

A COMPARATIVE STUDY OF THERMAL TOLERANCE, HYPOXIA
TOLERANCE AND WATER LOSS RESISTANCE
IN TWO FAMILIES OF INDO-PACIFIC
AMPHIBIOUS FISHES

by

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ABSTRACT

A COMPARATIVE STUDY OF THERMAL TOLERANCE, HYPOXIA TOLERANCE, AND WATER LOSS RESISTANCE IN TWO FAMILIES OF INDO-PACIFIC AMPHIBIOUS FISHES

Joni Elizabeth Wright

Two families of amphibious fishes, mudskippers (Family Gobiidae) and rockskippers (Family Blenniidae), occupy the mangal habitat of Hoga Island, Sulawesi, Indonesia. Tidepools serve as an aquatic refuge during diurnal low tide events; however, harsh conditions including high temperatures and low dissolved oxygen exist. Capable of bimodal respiration, these fishes are able to escape to land when tidepool environments become severe, although exiting tidepools to escape austere conditions may create a risk of dessication. Both families have the ability to utilize terrestrial environments; however, temporal resource partitioning of media has been observed between mudskippers and rockskippers. Three species of rockskippers, lined, *Istiblennius lineatus*, streaky, *I. dussumieri*, and Ambon, *Paralticus amboinensis*, along with common and barred mudskippers were collected from the mangals of Hoga Island. To elucidate the role physiological adaptations play in resource partitioning, I compared thermal tolerance, hypoxia tolerance, and water loss resistance between mudskippers and rockskippers. Rockskippers demonstrated higher aquatic thermal and hypoxia tolerance when compared to mudskippers. Common mudskippers showed no significant differences in thermal tolerance between media, while barred mudskippers revealed higher thermal

tolerance in air over water. It is likely that a combination of thermal and hypoxia tolerance contribute to resource partitioning between these groups.

CHAPTER I

AERIAL AND AQUATIC HEAT TOLERANCE OF AMPHIBIOUS INDO-PACIFIC FISHES

Introduction

Mangal habitats are often characterized by alternating periods of tidal exposure and inundation. During low tide, tidepools may be the only aquatic refuge available for resident fishes in these habitats; however, pool temperatures can become limiting in equatorial regions where water temperatures increase rapidly during mid-day. For example, on Hoga Island, Sulawesi, Indonesia (0.5°27.53'S 123°46.33'E), mid-afternoon tidepool temperatures may exceed 40°C, an increase of nearly 20°C from nighttime lows (Taylor et al., 2005). Fishes remaining in tidepool habitats must deal with rapidly increasing water temperatures, rising metabolic demand and concomitant decrease in oxygen levels (Gordon et al., 1985; Zander et al., 1999; Taylor et al., 2005), resulting in some of the most physiologically demanding aquatic conditions known. For some fishes, adaptations allowing an amphibious existence provide an opportunity to exit harsh tidepool conditions. Amphibious fishes residing in mangal habitats on Hoga Island include at least two mudskipper species (family Gobiidae) — common, *Periophthalmus kalolo*, and barred mudskippers, *P. argentilineatus* — and three rockskipper species (family Blenniidae) — lined, *Istiblennius lineatus*, streaky, *I. dussumieri*, and Ambon, *Paralticus amboinensis* rockskippers. While these fishes are syntopic, each family

exhibits varying degrees of terrestriality that may promote interspecific and intraspecific spatial and temporal partitioning of mangal habitats. Mudskippers are highly amphibious, spending much of their time on land (Gordon et al., 1978; Gordon et al., 1985). Common mudskippers, however, are more likely to be found on mangal mudflats, whereas barred mudskippers prefer to remain near mangrove prop roots in the upper mangals (Frith et al., 1976; Frith, 1977; Clayton, 1993). Both species emerge at low tide even during the heat of the day; although the need to keep respiratory surfaces moist probably keeps them near water (Graham, 1997). Conversely, observations of rockskipper behavior indicate a less terrestrial nature (Zander, 1972; Graham, 1973; Martin, 1995). Rockskippers spend little time in direct sunlight, exiting pools at night but not venturing far from water (Graham, 1973). Rockskipper behavior is not as well documented, and there are relatively few accounts of terrestrial behavior (Graham, 1997).

Differing emergence patterns between amphibious blennies and gobies no doubt reflect divergent strategies for dealing with changing water conditions from high to low tide and likely influence resource use between families. Most fishes are ideal ectotherms incapable of maintaining body temperatures independent of their medium. Consequently, temperature has a profound effect on fish physiology and behavior (Bennett and Beitinger, 1997; Beitinger et al., 2000) and may be a decisive factor in dictating the diurnal behavioral patterns seen in mudskipper and rockskipper species on Hoga Island.

Amphibious fishes, unlike their fully aquatic relatives encounter a unique set of thermal problems owing to their ability to move freely between air and water. The existing mudskipper and rockskipper literature shows an interesting dichotomy with the former group being much better studied than the latter group. Previous mudskipper

studies widely assume that temperature is the major abiotic factor dictating movement and distribution. However, most of these studies have focused more on physiology than behavior. For example, several studies specifically quantify temperature's effect on internal body temperature in sun and shade (Tytler and Vaughn, 1983), gonadal development (Shiota et al., 2003) and Critical Thermal Maxima (CTMax) under normoxic and hypoxic conditions (Taylor et al., 2005). Only a single study by Gordon et al. (1985) investigated mudskipper thermal preference range. In contrast, thermal studies on rockskippers have taken a more behavioral approach. Brown et al. (1992) reported temperatures at which rockskippers would leave tidepools, and Graham (1973) determined the upper lethal temperature in air for the eastern tropical Pacific Ocean rockskipper, *Mnierpes macrocephalus*.

Considering the unique ability of mudskippers and rockskippers to utilize both media, a study of temperature tolerance values in air and water could reveal a great deal about movement and behavior in both amphibious fish families inhabiting Hoga's mangals. The purpose of my research is to assess behavioral and physiological responses of Hoga's two mudskipper and three rockskipper species when exposed to increasing temperatures. The specific goals of my study are to quantify high temperature tolerance (i.e., CTMax) and ER temperatures of all five species in both air and water. I will interpret these results in light of the fishes' emergence behavior and distribution.

Materials and Methods

Collection and Maintenance of Fish

Common and barred mudskippers (n=34 and n=34, respectively) as well as lined, streaky, and Ambon rockskippers (n=32, n=33, and n=32, respectively) were collected from mangal habitats on Hoga Island, Sulawesi, Indonesia. Mudskippers were collected at night by immobilizing them with a bright light and capturing them in aquarium dip nets. Common mudskippers were collected from the open mudflats, while barred mudskippers were collected from the upper mangal areas. Rockskippers were netted from tide pools or rock ledges during daytime or nighttime. Following capture, fishes were transported to the Hoga Research Center and transferred into holding tanks. While mudskippers and rockskippers were housed separately, no attempt was made to separate species within families. Mudskippers were held in plastic holding tanks (121 × 39.6 × 30.8 cm) containing beach sand covered with clean seawater and at densities of 15 to 20 fish per tank. Emergent palm frond and driftwood pieces allowed fish free movement between air and water. Rockskippers were housed in round, plastic 5-L tanks at densities of 3 to 5 fish per tank. Airstones provided vigorous aeration to each tank. Mudskipper and rockskipper holding tank temperatures were kept between 26 and 28°C, with seawater changed twice daily and sand changed every 2-3 days to maintain habitat quality. Fish were fed TetraMin© flake food (rockskippers) or fresh chopped tuna (mudskippers) daily; however, animals were not fed 24 hours prior to or during trials. Upon trial completion, all fishes were released at site of capture. This study was approved by the UWF Animal Care and Use Committee (Appendix A).

Determination of Critical Thermal Maxima and Escape Temperature

High temperature tolerance estimates in air and water were determined for 16-18 individual fish from each of the five species (Table 1). Each fish was used only once in either an aerial or aquatic heat tolerance trial. Tolerance values were quantified using the Critical Thermal Methodology, CTM (Cowles and Bogert 1944; Beitinger et al. 2000) and expressed as CTMax temperatures (Cox 1974; Becker and Genoway 1979; Palidino et al. 1980; Beitinger et al. 2000). For each CTM trial, 5 to 10 fish were placed, one each, into 150-mL glass CTM chambers. In aquatic trials (Figure 1), CTM chambers were filled with clean, well-aerated seawater at 26°C, and air was bubbled through each beaker to keep oxygen levels high and promote uniform heating during trials. In aerial trials (Figure 2), a layer of filter floss covered by seawater-saturated Whatman filter paper was placed in the bottom of the CTM chambers to keep humidity levels high and prevent fish from coming in direct contact with the glass. Fish climbing the CTM chamber sides were gently shaken back to the bottom to prevent heat transfer from the glass resulting in the fish experiencing higher than the air temperature in the chamber. Transparent netting placed over the top of each CTM chamber prevented fish from escaping, while still allowing observation. Chambers were then suspended in a recirculating water bath and temperature in the bath was increased at a rate of $0.30^{\circ}\text{C}\cdot\text{min}^{-1}$ via a 1500-W heating element connected to an intermittent switch set at a 70% heating interval. Water or air temperature in each chamber was monitored continuously with a Sontek Model BAT-12 digital thermometer. Heating rates between 0.2 and $0.5^{\circ}\text{C}\cdot\text{min}^{-1}$ have been shown to be fast enough to prevent thermal

Table 1. Air And Water Critical Thermal Maxima

Common name Species	Air CTM (°C)			Water CTM (C°)		
	N	Mean	S.D.	N	Mean	S.D.
Family Blenniidae						
Ambon Rockskipper <i>Paralticus amboinensis</i>	16	38.8	0.71	16	40.7	0.42
Lined Rockskipper <i>Istiblennius lineatus</i>	16	38.7	0.52	16	40.7	0.55
Streaky Rockskipper <i>Istiblennius dussumieri</i>	17	38.9	0.83	16	41.1	0.61
Family Gobiidae						
Barred Mudskipper <i>Periophthalmus argentilineatus</i>	16	41.4	0.95	18	40.7	0.41
Common Mudskipper <i>Periophthalmus kalolo</i>	16	41.3	1.78	18	41.0	0.43

Note. Critical Thermal Maxima (CTMax) in air and water for three rockskipper species (Family Blenniidae), and two mudskipper species (Family Gobiidae) inhabiting mangal swamps around Hoga Island, southeast Sulawesi, Indonesia. Thermal tolerance values were determined for field acclimatized fish.

