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Respiratory allocation and standard rate of metabolism in the African lungfish, *Protopterus aethiopicus*

Ashley W. Seifert [a],*, Lauren J. Chapman [a,b,1]

[a] *Department of Zoology, University of Florida, Gainesville, FL 32611, USA*
[b] *Wildlife Conservation Society, 2300 Southern Boulevard, Bronx, NY 10460, USA*

Abstract

This paper quantifies the relationship between respiratory allocation (air vs. water) and the standard rate of metabolism (SMR) in the primitive air-breathing lungfish, *Protopterus aethiopicus*. Simultaneous measurements of oxygen consumed from both air and water were made to determine the SMR at ecologically relevant aquatic oxygen levels for juveniles 2 to 221 g. Total metabolic rate was positively correlated with body mass with a scaling exponent of 0.78. Aerial oxygen consumption averaged 98% (range = 94% to 100%) of total respiratory allocation under low aquatic oxygen levels. Measurements of oxygen consumption made across a gradient of dissolved oxygen from normoxia to anoxia showed that *P. aethiopicus* maintains its SMR despite a change in respiratory allocation between water and air.
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1. 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; Perry et al., 2001). Air-breathing in fishes dates to 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, 1995; Long, 1995; Graham, 1997; Perry et al., 2001). Early evolution of lungs and evidence of their widespread occurrence among ancient fishes is supported by the observation that bimodal breathing is present in extant representatives of mostly extinct groups, through the use of 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 lungfishes are of great interest because of their probable relationship as the sister group to

tetrapods (Tohyama et al., 2000). Recent evidence suggests that ventral lungs and a 2-cycle air-breathing circuit evolved within the Sarcopterygii, supporting the homology between lungfish and tetrapod lungs (Perry et al., 2001). 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 the energetics of these air breathers under ecologically relevant dissolved oxygen levels or the relationship between respiratory allocation (water vs. air) and body size.

The majority of early research on the African lungfish, *Protopterus 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 immediate post larval and early juvenile *Protopterus amphibius* depended on aquatic respiration for 70% of their oxygen demand, while fish between 300 and 500 g

* Corresponding author. Tel.: +1 352 392 7474; fax: +1 352 392 3704.
  E-mail address: aseifert@zoo.ufl.edu (A.W. Seifert).
[1] Current address: Department of Biology, McGill University, 1205 Dr. Penfield Avenue, Montreal, PQ, Canada H3Q 1B1.

obtained as little as 10–15% of their oxygen from water. For *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. Johansen and Lenfant (1967) found that one juvenile *Lepidosiren paradoxa* (150 g) used aquatic respiration to meet 64% of its metabolic demand. And, Oduleye (1977) found that 4 to 150 g juvenile *P. annectens* acquired about 94% of their oxygen aerially, however allocation was not reported in conjunction with size classes. These studies contributed to a physiological portrait of the Lepidosirenidae and Protopteridae as animals characterized by two distinct respiratory life stages, with small juveniles primarily dependent on aquatic respiration and an increased dependence on aerial respiration with growth (Jesse et al., 1967; Lenfant et al., 1970; McMahon, 1970; Johansen et al., 1976; Babiker, 1979). However, in these earlier studies, measurements of oxygen consumption in juvenile *P. aethiopicus* were made under conditions of near-saturated aquatic oxygen levels, an environment not reflective of observed natural ambient conditions at this life-history stage.

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 found 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. In this swamp habitat, dissolved oxygen concentration can be extremely low (Chapman et al., 2002a,b). Both Greenwood (1958) and Johnels and Svensson (1954) suggested that the shallowness of *P. aethiopicus* nests might be connected with the air-breathing behavior of the nestlings. In these nests, young had 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^{-1}, and between 0.98 and 2.66 mg L^{-1}, 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 are capable of aestivation 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.

The objective of this study was to examine the energetics of *P. aethiopicus* across a range of body sizes to detect ontogenetic shifts in respiratory allocation and to measure standard rate of metabolism. We hypothesized that based on their distribution in the hypoxic wetlands, juvenile lungfish would respire primarily aerially, regardless of size. We focused on juvenile lungfish over the size range typically found in the marginal hypoxic wetlands in the Lake Victoria

basin (nestlings to 40 cm TL, Goudswaard et al., 2001). We measured rate of oxygen consumption and relative allocation to air and water breathing across a range of aquatic oxygen levels reflective of the natural habitat.

