

Effects of respiratory media, temperature, and species on metabolic rates of two sympatric periophthalmid mudskippers *

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Abstract— Oxygen uptake rates in air and water were measured at 26.0 and 32.0°C for common mudskipper *Periophthalmus kalolo* collected from sun-exposed mudflats, and barred mudskipper *Periophthalmus argentilineatus* taken from shaded mangal zones on Hoga Island, Sulawesi, Indonesia. Mass-adjusted oxygen consumption rates between mudskippers were statistically similar, with both species exhibiting higher uptake in air than water. *Periophthalmus kalolo* at 26.0 and 32.0°C had respective uptake values of 0.295 and 0.358 mg g^{(0.75)⁻¹} hr⁻¹ in air, and 0.198 and 0.241 mg g^{(0.75)⁻¹} hr⁻¹ in water. *Periophthalmus argentilineatus* at 26.0 and 32.0°C had oxygen uptake values of 0.262 and 0.343 mg g^{(0.75)⁻¹} hr⁻¹ in air, and 0.199 and 0.246 mg g^{(0.75)⁻¹} hr⁻¹ in water. While metabolic rates increased significantly in both species following an acute increase in media temperature, the change was not large, indicating a reduced metabolic response to increasing environmental temperatures. Respective temperature quotients calculated from aerial and aquatic metabolic rate data were 1.38 and 1.39 for *P. kalolo*, and 1.56 and 1.42 for *P. argentilineatus*. The data reveal that *P. kalolo* and *P. argentilineatus* exhibit similar metabolic responses when exposed to changes in media and temperature, even though each experiences markedly different diel habitat temperature profiles and insolation patterns.

Introduction

Mudskippers (family Gobiidae: subfamily Oxudercinae) are amphibious fishes distributed along the Atlantic coast of Africa, and throughout the tropical Pacific. While occasionally found living in sandy (Stebbins & Kalk 1961) or rocky habitats (Gordon et al. 1969), most mudskipper species are associated with soft-bottom mangal or estuary environments that flood at high tide but are exposed to various degrees at low tide (Murdy 1989). Depending on tidal conditions, mudskippers can spend as much as 90% of their time emerged (Gordon et al. 1978, Gordon et al. 1985), making this group the most terrestrial of all known air-breathing fishes (Murdy 1989, Polgar & Crosa 2009). Although

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mudskippers experience a wide range of mangal air and water temperatures, it is while emerged, that they are subject to the greatest temperature extremes (Dunson 1977, Burhanuddin & Martosewojo 1979, Gordon et al. 1985). In some Southeast Asian mangals, for example, mudskippers may experience diel temperatures shifts of more than 10°C, and peak temperatures approaching 40°C—levels very near the fishes' physiological limit (Gordon et al. 1985, Taylor et al. 2005). Not surprisingly, mudskippers possess adaptations that support emergence and help mitigate high temperature effects, including resistance to evaporative water loss (Dabruzzi et al. 2011), unique ammonia excretion strategies (Gregory, 1977), and the ability to uptake a significant portion of their oxygen demand across skin and bucco-pharyngeal surfaces (Tamura et al. 1976). These adaptations allow mudskippers to move freely between pools and open mudflats at low tide, thereby gaining access to a wider array of food resources, and providing a means to effectively elude both aerial and aquatic predators (Nursall 1981).

Due to delineations in microhabitat conditions and competition for space, most mangroves are occupied by more than one mudskipper species (Nursall 1981, Swennen et al. 1995, Sayer 2005). Several studies document habitat partitioning of mudskipper fishes, in which each species occupies a well-defined mangal zone (Clayton 1993, Colombini et al. 1995, Takita & Ali 1999). The underlying mechanisms governing mudskipper habitat zonation are not well understood. Partitioning patterns have been linked to physical habitat conditions including substrate type (Chen et al. 2008) and food availability (Gibson 1982, Su & Lim 2016), or to physiological attributes such as thermal requirements (Gordon et al. 1985, Taylor et al. 2005), or oxygen extraction efficiency (Gordon et al. 1978, Clayton 1993). Various studies have attempted to link mudskipper location and movement patterns to oxygen uptake efficiency in water or air. Kok et al. (1998) found giant mudskippers *Periophthalmus schlosseri* Pallas, 1770 to be more efficient air breathers than Boddart's goggle-eyed goby *Boleophthalmus boddarti* Pallas, 1770. On the other hand, Tamura et al. (1976) found that Shuttles hopfish *Periophthalmus modestus* Cantor, 1842 and walking goby *Boleophthalmus pectinirostris* Linnaeus, 1758, both exhibit higher oxygen uptake in water than in air, and implied that air temperature had a greater influence on fish movements than oxygen uptake efficiency. Most mudskipper distribution studies are qualitative assessments that do not account for variation in habitat morphology and location, or competitive interactions resulting from differences in overall species composition (Clayton, 1993; Graham et al. 2007).

