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RESEARCH ARTICLE

Heart rate response and bimodal gas exchange in three developmental stages of the bullfrog *Lithobates catesbeianus* (Anura: Ranidae)

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ABSTRACT. Measuring cardiorespiratory variables can be challenging in developing animals, especially when they use bimodal gas exchange to maintain metabolic activity. In tadpoles, gas exchange may occur through the integument and gills when breathing in the water and through the lungs when breathing air, with varying contributions of each respiratory structure during development. The interaction between aquatic and air breathing results in a complex physiological response that may affect the cardiac cycle. Measuring the heart rate (f_H) together with aquatic and aerial gas exchange in anurans during their development can be challenging, since it may involve handling small animals and/or a certain degree of invasiveness (i.e., surgery to implant electrodes). Here, we evaluated concomitantly aquatic and aerial gas exchange, lung ventilation, and f_H in three stages of development of the bullfrog *Lithobates catesbeianus* (Shaw, 1802). We built a novel, non-invasive, closed respirometry system capable of measuring f_H, aerial and aquatic gas exchange simultaneously in animals of different sizes. Our integrative analysis revealed a decrease in the heart rate and an increase in oxygen consumption during the developmental stages of the bullfrog, but there was no adjustment of heart rate after or during air breathing. Moreover, tadpoles in metamorphosis showed higher oxygen consumption in air than in water, while aquatic breathing was responsible for releasing CO_2. Our results are consistent with those found in the literature, yet our study represents the first non-invasive investigation to evaluate bimodal gas exchange and heart rate simultaneously. Moreover, our setup holds potential for further advancements that would allow for controlled water and air composition. This tool could greatly facilitate the investigation of how cardiorespiratory physiology responds to varying environmental conditions.

KEY WORDS. Carbon dioxide production, closed respirometry, electrocardiogram, lung ventilation, oxygen uptake.

INTRODUCTION

The exchange of gases is essential for sustaining an organism's metabolic activities, involving the uptake of oxygen and the removal of carbon dioxide. Gas exchange between animals and the environment must occur constantly during all stages of development, even as distinct respiratory organs are being formed (Burggren and Doyle 1986a). During anuran development, these animals rely on a combination of respiratory organs, including the skin, gills (external or internal), and lungs, to extract oxygen from water and air (Burggren and West 1982). At the beginning of development, oxygen requirements for the embryo are met by diffusion through the skin (Burggren and Infantino 1994, Warkentin 2007), while larger larval stages utilize either aquatic respiration through gills and skin, and/or aerial respiration through lungs (if present) (Crowder et al. 1998). Bullfrog tadpoles use all three respiratory gas exchange surfaces during the premetamorphic and early metamorphic stages of development (Atkinson and Just 1975, Burggren and West 1982). However, tadpoles of other species develop their lungs well before metamorphosis, displaying

air-breathing behavior and lung inflation a few days after hatching (Schwenk and Phillips 2020).

Air breathing can be considered as an additional means to supply oxygen to meet metabolic demands, particularly in physiologically demanding environments. Various abiotic and biotic factors can influence the partial pressure of oxygen (PO_2) in water, leading to diverse respiratory responses of the animal (Burggren and Infantino 1994). Chronic hypoxic conditions in water can increase respiratory surface area, respiratory function of the gills, and skin vascularization (Pinder and Burggren 1983), while acute hypoxia might increase gill and lung ventilation (Wassersug and Seibert 1975, Feder and Wassersug 1984, Burggren and Infantino 1994). The interplay between aquatic and air breathing is highly intricate and mediated by receptors associated with gills and lungs, as lung ventilation inhibits gill ventilation, possibly to reduce oxygen loss from the blood into the water (West and Burggren 1983, Feder and Wassersug 1984).

Cardiorespiratory coupling consists of the interaction between the ventilatory cycle and the cardiac cycle, decreasing (bradycardia) or increasing (tachycardia) heart rate in intermittent air breathers (Pinder and Burggren 1983). Adult frogs exhibit tachycardia shortly after lung ventilation and bradycardia in response to environmental hypoxia, although tadpoles apparently do not alter their heart rate in response to ventilation or aquatic hypoxia (West and Burggren 1982, Burggren and Doyle 1986b). Heart rate alterations during development, from egg to adult, may differ among species (Burggren and Pinder 1991). Burggren and Doyle (1986b) showed a decrease in resting heart rate during bullfrog development, with hatched larvae displaying the highest resting heart rate, which decreases throughout development. However, the decrease in heart rate during development could be linked to allometric scaling rather than other factors associated with ontogeny or developmental processes (Burggren and Pinder 1991).

