

RESPIRATORY ALLOCATION AND THE RESTING RATE  
OF METABOLISM IN THE AFRICAN LUNGFISH  
*Protopterus aethiopicus*

By

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by

Ashley W. Seifert

I dedicate this thesis to my parents and my family. More than anyone else, their undying encouragement has helped keep me sane. Their love and belief in my potential pushed me to believe in myself.

I also dedicate this work to my grandparents, Evelyn and Nathan Epstein and Louise and Randolph Seifert. While Lou has had the opportunity to share in my triumphs along the way, I know that the others are smiling from somewhere in the cycle of life. I only hope that one day I can fulfill the destiny that all of you have seen for me. It is often I recall your memories and they keep me smiling one day at a time.

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Abstract of Thesis Presented to the Graduate School  
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RESPIRATORY ALLOCATION AND THE RESTING RATE  
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Air breathing in fishes has a very rich evolutionary history, having evolved independently many times in several different phylogenetic lineages. The ancient air-breathing lungfishes are of particular interest because of their proximate relationship to the ancestor of tetrapods. The use of lungs as the primary site of oxygen exchange and the seemingly increased physiological dependence upon aerial respiration across ontogeny provide an excellent opportunity to study the physiology of these fishes along an evolutionary vector similar to that which the tetrapods might have experienced as they began to invade terrestrial environments. However, there remain some key gaps in our understanding of the respiratory profile of lungfishes, in particular respiratory allocation, and metabolic demand of juveniles in the context of ecologically relevant aquatic oxygen levels.

This objectives of this study were to (1) investigate respiratory partitioning and quantify the resting rate of metabolism among juvenile *Protopterus aethiopicus* that vary

in body mass across a dissolved oxygen (DO<sub>2</sub>) range typical of that found within hypoxic wetlands and (2) quantify the effects of temperature on resting rates of metabolism of juvenile *P. aethiopicus* through calculation of Q<sub>10</sub> values across a range of temperatures representative of those found within their natural habitat.

Chapter 1 quantifies the relationship between respiratory allocation (air versus water) and the resting rate of metabolism (RRM) in the primitive air-breathing lungfish, *P. aethiopicus*. Simultaneous measurements of oxygen consumed from both air and water were made to determine the RRM at ecologically relevant levels of dissolved oxygen for juveniles 2 to 221 g. Aerial oxygen consumption averaged 98% (range = 94% to 100%) of total respiratory allocation. This finding contradicts the long-held belief that small juvenile lungfish primarily depend on aquatic respiration to meet their aerobic metabolic demand. Measurements of oxygen consumption made across a gradient of dissolved oxygen from normoxia to anoxia showed that *P. aethiopicus* maintains a stable RRM despite a change in respiratory allocation between water and air.

Chapter 2 examines the effect of temperature on the resting rate of metabolism in *P. aethiopicus*. We present a Q<sub>10</sub> value of 3.14 for six individuals ranging in body mass from 42 to 222 g across an ecologically relevant 10 °C temperature range. This is the first study to our knowledge to determine a Q<sub>10</sub> value for a non-teleost fish and the first to describe this relationship for an air-breathing fish. A comparison of literature-derived Q<sub>10</sub> values for both tropical and temperate fishes indicated a higher Q<sub>10</sub> in tropical species (n=3) than in temperate species (n=10) across an ecologically relevant thermal range.

## CHAPTER 1 GENERAL INTRODUCTION

The transition from water to land stands as a pivotal stage in vertebrate evolution. Any study of this transitional period undoubtedly alludes to the importance of the atmosphere as the new prime source of metabolic oxygen and fishes were the first vertebrates to rise to the surface and begin to break free of their bonds to aquatic existence.

Air breathing in fishes has a rich evolutionary history, having evolved independently numerous times in several different phylogenetic lineages and with various structures for gas exchange (Randall et al. 1981; Graham 1997). Air-breathing fishes are thought to have had their origins in the Late Silurian, based on fossil traces of lung-like structures in placoderms and ostracoderms (Gardiner 1980; Little 1990, Coates and Clack 1991; Gordon and Olson 1994; Long 1995; Graham 1997). The early evolution of lungs and their proposed widespread occurrence among ancient fishes are also strengthened by the observation that extant representatives of mostly extinct groups are bimodal breathers, who use either paired lungs (ancestral condition) or a single lung as the primary air-breathing organ (Randall et al. 1981; Burggren et al. 1986; Ultsch 1996). The phylogenetically ancient air breathers are of particular interest because of their proximate relationship to the ancestor of tetrapods. Their use of lungs as the primary site of oxygen exchange and their seemingly increased physiological dependence upon aerial respiration across ontogeny provide an excellent opportunity to study the physiology of these ancient fishes

along an evolutionary vector similar to that which the tetrapods might have experienced as they began to invade the land.

Surely the most infamous of air-breathing fishes are the lungfishes. Aside from the discovery of the coelacanth, *Latimeria chalumnae*, in the early part of the 19<sup>th</sup> century, the near simultaneous discovery of both *Lepidosiren paradoxa* and *Protopterus annectens* in the 1830's and the discovery of *Neoceratodus forsteri* in 1870 stand as three of the most important zoological discoveries of the last two centuries (Burggren and Johansen 1987). Their exquisite fossil record provides a good picture of the interspecific relationships of both extinct and extant forms. However, ever since their discovery, their taxonomic position in relation to the tetrapods has remained a source of rich scientific debate. Lungfishes belong to the Ceratodontimorpha and, along with *Latimeria* (the coelacanth), remain the only extant representatives of the Sarcopterygii, a group from which tetrapods later emerged. Within the Ceratodontimorpha, the Lepidosireniformes, comprised of the African family Protoperidae (four species) and the South American family Lepidosirenidae (one species), appear largely unchanged from the ancestral *Dipterus* of the Carboniferous period (Long 1995; Graham 1997). *Neoceratodus forsteri* is the lone species representative of the Ceratodontidae and is considered the most primitive of the three genera based on several phylogenetic characters (Marshall 1987). Of the three genera of lungfishes, two are obligate air-breathers, while the Australian species, *Neoceratodus forsteri*, uses aerial respiration as a supplementary means of gas exchange (Grigg 1965; Graham 1997). Fossil evidence and their relative morphological stability through evolutionary time allow for the conjecture that modern day

Lepidosireniformes may be physiologically similar to a tetrapod ancestor (Burggren et al. 1986).

Are these interesting creatures the closest extant relative of the tetrapods?

Regardless of when or whether scientists will come to answer this question, the fact remains that lungfish represent a very special organism for evolutionary biologists working from an ecological and physiological perspective. The respiratory and cardiovascular similarities that both *Lepidosiren paradoxa* and *Protopterus species* share with terrestrial amphibians make them interesting transitional species to investigate these systems in the context of vertebrate evolution.

Beginning with Homer Smith in the 1930's, the majority of early research on *Protopterus aethiopicus*, and lungfishes in general, centered on cardio-respiratory adaptations and the physiological effects of aestivation. Given that *P. aethiopicus* shifts from having strongly developed external gills as small juveniles to poorly developed internal gills and well-developed paired lungs as adults, researchers hypothesized a strong shift in respiratory allocation between air and water. Indeed, this is precisely what early research uncovered. A comparison of early studies that measured oxygen consumption in lungfishes yields a profile whereby juveniles use primarily aquatic respiration to meet metabolic demands, and adults rely almost exclusively on aerial respiration (Smith 1930; Sawaya 1946; Johansen and Lenfant 1967; Lenfant and Johansen 1968; Lenfant et al. 1970; McMahon 1970; Johansen et al. 1976; Babiker 1979). Most of these investigations centered exclusively on either juveniles or adults. However, a study done by Johansen et al. (1976) examined changes in respiratory allocation between water and air across a wide range in body size from 4 g to 500 g. Their experiments established a two-phase

respiratory profile for *P. amphibius* based on these experiments where the smallest juveniles relied on dissolved oxygen to meet metabolic demands, while larger animals acquired their oxygen aerially. Based on their findings they concluded that “the altered dependence on water and air-breathing during the life-cycle of *P. amphibius* will most likely set territorial limits to the distribution of larvae and young fish within the distribution range of the species” (Johansen et al. 1976. p. 399). This statement is striking in that it predicted the opposite of what is known of juvenile and adult African lungfish based on dissolved oxygen levels within their natural habitat.

A careful consideration of the ecology of juvenile lungfish highlights this interesting paradox. Juvenile species of African lungfish are born within nests that are found within swamp regions surrounding larger bodies of water or in portions of rivers where current is slow. Measurements of dissolved oxygen concentrations made by Greenwood (1958) at the surface of swamps surrounding Lake Victoria ranged from 1.01 to 1.71 mg l<sup>-1</sup> and within the breeding nests from 0.98 to 2.66 mg l<sup>-1</sup>. Research from the Lwamunda Swamp surrounding Lake Nabugabo (Uganda) found that juveniles remain within fringing swamps surrounding the main lake until they reach a body size of approximately 40 cm TL (Goudswaard et al. 2001). Individuals smaller than 40 cm TL are rarely found in the open waters of Lake Nabugabo where larger lungfish supported a thriving fishery before the introduction of Nile Perch in the 1960's. Dissolved oxygen levels in the Lwamunda Swamp are extremely low, and it is the younger juveniles (nestlings to juveniles of 40 cm TL) that are found under these hypoxic conditions (Goudswaard et al. 2001). With these ecological parameters in mind, it is curious that the youngest juveniles, who seemingly experience the greatest respiratory stress, would rely

mainly upon aquatic respiration, while adults who move into open water habitats would suddenly transform into animals dependant on aerial respiration. These previous findings set up an interesting paradox created by the seemingly opposing forces of dissolved oxygen levels and respiratory physiology of these animals.

If the transition from water to land in early tetrapods was mediated by hypoxic stress within ancient freshwater habitats, it was of great interest to me to determine if small, juvenile lungfish were primarily air-breathers and if their respiratory physiology was determined by strong environmental gradients. Given the ecological characteristics of Lake Nabugabo with a fringing hypoxic swamp surrounding a main lake of much higher DO<sub>2</sub>, and the findings of Johansen et al. (1976), I sought to resolve the above stated paradox and set out to examine the respiratory allocation of *Protopterus aethiopicus*. My objectives were to (1) investigate respiratory partitioning and quantify the resting rate of metabolism among juvenile *Protopterus aethiopicus* that vary in body mass across a dissolved oxygen (DO<sub>2</sub>) range typical of that found within hypoxic wetlands and (2) quantify the effects of temperature on resting rates of metabolism of juvenile *P. aethiopicus* through calculation of Q<sub>10</sub> values across a range of temperatures representative of those found within their natural habitat.

Chapter 2 explores respiratory allocation and the resting rate of metabolism in juveniles of the African lungfish (*Protopterus aethiopicus*). The objective of this chapter is to quantify respiratory allocation between water and air and the rate of oxygen consumption over the size range typically found in marginal hypoxic wetlands in the Lake Victoria basin (nestlings to 40 cm TL, Goudswaard et al. 2001). To meet this objective, the rate of oxygen consumption and relative allocation to air and water

breathing was measured across a broad range of dissolved oxygen concentrations including those reflective of the natural habitat. I also explored the effect of chamber acclimation time and chamber size on respiratory allocation and rate of oxygen consumption.

In Chapter 3, I quantify the relationship between temperature and the resting rate of metabolism in *Protopterus aethiopicus* through a temperature acclimation study. Fishes, as the most species-rich group of chordates, lack a comprehensive synthesis of metabolic data that includes both phylogenetically ancient (non-teleost) and advanced groups. In particular, studies examining metabolic rates and the effects of temperature in non-teleost fishes are completely absent from the literature. This chapter determined the  $Q_{10}$  value for *P. aethiopicus*. This  $Q_{10}$  value is discussed in relation to those discerned from the literature for both temperate and tropical teleosts.