2. Materials and methods

2.1. Study site

Specimens of *P. 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; see Randle and Chapman, 2004 for site map). Lake Nabugabo lies just south of the equator and is a small satellite lake (surface area = 24 km^2, 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). The dense interior of the swamp is characterized by numerous small lagoons and channels. These serve as important habitats for *P. aethiopicus* (<40 cm total length, Goudswaard et al., 2001). Dissolved oxygen concentrations within the swamp are severely hypoxic throughout the year. In a study of a small lagoon within the Lwamunda Swamp measurements were made during both wet and dry seasons at several microsites. Over a 1-year period monthly dissolved oxygen measurements in the upper 20 cm of water averaged 1.5 mg L^{-1} ($\approx$ 26.8 mm Hg) in the morning and 1.8 mg L^{-1} ($\approx$ 32.9 mm Hg) during the afternoon (Chapman et al., 2002b). Water temperatures at the sites averaged 22.3 °C in the morning and 23.8 °C in the early afternoon (Chapman et al., 2002b).

2.2. Collection and maintenance of P. aethiopicus

Individuals were live-captured using baited minnow traps set at various places within the lagoons of the Lwamunda Swamp and left overnight. Traps were collected the following morning and each captured lungfish placed in separate holding containers. This method was appropriate for catching individuals ranging in mass from 10 g to ~250 g. Smaller individuals (<5 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 (UF). Once at the University, individual lungfish were held in partitioned 208-L tanks with no two lungfish occupying the same space within a tank. Water temperature was maintained at 23 ± 0.5 °C, with a 12/12 photoperiod. Dissolved oxygen concentration averaged 8.16 ± 0.05 mg L^{-1} (147.2 ± 0.9 mm Hg). Temperature, dissolved oxygen, pH, conductivity, ammonia, and nitrite levels were measured weekly to maintain water quality. Individual fish were held under these conditions up to 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.

2.3. Respirometry

Oxygen consumption was measured using a two-phase respirometer on post-absorptive fish. Each phase of the respirometer contained an associated oxygen sensing probe (Ocean Optics FOXY® probe) and thermistor that provided for simultaneous measurement of oxygen consumption and temperature from both aerial and aquatic phases of the respirometer (see Seifert, 2004 for a complete description of respirometry set-up and oxygen sensing technology).

The probes were calibrated on a percent scale of oxygen saturation in the medium. Percent oxygen saturation was then converted to units of mm Hg (oxygen partial pressure, PO_2) 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. However, water circulated within the system and therefore mixing of the chamber was achieved in a flow-through manner. 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 (dechlorinated) 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_2 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 undetectable.

For respiratory measurements, individuals ($N=14$) were acclimated overnight to the system with free access to air. An overnight acclimation was used, because oxygen consumption measurements made after a 16-h acclimation were approximately two-fold lower than measurements made after 3 h of acclimation (Seifert, 2004). Prior to each run in the morning, the system was flushed with air-saturated water. This served to remove any metabolic wastes that may have accumulated overnight. The air chamber was then closed and the run initiated. Aquatic PO_2 concentration averaged 72.9 ± 3.4 mm Hg at the beginning of the trials. Oxygen measurements were made in air and water every 10 s over the course of the run and recorded automatically by 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 123.8 mm Hg. When the oxygen concentration in the air chamber approached this level, the stopper was removed and fresh air allowed to mix into the chamber and return the oxygen concentration to 100% saturation. This mixing did not increase aquatic PO_2 within the water chamber, where aquatic PO_2 was allowed to drop to the level at which aquatic oxygen consumption was negligible and respiratory allocation to air approached 100%.

Temperature was maintained throughout the run by placing the chambers inside of a Rubbermaid cooler that served as a temperature bath, and the entire apparatus was covered with a black sheet to shield the fish from disturbance. Runs lasted an average of 12 h. At the end of each run, individuals were drip dried and weighed within 0.1 g.