Measuring heart rate simultaneously with aquatic and aerial gas exchange can be challenging depending on the developmental stage, as it might involve small animals and/or a certain degree of invasiveness (such as electrode implanting surgery). Longhini et al. (2017) established a noninvasive technique for measuring heart rate and buccal movements in larger premetamorphic tadpoles, without accessing air breathing. In the present study, we examined bimodal gas exchange in developing tadpoles using an innovative non-invasive system that concurrently measures aquatic and aerial gas exchange, lung ventilation, and heart rate in three developmental stages of the bullfrog *Lithobates catesbeianus* (Shaw, 1802).

MATERIAL AND METHODS

Animals used and their maintenance

Measurements of heart rate and gas exchange in air and water were conducted on three groups representing different developmental stages of *L. catesbeianus*, following the classification of Gosner (1960). The stages, number of animals and mean body mass ± SD were as follows: Larval stages 28–36 (N = 5, 1.56 ± 0.19 g), characterized by the beginning of hind limb development; premetamorphosis, stages 39–41 (N = 15, 4.32 ± 0.18 g), with fully formed hindlegs; and metamorphosis, stages 42–43 (N = 5, 3.14 ± 0.24 g), with front limbs externalized. The animals were acquired from the bullfrog farm (Centro de Aquicultura da Unesp – CAUNESP) at the College of Agricultural and Veterinary Sciences, São Paulo State University (FCAV-UNESP) in Jaboticabal, São Paulo State, Brazil. All tadpoles were maintained under natural photoperiod (12:12 h light:dark) at 25 ± 1 °C in a tank (50 x 50 x 40 cm) filled with dechlorinated tap water continuously aerated by an air pump. The tadpoles were fed commercial fish food daily, except for 24 hours prior to measurements. The experimental approach was approved by the Ethics Committee on the use of Animals of University of São Paulo, campus Ribeirão Preto (CEUA-FFCLRP-USP, Protocol 17.5.119.59.3).

Heart rate, aerial and aquatic breathing

We constructed a unique apparatus capable of simultaneously measuring heart rate (f_H), aerial and aquatic gas exchange in animals of varying sizes. We used a plastic syringe (60 mL) in a horizontal position as a respirometer chamber, since closed respirometry can be performed with small animals using plastic syringes (Stevens 1992, Lighton 2008). Bimodal respiration was possible by filling the syringe partly with dechlorinated water and the other part with atmospheric air. The chamber volume was adjusted to a tadpoles' size, ranging from 10 to 15 mL air and from 15 to 20 mL water.

Heart rate was measured through non-invasive electrocardiogram (ECG) recordings following Longhini et al. (2017) and Altimiras and Larsen (2000). We used two pieces of wire (steam Ø 1.0 mm) placed in parallel (about 2 cm apart) and fixed perpendicularly at the bottom of the syringe (horizontally positioned). Both electrodes were connected to a differential AC amplifier (A-M Systems, model 1700, Sequim, WA, USA) by a cable (3' with 5-pin). The amplifier was configured to record with a gain of 10k, high cut-off 5Hz and lowcut-off 0.1–300 Hz (according to animal size). The signal was recorded (1 kHz sample rate) by a PowerLab acquisition system (ADInstruments, Sydney, Australia), using digital

filters (band-pass 100 Hz low and 5 Khz high) of Labchart software (version 8, ADInstruments) (Fig. 1). We used two systems to continuously measure gas exchange in water and air during the experiment (Fig. 1). Aquatic gas exchange: on the underside of the syringe, two aluminum tubes were glued (Ø 4.0 mm) to each end of the syringe to insert probe sensors for aquatic O_2 and CO_2. These sensors were connected to O_2 (Firesting) and CO_2 (Presens) analyzers which were connected to a computer and data recorded using the Pyron Oxygen logger (O_2) and the Presens measurement studio 2 (CO_2) software (Fig. 1). Air system: two aluminum connectors (Ø 3.0 mm) for both air inlet and outlet were glued to the top of the syringe. The outlet connector was connected by a hose to a desiccant box upstream to a gas analyzer (ADInstruments) and at the outlet of the gas analyzer was connected to the input of the respirometer, achieving close system respirometry. The gas analyzer was connected to the PowerLab acquisition system (Fig. 1).