Common mudskippers *Periophthalmus kalolo* Lesson, 1831 and barred mudskippers *Periophthalmus argentilineatus* Valenciennes, 1837 offer a good model system for studying comparative physiological adaptations of mudskippers inhabiting different mangal zones. These species are widely distributed throughout the Indo-Pacific where they are not only sympatric (i.e., inhabiting the same geographic area) across most of their range, but also syntopic (i.e., living within the same habitat) in mangrove systems (Murdy 1989). In addition, both species forage on similar diets of polychaetes, crustaceans, and insects (Burhanuddin & Martosewojo 1979, Murdy 1989, Clayton 1993). Frith (1977) described spatial segregation in sympatric populations of *P. argentilineatus* and *P. kalolo* in Thailand mangles, noting that *P. argentilineatus* occupy shaded upper mangal environments among mangrove prop roots, while *P. kalolo* remain in the lower, exposed mudflat zones. Oxygen uptake values have been determined for *P. argentilineatus* in air and water (Gordon et al. 1969, Steeger & Bridges 1995), however, no study to date has assessed metabolic uptake in both water and air for *P. kalolo*, and it is unclear what influence, if any, respiratory responses may have on spatial distribution of these fishes. This study quantifies effects of changing respiratory media and temperature on metabolic rates of *P. kalolo* and *P. argentilineatus* collected from Hoga Island, Sulawesi, Indonesia. Specific objectives of our study were to 1) quantify oxygen uptake in air and water for both mudskipper species at high and low diel temperatures encountered in their mangal habitats, 2) use these oxygen uptake values to assess metabolic temperature sensitivity, i.e., temperature quotients (Q_{10}) for both species in air and water, and 3) interpret results relative to fish behavior and zonation patterns in Hoga Island mangal habitats.

Materials and Methods

SITE DESCRIPTION

Periophthalmus kalolo and *P. argentilineatus* were collected from June to August 2004 from a mangal habitat located on the northwest edge of Hoga Island (05° 27.53S, 123° 46.33E) in the Wakatobi National Park, Sulawesi, Indonesia. The Hoga mangal habitat is unusual in that it rests on a compacted coral formation that prevents deep mangrove root penetration. As a result, the red, *Rhizophora stylosa*, and yellow, *Ceriops* sp., mangroves that dominate the mangal remain small (< 3m in height) and the area has only a superficial covering (a few cm to approximately 0.2 m) of fine mud. The lower mangal is predominately open flat expanses of thin mud and exposed coral, and sparsely populated (less than 2% cover) with small *Ceriops*. During low tide, pools form in coral depressions and experience a high degree of insolation and rapid midday temperature increase. Conversely, the upper mangal zone is marked by a distinct increase in *Ceriops* and *R. stylosa*, that provide approximately 70% cover. The many small, but persistent tide pools that form among shaded mangrove prop roots and pneumatophores at low tide are much less affected by high midday temperatures than the exposed mangal pools. Pool temperatures collected over a three year period in upper and lower mangrove zones, and reported in part by Taylor et al. (2005), show that exposed lower mudflat regions routinely experience diel temperature fluctuations of up to 11.5°C while upper shaded mangal areas show temperature shifts no greater than 6.5°C. High daytime temperatures in the lower and upper mangal pools average 36.3 and 29.7°C, respectively, while average nighttime pool temperatures remain nearly identical (25.3 and 25.2°C, respectively).

COLLECTION, TRANSPORT, AND MAINTENANCE OF MUDSKIPPERS

Fish collections were made at night by immobilizing mudskippers with a bright light and trapping them in aquarium dip nets. Fish were immediately transported to the Hoga Marine Research Center and transferred into plastic, screen covered holding tanks (121 × 40 × 31 cm). Holding tanks contained approximately 6 cm of clean beach sand covered with seawater (~33 ppt). Emergent palm frond and driftwood pieces in holding tanks allowed fish free movement between water and air. During the holding period, tanks were kept in the shade at temperatures between 25 and 28°C. Holding tank seawater was changed twice daily and sand changed every 2-3 days to maintain habitat quality. Mudskippers were held for no more than four days and were fasted for 48 h before metabolic trials to ensure measurements were taken in a post absorptive state. Each fish was used in only one aerial or aquatic experiment. Upon completion of trials, all fish were released at their site of capture.