Individuals, with the exception of a 2-g specimen, were all run in 5.1 cm (diameter) chambers. Chamber size was held constant (except for the smallest fish) despite the wide range in fish mass, because smaller specimens exhibited an elevated SMR when placed in smaller-volume chambers (Seifert, 2004). This chamber-size effect was detectable only when respiratory allocation to water was relatively high; it was not detectable when respiratory allocation was primarily aerial (Seifert, 2004). In this manuscript, we therefore report results for the smallest 2-g specimen only when respiratory allocation to air was high.

We refer to our measurements of oxygen consumption as standard rate of metabolism (SMR). Based on preliminary trials, we determined an appropriate chamber acclimation time to facilitate minimum levels of oxygen consumption. Our fish were post-absorptive juveniles, and we designed our set-up to minimize any external disturbance. Since movement of the fish during trials was almost exclusively associated with respiration and therefore considered a portion of maintenance metabolism, our final estimates probably do represent minimum rates for these fish (measured in the dark), and should be considered as the standard metabolic rate.

An additional experiment was run in the laboratory to investigate any changes in the SMR at near-saturated aquatic PO_2 compared to those at ecologically relevant PO_2 levels. These runs carried out on seven of the lungfish lasted 6 h or less and were terminated when PO_2 concentration in the water phase approached 108.4 mm Hg.

3. Results

In the first experiment, oxygen in the air chamber was never allowed to drop below 123.8 mm Hg, whereas fish were allowed to reduce the aquatic oxygen level over a much larger range. As aquatic PO_2 declined, lungfish reduced aquatic respiration to an average of $1.3 \pm 0.46\%$ percent (range $= 94$ to 100%, Table 1). We refer to this level as the plateau point where allocation to aquatic respiration was nearly zero, and fish relied almost entirely on atmospheric air. While there was some variation among individuals, the plateau level was positively correlated with body mass with smaller fishes reaching their plateau level at higher aquatic PO_2 levels ($r=0.717$, $p=0.004$).

While aerial consumption of oxygen over a range of aerial PO_2 fit a linear function, aquatic oxygen consumption fit an exponential function. To examine the degree to which the curve representing oxygen consumption from water departed from a linear relationship we fit an exponential function ($y=b_0 e^{b1*t}$) to individual aquatic-phase curves and compared the exponents (b_1) among individuals as a function of body mass (Table 1). The degree of curvilinearity was positively correlated with body mass ($r=0.598$, $p=0.031$), indicating a greater degree of curvilinearity in larger lungfish (Table 1). The smallest individual (2 g) was excluded from this analysis. The curvilinear function was fit to the line beginning with the

Table 1
Respiratory allocation measurements for *Protopterus aethiopicus*

Mass	Percent O_2 from air	Plateau PO_2 mm Hg	b_1
2	100	85.2	n/a
9	99	73.4	0.0003
22	100	57.2	0.0006
41.5	98.2	19.1	0.0004
50	97.7	4.7	0.0006
81	99.6	39.5	0.0002
87	100	10.6	0.0013
112	100	1.62	0.0024
134	97.7	35.5	0.0002
139.5	94	4.7	0.001
144	100	1.8	0.0021
206.5	97	8.7	0.0012
208	98.2	7.0	0.0011
221.8	100	0.0	0.0031

Column 1 represents the mass of each individual in grams. Column 2 represents percent oxygen acquired from air at the aquatic respiration plateau (representing the aquatic oxygen level at which individuals respired almost completely aerially). Column 3 represents the aquatic oxygen partial pressure (mm Hg) at this plateau point. Column 4 is the exponent b_1 from the curvilinear function $y = b_0 e^{b_1 * t}$ that represents the curvilinear nature of aquatic respiration observed for all fish in this study. b_1 is a measure of the degree to which the relationship between aquatic oxygen consumption and time (and therefore aquatic oxygen decline) departs from the expected linear relationship of metabolic regulators. All measurements were made in the laboratory at 23 °C.

starting aquatic PO_2 concentration and ending shortly after the plateau level. This allowed for comparison of the exponent (b_1) between individuals across their ranges of aquatic oxygen acquisition.