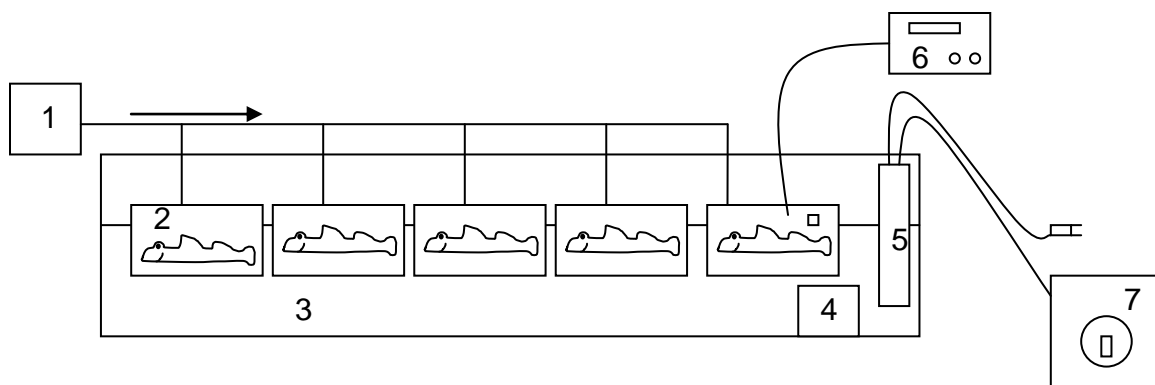


Figure 1. Apparatus to determine Critical Thermal Maxima (CTMax) in water with the following equipment: 1) aerator, 2) critical thermal chamber, 3) circulating water bath, 4) powerhead, 5) 1500 W heater, 6) digital thermocoupler, and 7) intermittent switch. Arrow indicates direction of air being pumped from the aerator to each chamber.

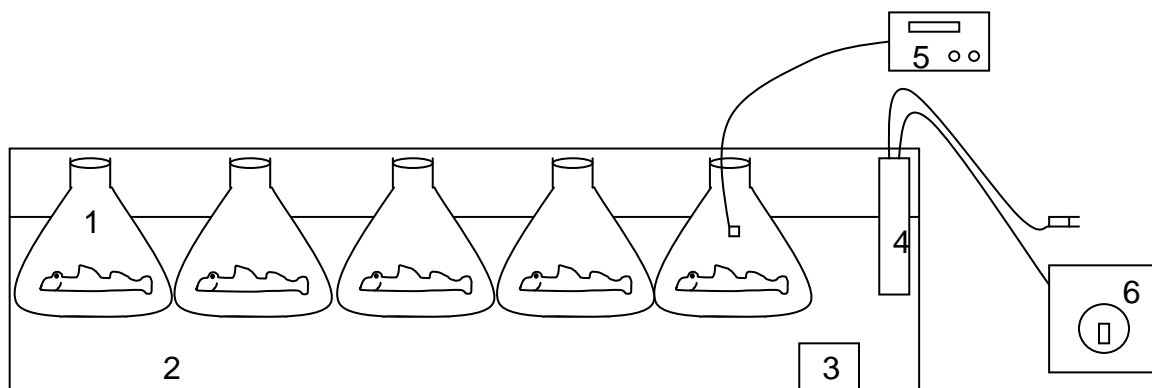


Figure 2. Apparatus to determine Critical Thermal Maxima (CTMax) in an aerial environment with the following equipment: 1) critical thermal chamber, 2) circulating water bath, 3) powerhead, 4) 1500 W heater, 5) digital thermocoupler, and 6) intermittent switch.

acclimation but slow enough to allow fish body temperature to track water temperatures for fish with mass and shape attributes similar to those in my experiments (Cox, 1974; Becker and Genoway, 1979). Likewise, Hutchinson (1961) found no appreciable differences between body temperature and air or water temperature when heating rates were maintained between 0.3 and 1.0 °C·min⁻¹.

Fish were carefully observed and temperatures recorded as each fish exhibited an ER and loss of equilibrium (LOE) (aquatic trials) or loss of righting response (LORR) (aerial trials). ERs, when present, were defined (for both aquatic and aerial trials) as persistent, strong attempts to leap from the CTM chamber lasting for at least 1 min (Taylor et al., 2005). LOE has been defined as the inability of the fish to maintain dorsoventral orientation for at least 1 min (e.g., Coutant, 1969; Bennett et al., 1998; Fangue et al., 2001) whereas LORR has been identified as the inability of the fish to right itself when turned on its side (Kaufmann and Bennett, 1989; Bennett and Beitinger, 1997; Hutchison, 1961). Upon LOE or LORR observation, the chamber temperature was recorded, the fish weighed (wet weight \pm 0.01g), measured (standard length \pm 0.1 cm) and returned to acclimation temperatures to recover. ER temperatures and CTMax in air and water were calculated for each species as the arithmetic mean of the collective thermal points at which the appropriate endpoint was reached (Lowe and Vance 1955, Cox 1974, Beitinger et al., 2000).

Statistical Analysis

Statistical comparisons of CTMax were made using three-factor (species \times media \times mass) Analysis of Variance (ANOVA). The CTM data were non-normally distributed

and so were normalized using a normal scores transformation prior to ANOVA analysis. To simplify the inclusion of mass into the ANOVA model, all fish species were grouped into one of four mass classes according to statistical quartiles (<0.64g, 0.64 – 1.16g, 1.16 – 2.61g, and >2.61g) prior to analysis. Differences in CTMax between media for each species were then compared using contrast analyses with a Bonferroni correction ($\alpha=0.01$). Statistical comparisons of mean escape temperatures in water for all five species were made using a one-factor, nested (by trial) ANOVA followed by a Tukey multiple comparison test (MCT) where significant differences were found. Rockskipper fishes displayed no escape response in air; therefore, Student's t-test was used to compare mean ER temperatures between the two mudskipper species and between media within each mudskipper species. All statistical decisions for ER were based on $\alpha = 0.05$.

Results

Fishes in my study showed differing patterns of thermal tolerance relative to media, family, and species (Figure 3). Three-factor ANOVA for CTMax (Table 1) found a significant interaction between species and media ($F_{4,155}=21.05$, $p<0.0147$), but no significant interactions were seen between species and mass ($F_{11,131} = 1.75$, $p=0.0689$), between media and mass ($F_{3,131}=0.61$, $p=0.6081$; Tables 2 and 3), or between species, media, and mass ($F_{7,131}=1.22$, $p=0.2956$). Contrast analyses showed that all three rockskipper species had significantly higher thermal tolerance in water than air ($p<0.0001$), whereas thermal tolerance of the barred mudskipper was significantly higher in air than water ($p=0.0013$), and common mudskipper showed no significant difference in tolerance between media ($p=0.0407$).

