrebs, Corp.). The catheter leads were passed through a hole made in the abdominal musculature and tunneled under the skin, exiting the lateral body wall skin ~2 cm rostral to the hindlimb. The left and right ventricle catheters were anchored with suture at the exit point, at the lateral midpoint, and dorsally on the animal. The sternum and abdominal wall were closed with 2-0 silk sutures (Surgical Silk, Medikrebs Corp.) followed by closure of the ventral skin. The animal was then placed on its ventral surface, and the ventricles and femoral catheters were attached to a rubber twist tie that was fixed to the dorsal surface. The anesthetic was reduced to 0% and the animal was monitored until it had taken five consecutive spontaneous breaths. The animal was then moved to a 200-L container in a 30°C in a walk-in room (Percival Scientific, Perry, IA) and allowed to recover for 24 h without access to water. After this recovery period, the animal was given a second injection of antibiotics (0.1 mL·kg^{-1} baytril), and water was added to its enclosure. All experiments were conducted 2 days after instrumentation.

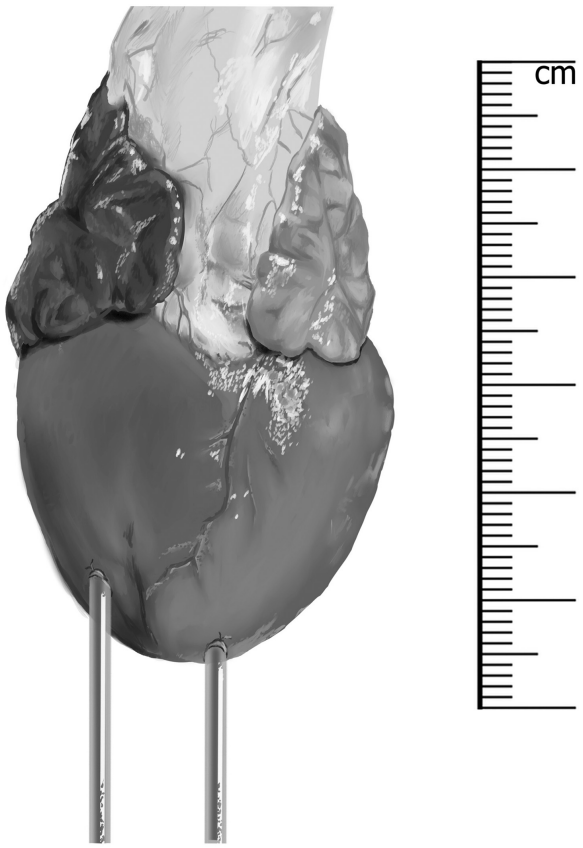

Figure 1. Drawing of the American alligator heart illustrating the placements of the intraventricular catheters. Artwork created by Kristofer Speer.

Experimental Chamber and Protocol

Twenty-four hours before the experiment, the animal was moved to a custom-made swim flume filled with water, where all experimental protocols were conducted (27). Water flow was generated with a 12-V trolling motor (Minn Kota Endura C2-30, Mankato, MN). Laminar flow was achieved with baffles (34 cm total height) constructed from 2 cm diameter polyvinyl chloride (PVC) pipes (15 cm length) glued together as previously described in a prior study using this custom flume (18). A custom-made acrylic lid was placed on the swim flume, such that it rested on the top of the water. The lid was constructed with a breathing chamber (87 cm long, 13 cm wide, and 9 cm tall) running the length of the middle of the lid, allowing the animal to surface and ventilate. After the lid was fitted into place, water was added to the top of the lid to ensure that the breathing chamber was sealed. Water temperature was set at 30°C using two submersible aquarium heaters (500 W, Hygger, Shenzhen City, China). A thermocouple was placed 4 cm into the water (BAT-12, Physitemp Instruments, Clifton, NJ) for continuous recording of water temperature during the experiment. Tubing was connected to a port in the breathing chamber and room air was passed through it at a rate of ~0.9 L·min^{-1}. Air passed through the breathing chamber and exited through a second port where air was subsampled for oxygen percentage with an oxygen analyzer (S-3AI, Ametek Applied Electrochemistry). The morning of each study, all catheters were passed through the lid via a 2-cm cut that ran the length of the breathing chamber, and the catheters were suspended above the chamber with helium-filled Mylar balloons. All catheters were connected to pressure transducers (ADInstruments model MLT0699) that were connected to a signal amplifier (Quad Bridge Amp, ADInstruments). A webcam (Stream Webcam HFR 1080p, China) pointed at the swim chamber, and red light was used to allow the investigators to monitor animals without disturbances. The pressure transducer was calibrated against a static column of water with the 30-cm water level representing zero. Signal outputs from the bridge amplifier, the thermocouple, and the oxygen analyzer were connected to a PowerLab 16/35 data acquisition system connected to a computer running LabChart Pro software (v.8.2, ADInstruments). Data were recorded at 40 Hz. All instruments were calibrated daily before each study and at the end of the protocol.

Hypoxic Exposure Protocol

At the beginning of the experimental protocol, animals were allowed to recover from any disturbance caused by connecting the catheters to the transducers for 3–4 h. Before progressing the protocol, all measured parameters had stabilized for at least 30 min. The breathing chamber air was then switched from room air to 5% O_2 for 30 min and responses were recorded. The gas composition was made using a gas mixer (GF-3mp, Cameron Instrument, Co., Port Aransas TX). Gas flow rate was set at 2 L·min^{-1} to ensure rapid turnover of air within the breathing chamber. During the exposure period, animals remained with their heads above water in the

breathing chamber. The gas flowing into the breathing chamber was then switched back to room air for recovery. All pressure parameters were allowed to return to baseline.