AERIAL RESPIRATION MEASUREMENTS

A Gilson single-valve differential respirometer was used to estimate routine aerial oxygen uptake for 32 *P. kalolo* and 32 *P. argentilineatus*. The manometric approach has proven effective in estimating metabolic rates of fishes under various environmental conditions including, during submersion (Mitz & Newman 1989, Wuenschel et al. 2004), when burrowed (Quinn & Schneider 1991), and while breathing air (Abel et al. 1987, Hill et al. 1996). For manometric respirometry approaches and assumptions see Gilson (1963), Cech (1990), and Lighton (2008). Manometric measures of fish metabolic rate are common (Chan 1990, Varo et al. 1993) and have accuracies consistent with polarographic (Peck & Moyano 2016) and Winkler titration methods (Lawton & Richards 1970, Peck & Moyano 2016). The respirometer used in our experiments was described by Lee et al (1987), and Chan (1990), and is specifically designed for measuring oxygen uptake in air-breathing mudskipper fishes. The respirometer was modified to accommodate mudskippers up to 6 cm in standard length by using 125-ml, glass Erlenmeyer sidearm flasks with Gilson-compatible ground glass necks as reaction and reference vessels (Haswell & Randall 1976). The Tygon[®] tubing was replaced with glass tubing to reduce the potential for leaks (Theodore et al. 1972).

The respirometer was comprised of five, single valve channels, each with its own reaction and reference flask. Prior to trials, flasks were washed with antibacterial soap, rinsed, and dried. A strip of Whatman® filter paper (3.0 × 0.5 cm) saturated with 20% KOH solution was added to the flask sidearm to remove carbon dioxide, and 5 ml of seawater was added to the flask to maintain humidity at 100%. The apparatus was assembled and pressure-tested without fish prior to each experimental run. Randomly selected mudskippers were then placed singly into one of four reaction flasks. The fifth flask, identical in all respects but containing no fish, was simultaneously run to control for non-fish respiration. Flasks were vented to the atmosphere and submerged into a constant-temperature, recirculating water bath for 45 minutes to allow fish and equipment to reach water bath temperatures. Trials were initiated by closing reaction flask vents and opening the main valve connecting the reference flask and manometer to the reaction chamber. Manometer fluid levels dropped as oxygen was consumed, and total consumption (μL) was determined by slowly adding air to the reaction flask via a calibrated, micrometer-controlled piston until manometer fluid returned to its starting level. Consumed oxygen was read directly from the micrometer at 10-minute intervals and converted to mg at standard temperature and pressure, dry. Total oxygen uptake was estimated as mg of oxygen consumed per hour (mg h^{-1}) for each mudskipper. A typical trial lasted approximately 2 hours.

AQUATIC RESPIRATION MEASUREMENTS

Routine aquatic oxygen uptake of 30 *P. kalolo* and 30 *P. argentilineatus* was quantified using flow-through respirometry techniques (see Cech 1990, Lighton 2008). For each trial, four randomly selected fish were placed, one each, into glass, 125-mL sidearm Erlenmeyer flasks capped with a #11 black rubber stopper. A control flask, identical in all respects but containing no fish, was simultaneously run to control for non-fish respiration. Clean, filtered, oxygen-saturated seawater at the appropriate treatment temperature entered at the bottom of the respirometer through a glass tube in the stopper, and exited through the flask sidearm. At the start of each trial, respirometer flow rates were set to between 5 and 20 ml min^{-1} depending on fish mass, such that the difference between inflow and outflow oxygen concentration (mg L) never fell below 85% saturation (Steffensen 1989, Cech 1990). Respirometer flasks were submerged into a constant-temperature, recirculating water bath for 60 minutes during which fish and equipment equilibrated to the appropriate treatment temperature, and outflow oxygen concentrations stabilized. Inflow and outflow oxygen concentrations were monitored at 30 minute intervals using Yellow Springs Instruments Model 55 oxygen meters, with values corroborated by Winkler titration (Cox 1990). Total oxygen uptake values were determined for each mudskipper as the mean difference between inflow and outflow oxygen content of the replicate samples, multiplied by the respirometer flowrate in liters per minute. Not including stabilization times, trials lasted between two and three hours.

