Ontogenetic Shifts in Oxygen Uptake in Common Mudskipper (Periophthalmus kalolo) and its Role in Microhabitat Selection

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Authors’ contributions
This work was carried out in collaboration among all authors. Author WAB designed the study protocol and performed the statistical analysis. Author JR wrote the first manuscript draft. Author NNK managed logistics and permitting. Author TFD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The common mudskipper, Periophthalmus kalolo is a tropical, amphibious fish that utilizes both air and water as a respiratory medium. Adult oxygen uptake in water and air is well studied, but requirements of post-metamorphosed fish are virtually unknown. Our study quantifies how ontogenetic shifts across mudskipper life stage affects microhabitat choice.

Study Design: metabolic rates in air and water were estimated for common mudskippers with mass values from 0.03 to 28.9 grams. Fish in each media were divided into 5 standard length classes (≤ 2.00, 2.01-4.00, 4.01-6.00, 6.01-8.00, ≥ 8.01 cm). Oxygen consumption for each class was calculated as the mean individual oxygen consumption for the group.

Place and Duration of Study: Hoga Research Laboratory, Wakatobi National Park, Sulawesi, Indonesia, between June 14 and August 26, 2005.

Methodology: Gilson manometry, and flow-through respirometry was used to determine respective aerial and aquatic oxygen uptake values for common mudskippers.

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**Results:** Mudskipper lengths varied by an order of magnitude (1.0 to 10.3 cm) and mass varied by three orders of magnitude (0.03 to 17.3 g). Mass-adjusted aerial metabolic rates of fish 2.00 cm or less in length, consumed up to ten times more oxygen than larger fish. Whereas, aerial consumption values for size classes 2.01 cm and above did not differ significantly from each other (ANOVA: $F_{4,112} = 40.29, P < 0.0001$; SNK, $\alpha = 0.05$).

Metabolic rates of submerged fish fell into two statistically distinct subsets. Mudskippers with lengths of 4.00 cm or less had significantly higher metabolic rates than fish in remaining size classes; however, mean oxygen uptake values of fish within subsets did not differ significantly (ANOVA: $F_{4,97} = 6.89, P < 0.0001$; SNK, $\alpha = 0.05$). When oxygen consumption values at each size class were compared, only the smallest mudskipper size class ($\leq 2.00$ cm) differed significantly between air and water ($t$-test: $df = 34$, $t = 3.44$, $P < 0.0001$). Mudskippers 2 cm in standard length or smaller consumed over seven times more oxygen in air than water. All other size classes had similar rates of oxygen uptake in air and water and air:water oxygen uptake ratios fell to approximately 1:1.

**Conclusions:** (1) Common mudskippers select different mangal habitats based on developmental life stage. (2) Small, post-metamorphosed fish <2 mm in standard length, prefer cool shaded mangal areas, whereas, larger mudskippers utilize sun-exposed zones. (3) Post-metamorphosed fish, consume 10X more oxygen than all other size groups, confirming that juveniles are well suited to aerial respiration oxygen shortly after hatching. (4) Emerged post-metamorphosed fish exploit cooler, wetter pool habitats, thereby ameliorating problems of desiccation, excretion, and/or predation. (5) Ontogenetic shifts are a key life-history event in *P. kalolo* that promotes wider mangrove habitat use, and plays an important role in establishing common mudskipper as a key mangal species.

**Keywords:** Mudskipper; metabolic rate; respirometry; habitat selection.

1. INTRODUCTION

The Common Mudskipper, *Periophthalmus kalolo*, (family Gobiidae) is an air-breathing fish common to mangrove (mangal) habitats throughout the Indo-Pacific. Post-metamorphic fish < 2.0 cm in standard length prefer shaded tidepool margins, whereas larger individuals aggregate in exposed lower intertidal mudflats [1,2], often in full sun. Indeed, larger fish may spend as much as 90% of their lifetime out of water [3,4]. Brillit [5], and Tyler and Vaughn [2] report that at low tide, small *P. kalolo* in Kuwait Bay, and Madagascar, respectively, seldom stray far from tidepools or wet mud. Tyler and Vaughn, [2] also found that small fish placed in the open preferentially move away from sun exposed areas toward wet, shaded zones.