Swim Protocol

After the hypoxic protocol, animals were allowed 1 h to recover before beginning a 5-min swimming protocol (1.55 ± 0.03 body lengths·s^{-1}). Swimming was induced by increasing the water flow rate while the animal's head was above water in the breathing chamber. In response to the increase in water speed, some animals submerged. If swimming was not induced, animals were tapped gently behind the rear limb with a thin aluminum rod to induce and sustain swimming. At the completion of the swim trial, the animals were allowed 30 min to recover before the study was ended. Animals were then removed from the swim flume, and euthanized by ventilation with 5% isoflurane followed by cranial pithing. Organ masses were then measured, and the heart was dissected to determine right and left atria masses, and the free wall mass of the right and left ventricles in eight alligators from each condition. All masses were measured to the nearest 0.01 g.

Measurements and Calculations

Mean values.

Heart rate was determined based on the pulsatile traces of either left or right ventricular pressures. Pressures for both ventricles and femoral pressure were taken as the mean of the peak systolic pressure response to all experimental manipulations. Because of the differing time frames of each protocol, the duration of the pressure and heart rate traces used for the analysis was different. The hypoxic response was taken as the mean values of the final 5 min of the hypoxic exposure, whereas the response to swimming was taken over a span of 30 s at the peak of the response.

Ventricle function.

To develop a more detailed understanding of the effects of the incubation conditions and each protocol, ventricular function was analyzed using LabChart Pro blood pressure module (v.8.2, ADInstruments). This module was used to measure maximum change in pressure over change in time ($\Delta P{\cdot}\Delta t^{-1}_{max}$), rate of pressure decline ($\Delta P{\cdot}\Delta t^{-1}_{min}$), and ventricle contractility, calculated as $\Delta P{\cdot}\Delta t^{-1}_{max}$ divided by the pressure (P) at the time of $\Delta P{\cdot}\Delta t^{-1}_{max}$. Units for ventricle contractility were 1·s^{-1}.

Statistics

Differences in body mass between alligator groups were assessed with ANOVA. Differences in organ masses were assessed with an ANCOVA with body mass as a covariate and condition as the independent variable. Differences between right and left atria and free ventricle wall masses were assessed with an ANCOVA with heart mass as a covariate and condition as the independent variable.

The effects of 5% O_2 exposure and of swimming on pressure parameters and heart rate were analyzed separately with an ANOVA for repeated measures with incubation condition as an independent variable. Fisher's least-significant difference (LSD) post hoc tests were used for pairwise comparisons between experimental groups. This analysis was also used for the assessment of differences in ventricular function in the right and left ventricles separately.

Statistica v13 software (StatSoft, Tulsa, OK) was used for all statistical analyses. Statistical significance was identified when $P \leq 0.05$. Data are presented as mean values ± SE.

Sample size for mass data was the 30 animals that were instrumented for the studies. For the physiological measurements, sample size decreased due to catheter failure. This included catheters that were nonfunctional before the start of the study and those that failed before or during the hypoxic exposure or the swim protocol.

RESULTS

Body mass was similar between the experimental groups (Table 1). Heart mass was significantly greater (+40%) in the H10 compared with the N21 animals (Table 1). Although body mass was a significant covariate (F value = 6.64, P = 0.0157) for heart mass, the incubation condition was also a significant independent factor (F value = 6.70, P = 0.0153) in the heart mass difference (Table 1). Furthermore, the right and left ventricle-free walls were significantly larger (+62% and +79%, respectively) in the H10 compared with the N21 animals. However, this difference was accounted for by heart mass, which was a significant covariate (F value = 108, $P \leq 0.01 \times 10^{-11}$ and F value = 150, $P \leq 0.01 \times 10^{-12}$, respectively). All other organ masses were similar between the groups.

Response to Acute 5% O_2

Systolic pressures in the right ventricle (RV P_{sys}) were significantly affected by exposure to hypoxia (F value = 12.88, $P \leq 0.00155$), and there was a significant interaction between incubation conditions of the juveniles and the response to hypoxic exposure (F value = 4.58, $P \leq 0.043$) (Fig. 2). Specifically, H10 juvenile alligators maintained relatively constant RV P_{sys} during the exposure ($P \leq 0.286$ post hoc LSD), whereas RV P_{sys} decreased significantly ($P \leq 0.00086$ post hoc LSD) in the N21-incubated juveniles (Fig. 3*A*). Before the acute hypoxia, systolic pressure in the left ventricle (LV P_{sys}) was significantly (F value = 5.78, $P \leq 0.025$) different between the groups, with the H10-incubated juveniles maintaining pressures that were ~22% lower than the N21-incubated juveniles (Fig. 3*B*). In addition, acute hypoxic exposure significantly (F value = 12.86, $P \leq 0.0017$) affected LV P_{sys} in an incubation condition-dependent manner, as represented by the significant, roughly 30% ($P \leq 0.0021$ post hoc LSD), increase in pressure in the H10-incubated juveniles, whereas the N21 juveniles were relatively constant ($P \leq 0.141$ post hoc LSD) (Fig. 3*B*). Interestingly, acute hypoxic exposure significantly (F value = 91.27, $P \leq 1.19 \times 10^{-9}$) increased femoral systolic arterial pressure (femoral P_{sys}) in both experimental groups (Fig. 3*C*). Furthermore, as indicated by the significant interaction (F value = 4.59, $P \leq 0.0424$) between incubation condition and the hypoxic exposure, the response of the H10 group was greater than the N21 group (Fig. 3*C*). Finally, acute hypoxic exposure significantly (F value = 94.44, $P \leq 5.69 \times 10^{-10}$) increased f_H in both groups to similar value, +71% and +110% in the N21 and H10 incubated juveniles, respectively (Fig. 3*D*).