Experimental protocol and data analysis

Each animal was individually measured at constant water (24 ± 0.3 °C) and air (25 ± 0.3 °C) temperature. All experiments were conducted on unanesthetized and unrestrained animals, which were kept in the respirometer chamber for at least 40 minutes before the measurements began. During the acclimatization period, the aerial phase was kept open to the surrounding air in the room. After the acclimatization period, all the water within the respirometer was gently replaced by air-saturated water at the same temperature, with care taken not to disturb the tadpole. Following this step, the respirometer was sealed (both air and water) to conduct measurements for 30–40 minutes. Rodgers et al. (2016) showed that background respiration caused by microbial growth was not significant for several hours of measurement. In our setup, measurements of background respiration for one hour have not yielded significant microbial oxygen consumption, suggesting that two 40-minute periods of freshly inserted water samples into the syringe did not lead to significant background respiration.

Occasionally during experiments, a tadpole would start floating just above the electrodes, which required adjustments to the amplifier's low-cut setting in order to enhance the signal quality. Additionally, when a tadpole ascended to the surface to breathe air, noise was generated and the signal was lost, but the signal was quickly re-established when the tadpole submerged itself again onto the electrodes. As a result, the animals maintained contact with the ECG electrodes for a significant portion of the experiment, providing nearly continuous f_H recordings throughout the procedure (Fig. 2A). Heart rate data analysis was carried out using Labchart software by applying the cyclic measurement tool with cyclic detection by ECG mode, adjusting the QRS width and filtering high pass with a 0.16Hz cut-off. We analyzed samples (at least one minute of continuous recordings) at

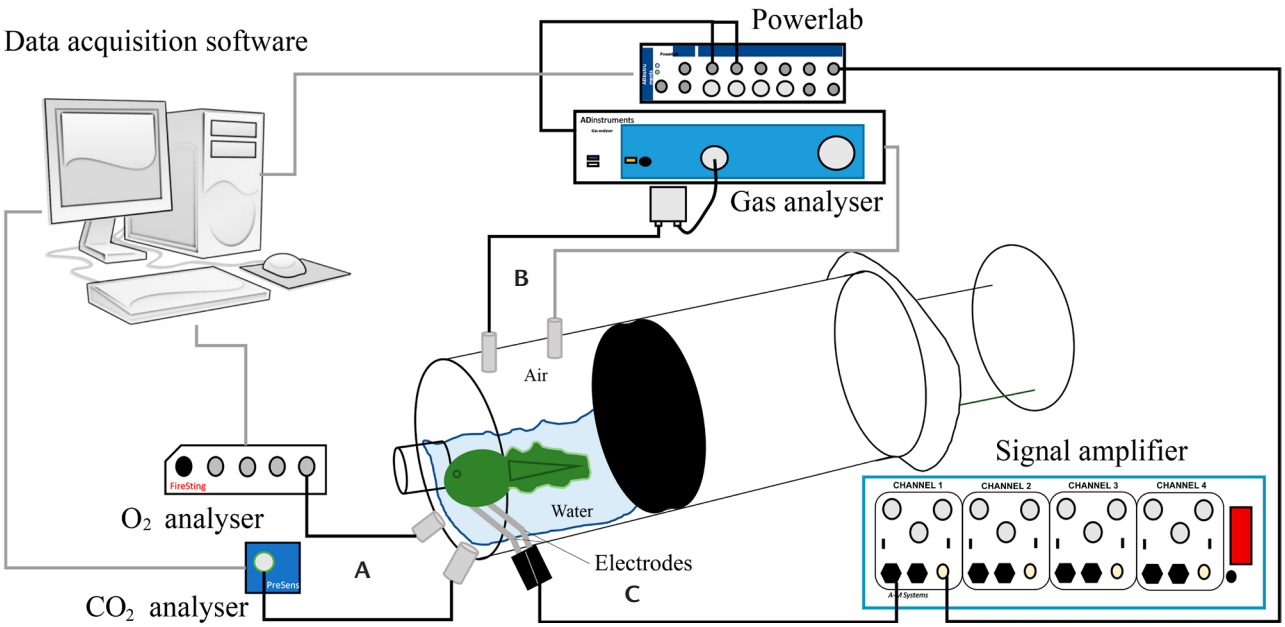

Figure 1. Scheme of non-invasive apparatus to measure gas exchange in water (A) and air (B), and heart rate (C).