CHAPTER 2  
RESPIRATORY ALLOCATION AND THE RESTING RATE OF METABOLISM IN  
*Protopterus aethiopicus*

**Introduction**

Air breathing in fishes has a rich evolutionary history, having evolved independently numerous times in several different phylogenetic lineages and with various structures for gas exchange (Randall et al. 1981; Graham 1997). Air-breathing fishes are thought to have had their origins in the Late Silurian, based on fossil traces of lung-like structures in placoderms and ostracoderms (Gardiner 1980; Little 1990; Coates and Clack 1991; Gordon and Olson 1994; Long 1995; Graham 1997). The early evolution of lungs and evidence of their widespread occurrence among ancient fishes is supported by the observation that extant representatives of mostly extinct groups are bimodal breathers, using either paired lungs (ancestral condition) or a single lung as the primary air-breathing organ (Randall et al. 1981; Burggren et al. 1986; Ultsch 1996). Despite air breathing having evolved independently several times in more derived fishes, primitive air breathers are of interest because of their proximate relationship to the ancestors of tetrapods. Their use of lungs as the primary site of oxygen exchange and *apparent* increased physiological dependence upon aerial respiration across ontogenetic stages provides an excellent opportunity to study the physiology of these ancient fishes along an evolutionary vector similar to that which tetrapods might have initially experienced during the transition to a terrestrial existence. However, very little is known about energetics of primitive air-breathers under ecologically relevant oxygen levels and the

relationship between respiratory allocation (water vs. air) and body size. In this study, I examined the energetics of juvenile African lungfish (*Protopterus aethiopicus*) across a range of body sizes to detect ontogenetic shifts in respiratory allocation and to measure resting rate of metabolism.

Of the three families of lungfishes, the Lepidosirenidae (comprised of one species, *Lepidosiren paradoxa*) and the Protopteridae (comprised of four species of *Protopterus*) are obligate air-breathers, whereas the Ceratodontidae (comprised of the Australian species, *Neoceratodus forsteri*), uses facultative air breathing as a supplementary means of gas exchange (Grigg 1965; Graham 1997). Obligate air-breathing lungfishes possess highly partitioned, paired lungs that originate from the esophagus, have separate pulmonary circuits, and circulatory systems with a high degree of septation in the heart, resembling that of extant amphibians (Burggren and Johansen 1987).

The majority of early research on *P. aethiopicus*, and lungfishes in general, focused on cardio-respiratory adaptations in larger fish (Sawaya 1946; Johansen and Lenfant 1968; Lahiri et al. 1970; Lenfant et al. 1970; McMahon 1970). The few studies that explored ontogenetic variation in respiratory strategies reported shifts in respiratory allocation with body size. For example, Johansen et al. (1976) found that small juveniles (3.7 g) of *Protopterus amphibius* relied on aquatic respiration to meet 70% of their oxygen requirement, whereas aquatic respiration accounted for as little as 10% of the oxygen uptake in larger specimens (300 g to 500 g). Similarly, Lenfant et al. (1970) found that aerial respiration accounted for nearly 97% of the total oxygen demand in large individuals of *Lepidosiren paradoxa*, but only 26% for small juveniles. The combination of these studies created a physiological portrait of the Lepidosirenidae and

Protopteridae as animals with two distinct respiratory life stages (Jesse et al. 1967; Lenfant et al. 1970; McMahon 1970; Johansen et al. 1976; Babiker 1979). Small, juvenile lungfish were thought to be primarily dependent on aquatic respiration for survival, while larger individuals became increasingly dependent on aerial respiration as they grew, ultimately relying on aerial respiration for over 90% of their metabolic demand.

This scenario, however, seems inconsistent within the ecological context of their habitats. In the field, nestling *P. aethiopicus* have been observed to begin air breathing at a length of 23 to 27 mm while still possessing external gills (Smith 1931; Greenwood 1987). At this size, they are associated with either floating mats of papyrus roots or amongst heavily vegetated patches within the swamp (Greenwood 1987). In lakes Victoria and Nabugabo (East Africa), Goudswaard et al. (2001) reported that *P. aethiopicus* smaller than 40 cm were very rare in open waters and almost exclusively associated with the fringing swamp. There, dissolved oxygen concentration can be extremely low (Chapman et al. 2002). Both Greenwood (1958) and Johnels and Svensson (1954) suggested that the shallowness of *P. aethiopicus* nests may be connected with the air-breathing behavior of the nestlings. In these nests, young have been observed resting on the roots near the surface (Greenwood 1987). Dissolved oxygen measurements made by Greenwood (1958) at the surface and at the bottom of the nests ranged between 1.01 and 1.71 mg l<sup>-1</sup>, and between 0.98 and 2.66 mg l<sup>-1</sup>, respectively. Although the smallest size of aestivating individuals is unknown, *Protopterus dolloi*, a West African lungfish species, has been found in cocoons at lengths of 40-50 mm (Johnels and Svensson 1954). The fact that individuals this small can aestivate suggests that they are capable of sustaining themselves solely on aerial respiration. In conditions such as these, and with

the capacity to acquire oxygen aerially, it would appear that small, juvenile lungfish might use aerial respiration in their natural environment to an extent previously unrecognized.

In earlier studies, measurements of oxygen consumption in small, juvenile *P. aethiopicus* were made under conditions of near-saturated dissolved oxygen, which do not reflect the observed natural ambient conditions at this life-history stage. The objective of this study was to quantify respiratory allocation between water and air and the rate of oxygen consumption in juvenile African lungfish (*Protopterus aethiopicus*) over the size range typically found in marginal hypoxic wetlands in the Lake Victoria basin (nestlings to 40 cm TL, Goudswaard et al. 2001). To meet this objective, we measured rate of oxygen consumption and relative allocation to air and water breathing across a range of dissolved oxygen concentrations reflective of the natural habitat. We also explored the effect of chamber acclimation time and chamber size on respiratory allocation and rate of oxygen consumption.

## **Materials and Methods**

### **Study Site**

Specimens of the African lungfish, *Protopterus aethiopicus* were obtained from a natural population within the Lwamunda Swamp that surrounds Lake Nabugabo, Uganda (31° 50' E; 31° 56' S; to 0° 20' E; 0° 25' S, Fig. 2-1). Lake Nabugabo lies just south of the equator and is a small satellite lake (surface area = 24 km<sup>2</sup>, mean depth = 4.5 m) that was isolated from Lake Victoria approximately 4000 years ago (Greenwood 1965). The lake lies within the extensive Lwamunda Swamp that was formerly a bay on the western shore

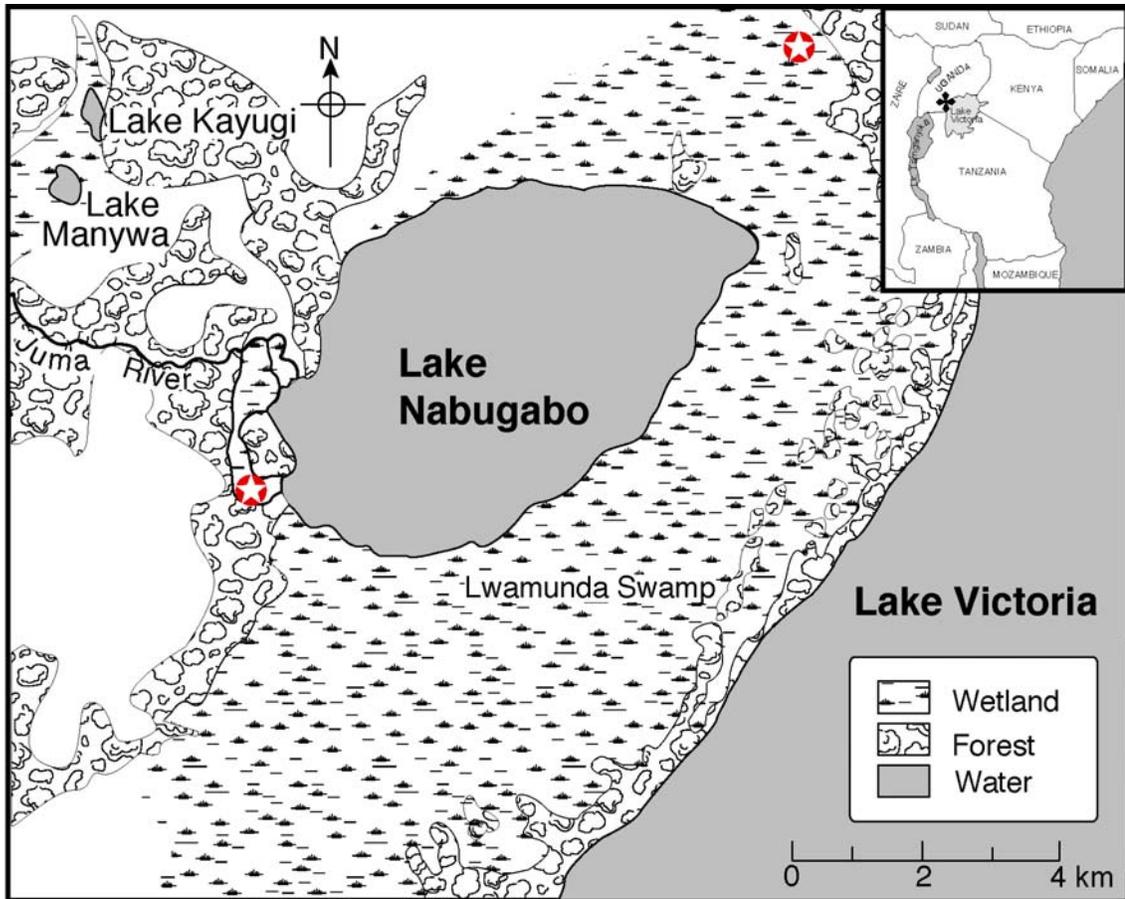


Figure 2-1. Lwamunda Swamp, Lake Nabugabo, Uganda. Red stars indicate two collection sites where specimens of *Protopterus aethiopicus* were captured.

of Lake Victoria (Worthington 1932; Greenwood 1965; Ogutu-Ohwayo 1993). The lake margin is dominated by hippo grass (*Vossia cuspidatum*), *Miscanthidium violaceum*, water lilies (*Nymphaea lotus* and *N. caerulea*), and small stands of papyrus (*Cyperus papyrus*). The dense interior of the swamp (dominated by *M. violaceum*) is characterized by numerous small lagoons and channels that serve as important habitats for *P. aethiopicus* (<40 cm total length, Goudswaard et al. 2001). Dissolved oxygen concentrations ( $\text{DO}_2$ ) at several sites within a small swamp lagoon were measured over a 1-year period. Monthly  $\text{DO}_2$  measurements in the upper 20 cm of water averaged  $1.5 \text{ mg l}^{-1}$  ( $\approx 26.8 \text{ mm Hg}$ ) in the morning and  $1.8 \text{ mg l}^{-1}$  ( $\approx 32.9 \text{ mm Hg}$ ) during the afternoon

(Chapman et al. 2002). Water temperatures at the sites averaged  $22.3^{\circ}\text{C}$  in the morning and  $23.8^{\circ}\text{C}$  in the early afternoon (Chapman et al. 2002).  $\text{DO}_2$  values in the Lwamunda swamp are particularly low in the dry season. In a survey of 12 small lagoons within the Lwamunda Swamp in the early dry season Chapman et al. (2002) reported an average  $\text{DO}_2$  level of only  $0.6\text{ mg l}^{-1}$  ( $\approx 11\text{ mm Hg}$ , upper 20 cm of water) in the early morning and  $1.3\text{ mg l}^{-1}$  ( $\approx 24\text{ mm Hg}$ ) in the mid-day period. In samples taken just above the sediments,  $\text{DO}_2$  averaged  $0.3\text{ mg l}^{-1}$  ( $\approx 5\text{ mm Hg}$ ) in the early morning and  $0.4\text{ mg l}^{-1}$  ( $\approx 7\text{ mm Hg}$ ) in the mid-day (Chapman et al. 2002).

### **Collection and Maintenance of *P. aethiopicus***

Individuals were live-captured using baited minnow traps set at various places within the lagoons and left overnight. Traps were collected the following morning and each lungfish placed in its own separate holding container. This method was appropriate for catching individuals ranging in mass from 10 g to  $\sim 250\text{ g}$ . Smaller individuals ( $< 5\text{ g}$ ) were obtained by dip netting vegetated areas within the lagoons and in all cases were caught in dense vegetation mats.

Individual lungfish were held in 1.2-l buckets with lids while in Uganda and then transported to the University of Florida. Once at the University, individual lungfish were held in partitioned 208.2-L tanks with no two lungfish occupying the same space within a tank. Water temperature was maintained at  $23 \pm 0.5^{\circ}\text{C}$ , with a 12/12 photoperiod. Dissolved oxygen concentration averaged  $8.16 \pm 0.05\text{ mg l}^{-1}$ . Temperature, dissolved oxygen, pH, conductivity, ammonia, and nitrite levels were measured weekly to maintain appropriate water quality. Individual fish were held under these conditions at least 1 year before experimental trials. Fish were fed once a week on fresh chicken or beef liver and,

in the case of the smallest individuals, blood worms. Our experimental protocols for *P. aethiopicus* were approved by the University of Florida (IACUC project #Z090).