Table 1. *Mass values for body mass, whole heart mass, right ventricle free wall, left ventricle free wall, right atrium, left atrium, liver, lung, and kidney of alligators incubated in 10% O_2 or 21% O_2*

Condition	Body, kg	Heart, g	RV, g	LV, g	RA, g	LA, g	Liver, g	Lung, g	Kidney, g
H10	5.75 ± 0.59 (14)	13.43 ± 1.45* (14)	2.72 ± 0.36** (8)	3.56 ± 0.60** (8)	1.15 ± 0.13 (8)	1.11 ± 0.22** (8)	61.318 ± 7.91* (14)	23.13 ± 1.77 (14)	15.80 ± 0.99 (14)
N21	5.24 ± 0.48 (16)	9.07 ± 0.85 (16)	1.66 ± 0.156 (8)	1.96 ± 0.18 (8)	0.93 ± 0.07 (8)	0.53 ± 0.05 (8)	59.57 ± 5.56 (16)	18.84 ± 1.41 (16)	14.41 ± 0.9 ± 8 (16)

Data are presented as mean values ± SE. Sample sizes are indicated in parentheses. Body, body mass; H10, 10% O_2; heart, heart mass; LA, left atrium; LV, left ventricle free wall; N21, 21% O_2; RA, right atrium; RV, right ventricle free wall. *Significant a difference between groups that was independent of the body mass; **significant difference in mass that was dependent on body mass based on the results from the ANCOVA.

In response to acute hypoxia, ventricular functional indexes were differentially affected and depended on the O_2 level that the juveniles experienced during incubation (Fig. 3, *E–H*). There was a significant (F value = 6.01, $P \leq 0.02310$) interaction between incubation condition and the response to acute hypoxia in RV maximal rate of pressure change (RV $\Delta P{\cdot}\Delta t^{-1}_{max}$). This was evident during the final 5 min of acute hypoxia, during which RV $\Delta P{\cdot}\Delta t^{-1}_{max}$ was unchanged in the H10 juvenile alligators, whereas the N21 group significantly ($P < 0.0168$ post hoc LSD) decreased $\Delta P{\cdot}\Delta t^{-1}_{max}$ by ~29% (Fig. 3*E*). Unlike the RV, before hypoxic exposure there was a significant (F value = 8.12, $P \leq 0.011$) effect of incubation condition on LV maximal rate of pressure change (LV $\Delta P{\cdot}\Delta t^{-1}_{max}$) (Fig. 3*F*), as values were ~50% lower in the H10 versus the N21 animals (Fig. 3*F*). During the final 5 min of the hypoxic exposure, LV $\Delta P{\cdot}\Delta t^{-1}_{max}$ was significantly (F value = 34.37, $P \leq 0.000015$) increased, by 73% and 40% in the H10 and N21 juvenile alligators, respectively, with no differences between the groups (Fig. 3*F*). The rate of RV pressure decline ($\Delta P{\cdot}\Delta t^{-1}_{min}$) was similar between the experimental groups