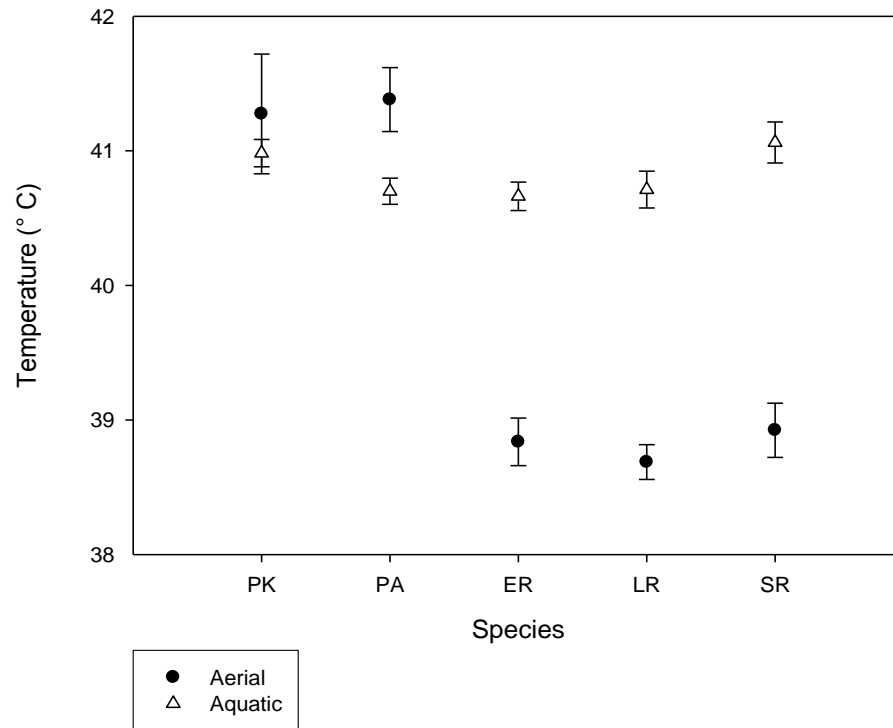


Figure 3. Mean aerial and aquatic Critical Thermal Maxima (CTMax) with standard error bars for five species of amphibious fishes on Hoga Island, Sulawesi, Indonesia. Species are represented as PK = *Periophthalmus kalolo*, PA = *Periophthalmus argentilineatus*, ER = *Paralticus amboinensis*, LR = *Istiblennius lineatus*, and SR = *Istiblennius dussumieri*. Aerial CTMax are denoted with filled circles, and aquatic CTMax are denoted with open triangles.

Table 2. Aerial Trial Species Wet Mass And Standard Length

Common Name Species	N	Wet Mass (g)			Standard Length (mm)		
		mean	S.D.	Range	Mean	S.D.	Range
Family Blenniidae							
Ambon Rockskipper <i>Paralticus amboinensis</i>	16	1.39	0.952	0.38 - 3.78	46.47	8.452	30.73 - 66.23
Lined Rockskipper <i>Istiblennius lineatus</i>	16	1.00	1.211	0.15 - 4.61	40.47	15.554	21.08 - 75.45
Streaky Rockskipper <i>Istiblennius dussumieri</i>	17	1.08	0.676	0.33 - 3.15	42.14	8.167	28.70 - 61.14
Family Gobiidae							
Barred Mudskipper <i>Periophthalmus argentilineatus</i>	16	3.18	0.958	1.65 - 4.59	49.89	16.464	22.61 - 71.69
Common Mudskipper <i>Periophthalmus kalolo</i>	16	3.04	1.076	1.76 - 5.86	60.39	8.675	46.18 - 79.86

Note. Mean wet mass and standard length determined for three species of rockskippers (Family Blenniidae) and two species of mudskippers (Family Gobiidae) on Hoga Island, Sulawesi, Indonesia used in aerial Critical Thermal Maxima (CTMax) trials.

Table 3. Aquatic Trial Species Wet Mass And Standard Length

Common Name	N	Wet Mass (g)			Standard Length (mm)		
Species		mean	S.D.	Range	mean	S.D.	Range
Family Blenniidae							
Ambon Rockskipper <i>Paralticus amboinensis</i>	16	1.11	0.486	0.51 - 2.40	43.32	8.222	28.53 - 60.45
Lined Rockskipper <i>Istiblennius lineatus</i>	16	0.80	1.064	0.21 - 4.64	36.67	12.700	22.49 - 77.38
Streaky Rockskipper <i>Istiblennius dussumieri</i>	16	0.73	0.895	0.09 - 3.60	33.30	11.718	16.05 - 64.20
Family Gobiidae							
Barred Mudskipper <i>Periophthalmus argentilineatus</i>	18	1.62	1.309	0.25 - 4.77	47.98	9.111	38.48 - 71.72
Common Mudskipper <i>Periophthalmus kalolo</i>	18	2.94	1.569	0.90 - 6.33	59.17	9.677	47.52 - 80.78

Note. Mean wet mass and standard length determined for three species of rockskippers (Family Blenniidae) and two species of mudskippers (Family Gobiidae) on Hoga Island, Sulawesi, Indonesia used in aquatic Critical Thermal Maxima (CTMax) trials.

When tested in water, all three rockskipper species and both mudskippers exhibited an ER. Statistical comparisons of ER, however, revealed no significant differences between species ($F_{4,66}=2.23$, $p=0.0753$). Barred and common mudskippers demonstrated well-defined escape responses in air; however, similar responses were notably absent in rockskipper species (Table 4). Temperatures necessary to elicit an ER in the two mudskippers were statistically indistinguishable ($t=0.14$, $p=0.7127$) and markedly high, occurring at 39.4 and 39.0°C, respectively. Intraspecific comparisons revealed that barred (ANOVA; $F_{1,32}=33.63$; $p<0.0001$) and common (ANOVA: $F_{1,32}=13.91$; $p=0.0007$) mudskippers both demonstrated ER at significantly higher temperatures in air when compared to water. Of course the lack of an aerial response precluded intraspecific comparisons in rockskippers.

Discussion

Water temperature exerts more control over fish physiology and behavior than any other single abiotic factor (Beitinger and Fitzpatrick 1979, Beitinger and Bennett 2000). Temperature responses almost certainly play a pivotal role in dictating rockskipper movements between media. Graham (1973) measured an upper lethal temperature (ULT) for the eastern tropical Pacific Ocean rockskipper of 40.3°C in water, but reports a much reduced ULT value of 32.0°C for the same species in air. Rockskippers in my study likewise displayed media-related differences in thermal tolerance, with CTMax values of Ambon, lined, and streaky rockskippers being higher in water than in air. In both media, CTMax values were somewhat higher than ULT estimates given by Graham (1973). It is important to note, however, that ULT estimates are commonly

Table 4. Air And Water Escape Response Temperatures

Common name Species	Air ER (°C)			Water ER (C°)		
	N	Mean	S.D.	N	Mean	S.D.
Family Blenniidae						
Ambon Rockskipper <i>Paralticus amboinensis</i>	16	N.R.		16	37.0	1.19
Lined Rockskipper <i>Istiblennius lineatus</i>	16	N.R.		16	38.3	0.83
Streaky Rockskipper <i>Istiblennius dussumieri</i>	17	N.R.		16*	37.5	1.08
Family Gobiidae						
Barred Mudskipper <i>Periophthalmus argentilineatus</i>	16	39.4	0.89	18	36.1	2.48
Common Mudskipper <i>Periophthalmus kalolo</i>	16	39.0	1.49	18	36.4	2.69

Note. Escape response (ER) values in air and water for three rockskipper species (Family Blenniidae), and two mudskipper species (Family Gobiidae) inhabiting mangals around Hoga Island, Sulawesi, Indonesia. N.R. = No escape responses were observed. Individuals which did not exhibit an escape response were not used in statistical analyses.

lower than CTM values (Brett, 1956; Bennett and Beitinger 1995). High aquatic tolerance values relative to air are consistent in all four rockskipper species tested to date, suggesting that amphibious blennies are better adapted to survive high temperature extremes when submerged.

Variability in thermal tolerance responses may also explain why rockskipper fishes are reluctant to expose themselves to potentially harmful daytime air temperatures. Rockskippers in my study did not attempt escape until water temperatures exceeded 37.0°C (Table 2). Similarly, Graham (1973) reports that in laboratory trials, eastern tropical Pacific Ocean rockskippers display an ER at temperatures between 35° and 37°C. Furthermore, field observations by Brown et al. (1991) found that the rockskipper Kirk's blenny, *Alticus kirki*, remained in tidepools even at temperatures as high as 36°C. Temperatures in Hoga Island tidepools never exceeded ca. 35°C, and so I had no opportunity to observe rockskipper ER in the field. However, while attempting to capture fish for laboratory trials, rockskippers were unwilling to leave tidepools, preferring instead to retreat into crevices or swim between pools via narrow openings rather than move over land. Curiously, while all amphibious blennies discussed here showed a well-developed aquatic ER, no rockskippers in my trials made any attempt to escape high air temperatures. Perhaps their ability to thrive in high water temperatures has significantly reduced any selective pressures on rockskipper fishes to develop an aerial ER.

In contrast to their reluctance to emerge during daytime, rockskippers voluntarily emerge from water for extended periods during nocturnal low tides (Graham, 1973; Martin, 1991) when air temperatures are well below the fishes' upper

aerial tolerance limits. Nighttime ambient temperatures in either media present no threat to rockskipper fishes suggesting that factors other than temperature are responsible for nocturnal emergence. One possible factor may be predation pressure. I have observed moray eels searching for prey in cracks and crevices of Hoga's tidepools at night, perhaps forcing rockskippers to seek refuge on land from these and other aquatic predators. Another possible motivation for nocturnal exodus from tidepools may be decreased oxygen availability when photosynthetic organisms switch over to respiration. Taylor et al. (2005) found that nighttime dissolved oxygen levels in Hoga Island tidepools routinely fall below 1ppm, and even brief periods of anoxia were not uncommon. Ikebe and Oishi (1996) agree that the frequency of terrestrial excursions by amphibious fishes is more likely affected by dissolved oxygen level than by temperature. It may be then, that rockskippers are forced to retreat to land when oxygen tensions fall to critical levels as the quickest access to free oxygen is aerial emergence (Zander et al., 1999; Aguilar et al., 2000).