TEMPERATURE QUOTIENT MEASUREMENTS

Metabolic thermal sensitivity of *P. kalolo* and *P. argentilineatus* was quantified as a temperature quotient, or Q_{10} value (Schmidt Neilson 1997). Metabolic rates used for Q_{10} calculations (Table 2) were derived from *P. kalolo* and *P. argentilineatus* in air acclimated at 26°C ($n=16$ each group), and *P. kalolo* and *P. argentilineatus* in water acclimated at 26°C and acutely exposed to 32.0°C ($n=15$ each group). The four treatments selected for these experiments approximate conditions routinely encountered by mudskippers in Hoga mangal habitats (Taylor et al. 2005). The low treatment temperature was maintained by cooling water in the recirculating water bath with an IceProbe® chiller, whereas the high temperature was produced using an Ebo-Jaeger® 200W submersible heater. A Glas-Col® Minitrol intermittent power supply was used to maintain treatment temperatures within $\pm 0.2^\circ\text{C}$ of the set point value. Mudskippers in high temperature trials were exposed to an acute $0.1^\circ\text{C}/\text{min}$ increase in temperatures from 26.0°C until reaching 32.0°C. Fish were then held at the higher temperature for an additional 45 minutes to allow fish and equipment to reach the appropriate

temperature before metabolic measurements were made. The Q_{10} values in air and water were calculated using the following equation (Schmidt-Nielsen 1997):

$$Q_{10} = (K_2 \div K_1)^{\frac{10}{T_2 - T_1}}$$

where, Q_{10} is the temperature quotient and K_2 and K_1 are mean metabolic rates at temperatures T_2 (32.0°C) and T_1 (26.0°C), respectively. At the end of each trial sequence, mudskippers were weighed (wet mass ± 0.1 g), measured (standard length ± 0.5 mm), and returned to holding tanks until released.

STATISTICAL ANALYSIS

Metabolic rates for common and barred mudskippers in air and water were adjusted for allometric mass effects by dividing total oxygen consumed (mg h^{-1}) by wet mass (g) raised to the power of 0.75 (Gillooly et al. 2001, Brown et al. 2004, Moses et al. 2008). Resulting metabolic values were reported as mass-adjusted metabolic rate (\pm SE) using the units $\text{mg hr}^{-1} \text{g}^{(0.75)-1}$ (see Table 2). Potential differences in treatment group mean mass values (i.e., mass values for *P. kalolo* and *P. argentilineatus* in air and water treatment groups) were tested using a one-way ANOVA. If appropriate, Tukey's multiple range test (MRT) was used to identify statistical relationships between treatment means. A three-way ANOVA was used to determine whether the experimental factors media, temperature, and species, or their interactions, had a statistically significant effect on mudskipper oxygen uptake. Statistical relationships between main effects mean values were explored using Tukey's post hoc test. All statistical decisions were based on an alpha level of 0.05.

Results

As a consequence of having selected mudskippers large enough to produce measurable oxygen uptake but small enough to fit easily into respirometer reaction flasks, fishes in all eight experimental treatment groups (separated by media, temperature, and species) were of similar size (Table 1). One-way analysis of variance confirmed that there were no statistically significant differences in mean standard length ($F_{7,123} = 0.05$, $P = 0.819$) or mean wet mass ($F_{7,123} = 1.41$, $P = 0.238$) between treatment groups.

Metabolic rates of common and barred mudskipper were not influenced by interactions between experimental factors. Three-way analysis of variance found no significant interactions between media and/or temperature, and/or species ($P \geq 0.236$ for all four interaction terms), but did detect statistically significant differences between main effect levels for two of the three experimental factors. Tukey post hoc tests ($\alpha = 0.05$) revealed that both species had significantly higher oxygen uptake in air than in water, with *P. argentilineatus* increasing oxygen uptake by ~35%, and *P. kalolo* uptake increasing by ~50%. In addition, both *P. kalolo* and *P. argentilineatus* exhibited a significant increase in oxygen uptake when media temperatures were increased from 26.0 to 32.0°C. Changes in oxygen uptake by *P. kalolo* and *P. argentilineatus* were statistically indistinguishable regardless of media or temperature, suggesting species had no significant effect on metabolic rate. It should be noted that resting and active metabolic rates were not determined for *P. kalolo* and *P. argentilineatus* in the present study, and that future experiments that include metabolic scope may provide a greater insight into the relationship between metabolic rate and habitat partitioning in these species.