### **Respirometry**

Oxygen consumption was measured using a two-phase respirometer (Fig. 2-2). The respirometer was constructed using PVC, oxygen impermeable vinyl tubing and brass compression fittings (Swagelok<sup>®</sup>). Several chambers of different sizes were built and were interchangeable within the system. The chamber where fish were placed consisted of a long length of PVC connected to a T-junction that served as an air chamber. The T-junction was constructed from clear PVC to allow periodic observation of fish during respiratory trials, while the air chamber itself was constructed from opaque PVC. The length of PVC that constituted the bulk of the water chamber was opaque. A 1.5 amp Mag-drive water pump, flow meter, oxygen-sensing probe, and thermistor were all placed in series for the water phase of the system. A stopper, with an oxygen probe, thermistor, and small fan inserted through it, was used to seal the air portion of the system. Thus, each phase of the respirometer contained an associated oxygen sensing probe and thermistor that provided for simultaneous measurement of oxygen consumption and temperature from both aerial and aquatic phases of the respirometer.

The respirometer utilized Ocean Optics<sup>®</sup> Oxygen Sensing technology to measure oxygen concentration in both water and air (Dunedin, FL; <http://www.oceanoptics.com>). Oxygen detection using these probes is based on excitation of a ruthenium complex suspended in a sol-gel matrix at the ends of the probes. Light is emitted from a blue LED source at ~470 nm that travels via fiber optic cables to the tip of the probes.

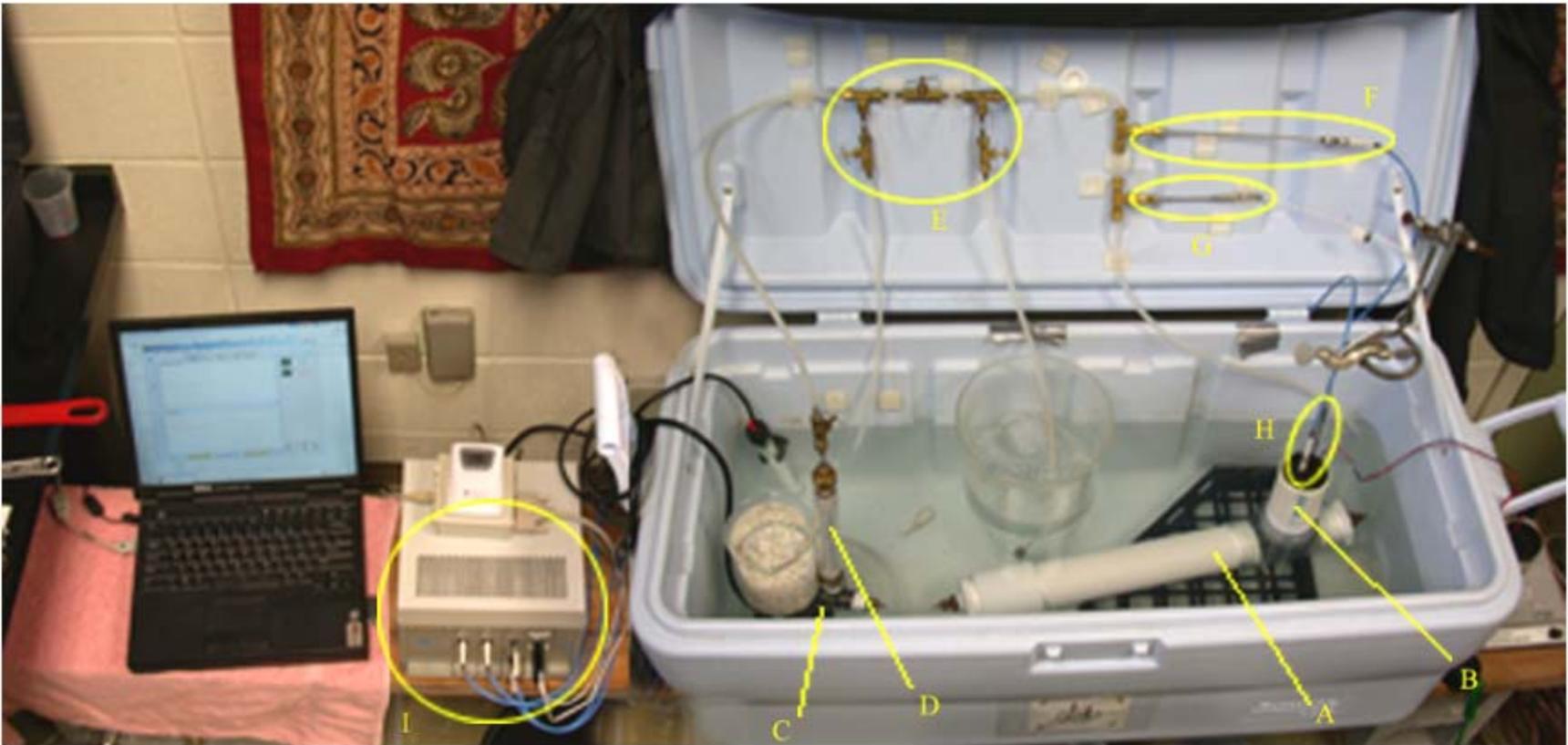


Figure 2-2. Respirometry system used to measure the resting rate of metabolism. The entire system is submerged in a water bath to maintain temperature. The system is connected to a laptop that records the data. (a) Water chamber; (b) air chamber; (c) 2.5 amp Mag Drive pump; (d) flowmeter; (e) three stop-cock compression fittings that allows the system to convert from a closed system to a flow-through system; (f) Ocean Optics oxygen sensing probe to measure  $DO_2$  in water phase; (g) thermistor measuring water temperature; (h) thermistor and Ocean Optics oxygen sensing probe to measure temperature and  $DO_2$  in air phase; (i) spectrophotometer.

The ruthenium is excited when exposed to the light, and emits energy at ~600 nm that is returned via the fiber optic cables to a spectrophotometer. As oxygen molecules come in contact with the tip of the probe, the energy associated with the excitation via the blue LED is quenched. This quenching (a non radiative transfer) results in a decrease of intensity. An inverse relationship therefore exists between the intensity of excitation associated with the ruthenium and the presence of oxygen in a given medium. This information in intensity units is converted to percent saturation of oxygen in the medium based on probe calibration (Krihak and Shahriari 1996).

The probes were calibrated to 0% DO<sub>2</sub> in water containing sodium sulfite. The probes were calibrated to 100% saturation for the water chamber in air-saturated water and for the air chamber in air. The probes were calibrated on a scale of percent saturation in the medium. Percent saturation was then converted to units of mg l<sup>-1</sup> with corrections for ambient air pressure and water vapor pressure. The respirometer was designed as a closed system with the capacity to flush out and replace the contents of either phase. This allowed the removal of any metabolic waste products that accumulated during the acclimation period.

Probes were calibrated prior to each run. Individual fish were placed in the system containing a mixture of aerated tap water and filtered tank water. Controls were run on the system with no fish present to determine biological activity of the water. In all cases microbial O<sub>2</sub> consumption in the water was determined to be negligible. Controls were also run to determine the diffusion of oxygen between the air and water phases under various levels of dissolved oxygen. These controls showed that the transfer of oxygen between the two phases was undetectably small.

The resting rate of metabolism (RRM) was measured both at the Makerere University Biological Field Station in Uganda and in our laboratory at the University of Florida (UF). Resting rate of metabolism was measured as a function of oxygen consumed over time. We refer to our measurements of oxygen consumption as RRM, although we believe our final estimates represent minimum rates for these fish (measured in the dark). Movement of the fish during trials was almost exclusively associated with respiration and therefore considered a portion of maintenance metabolism since it is necessary for survival. The protocols differed slightly as described below. For those fish measured in the field (N=16), individual fish were acclimated for 3-h periods inside the chamber with free access to air. After the acclimation period the air chamber was sealed and the run was initiated. Oxygen consumption was recorded over a DO<sub>2</sub> range from 7.21 ( $\approx$ 130 mm Hg) to 5.77 mg l<sup>-1</sup> ( $\approx$ 104 mm Hg) at 23° C. Measurements were made within 3 wks of capture, on post-absorptive fish, and we refer to these as our “field” estimates.

For those fish measured in the laboratory (N=14), individuals were acclimated overnight to the system with free access to air. The DO<sub>2</sub> concentration in the water chamber was allowed to drop to near 0.0 mg l<sup>-1</sup>, the approximate level at which aquatic oxygen consumption ceased. An overnight acclimation was used because oxygen consumption measurements revealed that the RRM measured after a 3-h acclimation dropped approximately two-fold when compared to measurements made after a 16-h acclimation. The effect of a short acclimation time on the RRM only became apparent after laboratory tests were run at the UF where temperature control over an extended period could be maintained. Prior to each run in the morning the system was flushed with air-saturated water until the DO<sub>2</sub> concentration in the system was greater than 2.57 mg l<sup>-1</sup>

( $\approx 46.4$  mm Hg). This served to remove any metabolic wastes that may have accumulated overnight and to raise aquatic  $\text{DO}_2$ . The air chamber was then closed and the run initiated. Oxygen measurements were made every 10 sec over the course of the run and recorded automatically by the computer software running the system. Each run was monitored and the percent saturation of oxygen in the air chamber was never allowed to drop below 608.0 mm Hg. When oxygen concentration within the air chamber approached 608.0 mm Hg, the stopper was removed and fresh air allowed to mix into the air chamber to return the oxygen concentration to 100% saturation. This mixing did not increase  $\text{DO}_2$  within the water chamber. Once the  $\text{DO}_2$  concentration within the water chamber reached a plateau representing zero uptake from water, the run was allowed to continue for 1 h and was then terminated. Oxygen consumption was recorded over a  $\text{DO}_2$  range between  $4.29 \text{ mg l}^{-1}$  ( $\approx 77.4$  mm Hg) and  $0.00 \text{ mg l}^{-1}$  at  $23^\circ \text{C}$  ( $\pm 0.5^\circ \text{C}$ ).

Temperature was maintained throughout the run by placing the entire system inside a Rubbermaid cooler that served as a temperature bath. Most of the system was submerged in the cooler and the entire apparatus was covered with a black sheet to shield the fish from any movements of the investigators. Individuals were starved for 7 d prior to each experiment to ensure that they were post absorptive. Runs lasted an average of 12 h. At the end of each run, individuals were drip dried and weighed (0.0 g).

An additional experiment was run in the laboratory to investigate any changes in the RRM at near saturated levels of  $\text{DO}_2$  compared to those at ecologically relevant  $\text{DO}_2$  levels. We used the overnight laboratory acclimation period to provide comparability with our runs at lower  $\text{DO}_2$ . Following acclimation, the entire chamber was flushed and the  $\text{DO}_2$  level in the chamber returned to near saturation. These runs lasted 6 h or less and

were terminated when DO<sub>2</sub> concentration in the water phase was less than 6.0 mg l<sup>-1</sup> (≈108.4 mm Hg).

Analysis of covariance was used to compare the bilogarithmic relationships of RRM and body mass among size groups and DO treatments. A t-test was used to compare means when body size was not required as a covariate.

## Results

### Field Laboratory Data

Measurements of oxygen consumption on lungfish taken within 3 wks of capture and measured at our field laboratory (N=16) at high DO<sub>2</sub> concentrations revealed a strong shift in respiratory allocation with body mass (Table 2-1). Individuals less than 55 g were almost exclusively water breathers (percent allocation from water  $95.7 \pm 0.9\%$ ). The allocation to air breathing increased from an average of  $4.3 \pm 0.9\%$  in individuals less than 55 g to  $50.7 \pm 9.0\%$  in individuals 55 to 111 g, and to  $79.7 \pm 2.7\%$  in individuals greater than 111 g (Table 2-1). Measurements of oxygen consumption also revealed a shift in the bilogarithmic relationship of RRM and body mass (Fig. 2-3). There was no difference in the slopes of the bilogarithmic relationship of RRM (mg O<sub>2</sub> hr<sup>-1</sup>) and body mass (g) between individuals < 83 g and ≥ 83 g ( $F = 4.187$ ,  $p = 0.063$ ). However, the intercepts (representing adjusted mean RRM) differed ( $F = 20.743$ ,  $p = 0.016$ ). Individuals < 83 g had a 5.4-fold higher adjusted mean RRM of 10.26 mg O<sub>2</sub> hr<sup>-1</sup> compared to individuals ≥ 83 g (1.89 mg O<sub>2</sub> hr<sup>-1</sup>, RRM adjusted to the mean mass of 82.0 g).