Compared to rockskippers, mudskippers demonstrate a more terrestrial lifestyle, frequently remaining outside of pools for extended periods during day or night and at all tidal stages (Martin, 1991). Even when pursued, mudskippers are inclined to escape over land. Gordon et al. (1969) found that aquatic ULT in a south China population of barred mudskippers¹ ranged from 33 to 35°C. The researchers argued that their relatively low thermal tolerance values motivated fish to leave warming tidepools. Without an aerial ULT estimate for comparison, however, it is difficult to assess whether daytime air temperatures would have provided a more amenable environment.

¹ Gordon et al. (1969) used the junior synonym *Periophthalmus sobrinus*

The aquatic tolerance values reported by Gordon et al. (1969) are considerably lower than comparable CTMax values for common and barred mudskippers on Hoga Island (41.0 and 40.7°C, respectively). As previously noted, the lower values may be partly attributable to methodology, but cooler acclimatization temperatures in the south China population (10 to 35°C; from Gordon et al. 1985) would have also produced lower temperature tolerance values. The CTMax data suggest that the ability to tolerate high temperature in aerial and aquatic environments affords common and barred mudskippers a more amphibious existence than that seen in rockskippers by allowing them to move freely between media in all but the most extreme thermal conditions.

Although common and barred mudskippers are syntopic across much of their range, the fishes distribute themselves differently across mangal zones. On Hoga Island, as well as on Surin Island, Thailand (Frith, 1977), common mudskippers² are most abundant on exposed lower mangal mudflats, whereas, barred mudskippers³ are more numerous in mangrove shaded pools in the upper mangal. Both species were similarly tolerant of high air temperatures; however, barred mudskippers exhibited significantly lower thermal tolerance in water (Table 1). While thermal tolerance differences can influence habitat selection in water-breathing tidepool fishes (Fangue et al., 2001), it is unclear if a relatively low aquatic CTMax adequately explains the proclivity of barred mudskippers for cooler shaded pools. It is more likely that temperature tolerance is one of several factors that influence microhabitat selection by mudskipper fishes (Gordon et al. 1985; Tytler and Vaughan 1983). For example, Tytler and Vaughan (1983) suggest

² Frith (1977) used the junior synonym *Periophthalmus koelreuteri*

³ Frith (1977) used the junior synonym *Periophthalmus vulgaris*

that common mudskipper⁴ and Boddart's goggle-eyed goby, *Boleophthalmus boddarti*, can effectively avoid overheating when emerged by selecting exposed microhabitats in Kuwait mangals that contribute to high evaporative cooling rates. Differences in water loss resistance (Tytler and Vaughan, 1983), excretion strategies (Gordon et al., 1969; Gordon et al., 1978) and foraging tactics (Clayton, 1993), among others, have been suggested as factors influencing microhabitat partitioning in mudskippers, but these lack empirical support. Regardless of the factors involved, reducing direct competition by habitat partitioning may be a mandatory condition of coexistence in austere mangal environments.

Amphibious fishes inhabiting Hoga Island's mangals exhibit a complex relationship with their thermal environment. These fishes are hyperthermic specialists that coexist within mangal habitats, but demonstrate markedly different tactics for dealing with high temperature extremes in water or air. Thermal tolerance and escape behavior of amphibious blennies complement nocturnal emergence behavior as well as explain the reluctance of these fishes to exit tidepools during the day. Conversely, thermal physiology and well developed ER assure that time and tide have little effect on mudskippers movements in either media. Ultimately, changing environmental temperatures act on physiology to dictate behavior in these species, resulting in novel behavioral patterns that not only enhance survival but also reinforce niche partitioning between families.

⁴ Tytler and Vaughan (1983) used the junior synonym *Periophthalmus koelreuteri*

CHAPTER II

A COMPARATIVE STUDY OF HYPOXIA TOLERANCE BETWEEN TWO FAMILIES OF AMPHIBIOUS FISHES

Introduction

Amphibious rockskippers (family Blenniidae) and mudskippers (family Gobiidae) can often be found taking refuge in mangal tidepools during low tide. Water temperatures in these pools can increase rapidly during daytime lows, increasing metabolic rates and at the same time exposing fish to hypoxic conditions as oxygen solubility decreases. Similarly, fish may experience severe hypoxia during nightly low tides when photosynthetic organisms shift to respiration, quickly depleting oxygen content in tidepool environments (Taylor et al., 2005; Martin, 1995; Congleton, 1980; Truchot and Duhamel-Jouve, 1980; Davenport and Woolmington, 1981; Steeger and Bridges, 1995; Schmidt-Nielsen, 1996; Fangue et al., 2001). Even at high tide, oxygen content in the burrows of submerged amphibious fishes may become severely reduced (Gordon et al., 1978; Chew and Ip, 1992). As bimodal breathers, amphibious fishes are uniquely adapted to deal with hypoxic waters by leaving tidepools and utilizing aerial oxygen directly, although the need to keep respiratory surfaces moist probably keeps them near water (Graham, 1997; Brown et al., 1992). Adaptations to air breathing may be morphological, physiological or behavioral, and allow these fishes to take advantage

of additional ecological resources to survive in locations that would otherwise be uninhabitable (Graham, 1997).

Both mudskippers and rockskippers possess modified gill structures suitable for aquatic as well aerial oxygen uptake. Although rockskippers are less well studied, they probably use air breathing tactics similar to those seen in mudskippers. Several studies have focused on gill examinations and relating morphology to respiratory capabilities among amphibious fishes (Tamura et al., 1976; Teal and Carey, 1967; Graham, 1973). Of the two families, mudskippers are thought to possess more advanced, or at least more effective, air breathing organs than rockskipper fishes (Graham, 1997). Major differences between the groups have been identified as variations in the structural architecture of the gill itself, and dissimilarities in cutaneous vascular tissue location or depth (Low et al., 1990). Notably, anecdotal accounts of terrestriality seem to correlate well with the degree of morphological sophistication in respiratory structures between amphibious fish species (Clayton, 1993).

Oxygen uptake physiology in air and water is well studied in mudskippers, but little is known about rockskipper fishes. A large number of studies have compared aerial and aquatic oxygen uptake of mudskippers (Chew and Ip, 1992; Steeger and Bridges, 1995; Tamura et al., 1976; Martin and Lighton, 1989). Often results of these studies are used to explain habitat partitioning by media preference where one mudskipper species exhibits higher oxygen uptake rates in air and the other in water (Kok et al., 1998; Takeda et al., 1999; Tamura et al., 1976). Relatively few studies estimate hypoxia tolerance in mudskippers, and only a single comment in the literature references hypoxia responses in rockskippers. Chew and Ip, (1992) observed that Boddart's goggle-eyed goby,

Boleophthalmus boddarti, becomes inert 30 minutes after oxygen levels fall below detectable limits in closed respirometry flasks. Similarly, eastern tropical Pacific Ocean rockskipper, *Mnierpes macrocephalus*, was observed to survive 40 minutes when forced to remain in hypoxic water (Graham, 1970). Unfortunately, dissolved oxygen concentrations were not reported. Only two studies empirically quantify low oxygen tolerance in mudskippers. Chinese mudskippers, *Periophthalmus cantonensis*, (Gordon et al., 1978) and gold-spotted mudskippers, *Periophthalmus chrysospilos*, (Chew et al., 1990) exhibit similar hypoxia tolerance values of 0.80 and 0.75mg/L⁻¹, respectively.

By exiting tidepools, amphibious fishes can avoid hypoxic water and gain access to aerial oxygen (Zander et al., 1999), but mudskippers and rockskippers show clear differences in their emergence patterns. Some mudskippers are known to spend 90% of their time out of water (Gordon et al., 1978); however, varying degrees of terrestriality are observed within the four mudskipper genera — *Boleophthalmus*, *Scartelaos*, *Periophthalmus*, and *Periophthalmodon* — with the two former considered to be more aquatic than the two latter (Clayton, 1993). While less well studied, emergence behavior has also been observed among rockskippers (Graham, 1997); for example, several studies indicate a nocturnal emergence pattern in istiblenniid rockskippers from the Red Sea (Martin, 1995; Zander, 1972). Differing emergence patterns between mudskippers and rockskippers are likely due, in part, to divergent tactics for dealing with the rigorous oxygen conditions that fishes encounter in mangal habitats. While many studies have assessed physiological or behavioral adaptations to hypoxia in syntopic mudskippers, no study to date has focused on syntopic distributions of both mudskippers and rockskippers.

The purpose of my study is to determine hypoxia tolerance, specifically Critical Oxygen Minima (COM) for five species of amphibious fishes on Hoga Island, Sulawesi, Indonesia. Mudskippers and rockskippers are syntopic fishes inhabiting areas throughout Southeast Asia and into West Africa. Two mudskipper species and three rockskipper species are found in abundance in mangal habitats on Hoga Island. The two mudskipper species are common, *Periophthalmus kalolo*, and barred, *Periophthalmus argentilineatus*, and the three rockskipper species are lined, *Istiblennius lineatus*, streaky, *Istiblennius dussumieri*, and Ambon, *Paralticus amboinensis*. All voluntarily exit tidepools and the aforementioned interfamilial dichotomy of media partitioning can be found. It is reasonable to assume that mudskippers and rockskippers will show different degrees of terrestriality based on habitat preference and conditions encountered therein. Investigation of hypoxia tolerance may provide better understanding into media as well as temporal partitioning between mudskippers and rockskippers.