Overall, temperature quotient values were quite similar between species as well as between media (Table 2). Metabolic rates for both mudskipper species were moderately insensitive to temperature increase, with Q_{10} in air and water ranging from approximately 1.4 to 1.5 (Table 2). Temperature quotients for *P. argentilineatus* were slightly higher in air than in water ($Q_{10} = 1.56$ and 1.42, respectively), while values for *P. kalolo* were similar in both water and air ($Q_{10} = 1.38$ and 1.39, respectively).

Table 1. Mean wet mass (g) and standard length (cm) for common (*Periophthalmus kalolo*) and barred (*Periophthalmus argentilineatus*) mudskippers used in air and water respirometry trials.

| Medium | Wet Mass (g) | | | Standard Length (cm) | |
|---------------------------|--------------|------|----|----------------------|------|
| | Mean | SE | N | Mean | SE |
| <i>P. argentilineatus</i> | | | | | |
| Air | 3.24 | 0.26 | 16 | 5.87 | 0.16 |
| Water | 3.47 | 0.20 | 15 | 5.66 | 0.29 |
| Pooled | 4.41 | 0.23 | 31 | 4.63 | 0.23 |
| <i>P. kalolo</i> | | | | | |
| Air | 3.17 | 0.24 | 16 | 5.98 | 0.18 |
| Water | 3.47 | 0.19 | 15 | 6.13 | 0.29 |
| Pooled | 4.61 | 0.24 | 31 | 4.69 | 0.24 |

Table 2. Mass adjusted oxygen uptake rates ($\text{mg g}^{(0.75)}\text{-1 hr}^{-1}$) at 26.0 and 32.0°C and temperature quotient values (Q₁₀) for common (*Periophthalmus kalolo*) and barred (*Periophthalmus argentilineatus*) mudskipper in air and water.

| Species | Mass-adjusted oxygen consumption at 26.0°C | | | Mass-adjusted oxygen uptake at 32.0°C | | | Q ₁₀ |
|---------------------------|--|-------|-------|---------------------------------------|-------|-------|-----------------|
| | N | Mean | SE | N | Mean | SE | |
| <i>Aerial</i> | | | | | | | |
| <i>P. argentilineatus</i> | 16 | 0.263 | 0.015 | 16 | 0.343 | 0.019 | 1.56 |
| <i>P. kalolo</i> | 16 | 0.295 | 0.016 | 16 | 0.358 | 0.016 | 1.38 |
| <i>Aquatic</i> | | | | | | | |
| <i>P. argentilineatus</i> | 15 | 0.199 | 0.016 | 15 | 0.246 | 0.020 | 1.42 |
| <i>P. kalolo</i> | 15 | 0.198 | 0.008 | 15 | 0.241 | 0.013 | 1.39 |

Discussion

Distribution and habitat preference of emerged *P. kalolo* and *P. argentilineatus* on the Hoga mangrove site were similar to patterns previously described for these species by Frith (1977) in western Thailand, and Nursall (1981) in Queensland, Australia. At all three sites, *P. kalolo* frequented pools on sun-exposed mudflats, whereas *P. argentilineatus* preferred cooler, shaded pools and mudflats. Likewise, shuttling between mudflat and pool habitats was evident in both species at all locations (Frith 1977; Nursall 1981). *Periophthalmus argentilineatus* and *P. kalolo* appear to be well adapted to any differences in insolation and tidal patterns each experience within their respective zones. It seems likely that the mudskippers gain some benefit from exploiting differences in mangal conditions. *Periophthalmus kalolo* is only one of a few species capable of inhabiting sun-exposed mangrove environments, whereas *P. argentilineatus* avoids the highest temperatures by taking advantage of cooler shaded pools. Although the mechanisms driving this segregation pattern are not obvious, species separation would decrease interspecific competition, thereby assuring that both fishes have nearly exclusive access to resources in their respective zones (see Taylor et al. 2005).