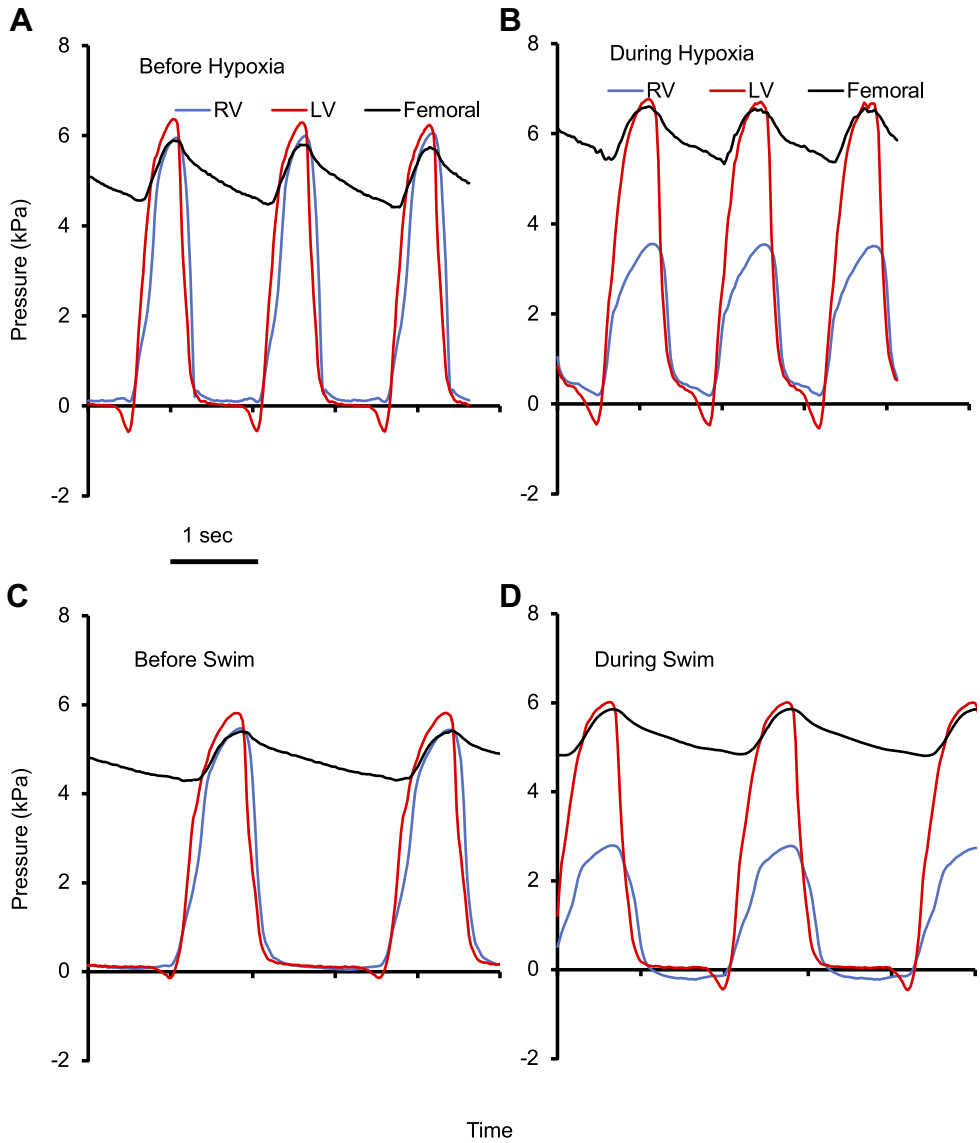

Figure 2. Representative traces of right and left intraventricular pressures taken from a normoxic incubated (N21) juvenile alligator. Right ventricle (RV) pressure is represented by the blue line while left ventricle (LV) pressure is represented by the red line. Traces are taken from the animals before (*A*), and during (*B*) acute hypoxia and before (*C*) and during (*D*) the final 5 min of a swimming bout.