Materials and Methods

Collection and Maintenance of Fish

Ambon rockskippers (n=17), lined rockskippers (n=15), streaky rockskippers (n=13), common mudskippers (n=14), and barred mudskippers (n=16) were collected on Hoga Island, Sulawesi, Indonesia (see Chapter I for collection methods). Mudskippers and rockskippers were housed separately; however, no attempt was made to separate species within families. Mudskippers were held in plastic holding tanks (121 × 39.6 × 30.8 cm) containing beach sand covered with clean seawater and at densities of 15 to 20 fish per tank. Rockskippers were housed in round, plastic 5-L tanks at densities of 3 to 5

fish per tank. Fish were fed TetraMin® flake food (rockskippers) or fresh chopped tuna (mudskippers) daily; however, animals were not fed 24 hours prior to or during trials. Upon trial completion, all fish were released at site of capture. This study was approved by the UWF Animal Care and Use Committee (Appendix A).

Determinations of Hypoxia Tolerance

Low-oxygen (hypoxia) tolerance values in water for 13-17 fish from each of the five species used in experiments were estimated using a nitrogen displacement system (Bennett and Beitinger, 1995). Hypoxic conditions were generated using a modification of a de-oxygenation system described by Smale and Rabeni (1995). Briefly, a Supreme Mag Drive ® model MD12 pump moved oxygen-saturated seawater through 2.5 cm diameter polyvinylchloride (PVC) tubing to the top of a transparent PVC oxygen extraction tube (5.1 cm ID x 1.7m). Seawater flowed down the extraction tube against an upward cascade of nitrogen bubbles generated by an air stone at the tube's base. Oxygen diffusing from seawater into the nitrogen bubbles was carried out of the system through a one-way valve. The counter-current gas/water flow removed oxygen at a rate of one half of the total oxygen concentration approximately every 20 min. Oxygen depletion half-times were held constant between trials by monitoring oxygen levels in the system using a YSI model 85 oxygen meter and adjustments in nitrogen were made as necessary. Partially deoxygenated water leaving the extraction tube then entered a splitter manifold where it was distributed among five 250-mL hypoxia (Erlenmeyer) flasks. The splitter manifold allowed fish to be removed from the system as Loss of Equilibrium (LOE) was observed without disrupting other fish in the system. Water leaving hypoxia flasks

flowed into an outflow manifold and back to the MagDrive pump inflow port. Oxygen depletion continued by recycling water through the system until LOE was observed in all fish.

In each trial five fishes — one of each species — were placed one each into hypoxia flasks. Before deoxygenation began, fishes were allowed to become accustomed to the flask environment for approximately one-half hour during which time fully oxygenated seawater circulated through the system. Flasks were sealed with rubber corks pierced with two pieces of buterate tubing attached to air line tubing, one for incoming and one for outgoing water flow. Fishes were carefully observed for escape response and LOE. Escape responses, when present, were defined (for both aquatic and aerial trials) as persistent, strong attempts to leap from the hypoxia chamber lasting for at least 1 min (Taylor et al., 2005). LOE has been defined as the inability of the fish to maintain dorsoventral orientation for at least 1 min (e.g., Coutant, 1969; Bennett et al., 1998; Fangue et al., 2001) Upon LOE, water was collected into a biological oxygen demand (BOD) bottle, then fixed and titrated via Winkler titration methods (Cox, 1990) to chemically determine the amount of oxygen in each chamber at LOE. Individuals were then transferred to flasks containing fully oxygenated water to recover. Mass ($\pm 0.01\text{g}$) and standard length ($\pm 0.1\text{cm}$) of each fish was measured and recorded.

Statistical Analysis

The arithmetic mean of all the LOE values for each species was determined as the critical oxygen minimum (COM). A one-way Analysis of Variance (ANOVA) was used to determine if differences in mass existed between species. COM and escape response

values between species were compared using a one-way ANOVA. Where significant differences were found, a Tukey multiple range test (MRT) was used to identify statistical relationships between treatment groups. All statistical decisions for ANOVA were based on an alpha value of 0.05.

Results

No significant differences in mass between species were found (ANOVA: $F_{4,70} = 1.56$, $p = 0.1934$; Table 5); therefore, mass was not considered to be a contributing factor in differences found in COM or escape responses between species. All rockskipper species reached significantly lower dissolved oxygen levels than mudskippers before succumbing to LOE (ANOVA: $F_{4,70} = 9.94$, $p < 0.0001$; Tukey, $\alpha = 0.05$; Table 6). While significant differences in escape responses were found between species (ANOVA: $F_{4,65} = 2.70$, $p = 0.0382$), Tukey's multiple comparison test (MCT) was unable to detect statistical differences in species means (Table 6).

Discussion

Physiological responses to acute hypoxia: oxygen tolerance

Dissolved oxygen (DO) levels are an important ecological condition contributing to distribution and survival of amphibious fishes living in Hoga Island tidepools. Not surprisingly, both mudskipper and rockskipper species in my study tolerated markedly hypoxic water with dissolved oxygen values below $1.0 \text{ mg} \cdot \text{L}^{-1}$. Hypoxia tolerance is poorly understood in amphibious fishes, with no hypoxia values known for rockskipper fishes, and only two mudskipper values reported in the literature. The Chinese

Table 5. Hypoxia Trial Species Wet Mass And Standard Length

Common Name	N	Mass (g)	S.D.	Length (cm)	S.D.
Species					
Family Blenniidae					
Ambon Rockskipper					
<i>Paralticus amboinensis</i>	14	3.63	6.652	5.01	2.411
Lined Rockskipper					
<i>Istiblennius lineatus</i>	16	1.81	2.471	4.54	1.725
Streaky Rockskipper					
<i>Istiblennius dussumieri</i>	17	0.59	0.334	4.29	3.461
Family Gobiidae					
Barred Mudskipper					
<i>Periophthalmus argentilineatus</i>	15	2.54	0.76	5.93	0.878
Common Mudskipper					
<i>Periophthalmus kalolo</i>	13	2.45	1.106	5.66	0.983

Note. Average (mean) wet masses in grams and standard lengths in centimeters were determined for three species of rockskippers (Family Blenniidae) and two species of mudskippers (Family Gobiidae) on Hoga Island, Sulawesi, Indonesia used in hypoxia tolerance trials. Sample sizes (N) are listed for each species.

Table 6. Escape Responses And Critical Oxygen Minima

Common Name Species	N	Escape Response (mg/L)	S.D.	COM (mg/L)	S.D.
Family Blenniidae					
Ambon Rockskipper					
<i>Paralticus amboinensis</i>	14	0.8	0.478	0.47	0.130
Lined Rockskipper					
<i>Istiblennius lineatus</i>	16	1.32	0.880	0.48	0.156
Streaky Rockskipper					
<i>Istiblennius dussumieri</i>	17	0.87	0.37	0.47	0.120
Family Gobiidae					
Barred Mudskipper					
<i>Periophthalmus argentilineatus</i>	15	1.34	0.475	0.70	0.154
Common Mudskipper					
<i>Periophthalmus kalolo</i>	13	1.12	0.525	0.68	0.167

Note. Average (mean) escape responses and Critical Oxygen Minima (COM) in $\text{mg} \cdot \text{L}^{-1}$ were determined for three species of rockskippers (Family Blenniidae) and two species of mudskippers (Family Gobiidae) on Hoga Island, Sulawesi, Indonesia used in hypoxia tolerance trials. Sample sizes (N) are listed for each species.

Chinese mudskipper exhibits a hypoxia threshold of $0.70\text{mg} \cdot \text{L}^{-1}$ (Gordon et al., 1978), similar to mudskippers in my study. Likewise, the gold-spotted mudskipper (Chew et al., 1990) exhibits a similar hypoxia threshold of $0.75\text{mg} \cdot \text{L}^{-1}$.

Rockskipper and mudskipper laboratory trials showed a clear statistical distinction in oxygen tolerance between the two families, with rockskipper's tolerating 30% lower oxygen levels than mudskippers. In harsh Hoga Island tidepools where oxygen is inherently low even under the best of conditions, a relatively small difference in oxygen tolerance between families ($0.20\text{mg} \cdot \text{L}^{-1}$ in my study) may be biologically relevant. Martin (1995) argues that the choice of media by amphibious fishes is likely dictated by physiological tolerance levels. Sensitivity to low dissolved oxygen may encourage mudskippers to regularly avoid hypoxic pools during daytime low tides, thereby promoting a high degree of terrestriality. Conversely, the ability of rockskippers to withstand dissolved oxygen levels as low as $0.50\text{mg} \cdot \text{L}^{-1}$ allows these fishes to exploit tidepool environments during typical daytime hypoxic conditions (Taylor et al., 2005). At night when photosynthesis ceases and oxic conditions in tidepools become critically hypoxic, or even anoxic (Taylor et al., 2005; Fanguie et al., 2001; Martin, 1995), both families are forced from tidepool environments.