Mudskipper success in mangal environments has been linked to respiratory efficiency in air and water (Chan 1990, Clayton 1993), yet oxygen uptake estimates for the group are frequently conflicting. Clayton (1993) found that of the five periophthalmid mudskipper species studied between 1969 and 1987, only Shuttles hopppfish, *P. modestus*, and the gold-spotted mudskipper, *P. chrysopilos* Bleeker, (1853) showed significant differences in oxygen uptake between media (Table 3). In both cases, the highest oxygen uptake rates were measured in water. Gordon et al. (1969) reported no significant difference between aerial and aquatic oxygen uptake in groups of small *P. argentilineatus* [= *P. sorbrinus*] acclimated at different salinities. A closer look at the Gordon et al. (1969) data, however, reveals higher uptake in air than water for all treatment groups (Table 3), with fish increasing their uptake by 10 to 60%. In the present study, mudskippers at 26.0°C exhibited approximately 50% higher oxygen uptake in air than in water, and aerial metabolic values at 32°C were approximately 45% higher than aquatic values (Table 2). A similar respiratory response was seen in the New Guinea mudskipper *Periophthalmus novaeguineensis* Eggert, 1935 [= *P. cantonensis*] tested at 30°C which exhibited 44% higher oxygen uptake in air than in water (Chang 1990).

Although the mechanisms resulting in differential oxygen uptake between media by *P. argentilineatus* and *P. kalolo* were not investigated directly, lower oxygen content and higher ventilation costs might explain lower oxygen uptake in water relative to air, whereas, higher aerial uptake during emergence could reflect increased activity levels in air, or shifts in physiological processes such as internal nitrogenous management. Regardless of the systems involved, efficient aerial oxygen extraction is consistent with high emersion tolerance, and a useful adaptation for periophthalmids occupying high mangal regions where long emersion times are common.

Mangrove thermal profiles are also a potentially important attribute shaping distribution and activity patterns of sympatric mudskipper species (Polgar & Crosa 2009). Diel changes in mangal air and water temperatures expose mudskippers to some of the harshest thermal conditions encountered by any fish (Stebbins & Kalk 1961, Clayton 1993, Chen et al. 2008). Mahadevan & Ravi (2015) have suggested that temperature extremes leave mudskippers vulnerable to temperature-mediated shifts in metabolic demand. When *P. argentilineatus* and *P. kalolo* were exposed to an acute temperature increase in the laboratory of 26 to 32°C (simulating diel temperature shifts encountered in the field), aerial and aquatic metabolic rates increased by ~25% in both species. Outside of the laboratory, mudskippers may mitigate some of the additional metabolic burden imposed by higher temperatures by wetting skin surfaces (Tytler & Vaughan 1983) or rolling in mud (Clayton 1993) to take advantage of evaporative cooling, or by altering skin color to reduce radiant heating rates (Stebbins & Kalk 1961, Chen et al. 2008). Mitigation tactics may be especially well

Table 3. Comparisons of aerial and aquatic oxygen uptake for five periophthalmid (subfamily: Oxudercinae) mudskipper species between 1969 and present.

| Species Authority Common Name | Wet Mass g | | | Temperature °C | Oxygen Uptake ml O ₂ ·kg·hr ⁻¹ | | Source |
|--|---------------|------|------|-------------------|---|-------|--------------------------|
| | Min | Mean | Max | | Air | Water | |
| <i>Periophthalmus argentilineatus</i> Valenciennes, 1837 Barred mudskipper | 2.2 | — | 9.2 | 20 | 63 | 65 | Milward, 1974 |
| <i>Periophthalmus argentilineatus</i> Valenciennes, 1837 Barred mudskipper | 1.0 | — | 15.0 | 24 | 94 | 84 | Gordon et al., 1969 |
| <i>Periophthalmus argentilineatus</i> Valenciennes, 1837 Barred mudskipper | 1.0 | 3.2 | 6.8 | 26 | 62 | 53 | Present Study |
| <i>Periophthalmus kalolo</i> Lesson, 1831 Common mudskipper | 0.9 | 4.6 | 7.7 | 26 | 73 | 49 | Present Study |
| <i>Periophthalmus modestus</i> Cantor, 1842 Sharles hopppfish | 4.0 | — | 8.0 | 20 | 97 | 167 | Tamura et al., 1976 |
| <i>Periophthalmus modestus</i> Cantor, 1842 Sharles hopppfish | 0.5 | 3.0 | 5.0 | 20 | 106 | 85 | Gordon et al., 1978 |
| <i>Periophthalmus chrysospilos</i> Bleeker, 1852 Gold-spotted mudskipper | 6.0 | — | 12.0 | 25 | 306 | 378 | Lee et al., 1987 |
| <i>Periophthalmus chrysospilos</i> Bleeker, 1852 Gold-spotted mudskipper | 20.0 | — | 25.0 | 29 | 48 | 88 | Natarajan & Rajulu, 1983 |
| <i>Periophthalmus novaequinaensis</i> Egbert, 1935 New Guinea mudskipper | 4.6 | — | 6.7 | 25 | 90 | 103 | Milward, 1974 |