Factors influencing size-dependent distribution of common mudskippers among mangal microhabitats are not well understood. Tamura and co-workers [6] suggested that recently post-metamorphic mudskippers are more effective at extracting oxygen from water than air, and must therefore remain closely associated with pool environments. However, histological examination has shown that epidermal vascularization in at least one periophthalmid, Shuttles Hoppfish (*Periophthalmus modestus*), is well-developed only 10 days post-hatch and is likely capable of supporting cutaneous aerial respiration [7]. Likewise, Clayton [8] has argued that high skin to gill area ratios in smaller mudskippers should support a greater dependence on cutaneous rather than branchial respiration leaving them better suited to aerial habitats than large fish. While numerous studies have assessed aquatic or aerial oxygen uptake in mudskippers larger than 5 cm standard length (E.g., [9,10,11,12]), no study to date has quantified metabolic rates in small *P. kalolo*, leaving open the question of how ontogenetic changes in body size affect relative oxygen extraction rates in air and water in early life stages.

To present study, we estimate aerial and aquatic oxygen uptake of Common Mudskipper across a range of sizes to identify ontogenetic shifts in metabolic rate. Oxygen uptake is measured between respiratory media across body size classes. Finally, we interpret metabolic rate data in light of environmental constraints and physiological adaptations to better understand factors influencing activity and distribution patterns of common mudskipper between upper and lower mangal zones.

2. MATERIALS AND METHODS

More than 200 mudskippers were collected for metabolic trials. Individual fish were run in either...
one air, or one water trial. A paired experimental design would have provided information for individual to fish however, studies were conducted in a remote field laboratory with limited the space and resources and a paired design wasn’t tenable.

Adult mudskippers were collected from a single environment type – open exposed tidal pools. Small mudskippers avoided sun exposed areas and were collected only in shaded upper mangrove zones. There was little if any overlap between the two groups.

Captured fish were taken to the Hoga Marine Research Center and transferred to covered, plastic holding tanks (121 × 39.6 × 30.8 cm) containing beach sand covered with 33% seawater. Emergent palm frond and driftwood pieces allowed fish free movement between air and water. Holding tank temperatures were kept between 25 and 28°C with seawater changed twice daily and sand changed every 2-3 days to maintain habitat quality. Mudskippers were fasted for 48 h prior to respirometry trials. Each fish was used in only one aerial or aquatic experiment and all fish were released to their site of capture at the end of the study.

2.1 Aerial Oxygen Uptake Protocols

Manometric respirometry [13,14,12] was used to estimate total resting routine metabolic rates in air for 117 Common Mudskippers. The respirometer design was similar to that of Taylor [12] and consisted of a 20-mL (fish ≤ 2.0 cm standard length), or 250-mL (fish ≥ 3.0 cm) glass respirometer flask connected by a manometer tube to a reference flask of equal volume. Seawater-saturated blotter paper maintained high humidity levels in both flasks, and a strip of 10% sodium hydroxide-saturated Whatman paper attached to the respirometer flask stopper removed expired carbon dioxide. An identical control (without fish) was run simultaneously during each trial to correct for non-fish respiration.

Fig. 1. Common mudskipper collected on Hoga Island (05°27.53’S, 123°6.33’E), Wakatobi national park, Sulawesi, Indonesia from June – August
Prior to each trial, the apparatus was pressure-tested to identify and eliminate system leaks and a randomly selected fish placed into the respirometer flask. Fish were allowed to adjust to the flask environment for 45 min at their experimental set point temperature at which time, the respirometer was sealed and the trial begun. As oxygen was consumed, respirometer flask pressure decreased causing manometer fluid displacement proportional to oxygen uptake. Manometer fluid was returned to its starting level by adding air slowly and in small amounts to the respirometer flask via a 10 cc Hamilton® gas-tight syringe. Measurements of total injected air were recorded every 30 min for three hours with total oxygen consumption (mg h$^{-1}$) estimated as milligrams of injected air added divided by total trial time. To account for cyclic changes in metabolic rate, trials were performed during late afternoon when fish were least active. In their natural habitat, mudskippers spend most of their time quietly resting on the sub-stratum. When placed into the respirometer, fish settled down quickly, and displayed only limited routine movement over the course of the trial.