The SMR was calculated as total oxygen consumed (mg O_2 h^{-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 reasonably good representation of aquatic PO_2 found in habitats used by juvenile lungfish in the field (Goudswaard et al., 2001; Chapman et al., 2002a,b). SMR was measured at an average PO_2 concentration of 24.9 mm Hg across all size classes. Total metabolic rate was positively correlated with body mass (linear regression, $r^2 = 0.9392$, $p = 0.031$, Fig. 1), with a scaling exponent of 0.78. Individuals showed a mean percent allocation to air breathing of 98.7 ± 0.4% at these low aquatic oxygen levels (Table 1).

Individuals were also run at high (near-saturated) aquatic PO_2 to test for an effect of oxygen availability on SMR (Fig. 2). We used analysis of covariance (ANCOVA) to test for a difference in the slopes and intercepts of the bilogarithmic relationships of the rate of oxygen consumption and body mass between low and high oxygen levels. There was no difference in slopes of the bilogarithmic relationship between SMR (mg O_2 h^{-1}) and body mass (g) between high and low aquatic PO_2 levels ($F = 3.177$, $p = 0.094$). In addition, the intercepts (representing the adjusted mean SMR) did not differ between the two oxygen levels ($F = 0.895$, $p = 0.357$). However, at high aquatic PO_2, individuals decreased their allocation to air breathing and increased allocation to aquatic respiration. Air breathing accounted for an average of 40.1 ± 4.1% of their total oxygen
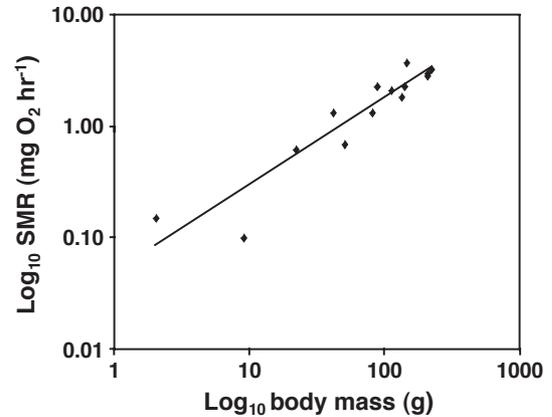

Fig. 1. The bilogarithmic relationship between SMR (mg O_2 h^{-1}) and body mass (g) for the African lungfish, *P. aethiopicus*. Data represent SMR at low aquatic oxygen levels (measurements made at plateau point where oxygen consumption in the aquatic phase was almost 0%; this was taken to represent ecological conditions because DO_2 levels would either not drop any lower or were in the range found in the field). Each diamond and square represents one measurement per individual. Measured at 23 °C.

consumption. Thus, there was a clear shift in respiratory allocation to increased oxygen uptake from water (paired $t = 12.02$, $p < 0.001$), although SMR remained stable.

We also measured pre-plateau SMR before oxygen acquisition from the aquatic phase reached a very low, stable level. The level of aquatic PO_2 at which the pre-plateau SMR was measured differed with each individual according to the plateau level. No difference in slopes of the bilogarithmic relationship between SMR (mg O_2 h^{-1}) and body mass (g) was found between pre-plateau levels and low levels of aquatic PO_2 ($F = 0.265$, $p = 0.612$), and there was no difference in the intercepts (representing the adjusted mean SMR) between groups ($F = 0.242$, $p = 0.626$). For the above two comparisons,
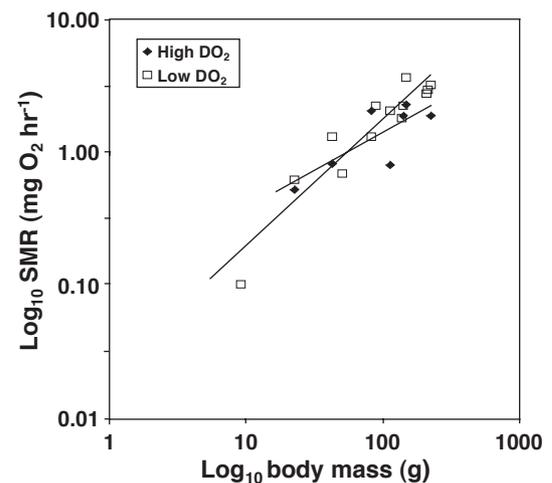