suitable to highly terrestrial *P. kalolo* that may experience long exposure periods to direct sunlight (Gordon et al. 1978). Conversely, *P. argentilineatus* may be less dependent on cooling tactics due to their greater proclivity for shaded mangrove areas (Nursall 1981).

Metabolic rate trials also revealed reduced temperature quotient responses for *P. argentilineatus* and *P. kalolo* mudskippers in both air and water (Table 2). The typical metabolic response of most poikilotherms, when faced with an acute increase in ambient temperature, is a doubling or tripling of metabolic rate with each 10°C increase in environmental temperature, i.e., a respective Q_{10} value equal to 2 or 3 (Schmidt-Neilsen 1997). Gordon et al. (1978), for example, reported Q_{10} values of 2.7 in water and 2.5 in air for *P. novaeguineensis* at temperatures between 20 and 30°C (Table 3). High metabolic sensitivity has also been reported in non-emergent intertidal fishes. Woolly sculpin, *Clinocottus analis* Girard, 1858, and the rockpool blenny, *Hypsoblennius gilberti* Jordan, 1882, for example, exhibit respective Q_{10} values of 3.3 and 2.6 following a 10°C seasonal temperature increase (Graham 1970). Conversely, Campbell & Davies (1975) estimated an aerial Q_{10} value of 1.23 at temperatures between 25 and 30°C for the air-breathing shanny, *Lipophrys pholis* Linnaeus, 1758. *Periophthalmus kalolo* and *P. argentilineatus*, in the present study also exhibited lower than expected Q_{10} values (between 1.38 and 1.56) in both media (Table 2). The temperature quotient is an index of metabolic thermal sensitivity. As previously noted, most intertidal fish exhibit a two to three fold increase in biological rate functions with every 10°C increase in ambient temperature. From an energetic perspective, exponential metabolic increases that may be tolerated in the short term can be detrimental in the long term. *Periophthalmus kalolo* and *P. argentilineatus* exhibit Q_{10} values less than two. The diminished Q_{10} responses in these species reduce the fishes' metabolic burden in hyperthermic mangal environments, where rapid temperature increases in air and water are common and food can be scarce (Taylor et al. 2005, Eme & Bennett 2010).

Spatial separation between mudskipper species within mangal habitats has been discussed in some detail (Frith 1977, Nursall 1981, Swennen et al. 1995; Takita et al. 1999, Mahadevan & Ravi 2015); however, the factors and interactions dictating partitioning are not immediately clear. Some mudskipper populations seem to segregate based on preferences for a specific substrate type, but as Clayton (1993) points out, a species living on soft mud in one area may occupy sand, or rocky shoreline at a nearby location. Air-breathing efficiency may influence species distribution in some areas, especially among the more terrestrial periophthalmid groups occupying high mangal zones with long emersion times (Tamura et al. 1976, Colombini et al. 1995, Kok et al. 1998). Often, however, no apparent differences in respiratory efficiency are noted among mudskipper species. For example, oxygen uptake for *P. argentilineatus* and *P. kalolo* in our study were statistically similar to one another. Currently available data seem to suggest that while specialized physiological adaptations, including a complex of air breathing structures (skin, gills, and bucco-pharyngeal cavity), cutaneous desiccation resistance, and a unique nitrogen management system are necessary for mudskipper survival in austere mangal environments, they do not reliably predict habitat partitioning patterns. Other physiological factors, such as desiccation and thermal relationships, may provide further insights into modeling mudskipper distributions, but may not provide plausible explanations in all cases. The main tenet of physiological ecology is that environmental conditions act on physiology to produce behavior (Fry 1946). If so, there may be no "uniform" pattern of mudskipper zonation, but rather each habitat arrangement is the unique result of broad environmental tolerances, being acted on by various environmental conditions existing within individual habitats.

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