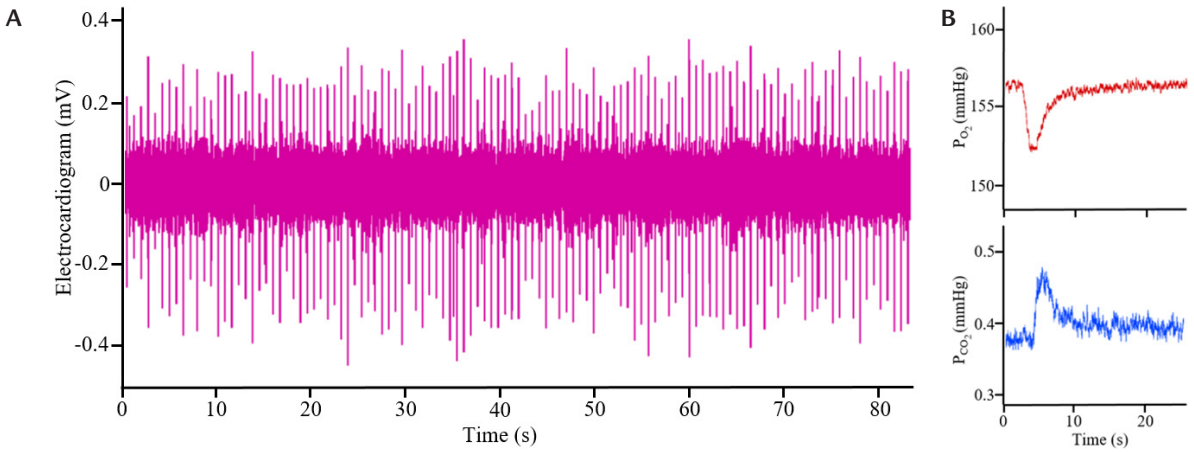

Figure 2. Representative data recording of electrocardiogram (A) and aerial ventilation (B) in a premetamorphic *Lithobates catesbeianus* at 25°C. In B the signals show a ventilatory event where the tadpole renewed the air in its lungs, resulting in a marked drop in PO_2 and an increase in PCO_2. Following the ventilatory event, the expired air was mixed with the remaining air within the closed respirometry system, resulting in a PO_2 slightly lower, and a PCO_2 slightly greater, than before ventilation.

intervals 5, 10, 20, 30 and 40 minutes. We considered the movements performed by a tadpole within a chamber to be sufficient to allow for mixing of water, but we did not test for the existence of an oxygen gradient within the aquatic phase (Rodgers et al. 2016).

Air and aquatic gases were continuously measured throughout all experiments. We used the rate of PO_2 decline and PCO_2 increase in air and water to calculate MO_2 and MCO_2 in air and water for each individual. Mass-specific MO_2 and MCO_2 (μmol gas. g^{-1}. h^{-1}) in the aerial and aquatic phases were calculated. Following Lefevre et al. (2011), the fall in PO_2 or the increase in PCO_2 during time (h), the volume of air or water (L: for the air phase, the volume of air in the closed system; for the aquatic water phase, the volume of water in the respirometer minus the tadpole's mass), the capacitance of O_2 and CO_2 (μmol $mmHg^{-1}$ L^{-1}) in air and water at experimental temperatures, and body mass (g) were used to calculate gas exchange. By constantly measuring the aerial phase, we were able to tally the occurrences of ventilatory events for each individual throughout an experiment (Fig. 2B).

To minimize visual disturbances to the animals, the respirometer was covered with an opaque material. After completing the experiment, the tadpoles were staged, gently dried using paper towels, and weighed to the nearest 0.001 g.

Statistical analysis

We adjusted a generalized linear model (GLM) to analyze all the measured variables. ANOVA test was performed to evaluate each GLMs and its components, and Tukey's post hoc test to obtain the pairwise comparisons. Specifically, we compared heart rate during experimental time and among the three groups, and gas exchange (MO_2 and MCO_2) in air, water and among tadpoles` stages. To examine the influence of body mass (g) on whole-body gas exchanges (μmol gas h^{-1}), we performed a linear regression between $\log_{10}$ of the MO_2 and MCO_2 in the air and water by $\log_{10}$ of body mass, adjusting two lines for aerial and aquatic gas exchanges. All models met the assumption of homogeneity of variance (Levene) and normality distribution (Shapiro-Wilk). The level of significance for all analyses was 0.05. Statistical analyses were conducted in Jamovi software, version 2.3 and GraphPad Prism, version 6 for Windows from GraphPad Software (San Diego, California USA), was used to plot the graphics.

RESULTS

Variability in heart rate (f_H) was observed throughout the experimental duration (Fig. 3A) across all stage groups, but without significant difference ($F_{4,115} = 0.62$, $p = 0.65$). Nevertheless, the f_H varied significantly among different developmental stages ($F_{4,115} = 12.6$, $p < 0.001$) (Fig. 3B), since the larval group showed a higher f_H than the premetamorphic (Tukey $p = 0.004$) and metamorphic (Tukey $p < 0.001$) groups. There was no significant difference between premetamorphic and metamorphic stages (Tukey $p = 0.07$) (Fig. 3B).