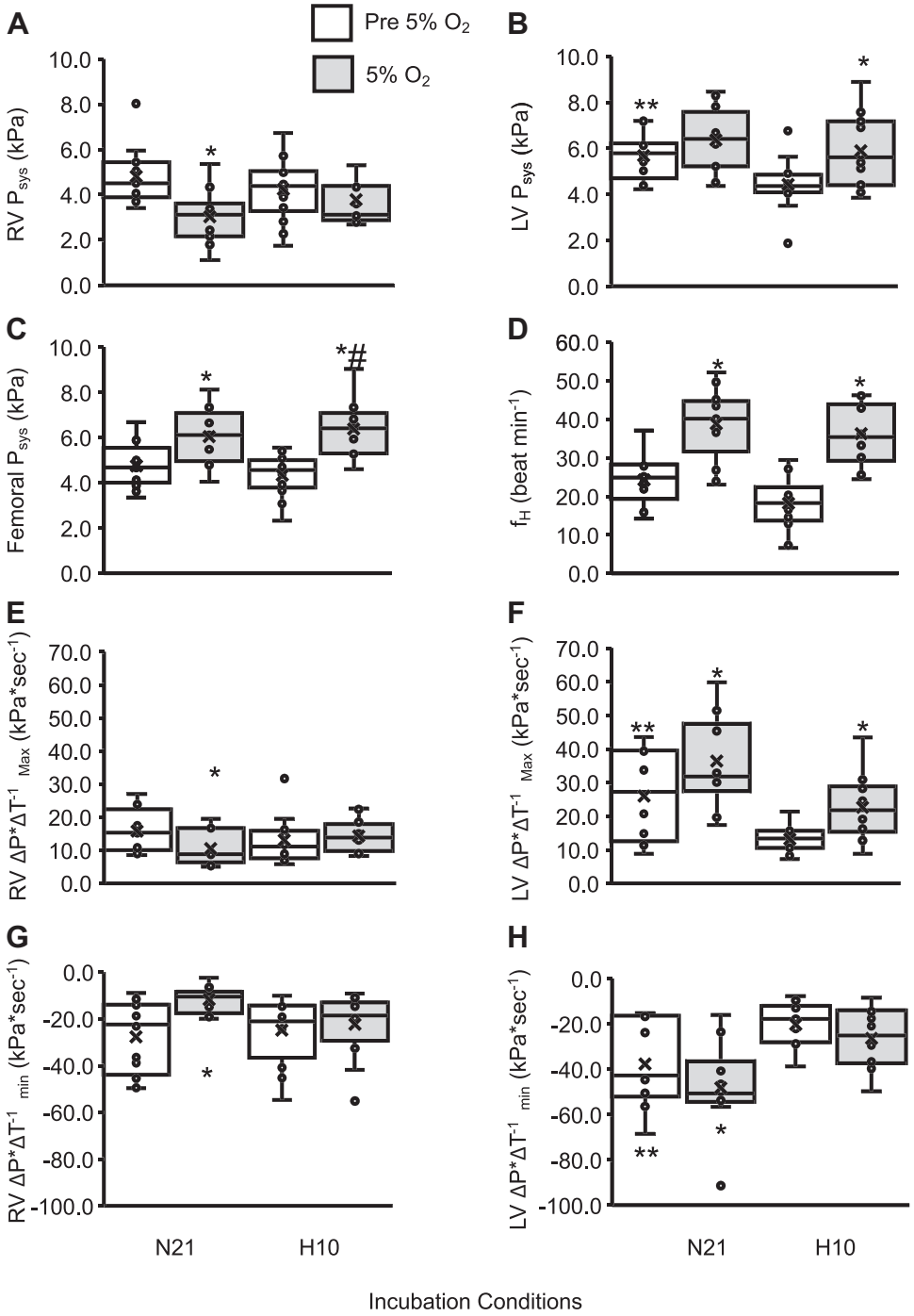

Figure 3. Cardiovascular response to the final 5 min of a 30-min exposure of 5% O_2 of mean peak systolic pressure in the right ventricle (RV P_{sys}) (*A*), left ventricle (LV P_{sys}) (*B*), the femoral artery (femoral P_{sys}) (*C*), heart rate (f_H) (*D*), right ventricle maximal change in pressure over time (RV) $\Delta P{\cdot}\Delta t^{-1}_{max}$ (*E*), left ventricle maximal change in pressure over time (LV) $\Delta P{\cdot}\Delta t^{-1}_{max}$ (*F*), right ventricle rate of pressure decline (RV) $\Delta P{\cdot}\Delta t^{-1}_{min}$ (*G*), and left ventricle rate of pressure decline (LV) $\Delta P{\cdot}\Delta t^{-1}_{min}$ (*H*), of alligators incubated under 10% O_2 (H10) or 21% O_2 (N21) conditions. Open columns represent the pretreatment values and the filled columns represent the treatment response. *Significant difference between pre- and posttreatment responses within an incubation condition; **differences between the groups in pre- or posttreatment values. #Significant difference in the response to treatment between the N21 and H10 animals. Sample size for RV function was n = 12 for the N21 animals and n = 14 for the H10 animals. Sample size for LV function was n = 13 for the N21 animals and n = 10 for the H10 animals. Sample size for femoral pressure was n = 14 for the N21 animals and n = 13 for the H10 animals.

before the hypoxic exposure. During the final 5 min of acute hypoxia, $\Delta P{\cdot}\Delta t^{-1}_{\text{min}}$ was significantly (F value = 5.99, $P \leq 0.023$ and $P \leq 0.0096$ post hoc LSD) lower, by ~54%, in the N21 incubated juveniles, although it was unchanged in the H10 group (Fig. 3*G*). Intriguingly, although acute hypoxic exposure had a significant (F value = 5.99, $P \leq 0.023$) effect on RV $\Delta P{\cdot}\Delta t^{-1}_{\text{min}}$, the interaction between incubation condition and hypoxic exposure was not significant (F value = 2.79, $P \leq 0.109$). Prior to hypoxic exposure, there was a significant (F value = 7.89, $P \leq 0.011$) difference in LV $\Delta P{\cdot}\Delta t^{-1}_{\text{min}}$n between the groups, with the N21 group exhibiting an ~84% greater value than the H10 group (Fig. 3*H*). During the final 5 min of acute hypoxia, only the N21 groups significantly ($P \leq 0.00255$ post hoc LSD) increased LV $\Delta P{\cdot}\Delta t^{-1}_{\text{min}}$: The N21 group LV $\Delta P{\cdot}\Delta t^{-1}_{\text{min}}$ increased by ~28%, whereas the change in the H10 group was not significant ($P < 0.064$) (Fig. 3*H*). RV contractility was relatively constant during acute hypoxia; however, LV contractility was significantly (F value = 7.29, $P \leq 0.0146$) different before the acute hypoxic exposure between the groups. Before acute hypoxia, the H10 animals maintained a LV contractility of 6.1 ± 0.5 s^{-1}; this value increased significantly ($P \leq 0.041$ post hoc LSD) to 8.2 ± 0.9 s^{-1} during

acute hypoxia, whereas the N21 animals maintained prehypoxic LV contractility of 12.7 ± 2.0 s^{-1}, which was similar ($P \leq$ 0.986 post hoc LSD) to the values (13.8 ± 1.9 s^{-1}) during the final 5 min of hypoxic exposure.

Responses to Swimming

It should be noted that f_H was slightly elevated before swimming compared with values during the prehypoxic treatment; however, all pressures were similar between the prehypoxic and preswim treatments. The 5-min swimming bout had no effect on RV P_{sys} in either group (Fig. 4*A*). In contrast, swimming significantly (F value = 21.44, $P \leq$ 0.00018) increased LV P_{sys} by ~48% and 25% in the H10 and N21 groups, respectively (Fig. 4*B*). Before the swim, femoral P_{sys} was slightly elevated in the N21 versus the H10 group (Fig. 4*C*). During swimming, both groups significantly (F value = 28.22, $P \leq$ 0.0000216) increased femoral P_{sys} by ~48% and 17% in the H10 and N21 groups, respectively (Fig. 4*C*). Although the relative change in femoral P_{sys} during swimming appeared to be different, the interaction between incubation conditions and swimming showed a trend toward a difference in the intensity of the response to swimming (F value = 2.80, $P \leq 0.10$). As noted before hypoxic exposure, preswimming f_H was significantly (F value = 4.70, $P \leq 0.041$)