Fig. 2. The bilogarithmic relationship between SMR (mg O_2 h^{-1}) and body mass (g) for the African lungfish, *P. aethiopicus*. Data were measured at high aquatic oxygen levels of (near saturation) and low levels (plateau point at which fish is respiring almost completely aerially; ecologically relevant). Neither the slopes or intercepts of the lines differed significantly (ANCOVA, $P > 0.05$). Each symbol represents one measurement per individual. Measured at 23 °C.

the 2-g individual was not included, because it had to be run in a different chamber size.

4. Discussion

This study measured both respiratory allocation to water and air, and the SMR for *P. aethiopicus*. Our results demonstrate that juvenile *P. aethiopicus* have a low SMR and high allocation to air breathing when respirometry measurements are made under ecologically relevant conditions of aquatic PO_2. Our measurements also demonstrate the ability of these fish to maintain their SMR over a range in aquatic oxygen partial pressures from saturation to hypoxia, despite shifts in respiratory allocation. Two additional findings to emerge from this study were that *P. aethiopicus* displays a curvilinear relationship describing the removal of oxygen from the aquatic medium (which was positively correlated with body mass), and that smaller juvenile lungfish appear to remove less total oxygen from the water before converting to an almost complete reliance on aerial respiration.

We estimated the standard rate of metabolism for juvenile *P. aethiopicus* 2 to 221 g collected from a natural population within the fringing swamps of Lake Nabugabo. Our findings collected in the laboratory indicated a strong positive relationship between SMR and body mass ($y = 0.7768x − 1.3004$). Chapman et al. (2002a) reported routine metabolic rates for 12 species of non-air-breathing fishes from the Lake Nabugabo region of Uganda adjusted to a mean mass of 3.9 g. The SMR for *P. aethiopicus* adjusted to the same body mass is lower than the routine metabolic rate for all 12 non-air-breathing species. Despite the difference between standard and routine measurements, the lower rates for *P. aethiopicus* are lower than could be explained by activity level alone. This supports the idea that air-breathing fishes generally have unusually low SMR when compared to non air-breathing species from similar habitats (Val and de Almeida-Val, 1995). In our study, individuals were held for up to 1 year in normoxic conditions prior to our metabolic rate experiments. One might anticipate even lower SMR values had our measurements been made on individuals held under field conditions prior to measurement.

Our results emphasize the importance of considering ecological conditions when measuring physiological parameters. A review of earlier studies of oxygen consumption in lungfishes suggested that juveniles used primarily aquatic respiration to meet metabolic demands while adults relied 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). In these earlier investigations, both total oxygen consumption and respiratory allocation were measured at high PO_2 levels. Our results of respiratory allocation at high (near saturated PO_2) aquatic oxygen levels support these earlier studies with juveniles acquiring approximately 60% of their oxygen uptake from water. However, this finding drastically differs from our measures of oxygen consumption at low aquatic oxygen levels where the mean allocation towards aerial

respiration for *P. aethiopicus* between 2 and 221 g averaged $98.7 \pm 0.46\%$. These latter measures are more reflective of field conditions, where juveniles are known to occupy very hypoxic habitats.

It is also clear from this study that *P. aethiopicus* is able to maintain a relatively constant SMR over a range of aquatic oxygen levels from normoxia to hypoxia. Measurements of SMR at normoxic conditions, pre-plateau levels, and with individuals respiring almost completely aerially did not differ. Although respiratory allocation differs across the range of aquatic PO_2 from normoxia to extreme hypoxia in the lungfish, adjustments in oxygen utilization seem to balance the overall uptake of oxygen to stabilize SMR. 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.