### UF Laboratory Data

The RRM of *P. aethiopicus* (N=14) measured in the laboratory comprised both aerial and aquatic oxygen consumption: Figs. 2-4 A,B illustrates these separate

components as oxygen consumed (mm Hg) over time (min) for two individuals. While aerial consumption of oxygen fit a linear function, aquatic oxygen consumption fit an exponential function (Figs. 2-4 A,B). To examine if the degree of curvilinearity within the water phase varied with body size, we fit an exponential function ( $y=b_0e^{b_1t}$ ) to individual curves and compared the exponents ( $b_1$ ) among individuals as a function of body mass. A comparison of the exponents indicated the degree to which the relationship was curved or departed from a linear relationship. The degree of curvilinearity was positively correlated with body mass ( $r = 0.598$ ,  $p = 0.031$ , Fig. 2-5). The smallest individual (2 g) was excluded from this analysis.

Lungfish reduced aquatic respiration to less than 1% of total oxygen consumed at low aquatic  $PO_2$  represented by the plateau point where aquatic respiration was nearly

Table 2-1. Respiratory allocation of *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, between water and air at high (130 mm Hg – 104 mm Hg) levels of  $DO_2$  and measured at an average temperature of 23° C. Measurements were made within 3 wks of capture at the Makerere University Biological Field Station, Uganda.

Mass (g)	Percent O <sub>2</sub> from Water	Percent O <sub>2</sub> from Air
17	96	4
21	97	3
27	94	6
55	68	32
77	67	33
83	19	81
89	65	35
107	61	39
108	19	81
111	46	54
120	25	75
123	31	69
149	16	84
160	14	86
169	20	80
180	16	84

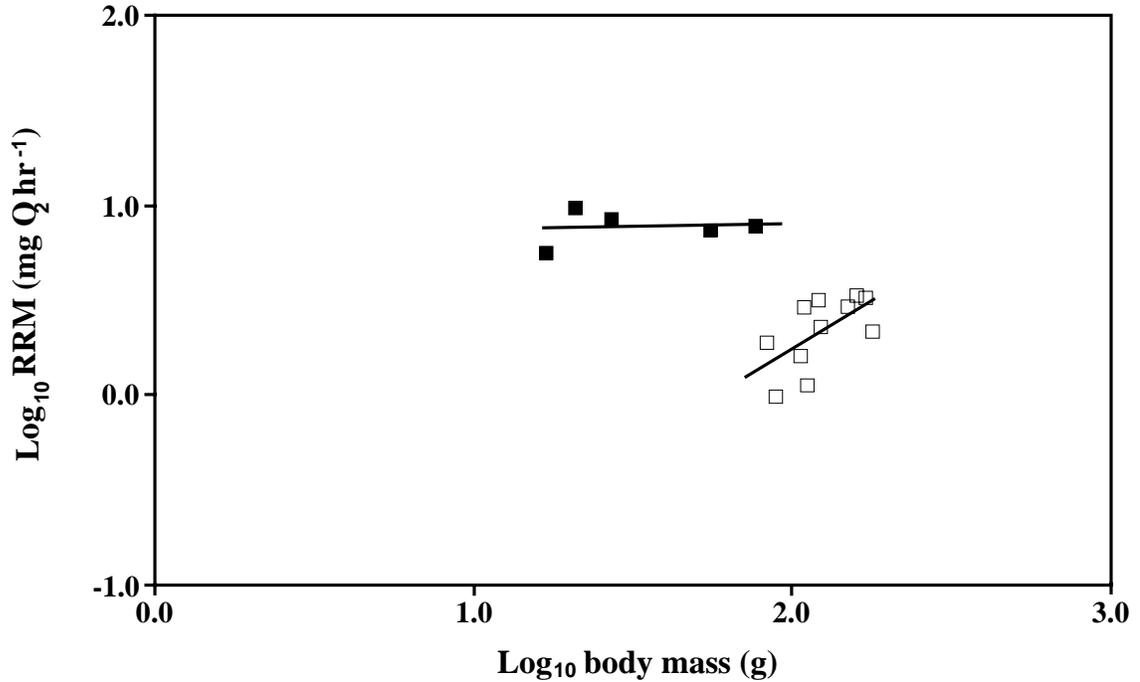
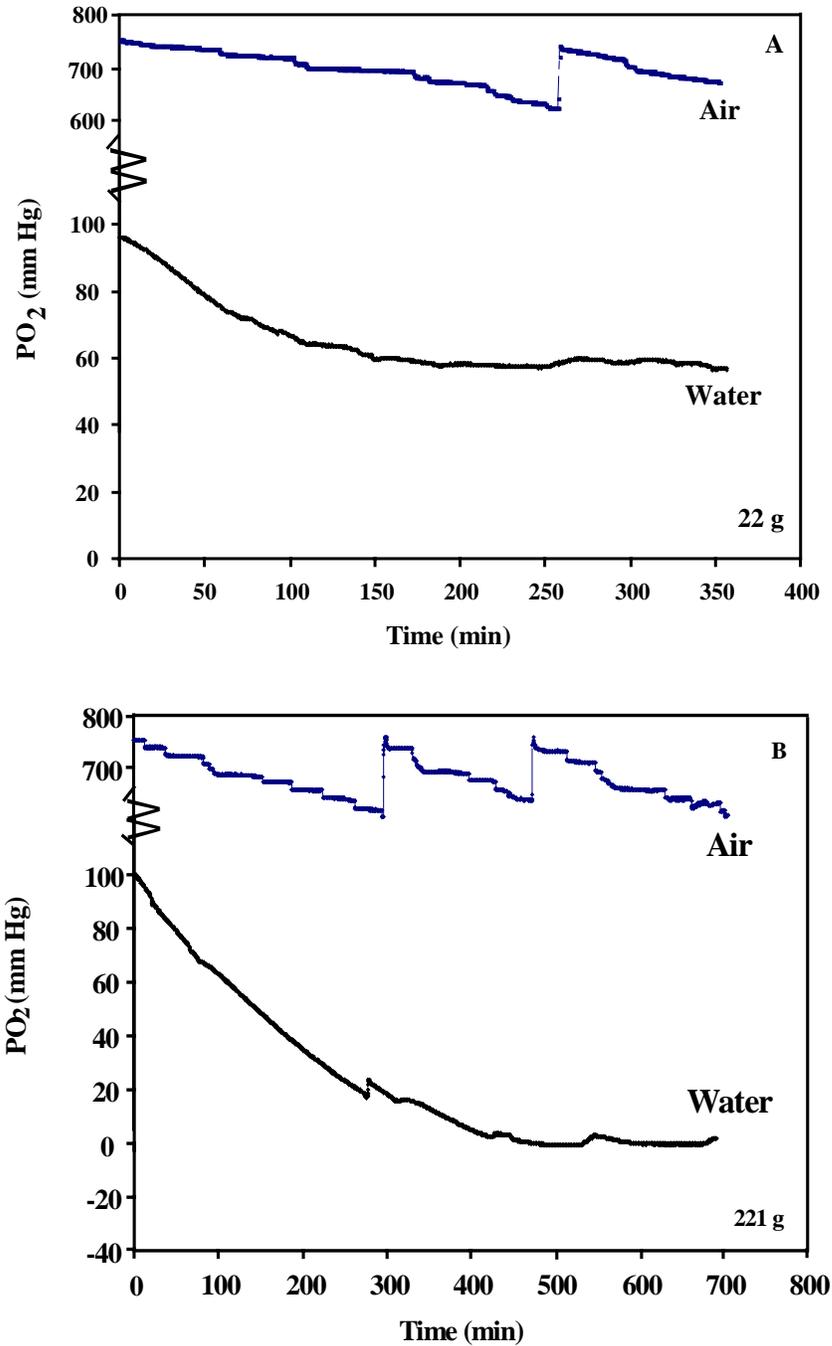


Figure 2-3. Bilogarithmic relationship between resting rate of metabolism (RRM) and body mass of *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, measured at high levels of DO<sub>2</sub> in the field [(7.21 (≈130 mm Hg) to 5.77 mg l<sup>-1</sup> (≈104 mm Hg) at 23° C]. Individuals were acclimated for 3 h prior to trials. Closed squares represent individuals ≤ 81 g, and open squares represent individuals ≥ 83 g. Individuals ≤ 81 g were measured in 2.5 cm chambers, and fish ≥ 83 g were measured in 5.1 cm chambers.



Figures 2-4. The decline in oxygen partial pressure over time for *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, in a two-phase, closed respirometer, where fish had access to both air and water simultaneously. The curvilinear function  $y=b_0e^{b_1t}$  for aquatic respiration is indicated for two representative individuals A)22 g , B)221 g).

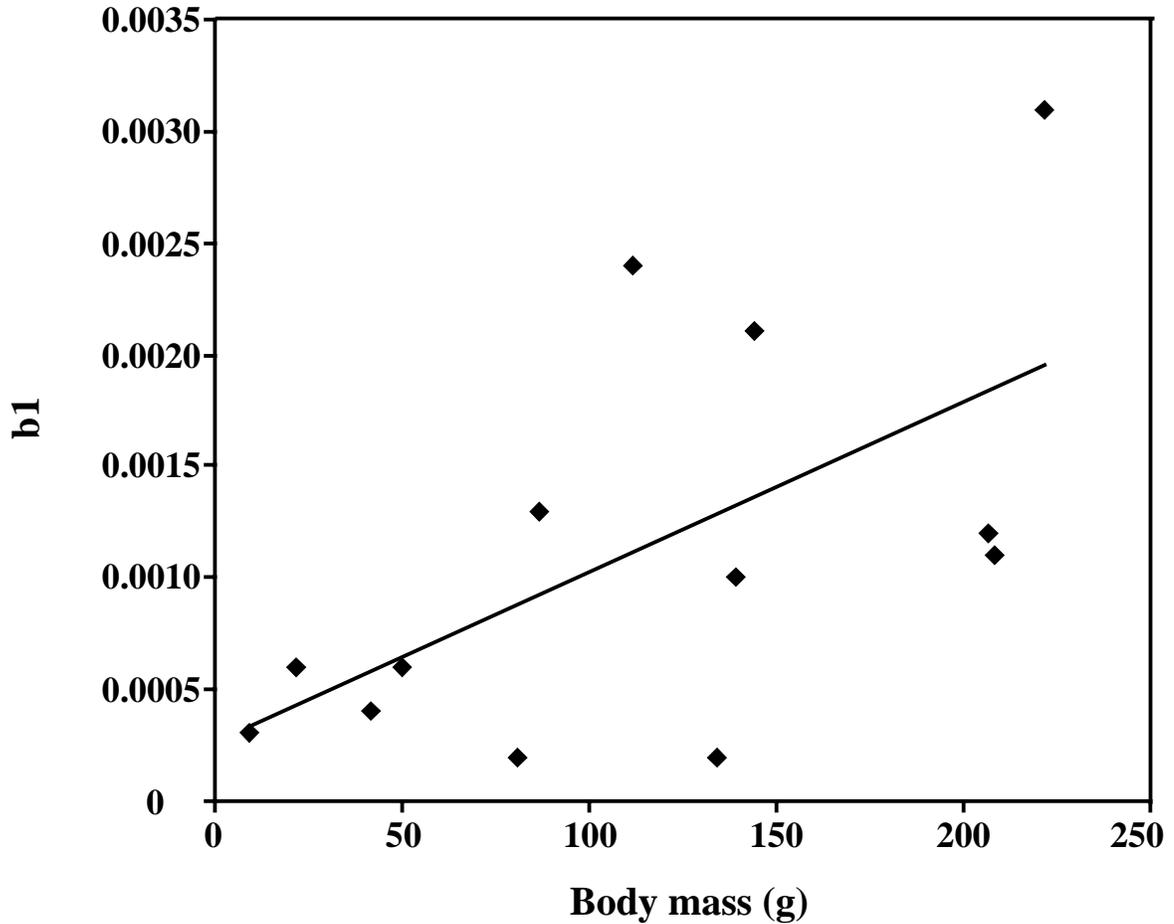


Figure 2-5. The relationship between the degree of curvilinearity ( $b_1$  from the equation  $y=b_1e^{b_0*t}$ ) and body mass (g) for *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda. Curvilinearity describes the decline in oxygen partial pressure over time in the aquatic phase of a two-phase, closed respirometer where fish had access to both air and water simultaneously.

zero on each graph (Table 2-2). Whereas some variation among individuals occurred, fish  $\geq 87$  g reached this plateau at a significantly lower  $DO_2$  level than individuals  $\leq 81$  g (t-test,  $t = 3.182$ ,  $p=0.030$ ). Active aquatic respiration ceased at a mean  $DO_2$  of  $0.48 \pm 0.2$   $mg\ l^{-1}$  ( $\approx 8.7$  mm Hg) for fish  $\geq 87$  g and  $2.58 \pm 0.7$   $mg\ l^{-1}$  ( $\approx 46.5$  mm Hg) for fish  $\leq 81$  g.