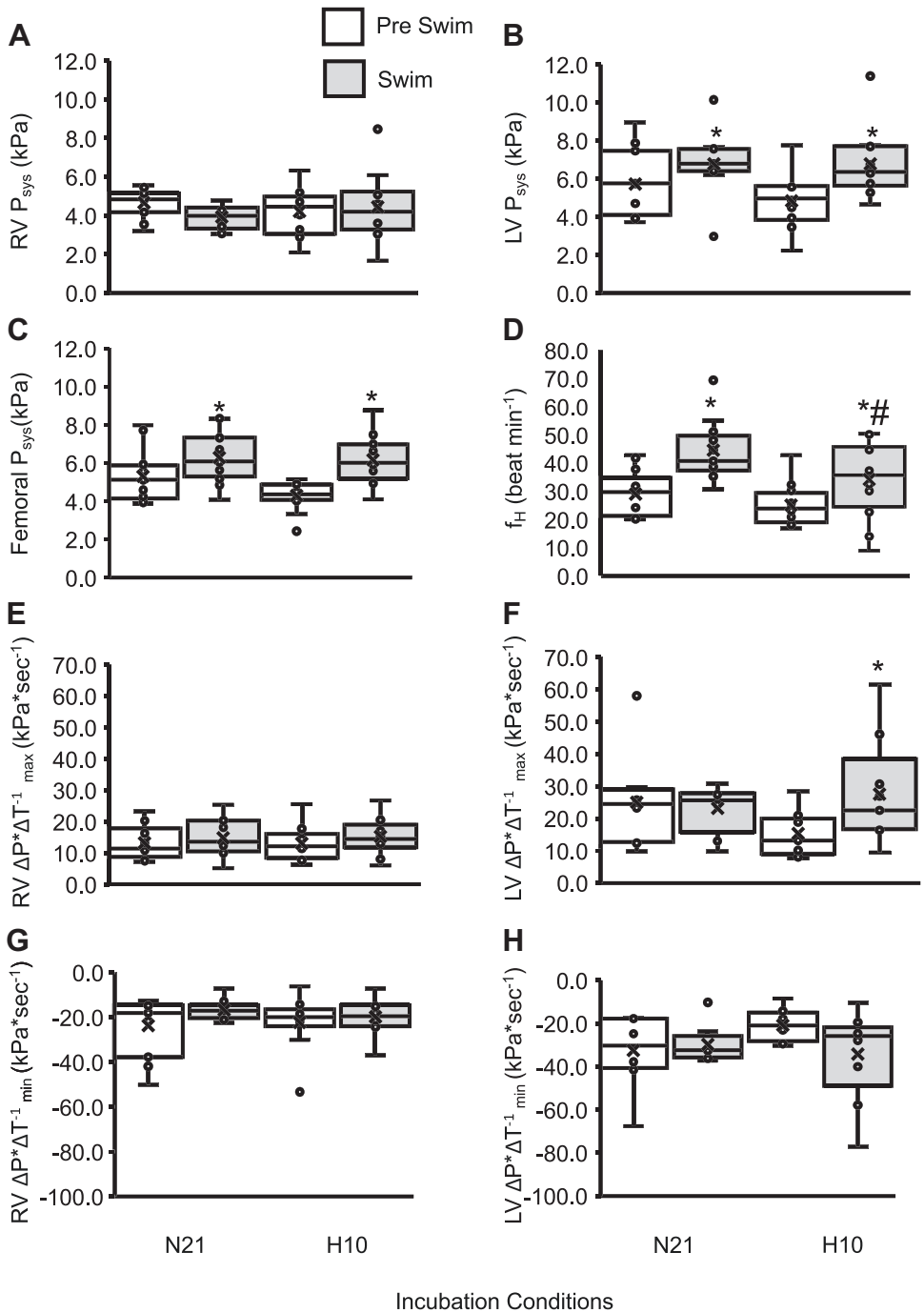

Figure 4. Cardiovascular response during a 5-min swimming bout of mean peak systolic pressure in the right ventricle (RV P_{sys}) (*A*), left ventricle (LV P_{sys}) (*B*), the femoral artery (femoral P_{sys}) (*C*), heart rate (f_H) (*D*), right ventricle maximal change in pressure over time (RV) $\Delta P{\cdot}\Delta t^{-1}_{max}$ (*E*), left ventricle maximal change in pressure over time (LV) $\Delta P{\cdot}\Delta t^{-1}_{max}$ (*F*), right ventricle rate of pressure decline (RV) $\Delta P{\cdot}\Delta t^{-1}_{min}$ (*G*), and left ventricle rate of pressure decline (LV) $\Delta P{\cdot}\Delta t^{-1}_{min}$ (*H*), of alligators incubated under 10% O_2 (H10) or 21% O_2 (N21) conditions. Open columns represent the pretreatment values and the filled columns represent the treatment response. *Significant difference between pre- and posttreatment responses within an incubation condition. #Significant difference in the response to treatment between the N21 and H10 animals. Sample size was for RV function was n = 12 for the N21 animals and n = 12 for the H10 animals. Sample size for LV function was n = 12 for the N21 animals and n = 10 for the H10 animals. Sample size for femoral pressure n = 14 for the N21 animals and n = 12 for the H10 animals.

higher in N21 versus the H10 groups (Fig. 4*D*). During swimming, f_H increased significantly (F value = 29.29, $P \leq 1.69 \times 10^{-5}$) in both groups by ~46% and 55% in the H10 and N21 animals, respectively (Fig. 4*D*).