Two additional findings that emerged from this inquiry into SMR in lungfish have begun to shed light on the physiological control mechanisms that may influence the maintenance of SMR over such a broad range in aquatic PO_2. First, the relationship between aquatic oxygen uptake and time was curvilinear. In addition, this relationship exhibited a positive correlation with increasing body mass. For a metabolic regulator breathing from one medium, the relationship between oxygen consumed per unit time should be linear given ample oxygen to meet the metabolic demand (Ultsch et al., 1981). Measurements made on both terrestrial air breathers and aquatic water breathers yield this result (Ultsch et al., 1981; McNab, 2002). The curvilinear nature of the relationship shown in the African lungfish suggests that the acquisition of oxygen from the aquatic phase may be fueled by a concentration 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. The diffusion of oxygen across multiple gas exchange surfaces may be ample to satisfy the metabolic demand at higher levels of dissolved oxygen. As PO_2 falls in the aquatic medium, the higher ratio of surface area to body mass in smaller individuals may allow for a greater efficiency in the removal of oxygen from water. Because larger individuals require more *total* oxygen to meet their metabolic demand, their lower surface area to body mass ratio may not be sufficient to remove oxygen from water at a constant rate and thus contribute to the higher degree of curvilinearity displayed.

As aquatic PO_2 falls, metabolic regulators maintain their SMR as a result of increased ventilatory effort, via a greater number of opercular movements, possibly coupled with greater perfusion of the lamellar surface (Ultsch et al., 1981). An inability to acquire the necessary oxygen to meet metabolic demands results in a curvilinear relationship, with the rate of oxygen extracted per unit time decreasing. In studies examining rate of gill ventilations in lungfish, there was no correlation between increasing hypoxia and the rate of aquatic respiration suggesting that lungfish are rather insensitive to aquatic PO_2 due to their bimodal mode of respiration (Johansen and Lenfant, 1968; Sanchez et al., 2001). Our observations further support these earlier studies. The

curvilinear relationship observed may reflect the lack of oxygen-sensitive chemoreceptors to falling aquatic PO_2 levels (Perry et al., 2005). As a bimodal breather, *P. aethiopicus* presumably uses the ease of arterial saturation with atmospheric oxygen to compensate for a loss in aquatic oxygen.

While the curvilinear relationship describing oxygen acquisition from water supports the ability of smaller individuals to extract oxygen more efficiently from water, our data also reveals that smaller individuals, when given the option, switch to almost complete aerial respiration, as indicated by the plateau levels, at higher aquatic PO_2. While a higher plateau level might appear inconsistent with the increased efficiency in aquatic oxygen uptake displayed by smaller individuals, it may reflect environmental pressures experienced by smaller lungfish in the field. The smallest juveniles (2 to 22 g) and post nestlings are associated with dense floating mats of papyrus roots, and the protection of dense vegetative areas of the inner swamp. These individuals are usually found within this vegetation, often near the surface, and based on our findings, are utilizing aerial respiration to meet metabolic demands. When held captive in tanks, these small individuals remain at the surface of the water column while the larger lungfish rest quietly at the bottom. When disturbed by breaking the water surface, or even upon approach, they exhibit a flight response that involves a burst of swimming activity where they attempt to escape the confines of the tank. While in the respirometer, the three smallest individuals were all observed resting at the water to air interface rather than seeking refuge further within the aquatic phase of the respirometer. Hence, the natural tendency to remain near the surface at a very small size is manifest here as a high plateau level. Again, the high rate of oxygen saturation from pulmonary respiration may simply make aquatic respiration unnecessary while these individuals are at rest. The high degree of arterial blood saturation could serve to pay off the oxygen debt incurred upon burst activity due to predator avoidance. Further studies examining activity and metabolic rate will help determine to what extent juveniles utilize both aquatic and pulmonary respiration to maintain metabolic rate during increased activity.

Lungfish display remarkable abilities in environmental tolerance that have helped them to survive since the emergence of tetrapods. Our findings, in conjunction with literature derived data for adults, reveal that their respiratory flexibility may be most evident at the juvenile stage when they inhabit the hypoxic nurseries of shoreline swamps. Future experiments will be necessary to define the role of the skin and gills in oxygen uptake and how this relates to changing levels in aquatic PO_2. In addition, further research examining the precise physiological mechanisms that assist *P. aethiopicus* to maintain their SMR over such a wide range of aquatic PO_2 that is characterized by shifts in respiratory allocation will help shed light on the evolutionary pressures driving pulmonary respiration in tetrapods.