Table 2-2. Respiratory allocation of *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, from air at the aquatic respiration plateau (representing the level of DO<sub>2</sub> at which individuals respired almost completely aerially). This plateau point was considered representative of ecologically relevant levels of DO<sub>2</sub>. Water temperature was approximately 23° C.

Mass	Percent O <sub>2</sub> from air
2.0	100.0
9.0	99.0
22.0	100.0
41.5	98.2
50.0	97.7
81.0	99.6
87.0	100.0
112.0	100.0
134.0	97.7
139.5	94.0
144.0	100.0
206.5	97.0
208.0	98.2
221.8	100.0

### Chamber Effect

In the field, chamber size was altered among specimens to maintain a similar ratio between chamber volume and fish mass following previously published protocols for other fishes. In the laboratory, three individuals (2, 9, and 22 g) were measured within a 2.5 cm (1-inch) PVC chamber (total water volume=266 ml), while the remaining individuals were measured in 5.1 cm (2-inch) PVC chambers (total water volume=1175 ml). To explore potential effects of chamber volume, we tested the 9- and 22-g fish in both 2.5 cm and 5.1 cm chambers to compare RRM. For these two individuals we replicated each chamber size twice at two different levels of DO<sub>2</sub> corresponding to two different ratios of respiratory allocation (pre-plateau and plateau). The RRM was calculated as two rates for each chamber size: from the portion of the curve describing the water phase before it plateaus when lungfish used both aquatic and aerial respiration (pre-plateau), and from the plateau of the curve when the lungfish consumed primarily

atmospheric oxygen (plateau). We adjusted RRM for each DO<sub>2</sub> regime by removing effects of body mass based on the equation described by Ultsch (1995),  $[\text{VO}_2(\text{adjusted}) = (\text{mean weight})^{b-1}(\text{observed weight})^{1-b}(\text{observed VO}_2)]$ . This allowed us to compare the adjusted mean RRM for each chamber size for both the pre-plateau and plateau. At pre-plateau DO<sub>2</sub> levels, individuals run in the 2.5 cm chamber had a 2.4-fold higher RRM when compared to the same individuals run in the 5.1 cm chamber (RRM, 2.5 cm:  $0.147 \pm 0.02$ , 5.1 cm:  $0.06 \pm 0.01$ ). At plateau DO<sub>2</sub> levels, RRM values were similar (RRM, 2.5 cm:  $0.046 \pm 0.01$ , 5.1 cm:  $0.045 \pm 0.01$ ). When these smaller lungfish were respiring from both media (which generally occurs above field-relevant DO<sub>2</sub> levels) there was a distinct chamber effect, with higher RRM values in the smaller chamber. When respiratory allocation was primarily aerial (at ecologically relevant levels of DO<sub>2</sub>), there was no detectable chamber effect.

### **Resting rate of metabolism**

The main objective of this study was to collect metabolic data for *Protopterus aethiopicus* at ecologically relevant DO<sub>2</sub> levels to provide a more realistic physiological portrait of how animals survive under natural conditions in their respective environments. The RRM was calculated as total oxygen consumed ( $\text{mg O}_2 \text{ hr}^{-1}$ ) from both the air phase and aquatic phase of the respirometer. We used the aquatic phase of the curve at its plateau where the lungfish is respiring almost completely aerially because this is a good representation of the level of DO<sub>2</sub> found in habitats used by small-sized lungfish in the field, and chamber effects were not detectable (Goudswaard et al. 2001; Chapman et al. 2002). With the exception of the 2-g individual, we used data for individuals run in the 5.1 cm chambers. Total metabolic rate was positively correlated with body mass

( $r^2=0.9392$ ,  $p=0.031$ , Fig. 2-6), with a scaling exponent of 0.78. Individuals showed a mean percent allocation to air breathing of  $98.7 \pm 0.48\%$ .

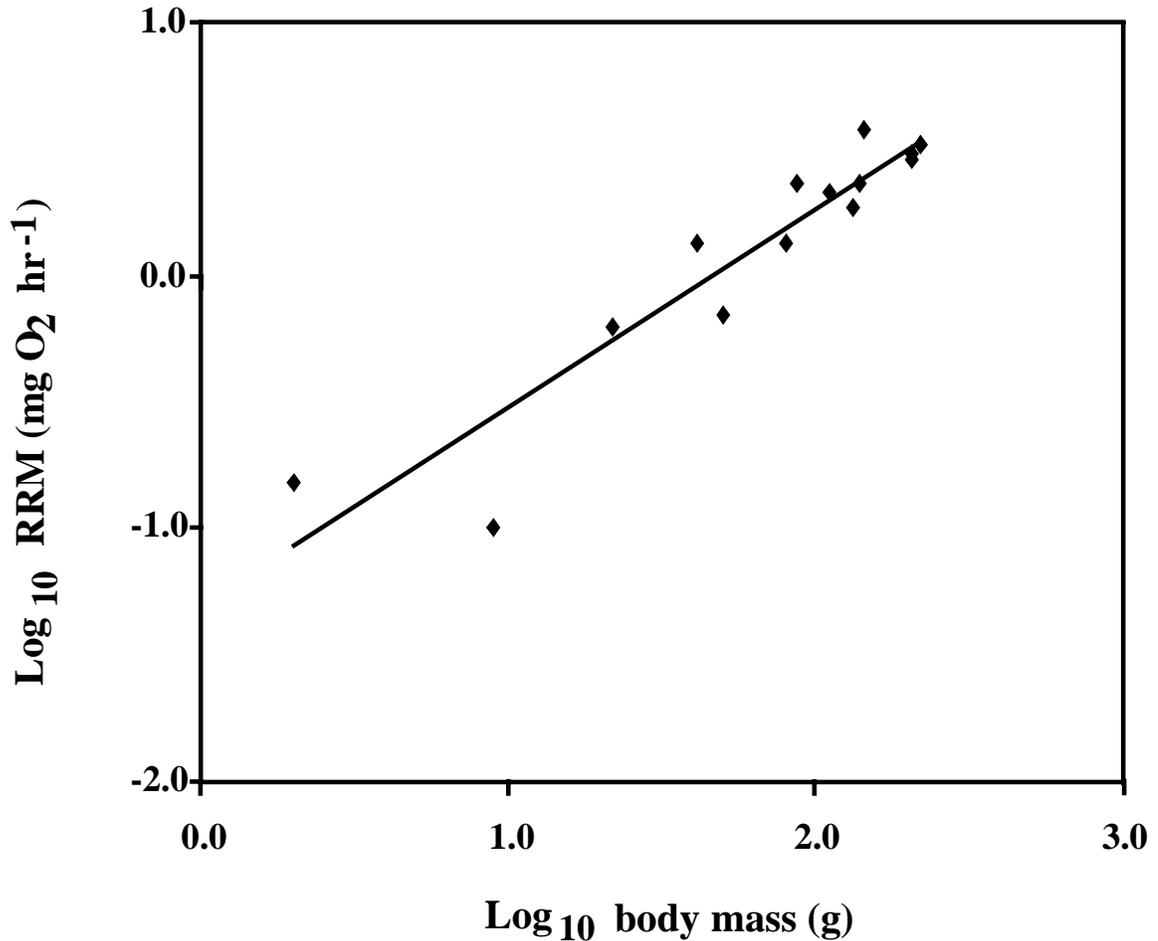


Figure 2-6. The bilogarithmic relationship between RRM ( $\text{mg O}_2 \text{ hr}^{-1}$ ) and body mass (g) for the African lungfish, *Protopterus aethiopicus*, collected from the Lwamunda Swamp, Uganda. Data represent RRM at low (ecologically relevant)  $\text{DO}_2$  levels (measurements made at plateau point where oxygen consumption in the aquatic phase was almost 0%; this was taken to represent ecological conditions because  $\text{DO}_2$  levels would either not drop any lower or were in the range found in the field). Measured at  $23^\circ \text{C}$ .

Individuals were also run at high (near saturated) levels of DO<sub>2</sub> to test for an effect of DO<sub>2</sub> on RRM. No difference in slopes of the bilogarithmic relationship between RRM (mg O<sub>2</sub> hr<sup>-1</sup>) and body mass (g) occurred between high and low levels of DO<sub>2</sub> (F = 3.177, p = 0.094). In addition, the intercepts (representing the adjusted mean RRM) did not differ between the two oxygen levels (F = 0.895, p = 0.357). However, respiratory allocation differed with body size and percent respiratory allocation from water decreased with an increase in body size for fish tested in both the “field” (r<sup>2</sup>=0.822, p<0.001) and the laboratory (r<sup>2</sup>=0.547, p=0.058).

We also measured pre-plateau RRM approximating bimodal respiration before oxygen acquisition from the aquatic phase plateaued. The level of dissolved oxygen at which the pre-plateau RRM was measured differed with each individual according to the plateau level. No difference in slopes of the bilogarithmic relationship between RRM (mg O<sub>2</sub> hr<sup>-1</sup>) and body mass (g) was found between pre-plateau levels and low levels of DO<sub>2</sub> (F= 0.265, p= 0.612). No difference in the intercepts (representing the adjusted mean RRM) occurred between groups (F= 0.242, p=0.626, Fig. 2-7). For the above two comparisons the 2-g individual was not included, because it had to be run in a different chamber size.

## Discussion

The results of this study clearly indicate the importance of considering ecological conditions when measuring physiological parameters. Juveniles between 2 and 221 g (including externally- gilled individuals) respired almost exclusively aerially at low

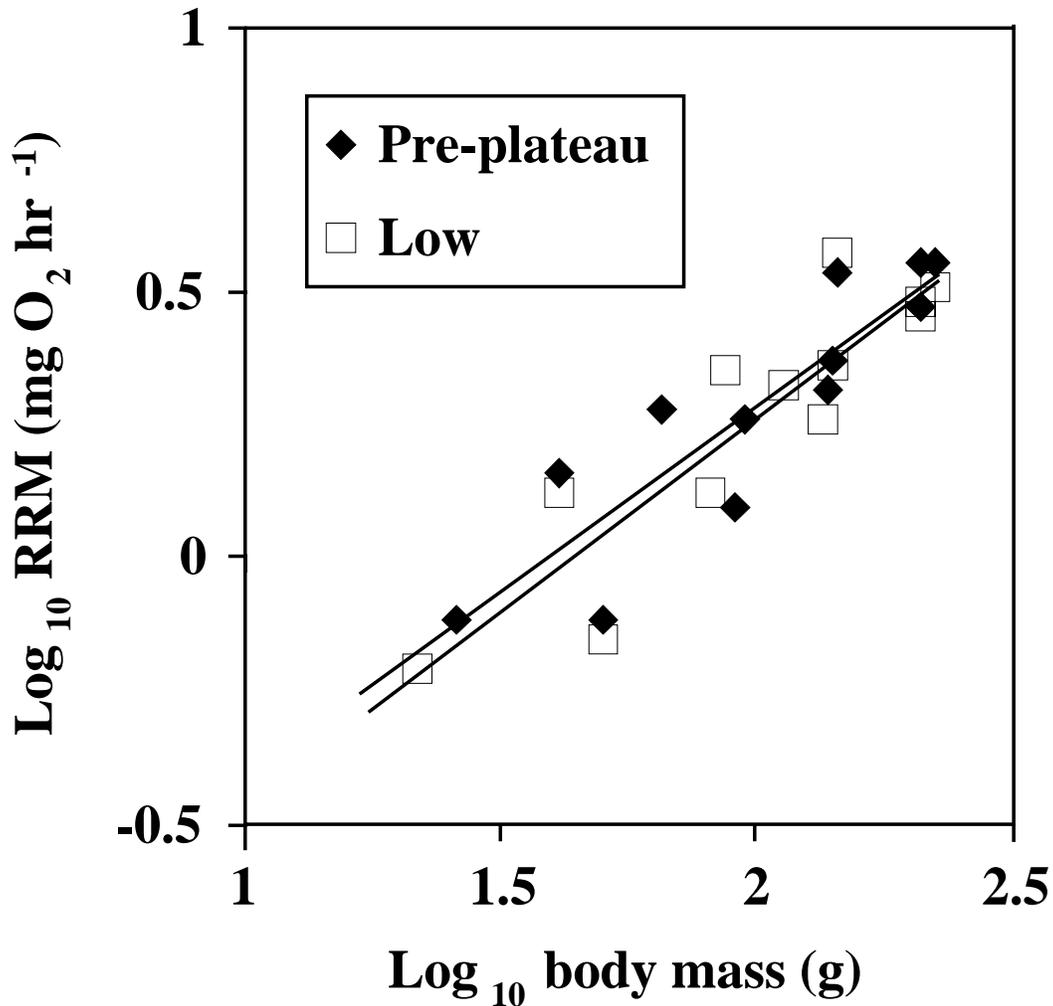


Figure 2-7. The bilogarithmic relationship between resting rate of metabolism (RRM) ( $\text{mg O}_2 \text{ hr}^{-1}$ ) and body mass (g) of *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, measured at levels of  $\text{DO}_2$  prior to the plateau in aquatic respiration versus low levels (plateau point at which fish is respiring almost completely aerially; ecologically relevant). Measured at  $23^\circ \text{C}$ .