Differences in morphological adaptations for oxygen uptake between families

Differences in hypoxia tolerance seen in amphibious fishes are likely influenced by structural (morphological) attributes of the respiratory system. While modifications in branchial support and cutaneous vascularization are common adaptations for both mudskippers and rockskippers, the degree of alteration varies. Branchial collapse is a

key limitation to gilled air breathers (Graham, 1970), so it is not surprising that amphibious fishes in general have thicker, sturdier lamellae (Brown et al., 1992). Likewise, cutaneous vascularization on the head, body (Zander, 1972) and bucco-pharyngeal cavities (Steeger and Bridges, 1995; Graham, 1997) are traits seen in both groups. While rockskippers are not well studied, they are generally believed to be less terrestrial than mudskippers (Graham 1997). For example, Brown et al. (1992) reported that secondary lamellae density was 20% lower in the rockskipper Kirk's blenny, *Alticus kirkii*, when compared to the Giant mudskipper *Periophthalmus schlosseri*. Even within mudskipper genera, morphological modifications in respiratory structures are thought to explain varying degrees of terrestriality observed between species (Clayton, 1993; Low et al., 1988; Chew and Ip, 1992).

Behavioral responses to induced hypoxia: escape responses

While the ability to leave the water when oxygen tensions reach critically low levels is a clear advantage to amphibious fishes (Graham and Lee, 2004), behavioral responses for Hoga Island species are not easily interpreted. Statistical differences in escape response values were detected with ANOVA ($P=0.0382$); however, Tukey's post-hoc test could not separate species means ($\alpha=0.05$). Nevertheless, some trends in the escape response data may be revealing. For example, escape responses of mudskippers and rockskippers in my study occurred at surprisingly low dissolved oxygen levels. Fish in both groups are clearly well adapted to leaving the water, yet made no attempt at escape until dissolved oxygen levels fell to approximately $1 \text{ mg} \cdot \text{L}^{-1}$ (Table 6), concentrations considered markedly hypoxic by Wannamaker and Rice (2000). By comparison, these values are similar to or even lower than LOE values

reported for wholly aquatic fishes which have little choice but to remain submerged as temperatures rise and oxygen levels fall (Smale and Rabeni, 1995).

Escape response data for amphibious fishes are sparse. Graham (1970) found that emergence times in the eastern tropical Pacific rockskipper increased as dissolved oxygen levels fell. Likewise, Ikebe and Oishi (1996) found that Shuttle's hopppfish, *Periophthalmus modestus*, cling to positions above the water line more frequently as DO levels decrease. Although methods and endpoints differ, both studies seem to infer that escape responses of amphibious fishes should be sensitive to dissolved oxygen levels. It is not immediately clear why responses of Hoga Island fishes are not seen until oxygen levels fall to within a few tenths of a milligram of potentially lethal levels. Perhaps the unnatural hypoxia apparatus environment prompted fish to remain submerged longer than they would have in natural surroundings. Conversely, it may be that a well developed hypoxia tolerance response is a necessary adaptation for mudskippers that may spend extended periods in hypoxic burrows (Gordon et al., 1978; Chew and Ip, 1992), as well as for less terrestrial (Graham, 1997) rockskippers that must routinely endure diurnal and nocturnal periods of severe hypoxia (Taylor et al., 2005). Indeed, streaky rockskippers in hypoxia trials were especially reluctant to bring escape responses on line. While these fish have an escape response mean value of 0.87 mg/l (second lowest value behind the Ambon rockskipper), the average does not take into account the 25% of fish that reached LOE levels without making an escape attempt. The low escape and LOE values may explain in part why streaky rockskippers are the most abundant rockskippers present in shallow, oxygen-deficient pools on Hoga Island. Increased sample sizes in laboratory studies, coupled with carefully collected water

quality data at the time of emergence, will likely be needed to clarify differences in escape response and provide a better understanding of emergence behavior by amphibious fishes on Hoga Island.

Resource utilization as a function of behavioral and physiological adaptation levels

In summary, it is probable that differences in physiological and behavioral responses between rockskippers and mudskippers reinforce resource partitioning and habitat use in Hoga mangals. In rockskippers, high tolerance of all but the most oxygen depleted waters is an adaptive characteristic for fishes that spend most of their time submerged in tidepools prone to daily hypoxic episodes. The austere oxic conditions likely exclude less tolerant fishes from the pool environment, thereby lessening predatory pressures while at the same time reducing interspecific resource competition. The ability to temporarily leave the tidepools affords rockskippers a means of escape from aquatic predators and nocturnal periods of anoxia. Mudskippers, on the other hand, spend most of their time emerged regardless of tidal stage or time of day (Martin, 1995). These fishes are more terrestrial than rockskippers and as a result, can move with impunity between media to avoid predators or forage for food. In addition, their ability to endure relatively low oxygen levels provides a margin of safety during times the fish is confined to its burrow or making short forays into hypoxic zones. Some authors have suggested that amphibious fishes represent a transition to land (Graham, 1997; Lee and Graham, 2002; Graham and Lee, 2004); however, I would argue that amphibiousness is an adaptation that ties fish to water in an environment that goes through regular cycles of drying and inundation.

CHAPTER III

WATER LOSS RESISTANCE IN TWO FAMILIES (FAMILY GOBIIDAE AND FAMILY BLENNIIDAE) OF AMPHIBIOUS FISHES

Introduction

Two groups of amphibious fishes, rockskippers (family Blenniidae) and mudskippers (family Gobiidae), occupy the mangal habitat of Hoga Island, Sulawesi, Indonesia. These fishes are capable of bimodal respiration allowing them to escape harsh tidepool conditions such as low dissolved oxygen (Gordon et al., 1978; Taylor et al., 2005), high temperatures (Graham, 1973; Brown et al., 1992), and fluctuating salinities (Brown et al., 1991; Kakuta and Murachi, 1992) by exiting to terrestrial refuges (Graham, 1997). While leaving tidepools may help air-breathing fishes escape intolerable aquatic conditions, it also introduces new difficulties that must be overcome in air. Although the air is humid in Hoga's mangals, there is an increased risk of desiccation when fishes are away from tidepools (Martin, 1991). It is likely that amphibious fishes have a low tolerance for dessication (Martin, 1993) despite their frequent outings onto land.

Because of their constant exposure to air and high rates of cutaneous water loss, amphibians have been the focus of several studies determining rates of evaporative water loss (EWL) (Young et al., 2006; Spotila and Berman, 1976). Due to terrestrial sojourns, mudskippers and rockskippers are subject to the same problems that

amphibians must face in regard to water loss across the skin; however, only two studies have assessed the rate of EWL for amphibious fishes. Graham (1973) measured rates of EWL for the eastern tropical Pacific rockskipper, *Mnierpes macrocephalus*, by determining percentage of water loss at death for fish exposed to various relative humidities and temperatures. Gordon et al. (1978) determined EWL measurements in the Chinese mudskipper, *Periophthalmus cantonensis*, by measuring weight changes in groups of fish exposed to sun or shade conditions in still or moving air. Additionally, he compared mudskipper values to the Indian bullfrog, *Rana tigerina*, exposed to the same conditions. Unfortunately, both of these studies utilized weight loss as the only indicator of EWL rates. With the influx of so many studies on amphibian and reptile water loss resistance, more advanced methods are available (Spotila and Berman, 1976; Young et al., 2005) and are frequently used to determine water loss resistance, although these have never been attempted with amphibious fishes.

While both mudskippers and rockskippers display volitional exit behavior from tidepools during low tide events (Graham, 1997), rockskippers tend to only exit tidepools at night (Graham, 1973), while mudskippers spend the majority of their time on land whether daytime or nighttime (Gordon et al., 1978; Gordon et al., 1985). This partitioning of resources between the two groups may be due to differing adaptive strategies based on differences in water loss resistance capabilities. The purpose of my study is to assess responses of common mudskippers, *Periophthalmus kalolo*, and Ambon rockskippers, *Paralticus amboinensis*, inhabiting Hoga's mangals to exposure to drying conditions. Determination of resistance to evaporative water loss in these two families of syntopic amphibious fishes may aid in ascertaining whether physiological

EWL attributes contribute to resource partitioning patterns between media on Hoga Island. I hypothesize that both groups will have high overall evaporative water loss values similar to those found in amphibian species; however, due to mudskipper's more terrestrial lifestyle, rockskippers will likely have lower cutaneous resistance to water loss values more closely resembling a free water surface when compared to mudskippers.

Materials and Methods

Collection and Maintenance of Fish

Common mudskippers (n=16) and Ambon rockskippers (n=12) were collected from mangal habitats on Hoga Island, Sulawesi, Indonesia. Both species were collected at night by immobilizing them with a bright light and capturing them in aquarium dip nets. Following capture, fishes were transported to the Hoga Research Center where they were transferred to holding tanks. Mudskippers were held in plastic holding tanks (121 × 39.6 × 30.8 cm) containing beach sand covered with clean seawater at densities of 15 to 20 fish per tank. Emergent palm frond and driftwood pieces allowed fish free movement between air and water. Rockskippers were housed in round, plastic 5-L tanks at densities of 3 to 5 fish per tank. Airstones provided vigorous aeration to each tank. Mudskipper and rockskipper holding tank temperatures were kept between 26 and 28°C, with seawater changed twice daily and sand changed every 2-3 days to maintain habitat quality. Fish were fed TetraMin® flake food (rockskippers) or fresh chopped tuna (mudskippers) daily; however, animals were not fed 24 hours prior to or during

trials. Upon trial completion, all fishes were released at site of capture. This study was approved by the UWF Animal Care and Use Committee (Appendix A).