2.2 Aquatic Oxygen Uptake Protocols

Estimates of total aquatic resting routine oxygen uptake were quantified in 92 common mudskippers using flow-through respirometry techniques [14]. For each trial, a fish was randomly selected from the holding tank and transferred into 25 mL (fish ≤ 3.00 cm standard length) or 250 mL (fish ≥ 3.01 cm) glass respirometer flask. Filtered seawater was pumped through the respirometer flask at rates between 3 and 20 mL min$^{-1}$. Flow rates varied with fish mass so that oxygen content of water leaving the flask never fell below 15% of total inflow oxygen concentration [14]. Fish were allowed to adjust to the flask environment for 45 min, after which three respirometer outflow water samples were collected in biological oxygen demand (BOD) bottles. Bottles were filled slowly from the bottom and allowed to overfill at least two times their volume to minimize the chance of atmospheric oxygen uptake [15]. Oxygen concentration of each BOD sample was determined using standard Winkler titration techniques [15]. A control flask, identical in all respects but containing no fish, was included in each trial to allow correction for oxygen consumption from non-fish sources. Total oxygen uptake (mg h$^{-1}$) was determined as the mean difference in oxygen content (mg L$^{-1}$) between water leaving the control flask and respirometry flask, multiplied by the respirometer flow rate (L h$^{-1}$).

Aerial and aquatic respirometers were held at a constant temperature by submerging the respirometer into a temperature controlled, recirculating water bath maintained at 26.5 ± 0.09 C. The experimental temperature was based on the average temperatures encountered in Hoga's mangal habitat. Fishes were carefully observed during experiments, and trials in which individuals exhibited more than intermittent movements were omitted. At the end of each trial, mudskippers were weighed (wet mass ± 0.001 g), measured (standard length ± 0.01 mm), and returned to their holding tank.

Mass-adjusted oxygen consumption rates were calculated as the total oxygen consumed (mg h$^{-1}$) divided by wet mass raised to the power of 0.80. The mass adjustment exponent of 0.80 is commonly used in fish respirometry to correct for allometric relationships between mass and metabolic rate [16], and replaces the practice of dividing total metabolic rate by mass alone [14]. All values were corrected to standard temperature and pressure.

Fish in each media were grouped into one of 5 size classes (≤ 2.00, 2.01-4.00, 4.01-6.00, 6.01-8.00, ≥ 8.01 cm in SL) and final oxygen consumption measures for each size class reported as the arithmetic mean of the collective mass-adjusted oxygen consumption rates of individual fish. Mass-adjusted oxygen consumption values in air and water were plotted on fish mass to identify differences across size classes or between respiratory media.

Mass-adjusted oxygen consumption between size classes, within media were explored using one-way analysis of variance (ANOVA). Where significant changes were found, a Student-Newman-Keuls (SNK) multiple range test (MRT) was used to identify statistical relationships between size groups. Statistical decisions for within media ANOVA and SNK MRT comparisons were based on an alpha level of 0.05. Comparisons of mass-adjusted metabolic rates within size classes between media were made using Student’s independent t-test and all statistical decisions based on a Bonferroni corrected alpha level of 0.01.
Fig. 2. Mass adjusted oxygen consumption on mass and length for common mudskipper collected on Hoga Island Wakatobi national park, Sulawesi, Indonesia from June – August

3. RESULTS AND DISCUSSION

Aerial and aquatic metabolic rates of mudskippers between 1.0 and 10.3 cm in length exhibited mass-adjusted aerial metabolic rates spanning three orders of magnitude (0.03 to 17.3 g). Fish with SL of 2.00 cm or less in length, consumed up to ten times more oxygen than larger fish. whereas, aerial consumption values for size classes 2.01 cm and above did not differ significantly from each other (ANOVA: F4,112 = 40.29, P < 0.0001; SNK, α = 0.05).