Acknowledgments

References

Babiker, M.M., 1979. Respiratory behaviour, oxygen consumption and relative dependence on aerial respiration in the African lungfish (*Protopterus annectens*; Owen) and an air-breathing teleost (*Clarias lazera* C.). Hydrobiologia 65, 177–187.

Burggren, W.W., Johansen, K., McMahon, B., 1986. Respiration in phyletically ancient fishes. In: Foreman, R.E. (Ed.), Evolutionary Biology of Primitive Fish. Plenum Press, New York, pp. 217–252.

Chapman, L.J., Chapman, C.A., Nordlie, F.G., Rosenberger, A.E., 2002a. Physiological refugia: swamps, hypoxia tolerance and maintenance of fish diversity in the Lake Victoria region. Comp. Biochem. Physiol. A 133, 421–437.

Chapman, L.J., Nordlie, F.G., Seifert, A.W., 2002b. Respiratory oxygen consumption among groups of *Pseudocrenilabrus multicolor victoriae* subjected to different oxygen concentrations during development. Comp. Biochem. Physiol. A 61, 242–251.

Coates, M.I., Clack, J.A., 1991. Fish-like gills and the breathing in the earliest known tetrapod. Nature 352, 234–236.

Gardiner, B., 1980. Tetrapod ancestry: a reappraisal. In: Panchen, A.L. (Ed.), The Terrestrial Environment. Academic Press, London, pp. 177–185.

Gordon, M.S., Olson, E.C., 1995. The Invasions of the Land. Columbia University Press, New York.

Goudswaard, K.P.C., Witte, F., Chapman, L.J., 2001. Decline of the African lungfish (*Protopterus aethiopicus*) in Lake Victoria (East Africa). Afr. J. Ecol. 39, 1–11.

Graham, J.B., 1997. Air Breathing Fishes: Evolution, Diversity and Adaptation. Academic Press, San Diego, CA.

Greenwood, P.H., 1958. Reproduction in the East African lungfish *Protopterus aethiopicus* Heckel. Proc. Zool. Soc. Lond. 130, 547–567.

Greenwood, P.H., 1965. The cichlid fishes of Lake Nabugabo, Uganda. Bull. Mus. Natl. Hist. Nat., Zool. 12, 315–357.

Greenwood, P.H., 1987. The natural history of African lungfishes. J. Morphol. 1, 163–179 (suppl).

Jesse, M.J., Shub, C., Fishman, A.P., 1967. Lung and gill ventilation of the African lungfish. Res. Physiol. 3, 267–287.

Johansen, K., Lenfant, C., 1967. Respiratory function in the South American lungfish, *Lepidosiren paradoxa* (Fitz). J. Exp. Biol. 46, 205–218.

Johansen, K., Lenfant, C., 1968. Respiration in the African lungfish *Protopterus aethiopicus*: II. Control of breathing. J. Exp. Biol. 49, 453–468.

Johansen, K., Lomholt, J.P., Maloiy, G.M.O., 1976. Importance of air and water breathing in relation to size of the African lungfish *Protopterus amphibius* (Peters). J. Exp. Biol. 65, 395–399.

Johnels, A.G., Svensson, G.S.O., 1954. On the biology of *Protopterus annectens* (Owen). Ark. Zool. 7, 131–158.

Lahiri, S., Szidon, J.P., Fishman, A.P., 1970. Potential respiratory and circulatory adjustments to hypoxia in the African lungfish. Fed. Proc. 29, 1141–1148.

Lenfant, C., Johansen, K., 1968. Respiration in the African lungfish *Protopterus aethiopicus*: I. Respiratory properties of blood and normal patterns of breathing and gas exchange. J. Exp. Biol. 49, 437–452.

Lenfant, C., Johansen, K., Hanson, D., 1970. Bimodal gas exchange and ventilation–perfusion relationship in lower vertebrates. Fed. Proc. 29, 1124–1129.