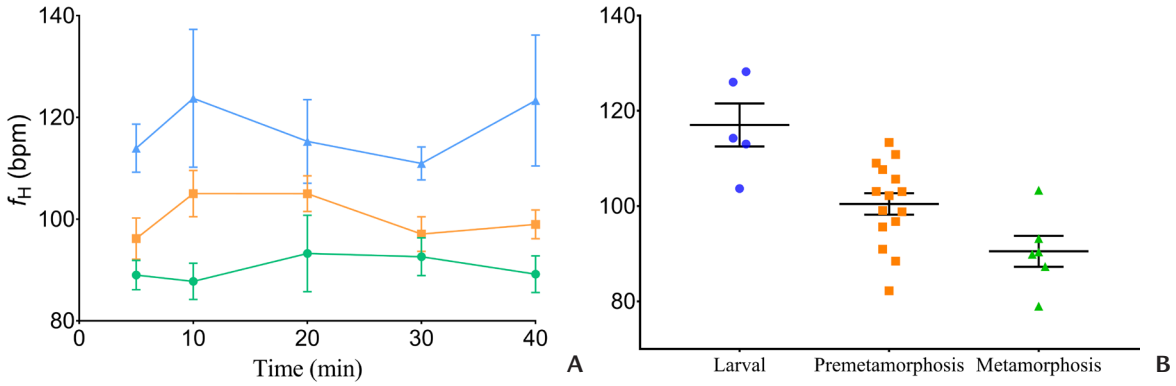

Figure 3. Heart rate (f_H) variation during the experiment (A) and individual f_H (B) in larval (blue line and points), premetamorphosis (orange line and points) and metamorphed individuals (green line and points) of *Lithobates catesbeianus*. All bars represent mean ± s.e.m.

While animals remained submerged on top of the ECG electrodes for most of the time, tadpoles periodically surfaced to breathe air. This continuous behavior allowed for the simultaneous measurement of aerial and aquatic gas exchange. Ventilation events (mean ± SE) pertaining to air intake were observed in premetamorphic (10.6 ± 0.82) and metamorphosed tadpoles (15 ± 3.83). While larval-stage tadpoles also engaged in aerial gas exchange, the system sensitivity was inadequate for detecting ventilatory events in this stage. Furthermore, no discernible heart rate adjustments were noted after or during air ventilation.

There were significant differences in mass-specific oxygen consumption among stage groups and between aerial and aquatic phases (Table 1). In the aerial phase, tadpoles in metamorphosis consumed more oxygen than those in premetamorphosis (Tukey $p < 0.001$) and larval (Tukey $p < 0.001$) stages (Fig. 4A, Table 2). In the aquatic phase, premetamorphic tadpoles showed higher oxygen consumption only than larvae (Tukey $p = 0.004$). Moreover, only metamorphic tadpoles did take up significantly more oxygen in air than in water (Tukey $p < 0.001$) (Fig. 4A). Tadpoles in all stages released more CO_2 in water than air (Tables 1, 2) (Fig. 4B), with premetamorphic (Tukey $p < 0.001$)

Table 1. GLM analysis of gas exchange (µmol gas g^{-1} h^{-1}) in air and water among stage groups.

	df	Sum of Square		F		P	
		MO_2	MCO_2	MO_2	MCO_2	MO_2	MCO_2
Model	5	219.3	111.09	23.9	47.43	<.001	<.001
Groups	2	90.8	8.71	24.7	9.3	<.001	<.001
Medium	1	87.2	67.04	47.5	143.12	<.001	<.001
Group * Medium	2	69.4	6.36	18.9	6.79	<.001	0.003
Residuals	46	84.6	21.55				
Total	51	303.9	132.64				