Swimming did not affect the right ventricle function indices, RV $\Delta P{\cdot}\Delta t^{-1}_{max}$, or RV $\Delta P{\cdot}\Delta t^{-1}_{min}$ for either group. However, contractility of the RV did increase significantly (F value = 4.64, $P \leq 0.04$ and $P \leq 0.0165$ post hoc LSD) in the N21 group from a preswim value of 5.6 ± 1.0 to 19.6 ± 6.8 s^{-1}. Left ventricle function indices did exhibit a response to swimming. Specifically, there was a significant (F value = 4.80, $P \leq 0.045$) interaction between incubation condition and swimming in the LV $\Delta P{\cdot}\Delta t^{-1}_{max}$ response, with the H10 group significantly ($P \leq 0.014$ post hoc LSD) increasing by ~80% during the swim, whereas the N21 group maintained constant values (Fig. 4*F*). Like the RV, LV $\Delta P{\cdot}\Delta t^{-1}_{min}$ values for all animals were unaffected by swimming. There was a significant (F value = 6.55, $P \leq 0.022$) interaction between incubation condition and swimming, with LV contractility of the N21 group decreasing from 12.7 ± 2.2 s^{-1} to 8.2 ± 1.5 s^{-1}, whereas the H10 animals maintained relatively constant values from 9.2 ± 2.7 to 11.5 ± 4.2 s^{-1} during the swim.

DISCUSSION

As established in earlier studies, developmental hypoxia results in cardiac enlargement in juvenile alligators. We now provide data on ventricular function by measuring pressure dynamics and heart rate of surgically recovered juvenile alligators that had been incubated under 10% O_2 (H10) or 21% O_2 (N21) conditions. Our findings demonstrate that the plasticity of the developing heart results in lasting effects on cardiac function, but the effects are ventricle specific. H10 juveniles maintained right ventricle (RV) function during acute hypoxia, whereas N21 juveniles showed decreased RV function. In contrast, left ventricle (LV) functional parameters are lowered by developmental hypoxia when the H10 and N21 juveniles are compared. Finally, as stated by other investigators, blood can flow from the RV into the left aorta when RV pressure exceeds systemic pressure (20, 28, 29). Our findings of intraventricular pressures in surgically recovered animals demonstrate that blood flow in the left aorta must originate from the left ventricle during acute hypoxia and swimming, as previously suggested in alligators under control conditions (20).

Comparison With Previous Studies

Intraventricular pressures have been reported in previous studies on similar-sized crocodilians (19, 20, 28, 29), and the systolic pressures in the two ventricles during the control situation of the present study were below, similar, and above those reported earlier. Some of the earlier studies report on a single or very few individuals, making it difficult to compare those results directly with our measures. In addition, differences between studies are likely due to the invasiveness of the proceeding surgery, and the choice of anesthesia as well as the duration of the subsequent recovery period of prior studies. In our study, the alligators were studied 2 days after surgery, and the animals appeared to exhibit normal behaviors and would readily swim in the swim tunnel.

Acute Hypoxic Response

To simulate the blood oxygen conditions experienced by a diving crocodilian (30), we investigated the functional changes in ventricular and systemic pressures that accompany bouts of 5% O_2. In the current study, there was a clear effect of developmental programming on the cardiac functional phenotype persisting into juvenile life (Fig. 3, *A*–*H*). Specifically, right ventricular functional indices RV P_{sys}, $\Delta P{\cdot}\Delta t^{-1}_{max}$, and $\Delta P{\cdot}\Delta t^{-1}_{min}$ were constant in the H10 group during acute hypoxia, and these same indices were depressed in the N21 group (Fig. 3, *A*, *E*, and *G*). In a concurrent study (Crossley JL, Smith B, Tull M, Elsey RM, Wang T, Crossley DA; unpublished observations), we found that 5% O_2 was sufficient to decrease arterial P_{O_2} by ~70%, and we previously reported that a 20-min exposure to 5% O_2 had no appreciable effects on blood lactate concentrations (19), a finding we found after 30 min of exposure in the parallel study (J.L. Crossley, B. Smith, M. Tull, R.M. Elsey, T. Wang, and D.A. Crossley, unpublished observations). The current study provides novel data regarding ventricular function in recovered animals. A prior study of anesthetized American alligators reported a similar dampened RV P_{sys} response to 5% O_2 in H10 versus N21 juvenile animals (19). Interestingly, chronically hypoxic fetal sheep increase stroke volume of the right ventricle and may also increase intraventricular pressures (31). In addition, anesthetized sheep that were subjected to fetal anemic-induced hypoxia increased left ventricle function during acute hypoxia as adults, whereas a nonanemic group decreased left ventricle function during acute hypoxia as adults (32). Broberg et al. (32) suggested that the improved LV function during acute hypoxia may be based on the increased coronary conductance in the anemic animals (33) or an increase in subcellular calcium handling. However, these parameters have not been investigated in the programmed crocodilian heart.