Evaporative Water Loss (EWL)

Rates of water loss were determined using an open flow evaporative water loss (EWL) system described by Young et al. (2006). Dry compressed air was passed across dryrite chemical desiccant and pumped at a rate of approximately $0.25 \text{ cm} \cdot \text{s}^{-1}$ through a clear polyvinylchloride (PVC) EWL chamber (4 cm diameter \times 12 cm) containing a single fish. Measurements were made in the shade at air temperatures between 25 and 27°C. Humidity and temperature values of the incurrent and excurrent air were measured using Tecpel© model DTM-321 humidity meter ($\pm 0.3\%$ RH) every 2–5 min until relative humidity remained constant for a minimum of 10 min. Before the fish were removed from the chamber, skin temperature was measured using a Kintrex© model IRT0421 non-contact infrared thermometer. The fish were then removed from the chamber, weighed ($\pm 0.1\text{g}$) and standard length ($\pm 0.1 \text{ cm}$) determined.

Rates of surface specific EWL was determined from equations of Bernstein et al. (1977) using an empirical formula based on mass (McClanahan and Baldwin 1969) in conjunction with tables of standard saturated vapor density (List, 1971). Values were then converted to total resistance to EWL (R_t) by determining the difference between the water vapor density at the skin and in the chamber and dividing it by the surface specific EWL rate. R_t was calculated by using two equations of Spotila and Berman (1976). These equations are summarized below.

$$1. \quad R_t = (VD_s - VD_e) \div EWL_{sa}$$

$$2. \quad R_t = R_c + R_b$$

In equation 1, VD_s is the water vapor density at the skin and VD_e is the water vapor density of the air in the chamber. EWL_{sa} is the surface area specific rate of water loss. In equation 2, R_t is the sum of boundary layer resistance (R_b) and cutaneous resistance (R_c). R_b originates from a layer of air which surrounds an object. This resistance is determined by the ambient environmental conditions, in addition to the size and shape of the animal. R_c is the residual resistance after the removal of boundary layer effects. The physiological and structural characteristics of an animal's exposed skin contribute to any cutaneous resistance that occurs.

R_b for live animals is calculated by estimating R_t on a free water surface, such as a 3% agar (97% water) model of the animal which is similar in shape and size. Models of this type are referred to as free water surface models since they are shown to lose water at the same rate as a free water surface ($R_c = 0 \text{ s cm}^{-1}$). Spotila and Berman (1976), Schwarzkopf and Alford (1996), and Navas and Araujo (2000) successfully used agar models as null models in water relation and thermal ecology investigations of amphibians. In the present study, several fish of various sizes were frozen and all of the surfaces except the ventral surface were placed in a clay putty mold. During amphibian trials, ventral surfaces are not exposed during experimentation due to the fishes' proclivity to take a water conserving stance (Shoemaker et al. 1992; Young et al., 2005). Agar was poured into the mold and left to set. Agar models were placed in the experimental chamber to determine R_c , which is assumed to be equal to R_t for a free water surface. Agar models were placed into EWL chamber and R_b determined. Finally, fish cutaneous resistance was calculated from the equation below.

$$R_c = R_t - R_b$$

R_t is the surface specific resistance calculated from the fish and R_b is the similar value calculated from the agar model. The R_t value for the population was determined as the arithmetic mean of the collective experimental endpoints for each species. Cutaneous resistance (R_c) can be obtained by rearranging equation 2 and subtracting R_b (total resistance for the 3% agar model) from R_t (total resistance calculated for live animal of similar size) as seen in equation 3. The R_c values should be considered sufficient estimates of cutaneous water loss resistance since they are based on calculations using rates of EWL inclusive of a pulmonary water loss component.

Due to erroneous results indicated by EWL measurements which were too high for each species, statistical analyses for this study were unnecessary.

Results

Rates of cutaneous evaporative water loss determined for Ambon rockskippers were $208.63 \text{ sec} \cdot \text{cm}^{-1}$. Rates of cutaneous evaporative water loss determined for common mudskippers were $3,082.65 \text{ sec} \cdot \text{cm}^{-1}$.

Discussion

Amphibians demonstrate cutaneous evaporative water loss rates ranging between 0 and $63 \text{ cm} \cdot \text{sec}^{-1}$ (Young et al., 2005). Cutaneous evaporative water loss rates determined for amphibious fishes in my study were markedly higher (one to two-fold) than amphibians indicating that determinations from my study were likely erroneous. As this was a pilot study for EWL measurement of amphibious fishes, experimental errors might have been compounded by several factors including but not limited to flow rates and chamber size. Low flow rates were necessary for accurate

EWL measurement, but were difficult to control. The large size of EWL chambers limited the scope of my study and contributed to unreliable measurements. Due to large EWL chambers, the smallest Hoga Island amphibious fishes were excluded from testing procedures. Large chambers may have also contributed to experimental errors by allowing the fishes room for movement. Young et al. (2005) found that relative humidity values were twice as high when animals shifted position in the chamber. We observed that relative humidity fluctuated drastically when fish in the chamber moved, thereby increasing the amount of time necessary to obtain stabilization periods. Long stabilization intervals exposed fishes in the chamber to unusually long drying-out times which the fishes would not experience in their natural habitats.

Amphibians have been observed to assume a water-conserving posture and they maintain this posture during EWL experiments. In addition, the water-conserving posture is necessary for valid surface area determinations (Young et al., 2006). Martin (1993) reported that while out of water, amphibious fishes are often observed in what may be a water-conserving posture by balancing on spread pectoral fins. While amphibious fishes do probably have to cope with drying out, because they have immediate and continuous access to water, it is likely that they never evolved water-conserving postures. During future EWL studies, it would be necessary to anaesthetize animals to prevent movement during trials.

Based on previous EWL studies on amphibians, it is probable that differences in physiological EWL attributes play a role in resource partitioning between families. Wygoda (1984) and Shoemaker et al. (1992) determined that a relationship between lifestyle and cutaneous water loss resistance exists. Young et al. (2005) found that

EWL resistance is indicative of species' ecological habitat. Amphibians which live in moist environments have EWL rates similar to a free-water surface, while arboreal amphibians which have less access to water and are exposed to wind demonstrate significantly higher EWL rates (Spotila and Berman, 1976; Young et al., 2005).

Because resource partitioning between gobiids and blenniids is observed in the mangals of Hoga Island, future studies are necessary to obtain reliable methods for EWL rate determination in these animals. Resistance to water loss may be important in determining their overall ecological strategies.

REFERENCES

- Aguilar, N. M., A. Ishimatsu, K. Ogawa, and K. K. Huat. 2000. Aerial ventilatory responses of the mudskipper, *Periophthalmodon schlosseri*, to altered aerial and aquatic respiratory gas concentrations. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 127:285-292.
- Becker, C. D. and R. G. Genoway. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environmental Biology of Fishes* 4:245-256.
- Beitinger, T. L. and W. A. Bennett. 2000. Quantification of the role of acclimation temperature in temperature tolerance of fishes. *Environmental Biology of Fishes* 58:277-288.
- Beitinger, T. L., W. A. Bennett, and R. W. McCauley. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environmental Biology of Fishes* 58:237-275.
- Beitinger, T. L. and L. C. Fitzpatrick. 1979. Physiological and ecological correlates of preferred temperature in fish. *American Zoology* 19:319-329.
- Bennett, W. A., and T. L. Beitinger. 1995. Overview of techniques for removing oxygen from water and a description of a new oxygen depletion system. *The Progressive Fish-Culturist* 57:84-87.

- Bennett, W. A., and T. L. Beitinger. 1997. Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. *Copeia* 1997(1):77-87.
- Bennett, W. A., R. W. McCauley, and T. L. Beitinger. 1998. Rates of gain and loss of heat tolerance in channel catfish. *Transactions of the American Fisheries Society* 127:1051-1058.
- Bernstein, M. H., D. M. Hudson, J. M. Stearns, and R. W. Hoyt. 1977. Measurement of evaporative water loss in smaller animals by dew-point hygrometry. *Journal of Applied Physiology* 43:382-385.
- Brett, J. R. 1956. Some principles in the thermal requirements of fishes. *The Quarterly Review of Biology* 31:75-87.
- Brown, C. R., M. S. Gordon, and H. G. Chin. 1991. Field and laboratory observations on microhabitat selection in the amphibious Red Sea rockskipper fish, *Alticus kirki* (Family Blenniidae). *Marine Behavior and Physiology* 19:1-13.
- Brown, C. R., M. S. Gordon, and K. L. M. Martin. 1992. Aerial and aquatic oxygen uptake in the amphibious red sea rockskipper fish, *Alticus kirki* (Family Blenniidae). *Copeia* 1992(4):1007-1013.
- Chew, S. F. and Y. K. Ip. 1992. Biochemical adaptations of the mudskipper *Boleophthalmus boddarti* to a lack of oxygen. *Marine Biology* 112:567-571.
- Chew, S. F., A. L. L. Lim, W. P. Low, C. G. L. Lee, K. M. Chan, and Y. K. Ip. 1990. Can the mudskipper, *Periophthalmus chrysopilus*, tolerate acute environmental hypoxic exposure? *Fish Physiology and Biochemistry* 8(3): 221-227.
- Clayton, D. A. 1993. Mudskippers. *Oceanography and Marine Biology Review* 31: 501-577.