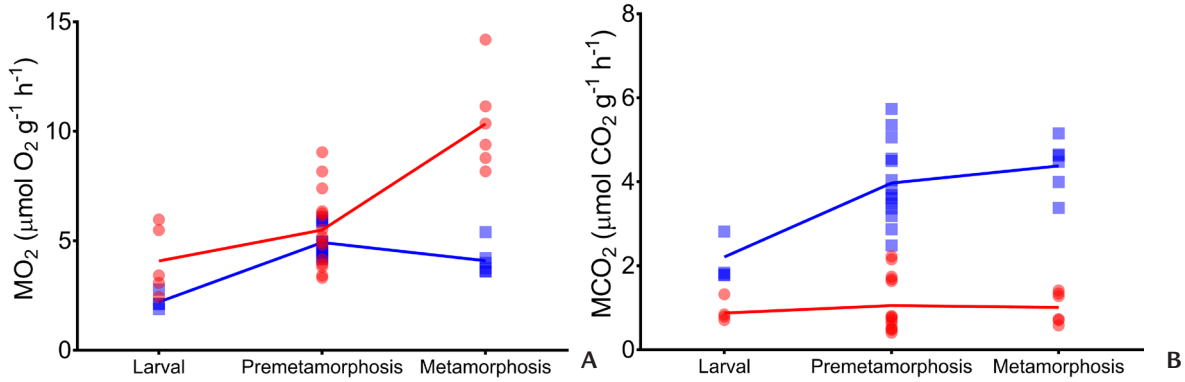

Figure 4. Mass-specific oxygen consumption (A) and carbon dioxide released (B) for aerial (red lines and points) and aquatic (blue lines and points) gas exchange during development of *Lithobates catesbeianus*.

Table 2. Results of Tukey post-hoc comparison tests regarding gas exchange (μmol gas g^{-1} h^{-1}) in air and water among stage groups.

Group	df	Comparisons			Difference		SE		t		p Tukey	
		Medium	Group	Medium	MO_2	MCO_2	MO_2	MCO_2	MO_2	MCO_2	MO_2	MCO_2
Larval	46	Air	Larval	Water	1.8723	-1.3341	0.858	0.433	2.1833	-3.082	0.265	0.038
Larval	46	Air	Metamorphosis	Air	-6.2634	-0.133	0.821	0.414	-7.6287	-0.321	<.001	1
Larval	46	Air	Metamorphosis	Water	-0.0111	-3.5033	0.821	0.414	-0.0136	-8.453	1	<.001
Larval	46	Air	Premetamorphosis	Air	-1.4172	-0.178	0.7	0.353	-2.0241	-0.504	0.345	0.996
Larval	46	Air	Premetamorphosis	Water	-0.8462	-3.0959	0.7	0.353	-1.2086	-8.76	0.83	<.001
Larval	46	Water	Metamorphosis	Water	-1.8834	-2.1692	0.821	0.414	-2.294	-5.234	0.217	<.001
Larval	46	Water	Premetamorphosis	Water	-2.7185	-1.7617	0.7	0.353	-3.8827	-4.985	0.004	<.001
Metamorphosis	46	Air	Larval	Water	8.1356	-1.2012	0.821	0.414	9.9091	-2.898	<.001	0.06
Metamorphosis	46	Air	Metamorphosis	Water	6.2522	-3.3703	0.783	0.395	7.9869	-8.529	<.001	<.001
Metamorphosis	46	Air	Premetamorphosis	Water	5.4171	-2.9629	0.655	0.331	8.271	-8.962	<.001	<.001
Premetamorphosis	46	Air	Larval	Water	3.2895	-1.1561	0.7	0.353	4.6981	-3.271	<.001	0.023
Premetamorphosis	46	Air	Metamorphosis	Air	-4.8461	0.045	0.655	0.331	-7.3993	0.136	<.001	1
Premetamorphosis	46	Air	Metamorphosis	Water	1.4061	-3.3253	0.655	0.331	2.1469	-10.058	0.282	<.001
Premetamorphosis	46	Air	Premetamorphosis	Water	0.571	-2.9179	0.495	0.25	1.1533	-11.676	0.856	<.001
Premetamorphosis	46	Water	Metamorphosis	Water	0.8351	-0.4074	0.655	0.331	1.2751	-1.232	0.797	0.819

and metamorphic (Tukey $p < 0.001$) animals secreting more CO_2 in water than the larval stage. On the other hand, in the aerial phase there were no significant differences among stage groups (Table 2) (Fig. 4B).

There was a positive correlation between MO_2 ($R^2 = 0.77$, $F_{1,24} = 82.19$, $p < 0.0001$) or MCO_2 ($R^2 = 43$, $F_{1,24} = 82.19$, $p < 0.0001$) and body mass only in the aquatic phase (Fig. 5), but not in the aerial phase. Slopes representing aerial and aquatic gas exchange were not significantly different in both media, but differences between intercepts were significant for MO_2 ($F_{1,49} = 16.28$, $p = 0.0002$) and MCO_2 ($F_{1,49} = 157.9$, $p < 0.0001$) (Fig. 5). Tadpoles in the premetamorphic stage showed a great body mass, but decreased air breathing (Fig. 5A). When removing premetamorphosis data from the analysis, there was a significant positive correlation ($R^2 = 0.96$, $F_{1,9} = 237.3$, $p < 0.0001$) between MO_2 in the aerial phase and body mass.