Functional phenotype of the LV exhibited an opposite pattern to that of the RV (Fig. 3, *B*, *F*, and *H*). Specifically, the H10 group increased LV P_{sys} during acute hypoxia, which was absent in the N21 group (Fig. 3*B*). Given that, during acute hypoxia in both groups, RV pressure was lower than the increase in femoral P_{sys} (Fig. 3, *A* and *C*), recovered American alligators are hemodynamically unable to shunt blood from the RV through the left aorta in response to hypoxia. Compared with the prehypoxic LV functional indices, the N21 animals maintained greater LV P_{sys}, $\Delta P{\cdot}\Delta t^{-1}_{max}$, and $\Delta P{\cdot}\Delta t^{-1}_{min}$ than the H10 group (Fig. 3, *B*, *F*, and *H*). These findings differ from the lack of changes in anesthetized American alligators subjected to similar acute hypoxic bouts (19). Although differences in adrenergic receptor tone could account for the differences between the current and previous studies, the use of anesthesia conceivably played a role in the noted differences.

Although it is beyond the scope of the current study, an investigation of adrenergic tone on ventricular function and the capacity to modulate this tone is warranted. Indeed, Joyce et al. (18) reported a differential β-adrenergic tone on blood flow in the left aorta during swimming in juvenile American alligators that were subjected to hypoxic incubation versus those incubated in normoxia. Thus, the capacity to increase β-adrenergic tone during hypoxia may be the

basis for the capacity to increase LV P_{sys} in the H10 group only (Fig. 3*B*).

It is important to note that, although $\Delta P{\cdot}\Delta t^{-1}_{max}$ values during acute 5% O_2 exposure were statistically similar between the H10 and N21 groups, the H10 juveniles increased $\Delta P{\cdot}\Delta t^{-1}_{max}$ ~70%, versus 40% in the N21 group. This further suggests a possible increase in regulatory capacity in the H10 group (Fig. 3*F*). Finally, the rate of myocardial relaxation, $\Delta P{\cdot}\Delta t^{-1}_{min}$, was constant in the H10 group, although this rate increased for the N21 animals (Fig. 3*H*). The constancy of $\Delta P{\cdot}\Delta t^{-1}_{min}$, which represents a metric of ventricle myocardial calcium sequestration rate, in the H10 group, suggests limited capacity to modulate calcium efflux in the LV of this group (34). It should be noted that both groups of experimental juvenile animals increased $\Delta P{\cdot}\Delta t^{-1}_{min}$ by ~28% (Fig. 3*H*).

Swimming

We have previously used voluntary swimming as a means to increase oxygen demand and cardiac performance (18, 27). However, swimming had no impact on RV indices of function (Fig. 4, *A*, *E*, and *G*) in this study. Conversely, during swimming, LV P_{sys} increased in both groups to a similar level (Fig. 4*A*). Although additional studies are needed, the finding suggests that the LV P_{sys} during swimming lacks plasticity in American alligators. Interestingly, as with acute hypoxia, femoral P_{sys} was higher than the RV P_{sys}, indicating that left aortic blood flow in both groups originates from the LV and the animals continued to perfuse the pulmonary circulation. This is particularly relevant because we observed that some animals from both experimental groups swam while submerged. Additional studies are needed to investigate whether the lack of a LV P_{sys} difference between the groups reflects a lack of plasticity in the regulatory mechanisms that impact afterload. In a prior study, we found that embryonic American alligators lack plasticity of α-adrenergic tone, a key contributor to afterload (6). If this persists in juvenile alligators, other mechanisms that regulate left ventricle function during swimming possess a degree of developmental phenotypic plasticity, as we found that it did differ between the experimental groups. As further evidence that left ventricle function is amenable to altering the functional phenotype if exposed to hypoxic incubation $\Delta P{\cdot}\Delta t^{-1}_{max}$ increased during the swim bout in the H10 group only, and $\Delta P{\cdot}\Delta t^{-1}_{min}$ in the left ventricle exhibited a trend ($P = 0.062$) toward increasing in only the H10 group as well (Fig. 4, *F* and *H*). Collectively, the data suggest increases in indices of myocardial contractility in the juvenile alligators that experienced hypoxia as embryos.

Perspectives and Significance

Our present assessment of right and left ventricle function in recovered juvenile American alligators subjected to hypoxia during incubation suggests that the cardiac phenotype is dictated, in part, by developmental oxygen. However, this plasticity is chamber specific with the right ventricle exhibiting more developmental phenotypic plasticity. Importantly, we observed the greatest difference between developmental programming when the alligators were exposed to hypoxia. The mechanism that enabled H10 juvenile alligators to maintain RV function during acute hypoxia was not explored. Interestingly, LV of the H10 group was capable of increasing P_{sys} during acute hypoxia, whereas the N21 group lacked this capacity. Finally, our simultaneous measurements of ventricular and systemic pressures in response to hypoxia and swimming indicated that blood flow in the left aorta must originate primarily from the LV in American alligators.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.L.C., T.L., M.T., T.W., and D.A.C.2nd conceived and designed research; J.L.C., T.L., M.T., and D.A.C.2nd performed experiments; J.L.C., T.W., and D.A.C.2nd analyzed data; T.W. and D.A.C.2nd interpreted results of experiments; J.L.C. and D.A.C.2nd prepared figures; J.L.C., T.L., and D.A.C.2nd drafted manuscript; J.L.C., T.L., M.T., R.M.E., T.W., and D.A.C.2nd edited and revised manuscript; J.L.C., T.L., M.T., R.M.E., T.W., and D.A.C.2nd approved final version of manuscript.

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