Metabolic rates of submerged fish fell into two statistically distinct subsets. mudskippers with lengths of 4.00 cm or less had significantly higher metabolic rates than fish in remaining size classes; however, mean oxygen uptake values of fish within subsets did not differ significantly (ANOVA: F4,87 = 6.89, P < 0.0001; SNK, α = 0.05). When oxygen consumption values at each size class were compared, only the smallest mudskipper size class (2.00 cm) differed significantly between air and water (t-test: df = 34, t = 3.44, P = 0.0001). Mudskippers less than or equal to 2 cm in SL consumed over seven times more oxygen in air than water. All other size classes had similar rates of oxygen uptake in air and water and air:water oxygen uptake ratios fell to approximately 1:1 (Table 1).

Tamura [6] concluded that P. kalolo distributions in mangal environments are related to differing metabolic requirements in air and water, leading the authors to suggest that small fish were better suited to aerial oxygen uptake. Our data, however, suggest that small mudskippers retain proportionally high oxygen uptake in water. As a result, smaller fish are more likely to seek out pool microhabitats. Access to water would reduce desiccation risk while also lowering threats from aerial predators. Common mudskippers less than 2 cm in SL had mass-adjusted metabolic rates ten times higher than seen in larger fish, — an observation consistent with existing morphological [8] and histological [7] evidence in periophthalmids.

The high cost of emergence restricts small common mudskippers to short emergence times and requires they remain near tidepools. Only later, as aerial metabolic rates decrease do fish begin to move into more exposed mangal areas. Tytler and Vaughn [2] found that small common mudskippers from Kuwait Bay remained in shallow pools or wet mud until reaching sizes between 1.5 and 2.5 cm in SL after which, they could be found moving into drier microhabitats. Likewise, Brillet [5] noted that small Common Mudskippers in Madagascar were never found more than a few centimeters away from
mangrove tidepools and it was not until reaching SLs of 2 cm or more that fish ventured into drier exposed areas. Common Mudskippers in our study showed a similar distribution at low tide, with fish less than 2 cm in length remaining close to pools in upper mangal regions near mangroves, and larger fish most numerous in lower mangal mudflats. High mass-adjusted metabolic rates noted for small mudskippers in both air and water might be explained by demands of rapid growth or higher activity levels, but it is unclear why the increase becomes more pronounced when fish are emerged.

High surface area to volume ratios of small mudskippers are believed to support greater amphibious behavior and set the pace of physiological processes. Dry season temperatures on Hoga can be extreme with daytime air temperatures increasing by as much as 12°C in exposed areas. High temperatures not only elevate metabolic rate but also promote high rates of evaporative water loss in mudskippers [2]. Small mudskippers may avoid problems associated with extreme temperatures when emerged, by remaining in shaded upper mangals where daytime temperatures are on average 5°C lower than those experienced by fish on exposed mudflats. Their proximity to pools may have the added advantage of allowing small fish to quickly move between air and water thus effectively reducing both aerial and aquatic predatory pressure [5]. In addition, spatial separation of small from large common mudskippers may reduce direct competition and intraspecific cannibalism, allowing fish to more effectively exploit mangal resources.

The high cost of being amphibious reinforces a close association with pool environments. Small P. kalolo likely benefit from inhabiting upper mangal regions. Our data suggest that temperature relationships dictate mudskipper amphibious behavior and set the pace of physiological processes. Dry season temperatures on Hoga can be extreme with daytime air temperatures increasing by as much as 12°C in exposed areas. High temperatures not only elevate metabolic rate but also promote high rates of evaporative water loss in mudskippers [2]. Small mudskippers may avoid problems associated with extreme temperatures when emerged, by remaining in shaded upper mangals where daytime temperatures are on average 5°C lower than those experienced by fish on exposed mudflats. Their proximity to pools may have the added advantage of allowing small fish to quickly move between air and water thus effectively reducing both aerial and aquatic predatory pressure [5]. In addition, spatial separation of small from large common mudskippers may reduce direct competition and intraspecific cannibalism, allowing fish to more effectively exploit mangal resources.