DISCUSSION

Our integrative analysis revealed noteworthy variations in heart rate and oxygen consumption among different development stages of *L. catesbeianus*. The findings showed a decrease in heart rate and an increase in air breathing as de-

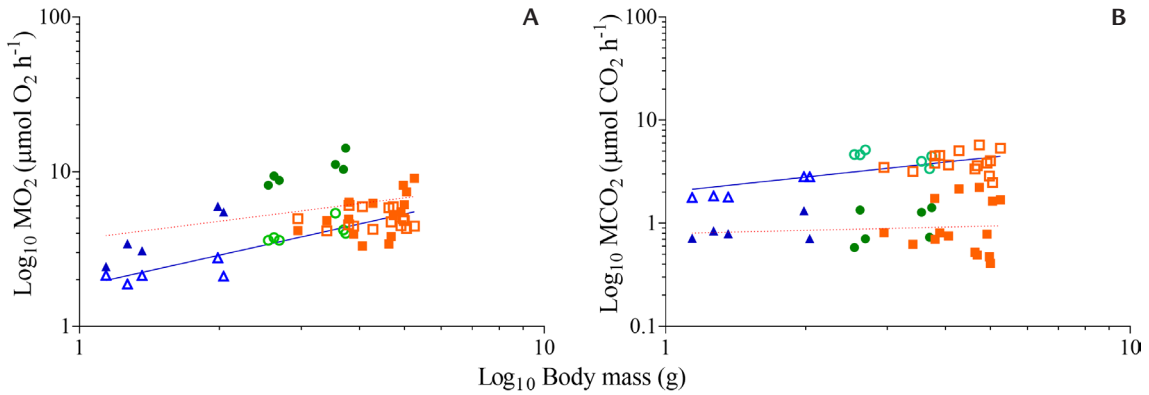

Figure 5. Relationship between Log_{10} whole-body oxygen consumption (A) and carbon dioxide release (B) (μmol h^{-1}) in air (filled symbols) and water (open symbols), and Log_{10} body mass (g) in larval (blue triangles), premetamorphic (orange squares) and metamorphic (green circles) stages of *Lithobates catesbeianus*. Each point represents a measurement from a single animal. The regression lines correspond to aerial (red) and aquatic (blue) gas exchange. Dotted lines represent no significant correlation.

velopment progressed. Specifically, the larval group exhibited a higher heart rate compared to both the premetamorphic and metamorphic groups, likely attributed to alterations in the intrinsic frequency of the cardiac pacemaker and allometric scaling effects (Burggren and Doyle 1986b, Burggren and Pinder 1991). Moreover, the process of metamorphosis entails significant body reorganization in tadpoles, including reshaping and repositioning of the heart to accommodate the new body plan (Sandoval et al. 2022). These transformations within the cardiovascular system also contribute to the reduction in heart rate as the tadpole undergoes its transition into an adult form. While fluctuations in f_H were observed across all groups during the experiment, these variations lacked significance, suggesting that the animals were not in a stressed state.

Tadpoles of some species inflate their lungs very early in development (Crowder et al. 1998, Phillips et al. 2020, Schwenk and Phillips 2020) and employ strategies like bubble-sucking or breach-breathing to take in air. Bubble-sucking is often utilized by smaller tadpoles, while breach-breathing is favored by larger ones, involving breaking the water's surface tension (Schwenk and Phillips 2020). We observed ventilatory events in intermediate-stage tadpoles (larval group) using bubble-sucking, while the system only detected such events in larger stages (premetamorphic and metamorphic groups). Smaller tadpoles employing bubble-sucking might move smaller gas volumes, potentially falling below the system's detection threshold.

During the larval stage, tadpoles exhibited similar oxygen consumption rates in both air and water, indicating effective bimodal respiration catering to their metabolic needs. Interestingly, this trend persisted in the premetamorphic stage, with a slight upturn in aquatic oxygen consumption. It is likely that at this stage, the skin plays a prominent role in gas exchange due to ongoing limb formation in the cranial part of the abdominal cavity, possible hindering pulmonary ventilation and air breathing. However, as limbs become externalized in the metamorphic stage, air breathing becomes dominant, enabling a transition from aquatic to terrestrial environments (McDiarmid and Altig 1999).

The results showed that the release of CO_2 was consistently higher in water across all developmental stages. This suggests a significant contribution of cutaneous gas exchange in water during this stage, concomitantly to gill involution during metamorphosis. Furthermore, as air breathing becomes more effective (metamorphic stage), respiratory organs exhibit specialization: lungs primarily handle oxygen uptake, while skin primarily facilitates CO_2 release into water (Burggren and West 1982).