concentrations of  $\text{DO}_2$ , which is contrary to earlier studies on small juveniles at high oxygen levels. *Protopterus aethiopicus* also showed a remarkable ability to maintain its RRM over the entire range of  $\text{DO}_2$  from 100% saturation to near anoxic conditions. The relationship between aquatic gas exchange and  $\text{DO}_2$  is described by a curvilinear relationship with the degree of curvilinearity increasing as a function of body mass. This result, along with both the findings related to acclimation time and chamber size, stress

the importance of protocol in determining the RRM and respiratory allocation to water and air in air-breathing fishes. These results differ from previous studies where both total oxygen consumption and respiratory allocation were measured at high DO<sub>2</sub> levels. To better understand the importance of these results, they need be examined in light of what is known of respiration in lungfish.

### **Metabolic Profile**

A comparison of previous studies that measured oxygen consumption in lungfishes, yielded a profile whereby juveniles used primarily aquatic respiration to meet metabolic demands, and adults relied almost exclusively on aerial respiration to meet metabolic oxygen demands (Smith 1930; Sawaya 1946; Johansen and Lenfant 1967; Lenfant and Johansen 1968; Lenfant et al. 1970; McMahon 1970; Johansen et al. 1976; Babiker 1979). Johansen et al. (1976) reported that immediate post larval and early juvenile *Protopterus amphibius* depended on aquatic respiration for 70% of their oxygen demand, whereas fish between 300 and 500 g obtained as little as 10-15% of their oxygen from water (Johansen et al. 1976). When measuring respiratory allocation between water and air in *Protopterus annectens* Babiker (1979) found that small specimens < 200 g utilized less than 10% aerial respiration to meet metabolic demands, whereas individuals > 400 g utilized aerial respiration for 83% of their total oxygen uptake. In the only other published study to measure juvenile lungfish and report body mass, Johansen and Lenfant (1967) found that one juvenile *L. paradoxa* (150 g) used aquatic respiration to meet 64% of its metabolic demand. Oduleye (1977) found that 4 to 150 g juvenile *P. annectens* acquired about 94% of their oxygen aurally, however variation among size classes was not reported in this study.

These previous findings mirror RRM measures that we made on lungfish within 3 wks of capture under high levels of DO<sub>2</sub>, when juvenile *P. aethiopicus* (< 83 g) used aquatic respiration to account for  $84 \pm 7\%$  of their total oxygen uptake. Similarly, measurements in our laboratory at UF after long-term acclimation to normoxia and taken at high DO<sub>2</sub> indicated higher allocation to aquatic respiration with juveniles < 83 g acquiring approximately  $66 \pm 10\%$  oxygen uptake from water. These results differed from laboratory measures at low aquatic oxygen levels that indicated a mean allocation towards aerial respiration for individuals between 2 and 221 g of  $98.7 \pm 0.46\%$ .

Our laboratory measurements of oxygen consumption made across a gradient of DO<sub>2</sub> from normoxia to anoxia showed that *P. aethiopicus* maintains its RRM despite a change in respiratory allocation between water and air. While respiratory allocation differed depending on size, with smaller individuals acquiring more oxygen from the aquatic phase at higher levels of DO<sub>2</sub>, *P. aethiopicus* displays a remarkable ability to maintain its RRM despite these drastic changes in respiratory allocation. Larger individuals still satisfy the bulk of their oxygen demand aerially, while the smallest individuals apparently rely more on aquatic respiration at higher levels of dissolved oxygen. This difference may represent the ability of the gills to efficiently extract oxygen from water in smaller individuals. Based on the curvilinear relationship between aquatic oxygen consumption and increasing body size, it would appear as if smaller individuals are more capable of satisfying their oxygen demand via the gills and or skin. The curvilinear pattern of oxygen uptake observed in *P. aethiopicus* in the aquatic phase of respiration may indicate a passive adjustment to falling levels of DO<sub>2</sub> that would support active adjustments to aerial respiration as the primary mechanism to maintain a relatively

constant RRM. For a metabolic regulator breathing from one medium, the relationship between oxygen consumed as a function of time would be linear on a given ample oxygen to meet the metabolic demand. Measurements made on both terrestrial air breathers and aquatic water breathers yield this result (McNab 2002). The exponential nature of the relationship shown here suggests that the acquisition of oxygen from the aquatic phase may be fueled by a diffusion gradient, maintained across the gills, skin, or both, and related to total respiratory surface area. Smaller individuals have a larger surface area to body mass ratio than do larger individuals and thus a passive diffusion of oxygen across multiple gas exchange surfaces may be ample to satisfy the oxygen demand at higher levels of dissolved oxygen. A large diffusion gradient that would help to facilitate oxygen uptake in hypoxic water would cause increased rates of oxygen diffusion under higher levels of  $DO_2$  due to the passive nature of the diffusion gradient.

Although aquatic respiration in the lungfish has been assumed to occur mainly at the gills, recent studies on *Lepidosiren paradoxa* have indicated that the skin may play a more prominent roll in the aquatic exchange of gases (Abe and Steffensen 1996; Sanchez and Glass 2001). No direct experiment has been performed on lungfishes to determine the contributions of both skin and gills to aquatic respiration. However, Sacca and Burggren (1982) investigated this possibility in *Calamoichthys calabaricus*, a primitive air-breathing polypterid and determined that the skin and gills contributed 32% and 28%, respectively, to oxygen uptake. Spaces between the large, thick ganoid scales of *C. calabaricus* therefore permitted aquatic gas exchange suggesting the relatively smaller scales of *P. aethiopicus* would pose little resistance to oxygen uptake. In fact, in similar shaped, elongate fishes, such as *Electrophorus* (Farber and Rahn 1970), and

*Saccobronchus* (Singh and Hughes 1971), which are all capable of air breathing, the skin has been shown to be an equally important gas exchange organ. The frequency of gill ventilation in lungfishes is known to remain constant with increasing hypoxia, and thus it seems reasonable to suggest that the increase in aquatic respiration can be attributed to diffusion across the skin (Johansen and Lenfant 1968; Sanchez et al. 2001). Furthermore, when burrowing in soft mud, the gills of *P. aethiopicus* would be severely compromised, although burrowing individuals efficiently excrete both CO<sub>2</sub> and metabolic wastes. Under conditions of extreme hypoxia, it is possible that the skin plays an even greater role in oxygen exchange. Our observations of individuals before and after trials indicated that *P. aethiopicus* might actively alter vascularization patterns to the skin. The junction of both the pelvic and pectoral fins with the body were always very red, and the color intensified in some individuals over the course of a trial. This red color also extended along portions of the appendages. In some cases, lighter-colored individuals showed an increase in vascularization over extensive portions of the body. Lenfant and Johansen (1968) documented a progressive vasodilation of the skin in response to prolonged air exposure. An increased vascularization of the skin could facilitate increased oxygen uptake and/or increased offloading of carbon dioxide. Future investigations comparing total respiratory surface area to body mass in the lungfish along with direct measurements of gas exchange across the skin will be needed to determine more clearly the role of the skin in oxygen uptake in *P. aethiopicus*.

### **Methodological Issues**

In the process of evaluating the RRM in *P. aethiopicus*, two methodological issues were discovered that influence metabolic rate measurements in this species: chamber size and acclimation time. Both a change in chamber size with body mass and the short

acclimation time used in the field led to differences between our field and laboratory measurements of RRM in *P. aethiopicus*. At our field lab, small juveniles exhibited elevated metabolic rates (when total metabolic rate was graphed as a function of body size) compared to larger individuals, with small juveniles having a 5.9-fold higher RRM than those individuals  $\geq 83$  g. However, the shift was coincident with our shift from a small to large chamber size. When we evaluated chamber size effects in the laboratory, we found that use of a smaller chamber size for fish  $< 22$  g coincided with an elevation in the RRM at high  $\text{DO}_2$ , but not when fish were breathing primarily atmospheric air (plateau level). Thus, we attribute the shift in adjusted mean RRM that we observed between small and large individuals in our field lab to reflect, at least in part, the change in chamber size. The smaller chambers may be perceived by smaller individuals as confining and illicit a stress response in these smaller individuals. Similar results were found, in both *Protopterus amphibius* (Johansen et al 1976) and in *Protopterus annectens* (Babiker 1980) with elevated RRM in smaller individuals. Methods used in these studies indicated that alternate chamber sizes were also used. Many previous studies also report a short acclimation time in the chamber. Our acclimation comparisons in the laboratory revealed at least a two-fold decrease in RRM between a 3-h and 16-h acclimation period. This suggests that chamber acclimation time should be carefully considered in any design or meta-analysis of fish metabolic rates.

Both methodological issues may reflect behavioral responses. A short acclimation time potentially increases stress levels. Within the various chambers the smallest individuals were seen to hover at the water/air interface, whereas large individuals spent the majority of their time hidden within the opaque portion of the water chamber except

when moving to breathe air. Prodding of smaller individuals both in the chamber and in holding tanks elicited a burst of activity. This behavior resembled an escape mechanism or predator avoidance. While all juveniles measured in this experiment were from the swamp, the smallest fish, particularly the gilled individuals, were often found associated with dense vegetation in the field. When disturbed in the field, they behaved in a similar manner. Their reflex is to retreat rapidly to the bottom of the water column, and this behavior occurs when an animal is confined or threatened. The close confines of the smaller chamber could have increased the stress level of these individuals enough to account for the increase in metabolic rate. The effect disappeared when individuals switched to an almost complete dependence on aerial respiration. This suggests that the increase in RRM may be attributed to an increase in aquatic respiration brought about by a stress response to a confined chamber. This conclusion is reinforced by the finding that chamber size apparently had no effect on aerial respiration.

It is clear from this study that *Protopterus aethiopicus* is able to maintain a relatively constant RRM over a range of  $DO_2$  levels from normoxia to hypoxia. Measurements of the RRM at normoxic conditions, pre-plateau levels, and with individuals respiring almost completely aurally did not differ. Although respiratory allocation differs across the range of dissolved oxygen from normoxia to extreme hypoxia, adjustments in oxygen uptake serve to balance the overall uptake of oxygen to stabilize RRM. Since smaller juvenile specimens of *Protopterus aethiopicus* are clearly taking in higher amounts of dissolved oxygen at near saturated levels of  $DO_2$ , it will be interesting to determine if these adjustments are passive or active and contribute to surplus energy uptake, or if they are related to both carbon dioxide excretion and

nitrogenous waste removal. Future experiments will be necessary to define the role of the skin in oxygen uptake and how this relates to changing levels of  $\text{DO}_2$ .

CHAPTER 3  
THE RELATIONSHIP BETWEEN TEMPERATURE AND THE RESTING RATE OF  
METABOLISM IN *Protopterus aethiopicus*

**Introduction**

Few of all environmental factors known to affect metabolism are as significant as temperature. The study of this relationship, from both a molecular and whole organism perspective, yields insight into the intimate connection between animal energetics and the environment. A derivation from first principles of the association between temperature and the resting rate of metabolism has been a topic of rich conversation ever since August Krogh and his co-workers attempted to formulate such a relationship for poikilothermic organisms (Krogh 1914, 1916). His quantitative expression of this relationship for a single species, the goldfish (*Cassius auratus*) led to his derivation of what came to be known as “Krogh’s normal curve” (Ege and Krogh 1914). The normal curve expressed an exponential increase in metabolic rate as a function of increasing temperature.