Table 1. Mass-adjusted oxygen consumption (mg 0.8 h−1), and total oxygen consumption (mg h−1) rates in air and water for common mudskippers (Periophthalmus kalolo). Standard length (SL), and wet mass (g) for all size classes in air and water as well as air:water oxygen uptake ratios. Oxygen consumption estimates with like superscripts are statistically indistinguishable.

| Size class (cm) | SL (cm) | Mass (g) | Total O2 uptake | Mass-adjusted O2 uptake | Air:Water Ratio |
|----------------|---------|----------|-----------------|------------------------|----------------|
|                | n       | Mean ± SE| Mean ± SE       | Mean ± SE              | Mean ± SE      |                 |
| **Air**        |         |          |                 |                        |                |
| ≤ 2.00         | 29      | 1.34 ± 0.033| 0.07 ± 0.007  | 0.33 ± 0.039          | 3.80 ± 0.464ab| 1.34 ± 0.033  |
| 2.01-4.00      | 35      | 3.05 ± 0.106| 0.42 ± 0.050  | 0.21 ± 0.021          | 0.51 ± 0.058bc| 3.05 ± 0.106  |
| 4.01-6.00      | 28      | 5.04 ± 0.106| 1.78 ± 0.138  | 0.40 ± 0.029          | 0.27 ± 0.023bc| 5.04 ± 0.106  |
| 6.01-8.00      | 14      | 6.78 ± 0.151| 4.31 ± 0.300  | 0.85 ± 0.067          | 0.27 ± 0.024bc| 6.78 ± 0.151  |
| ≥ 8.01         | 11      | 9.37 ± 0.147| 11.69 ± 0.786 | 2.02 ± 0.197          | 0.29 ± 0.028bc|                 |
| **Water**      |         |          |                 |                        |                |
| ≤ 2.00         | 7       | 1.29 ± 0.112| 0.06 ± 0.010  | 0.05 ± 0.011          | 0.51 ± 0.109bc| 7.5:1          |
| 2.01-4.00      | 28      | 3.18 ± 0.102| 0.56 ± 0.057  | 0.24 ± 0.027          | 0.40 ± 0.036bc| 1.3:1          |
| 4.01-6.00      | 28      | 4.92 ± 0.118| 1.69 ± 0.137  | 0.41 ± 0.032          | 0.28 ± 0.020bc| 1:1            |
| 6.01-8.00      | 17      | 7.03 ± 0.149| 5.13 ± 0.264  | 0.90 ± 0.050          | 0.25 ± 0.015bc| 1:1            |
| ≥ 8.01         | 12      | 9.01 ± 0.215| 11.09 ± 1.076 | 1.78 ± 0.180          | 0.26 ± 0.018bc| 1:1            |
4. CONCLUSION

Common mudskipper, *Periophthalmus kalolo*, occupy different mangal habitat types depending on developmental life stage. Small, post-metamorphosed mudskippers less than 2 mm in SL were found in cooler shaded mangal pools, whereas, larger mudskippers utilize sun-exposed zones.

Oxygen uptake by small post-metamorphosed fish, was an order of magnitude higher than all other size groups, thus confirming that juvenile common mudskippers are well suited to meet aerial oxygen uptake demand shortly after hatching. Perhaps more importantly, emerged post metamorphosed fish successfully exploit cooler and wetter pool margins thereby ameliorating problems associated with desiccation, excretion, and/or predation. Ontogenetic shifts are a key life-history event in *P. kalolo* that, not only enhances survival, but also promotes wider use of mangrove habitats by the species. The ability to exploit both behavioral and physiological adaptations plays an important role in establishing the common mudskipper as a keystone mangal species

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All fish used in this study were treated in accordance with ethics guidelines approved by the University of West Florida Animal Care and Use Committee (Protocol 2020-006).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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