The observed variation in gas exchange can be attributed to changes in animal mass (see Material and Methods). Our results demonstrated a positive relationship between animal body mass and gas exchange (MO_2 and MCO_2) in water, a relationship well-documented in the literature (Lighton 2008, Kozlowski et al. 2020). However, the absence of correlation between body mass and air breathing was due to animals in the premetamorphic stage. These animals presented higher body mass coupled with lower metabolic rates, likely influenced by tissue reorganization processes and the potential interference of developing limbs on pulmonary ventilation. Conversely, metamorphic stage tadpoles exhibit lower mass due to tail absorption, resulting in a positive correlation between body mass and metabolic rate when the premetamorphic stage data is excluded from the analysis.

Studies investigating heart rate in conjunction with aquatic and aerial breathing have been conducted in certain fish species (Burggren 1979, Sacca and Burggren 1982, Barrionuevo and Burggren 1999, Altimiras and Larsen 2000). While increased heart rate linked to aerial respiration has been noted in air breathing fish (Singh and Hughes 1971), tadpoles, on the other hand, do not seem to display ventilatory tachycardia associated with air breathing (West and Burggren 1982). In our study, we similarly did not observe an adjustment in f_H linked to ventilatory events. This could be attributed to the tadpoles' less developed nervous system for cardiorespiratory regulation, immature peripheral reflexes governing heart rate, or the dominance of skin-mediated gas exchange, which might keep breathing rates constant in water (Buggren and Doyle 1986b).

Longhini et al. (2017) were the first to use a non-invasive approach to measure f_H and gill ventilation in premetamorphic tadpoles of *L. catesbeianus*. Our study builds on this by demonstrating the feasibility of simultaneously measuring aerial and aquatic gas exchange alongside heart rate in conscious tadpoles. Although some data on cardio-respiratory physiology in frog larvae exist, comparing our results with those of prior studies is challenging due to methodological disparities. These differences involve analyzed larval stages, temperature conditions, experimental setups, invasiveness levels, and acclimatization times. For example, Burggren and Doyle (1986b) recorded f_H in various bullfrog developmental stages at 20 °C, obtaining 40 – 50 bpm in larval, < 40 bpm in premetamorphic, > 40 bpm in metamorphic tadpoles, and 30 bpm in adults. Longhini et al. (2017) measured a f_H of < 20 bpm in premetamorphic stages at 15 °C, < 60 bpm at 25 °C and < 80 bpm at 30 °C. West and Burggren (1982) showed that pre-metamorphic bullfrogs increased lung ventilation

from 12 to 51 events per hour during increasing hypoxia (O_2 decreasing from 82 to 21 mmHg), without altering f_H (~50 bpm at 20 °C). Burggren and West (1982) also quantified MO_2 and MCO_2 across skin, gills, and lungs in different developmental stages. They did not find lung ventilation in larval stages, but skin and gill MO_2 (3.4 and 2.3 μmol O_2. g^{-1}. h^{-1}, respectively) and MCO_2 (2.6 and 1.7 μmol CO_2. g^{-1}. h^{-1}, respectively) gas exchange, while premetamorphic gas exchange through skin, gills, and lungs were, respectively, MO_2 4.6, 1.2 and 1.2 μmol O_2. g^{-1}. h^{-1}, and MCO_2 4.2, 3.0 and 0.2 μmol CO_2. g^{-1}. h^{-1}. In post-metamorphic animals without gills, cutaneous and pulmonary gas exchange contributed (MO_2 0.7 and 4.3 μmol O_2. g^{-1}. h^{-1}; MCO_2 4.9 and 0.6 μmol CO_2. g^{-1}. h^{-1}, respectively). Despite methodological disparities, our findings align with existing literature in response patterns. This suggests that our experimental setup is dependable for assessing the cardio-respiratory system, even while concurrently measuring oxygen consumption in aquatic and aerial phases.

While there is some literature on *L. catesbeianus* tadpole's gas exchange and heart rate, our study stands out as the first non-invasive investigation to concurrently evaluate bimodal gas exchange and heart rate across different developmental stages. Furthermore, our approach holds the potential for further enhancements, enabling controlled modifications in water composition (such as pH, temperature, or pollutant levels) and air composition (such as gas concentrations), ultimately reducing extraneous factors that affect the capture of electromyogram signals associated with gill ventilation or to measure f_H in smaller animals. Through these enhancements, larval model organisms could serve as valuable tools for exploring cardiorespiratory physiology under varying environmental conditions.

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