Winberg (1956), examining resting metabolic rates in fishes, compared values drawn from the literature to Krogh’s normal curve in an effort to compare metabolic rates from different species measured at different temperatures. Using the normal curve for orientation, Winberg (1956) derived a series of coefficients that could be used to adjust metabolic rates of temperate fishes by applying the Van’t Hoff equation,

$$[Q_{10}=(K_2/K_1)^{(10/t_2-t_1)}]$$

where  $K_1$  and  $K_2$  are the metabolic rates measured at two different temperatures  $t_1$  and  $t_2$ , for changes in temperature across intervals of 10 °C. This permitted adjustment of

metabolic rates measured at a specific temperature to a second specific temperature of biological interest and a description of how an individual's resting metabolism responded to an increase or decrease in temperature. As long as an ectotherm is acclimated for an adequate length of time to each new temperature, this rate reflects the long-term compensatory effects associated with metabolism affecting a homeostatic balance within the individual despite a change in environmental conditions.

The  $Q_{10}$  coefficients for fishes derived by Winberg were based largely on data collected for temperate fishes. The degree to which these coefficients apply to metabolic data collected for fishes that inhabit tropical or polar waters and that experience a smaller range of natural temperature fluctuation is largely unknown. Researchers investigating species from these systems are faced with the decision to calculate their own  $Q_{10}$  values or to use published values measured on other species. Based on the experiments of Johnston et al. (1991) and Clarke and Johnston (1999), utilizing  $Q_{10}$  relationships for organisms with different lifestyles and/or from unrelated taxa can clearly lead to erroneous adjustments to calculated metabolic rates.

Fishes, as the most species-rich group of chordates, lack a comprehensive synthesis of metabolic data that includes both ancient relic fishes (non-teleost) and teleosts, and that adequately represents tropical, temperate, and polar regions. In particular, studies examining metabolic rates and the effects of temperature in non-teleost fishes are completely absent from the literature. The objective of this study was to determine the  $Q_{10}$  value for the African lungfish, *Protopterus aethiopicus*, an ancient air-breathing fish from a tropical freshwater habitat. Here we present the  $Q_{10}$  values for six individuals ranging in body mass from 42 to 222 g evaluated across a biologically

relevant 10 °C temperature range. These  $Q_{10}$  values are discussed in relation to values drawn from the literature for both temperate and tropical teleosts.

### **Materials and Methods**

Six juvenile lungfish ranging in size from 44 to 222 g were used in this study. Individuals had been housed at the University of Florida (UF) since their collection in June 2001 from Lake Nabugabo, Uganda. Lake Nabugabo lies just south of the equator and is a small satellite lake (surface area = 24 km<sup>2</sup>, mean depth = 4.5 m) that was isolated from Lake Victoria approximately 4000 years ago (Greenwood 1965). The lake lies within the extensive Lwamunda Swamp that was formerly a bay on the western shore of Lake Victoria (Worthington 1932; Greenwood 1965; Ogutu-Ohwayo 1993). In the Lake Nabugabo system, Goudswaard et al. (2001) found that 90% of the lungfish captured in minnow traps in the swamp were between 20 cm and 45 cm TL. Larger lungfish in the system are found in more open waters (L. Chapman, personal observations). Water temperatures measured throughout the year and within the swamp averaged between 21.3 °C and 25.2 °C in the early morning and between 21.3 °C and 27.2 °C in the early afternoon (Chapman et al. 2002).

Prior to metabolic trials, individuals were held at 23 °C ±0.5 °C (12/12 photoperiod) in four partitioned 20-gallon tanks with no two lungfish occupying the same space within a tank. Tank temperatures were controlled with aquarium heaters, and temperatures were measured daily. Dissolved oxygen, pH, conductivity, ammonia, and nitrite levels were measured weekly to maintain appropriate water quality, and tanks were maintained on a 12/12 photoperiod. Individuals were fed fresh chicken liver once a week. Our experimental protocols for *P. aethiopicus* were approved by the University of Florida (IACUC project #Z090).

Six individuals were acclimated across the temperature range of 20 °C to 30 °C over a 5-month period beginning in January 2003. Individuals initially at 23 °C were gradually increased to 30 °C over a 1-wk period. Once at 30°C, individuals were acclimated for at least 2 wks with a daily fluctuation in temperature of less than  $\pm 0.5$  °C. Each lungfish was assigned a number, and a random number table was used to select the order in which individuals were measured for their resting rate of metabolism (RRM). For fishes the resting rate of metabolism is approximately the same as the standard rate of metabolism (SRM) and is “the respiration rate of an unfed fish resting quietly in the experimental chamber” (Clarke 1991, 1993). Here we use the (RRM), which we believe accurately reflects the standard rate of metabolism for *Protopterus aethiopicus*. Individuals were starved for 1 wk prior to their metabolic run. The RRM was measured via the measurement of oxygen consumption in a two-phase respirometer following the protocol described in Chapter 2. Oxygen consumed from both water and air was measured simultaneously. Respirometer temperature was maintained to within  $\pm 0.5$  °C during each experimental run.

Once all six individuals were measured for the RRM, individuals were lowered to the next temperature regime over a 3-d period and again acclimated for at least a 2-wk period. In this way, the above metabolic experiments were performed at 30 °C, 25 °C, and 20 °C.

### Statistics

The resting rate of metabolism (RRM) for each individual was calculated as an adjusted mean by controlling for the effects of body mass based on the equation described by Ultsch (1995) [ $VO_2$  (adjusted) = (mean weight)<sup>b-1</sup>(observed weight)<sup>1-b</sup>(observed  $VO_2$ )]. We used the scaling exponent  $b=0.78$  from Chapter 2 that describes

the bilogarithmic relationship between metabolic rate and body mass for this population of lungfish across a broad size range of 14 juvenile fish. Repeated measures analysis of variance was used to calculate the adjusted mean resting rate of metabolism at each water temperature. Following Potvin et al. (1990), Mauchly's criterion was used to test for the compound symmetry of the variance-covariance matrix. The  $Q_{10}$  value relating RRM to water temperature over a 10°C range was calculated using the equation:  $Q_{10}=(K_2/K_1)^{(10/(t_2-t_1))}$ , where  $K_2$  and  $K_1$  are metabolic rates at temperatures  $t_2$  and  $t_1$ . The determination of the  $Q_{10}$  value indicates an adjustment in RRM to temperature changes across a known temperature gradient (Winberg 1956) and was determined for *Protopterus aethiopicus* over the range of 20 °C to 30 °C. All errors reported are standard errors.

### Results

*Protopterus aethiopicus* showed a positive bilogarithmic relationship between the rate of oxygen consumption and body mass (range=44 to 222 g) at all three water temperatures (20 °C:  $r^2=0.798$ ,  $P=0.016$ ; 25 °C:  $r^2=0.847$   $P=0.009$ ; 30 °C:  $r^2=0.862$ ,  $P=0.007$ , Figure 3-1). Because of the relatively small sample size for which we could repeatedly measure metabolic rates across a temperature gradient, we used the slope of the bilogarithmic relationship described in Chapter 2 (0.78) to derive adjusted mean resting metabolic rates at each of the three temperatures. Adjusted mean RRM ranged from 0.00833 mg O<sub>2</sub> hr<sup>-1</sup> at 20°C to 0.01567 mg O<sub>2</sub> hr<sup>-1</sup> at 25°C and 0.02617 mg O<sub>2</sub> hr<sup>-1</sup> at 30°C (adjusted to a mean body mass of 110.5 g, Fig 3-2).

The mean RRM at each temperature interval was significantly different from the preceding interval (20 °C vs 25 °C:  $p=0.006$ , and 25 °C vs 30 °C:  $p=0.019$ , repeated contrasts, Fig. 3-2).

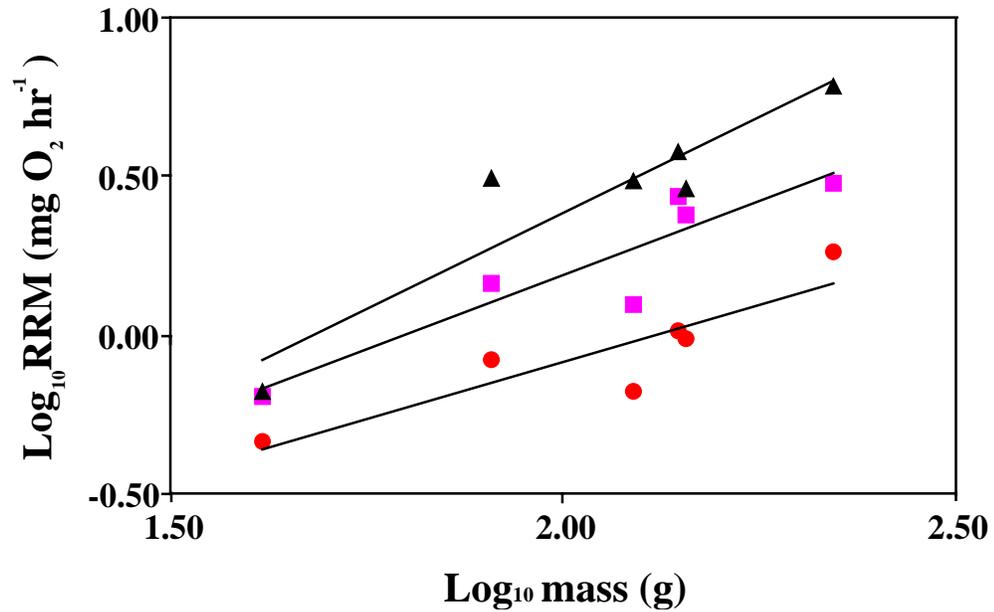


Figure 3-1. Bilogarithmic relationship between resting metabolic rate (RRM) and body mass for six individuals of *Protopterus aethiopicus* from the Lwamunda Swamp, Uganda, measured at three different temperatures: 30 °C, 25 °C, and 20 °C. Lungfish were acclimated for at least 2 wks prior to measurement. Triangles represent 30 °C, squares represent 25 °C, and closed circles represent 20 °C.

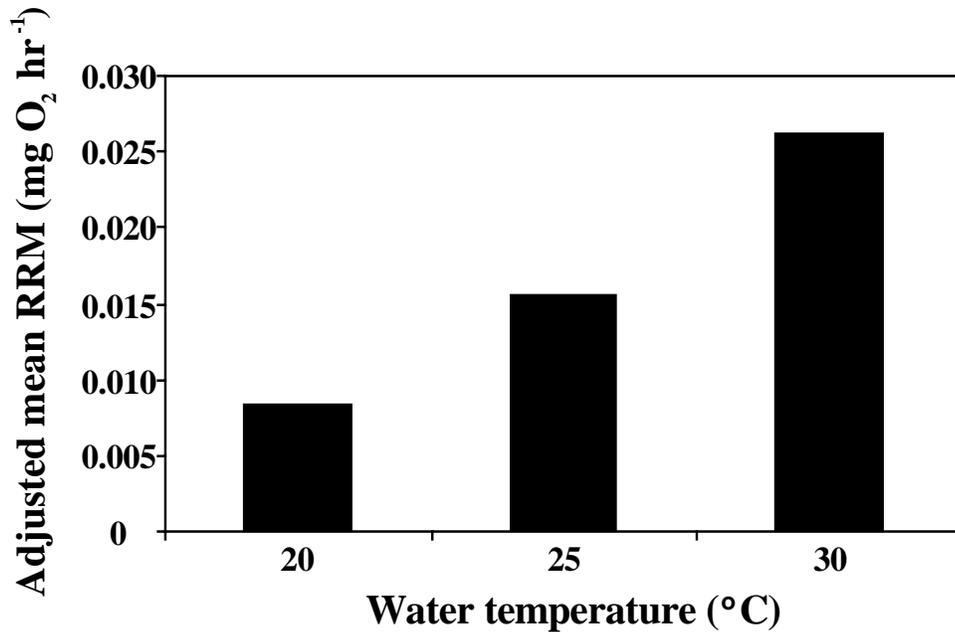


Figure 3-2. Mean resting rate of metabolism adjusted to 110.5 g fish (mean for six individuals) for three temperatures: 30 °C, 25 °C, and 20 °C. A within-subject contrast found the mean RRM at each temperature interval to differ significantly from the preceding interval: 20 °C vs 25 °C  $p=0.006$ , and 25 °C vs 30 °C  $p=0.019$ . Error bars are less than  $\pm 0.002$ .

The relationship between RRM and temperature was calculated as the  $Q_{10}$  value for the temperature interval of 10°C between 20°C and 30°C. The  $Q_{10}$  value was 3.14.