Little, C., 1990. The Terrestrial Invasion. Cambridge University Press, Cambridge.

Long, J.A., 1995. The Rise of Fishes: 500 Million Years of Evolution. Johns Hopkins University Press, Baltimore.

McMahon, B.R., 1970. The relative efficiency of gaseous exchange across the lungs and gills of an African lungfish *Protopterus aethiopicus*. J. Exp. Biol. 52, 1–15.

McNab, B.K., 2002. The Physiological Ecology of Vertebrates. Cornell University Press, London.

Oduleye, S.O., 1977. Unidirectional water and sodium fluxes and respiratory metabolism in the African lungfish *Protopterus annectens*. J. Comp. Physiol. 119, 127–139.

Ogutu-Ohwayo, R., 1993. The effect of predation by Nile perch, *Lates niloticus* L., on the fish of Lake Nabugabo, with suggestions for conservation of endangered endemic cichlids. Conserv. Biol. 7, 701–711.

Perry, S.F., Wilson, R.J.A., Straus, C., Harris, M.B., Remmers, J.E., 2001. Which came first, the lung or the breath? Comp. Biochem. Physiol. A 129, 37–47.

Perry, S.F., Gilmour, K.M., Vulesevic, B., McNeil, B., Chew, S.F., Ip, Y.K., 2005. Circulating catecholamines and cardiorespiratory responses in hypoxic lungfish (*Protopterus dolloi*): a comparison of aquatic and aerial hypoxia. Physiol. Biochem. Zool. 78, 325–334.

Randall, D.J., Burggren, W.W., Farrell, A.P., Haswell, M.S., 1981. The Evolution of Air Breathing in Vertebrates. Cambridge University Press, Cambridge.

Randle, A.M., Chapman, L.J., 2004. Habitat use by the African anabantid fish *Ctenopoma muriei*: implications for costs of air-breathing. Ecol. Freshw. Fish 13, 37–45.

Sanchez, A., Sonncini, R., Wang, T., Koldkjaer, P., Taylor, E.W., Glass, M.L., 2001. The differential cardio-respiratory responses to ambient and systemic hypoxaemia in the South American lungfish, *Lepidosiren paradoxa*. Comp. Biochem. Physiol. A 130, 677–687.

Sawaya, P., 1946. Sobre a biologia de alguns peixes de respiracao aerea (*Lepidosiren paradoxa* Fitzinger e *Arapaima gigas* Cuvier). Bol. Fac. Univ. San Paulo (Zool.) 11, 255–285.

Seifert, A.W., 2004. Respiratory allocation and the resting rate of metabolism in the African lungfish, *Protopterus aethiopicus*. Master of Science Thesis, University of Florida, USA.

Smith, H.W., 1930. Metabolism of the lungfish *Protopterus aethiopicus*. J. Biol. Chem. 88, 97–130.

Smith, H.W., 1931. Observations on the African lungfish, *Protopterus aethiopicus*, and the evolution from water to land environments. Ecology 12, 164–181.

Tohyama, Y., Ichimiya, T., Kasama-Yoshida, H., Cao, Y., Hasegawa, M., Kojima, H., Tamai, Y., Kurihara, T., 2000. Phylogenetic relation of lungfish indicated by the amino acid sequence of myelin DM20. Mol. Brain Res. 80, 256–259.

Ultsch, G.R., 1996. Gas exchange, hypercarbia and acid–base balance, paleoecology, and the evolutionary transition from water-breathing to air-breathing among vertebrates. Palaeogeogr. Palaeoclimatol. Palaeoecol. 123, 1–27.

Ultsch, G.R., Jackson, D.C., Moalli, R., 1981. Metabolic oxygen conformity among lower vertebrates: the toadfish revisited. J. Comp. Physiol. B 142, 439–443.

Val, A.L., de Almeida-Val, V.M.F., 1995. Fishes of the Amazon and their Environments. Springer-Verlag, Berlin.

Worthington, E.B., 1932. Scientific results of the Cambridge expedition to the East African lakes, 1930-1. Fishes other than Cichlidae. J. Linn. Soc. Lond. Zool. 38, 121–134.