### **Discussion**

The primary focus of this study was to examine the response in RRM of *Protopterus aethiopicus* within a biologically relevant range of water temperatures to gain insight into how this tropical, freshwater, non-teleost fish responds to changes in temperature. This was accomplished by accurate determination of the RRM, adequate acclimation to temperature for each individual in order to remove the effects of any short-term compensatory mechanisms in response to temperature, and a review of those studies from the literature that followed these criteria. *Protopterus aethiopicus* exhibited a strong, positive increase in the resting rate of metabolism (RRM) with an increase in temperature across an ecologically relevant temperature range. To our knowledge, this study is the first to quantify the relationship between temperature and the resting rate of metabolism for a non-teleost fish, and the first study to examine this relationship in an air-breathing fish.

Previous studies that examined the effect of temperature on metabolic rate in fishes are limited and in most instances focused on deviations from Krogh's normal curve. Clarke and Johnston (1999) sought to refine this relationship for teleost fishes utilizing literature-derived data that met a specific set of criteria, similar to those listed above (i.e. accurate determination of RRM, adequate acclimation time, etc. See Clarke and Johnston 1999 for full description of criteria). Because they focused on describing the effects of temperature as a general relationship among as many species as possible, they included measurements made on the same species from different studies at different temperatures. Using an Arrhenius model to compare resting metabolic rates adjusted for a

50 g fish across a temperature range, they found that tropical fish require 6.2 times more oxygen at 30 °C than do polar fish at 0 °C (Clarke and Johnston 1999). This relationship explained 59% of the variance among 69 species of teleosts, with the remaining variance concluded to be the combined effect of phylogeny, ecology, and/or lifestyle (Clarke and Johnston 1999). Clarke and Johnston (1999) also examined taxonomic variation within this relationship. Using the same species data pooled by taxonomic order, they detected no difference in slopes of the relationship between temperature and RRM among orders. Clarke and Johnston (1999) also derived a  $Q_{10}$  value of 1.83 from their curve based on 69 species of teleosts, and reported a median  $Q_{10}$  value of 2.40 from 14 published values on individual species that were exposed to different temperature regimes. However, they did not explicitly explore  $Q_{10}$  values for individual species in different thermal habitats.

Studies that measure the resting rate of metabolism within the same species across a gradient of temperatures directly examine the effects of temperature upon metabolism by attempting to remove compensatory short-term mechanisms that occur in non-acclimated organisms. It has been shown that animals that are exposed to abrupt temperature changes without adequate acclimation, respond metabolically in a fashion that tends to inflate  $Q_{10}$  values when compared with acclimated animals (Holeton 1974). Thus, an accurate determination of  $Q_{10}$  values on temperature acclimated fish provides a means to compare different species from different environmental temperature regimes.

Studies of  $Q_{10}$  values and comparisons of these values across species are useful in illustrating (a) if the rate of change follows the standard derived relationships calculated from interspecific data, and (b) whether the rate of change varies among species, or groups of ecologically or phylogenetically related taxa. We found 10 published studies

that met our criteria for comparing effects of temperature on the resting rate of metabolism within the same species (Table 3-1). Eight of these studies involved fishes that occur in temperate zones, while this study and the remaining two studies examined tropical species. Mean masses were available from many of these published studies (tropical  $n=3$  and temperate  $n=8$ ). We tested the effect of mass as a covariate against  $Q_{10}$  by running an ANCOVA. The effect of mass was not significant ( $p=0.170$ ). Therefore we removed mass from our model and used a t-test to test for a difference between the  $Q_{10}$  values between the two groups. Removal of the unusually high  $Q_{10}$  value for *Anguilla rostrata* leads to a mean  $Q_{10}$  value for temperate species of 2.43, a value significantly lower than the mean value for the three tropical species ( $Q_{10}=2.95$ ,  $t=3.85$ ,  $p=0.027$ ). When the *A. rostrata* value is included, the mean  $Q_{10}$  for the tropical fish group does not differ from the temperate group (mean  $Q_{10}$  for temperature species=2.54,  $t=1.39$ ,  $p=0.191$ ). Sample size was insufficient to perform a phylogenetic contrast analysis. However, both tropical and temperate species represent a broad range of lineages. Assuming that the *A. rostrata* value is not representative of the group, we can speculate on the apparent differences in  $Q_{10}$  values between the sample of temperate and tropical species available. It may reflect the lower degree of thermal variability that is experienced throughout the year in tropical species. For *P. aethiopicus* from Lake Nabugabo, daytime water temperatures from the swamp where juveniles are exclusively found range between 21.3°C and 27.2°C (Chapman et al. 2002, based upon 1 year of monthly samples). This is far smaller than the ranges of temperatures experienced seasonally in temperate climates. It is possible that temperate species might possess a greater ability to maintain resting metabolism across their natural temperature gradient.

Table 3-1.  $Q_{10}$  values calculated for various species of teleost fishes. Resting rates of metabolism were provided by Andrew Clarke (British Antarctic Survey) adjusted to a 47 g fish.  $Q_{10}$  values were calculated across temperature ranges listed. Citations indicate initial source of raw metabolic data. All studies included measured resting rate of metabolism ( $\text{mg O}_2 \text{ l}^{-1} \text{ hr}^{-1}$ ) and acclimated individuals for sufficient periods of time prior to measurement at different temperatures. Means are for tropical and temperate  $Q_{10}$  values and are significantly different ( $p=0.027$ ). \*In calculating the mean  $Q_{10}$  for temperate species, *Anguilla rostrata* was not included as described in the text.

Species	Temp range (°C)	$Q_{10}$	Mean	Study
Tropical				
<i>Colossoma macropomum</i>	20-30	2.97		Saint-Paul et al. 1988
<i>Oreochromis mossambicus</i>	19-28	2.74		Caulton 1978
<i>Protopterus aethiopicus</i>	20-30	3.14		this study
			2.95	
Temperate				
<i>Scophthalmus maximus</i>	6.0-22.0	3.06		Mallekh and Lagardere 2002
<i>Gambusia affinis</i>	10.0-30	2.24		Cech et al. 1985
<i>Anguilla anguilla</i>	20.0-27.0	2.48		Degani et al. 1989
<i>Anguilla rostrata</i>	15.0-25.0	3.67		Degani & Gallagher 1985
<i>Cyprinus carpio</i>	10.0-30.0	2.60		Beamish 1964
<i>Carassius auratus</i>	10.0-30.0	2.29		Beamish and Mookherji 1964
<i>Catostomus commersonii</i>	10.0-30.0	2.44		Beamish 1964
<i>Oncorhynchus nerka</i>	5.0-20.0	2.02		Brett & Glass 1973
<i>Salvelinus fontinalis</i>	10.0-20.0	2.84		Beamish 1964
<i>Pleuronectes platessa</i>	2.0-22.0	2.06		Fonds et al. 1992
<i>Platichthys flesus</i>	2.0-22.0	2.24		Fonds et al. 1992
			2.43*	

Comparing our derived  $Q_{10}$  values for both temperate and tropical species to the  $Q_{10}$  value of 1.83 derived by Clarke and Johnston (1999) for all teleosts (whether polar, temperate or tropical), highlights the problems associated with using published  $Q_{10}$  values to adjust metabolic rates in the absence of species-specific values. It is a common practice to adjust metabolic rates using Winberg's  $Q_{10}$  coefficients derived from temperate species based on Krogh's normal curve, and it has been argued that a  $Q_{10}$  of 2

is an appropriate adjustment for this relationship among diverse species (Fry and Hochachka 1970, Miller and Mann 1973). The derived  $Q_{10}$  values presented here clearly show how a generalized application of between-species  $Q_{10}$  values can lead to erroneous determinations when adjusting metabolic rates. Furthermore, while within-species  $Q_{10}$  values show variation, a lack of appropriate data for a diverse array of fishes confounds understanding the source of this variation.

Although the number of fish species for which  $Q_{10}$  data are available and their phylogenetic diversity is small, this comparison highlights the need for further examination of phylogenetic and geographic trends in  $Q_{10}$  data. With the exception of this study, all previous data represent teleost fishes. Preliminary examination of resting metabolic rates in non-teleost fishes suggests a low resting rate of metabolism to be a primitive character (A. Seifert, unpublished data). It will be of interest to investigate if a lower thermal sensitivity in terms of  $Q_{10}$  is correlated with taxonomic position or if it is primarily a function of habitat. In addition, future work that examines effects of temperature outside of the range experienced in nature will help to address whether metabolic compensation approaches the higher and lower bounds of thermal tolerance. A few studies suggest that there is metabolic depression above some critical temperature, but mechanisms for these observations are not well understood (Saint-Paul 1983). Further investigations will shed light on the contributions of protein turnover, membrane homeostasis, and cellular ion balance to metabolism. Whereas these processes presumably play an important role, it is unclear what the individual contributions of these processes are to maintenance metabolism or their relative relationship to temperature and lifestyle (Clarke and Johnston 1999). A more complete picture of the relationship

between temperature and its effect on resting metabolic rate across all groups of fishes will yield a deeper understanding of the effect of environmental factors upon physiological processes in ectotherms.

## CHAPTER 4 SUMMARY AND CONCLUSIONS

This study quantified respiratory allocation and the resting rate of metabolism (RRM) in the African lungfish, *Protopterus aethiopicus*, under ecologically relevant levels of dissolved oxygen. Simultaneous measurements of oxygen consumed from both air and water were used to measure RRM of juvenile lungfish across the size range typically found in hypoxic swamps of the Lake Victoria basin. I also measured the effects of temperature on the resting rate of metabolism and compared calculated  $Q_{10}$  values to those calculated for temperate and tropical fishes. The following section summarizes the major findings of this study.

1. Aerial oxygen consumption measured at hypoxic  $DO_2$  levels reflective of the natural habitat averaged 98% (range = 94% to 100%) of total respiratory allocation for juvenile lungfish between 2 and 221 g (including externally-gilled individuals). This finding contradicts the long-held belief that small juvenile lungfish primarily depend on aquatic respiration to meet their aerobic metabolic demand, but is supported by ecological data that suggest individuals < 40 cm TL primarily are found in hypoxic wetlands.
2. Total metabolic rate was positively correlated with body mass with a scaling exponent of 0.78.
3. Measurements of oxygen consumption made across a gradient of dissolved oxygen from normoxia to anoxia showed that *P. aethiopicus* generally maintains its RRM despite a change in respiratory allocation between water and air.
4. The relationship between aquatic gas exchange and  $DO_2$  is described by a curvilinear relationship with the degree of curvilinearity increasing as a function of body mass. There is as yet no explanation for this phenomenon.
5. In the process of evaluating the RRM in *P. aethiopicus*, two methodological issues were discovered that influence metabolic rate measurements in this species: chamber size and acclimation time. Both a change in chamber size with body mass and the short acclimation time used in the field led to differences between our field

lab and laboratory measurements of RRM taken at UF in *P. aethiopicus*. A decrease in chamber size led to an increase in RRM. An increase in acclimation time from 3 h to 16 h led to a two-fold decrease in RRM.

6. *Protopterus aethiopicus* exhibited a strong, positive increase in the resting rate of metabolism (RRM) with an increase in temperature across an ecologically-relevant temperature range. The  $Q_{10}$  value for the temperature interval of 20°C and 30°C was 3.14. To our knowledge, this study is the first to quantify the relationship between temperature and the resting rate of metabolism for a primitive, non-teleost fish, and the first study to examine this relationship in an air-breathing fish.
7. A comparison of literature-derived  $Q_{10}$  values for both tropical and temperate fishes indicated a higher  $Q_{10}$  in tropical species (2.95, n=3) than in temperate species (2.43, n=10) across an ecologically relevant thermal range. This difference may reflect the lower degree of thermal variability that is experienced throughout the year in tropical species.

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## BIOGRAPHICAL SKETCH

Ashley Winn Seifert was born on April 6<sup>th</sup>, 1976, in Syosset, New York. He is one of three children of Randolph Winn Seifert and Janine Epstein Seifert. Raised in a liberal-minded atmosphere, he was taught to search for his own answers to the mysteries of life. Following a whim, he attended Bowdoin College in Brunswick, Maine, where he earned an A.B. in biology with a minor in philosophy. After graduating, his interests led to a now coincidental meeting and consequent friendship with Dr. Karel Liem who suggested an avenue for potential study with Dr. Lauren Chapman. After working as her research assistant for one year he enrolled in the graduate program in the Department of Zoology at the University of Florida where he completed his Master of Science degree. He is still seeking answers and still following whims.