- Congleton, J. L. 1980. Observations on the responses of some southern California tidepool fishes to nocturnal hypoxic stress. *Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology* 66:719-722.
- Coutant, C. C. 1969. Temperature, reproduction, and behavior. *Chesapeake Science* 10:261-274.
- Cowles, R. B. and C. M. Bogert. 1944. A preliminary study of the thermal requirements of desert reptiles. *Bulletins of the American Museum of Natural History* 83:265-296.
- Cox, D. K. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. p. 158-163. *In: Thermal Ecology*. J. W. Gibbons and R. R. Sharitz (eds.). National Technical Information Service, Springfield, VA.
- Cox, D. K. 1990. *Laboratory Manual of General Ecology*. W. C. Brown Publishers, Dubuque, Iowa.
- Davenport, J. and A. D. Woolmington. 1981. Behavioural responses of some rocky shore fish exposed to adverse environmental conditions. *Marine Behavior and Physiology* 8:1-12.
- Fangue N. A., K. E. Flaherty, J. L. Rummer, G. Cole, K. S. Hansen, R. Hinote, B. L. Noel, H. Wallman, and W. A. Bennett. 2001. Temperature and hypoxia tolerance of selected fishes from a hyperthermal rockpool in the Dry Tortugas, with notes on diversity and behavior. *Caribbean Journal of Science* 37:(1-2)81-87.
- Frith, D.W. 1977. A preliminary list of macrofauna from a mangrove forest and adjacent biotopes at Surin Island, western peninsular Thailand. *Phuket Marine Biological Center* 17:1-14.

- Frith, D. W., R. Tantanasiwong, and O. Bhatia. 1976. Phuket Marine Biological Center 10:1-37.
- Gordon, M. S., I. Boetius, D. H. Evans, R. McCarthy, and L. Oglesby. 1969. Aspects of the physiology of terrestrial life in amphibious fishes. I. The mudskipper, *Periophthalmus sobrinus*. Journal of Experimental Biology 50: 141-149.
- Gordon, M. S., D. J. Gabaldon, and A. Y.-W. Yip. 1985. Exploratory observations on microhabitat selection within the intertidal zone by the Chinese mudskipper fish *Periophthalmus cantonensis*. Marine Biology 85:209-215.
- Gordon, M. S., W. W.-S. Ng, and A. Y.-W. Yip. 1978. Aspects of the physiology of terrestrial life in amphibious fishes. III. The Chinese mudskipper *Periophthalmus cantonensis*. Journal of Experimental Biology 72:57-75.
- Graham, J. B. 1970. Preliminary studies on the biology of the amphibious clinid *Mnierpes macrocephalus*. Marine Biology 5:136-140.
- Graham, J. B. 1973. Terrestrial life of the amphibious fish *Mnierpes macrocephalus*. Marine Biology 23:83-91.
- Graham, J. B. 1997. Air-breathing fishes: evolution, diversity, and adaptation. Academic Press, San Diego.
- Graham, J. B. and H. J. Lee. 2004. Breathing air in air: In what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? Physiological and Biochemical Zoology 77(5):720-731.
- Hutchison, V. H. 1961. Critical thermal maxima in salamanders. Physiological Zoology 34:92-125.

- Ikebe, Y. and T. Oishi. 1996. Correlation between environmental parameters and behaviour during high tides in *Periophthalmus modestus*. Journal of Fish Biology 49:139-147.
- Kakuta, I. and S. Murachi. 1992. Responses of the blenny *Istiblennius enosimae* exposed to asphyxic environments—I. Effects on heart rate and urine flow. Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology 102(2): 295-298.
- Kaufmann, J. S. and A. F. Bennett. 1989. The effect of temperature and thermal acclimation on locomotor performance in *Xantusia vigilis*, the desert night lizard. Physiological Zoology 62:1047-1058.
- Kok, T. W. K., C. B. Lim, T. J. Lam, and Y. K. Ip. 1998. The mudskipper *Periophthalmus schlosseri* respire more efficiently on land than in water and vice versa for *Boleophthalmus boddarti*. Journal of Experimental Zoology 280:86-90.
- List, R. J. 1971. Smithsonian Meteorological Tables Publication 4014. Smithsonian Institution, Washington, D.C.
- Lee, H. J. and J. B. Graham. 2002. Their game is mud. Natural History 9:42-47.
- Low, W. P., D. J. W. Lane, and Y. K. Ip. 1988. A comparative study of terrestrial adaptations in three mudskippers—*Periophthalmus chrysospilos*, *Boleophthalmus boddarti* and *Periophthalmodon schlosseri*. Biological Bulletin 175:434-438.
- Low, W. P., D. J. W. Lane, and Y. K. Ip. 1990. A comparative study of the gill morphometry in three mudskippers—*Periophthalmus chrysospilos*, *Boleophthalmus boddarti* and *Periophthalmodon schlosseri*. Zoological Science 7:29-38.

- Lowe, C. H. and V. J. Vance. 1955. Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*. Science 122: 73-74.
- Martin, K. L. M. 1991. Facultative aerial respiration in an intertidal sculpin, *Clinocottus analis* (Scorpaeniformes: Cottidae). Physiological Zoology 54(5):1341-1355.
- Martin, K. L. M. 1993. Aerial release of CO₂ and respiratory exchange ratio in intertidal fishes out of water. Environmental Biology of Fishes 37:189-196.
- Martin, K. L. M. 1995. Time and tide wait for no fish: intertidal fishes out of water. Environmental Biology of Fishes 44:165-181.
- Martin, K. L. M. and J. R. B. Lighton. 1989. Aerial CO₂ and O₂ exchange during terrestrial activity in an amphibious fish, *Alticus kirki* (Blenniidae). Copeia 1989(3):723-727.
- McClanahan, L. J. and R. Baldwin. 1969. Rate of water uptake through the integument of the desert toad, *Bufo punctatus*. Comparative Biochemistry and Physiology 28:381-389.
- Navas, C. A. and C. Araujo. 2000. The use of agar models to study amphibian thermal ecology. Journal of Herpetology 34:330-334.
- Palidino, R. V., J. R. Spotila, J. P. Schubauer, and K. T. Kowalski. 1980. The critical thermal maximum: a technique used to elucidate physiological stress and adaptation in fishes. Review Canada Biology 39:115-122.
- Schmidt-Nielsen, K. 1996. Animal Physiology: Adaptation and Environment, 5th ed. Cambridge University Press, Cambridge, United Kingdom.

- Schwarzkopf, L. and R. A. Alford. 1996. Desiccation and shelter-site use in a tropical amphibian: comparing toads with physical models. *Functional Ecology* 10:193-200.
- Shiota, T., A. Ishimatsu, and K. Soyano. 2003. Effects of temperature on gonadal development of mudskipper (*Periophthalmus modestus*). *Fish Physiology and Biochemistry* 28:445-446.
- Shoemaker, V., S. S. Hillman, S. D. Hillyard, D. C. Jackson, L. L. McClanahan, P. C. Withers, and M. L. Wygoda. 1992. Exchange of water, ions and respiratory gases in terrestrial amphibians, p. 125-150. *In: Environmental Physiology of the Amphibians*. M. E. Feder (ed.). University of Chicago Press, Chicago.
- Smale, M. A. and C. F. Rabeni. 1995. Hypoxia and hyperthermia tolerances of headwater stream fishes. *Transactions of the American Fisheries Society* 124:698-710.
- Spotila, J. R. and E. N. Berman. 1976. Determination of skin resistance and the role of the skin in controlling water loss in amphibians and reptiles. *Comparative Biochemistry and Physiology* 55A:407-411.
- Steeger, H. U., and C. R. Bridges. 1995. A method for long-term measurement of respiration in intertidal fishes during simulated intertidal conditions. *Journal of Fish Biology* 47:308-320.
- Takeda, T., A. Ishimatsu, S. Oikawa, T. Kanda, Y. Ishida, and K. H. Khoo. 1999. Mudskipper *Periophthalmodon schlosseri* can repay oxygen debts in air but not in water. *Earth and Planetary Science Letters* 144(1-2):9-19.

- Tamura, S. O., H. Morii, and M. Yuzuriha. 1976. Respiration of the amphibious fishes *Periophthalmus cantonensis* and *Boleophthalmus chinensis* in water and on land. *Journal of Experimental Biology* 65:97-107.
- Taylor, Josi R., M. M. Cook, A. L. Kirkpatrick, S. N. Galleher, J. Eme, and W. A. Bennett. 2005. Thermal tactics of air-breathing and non air-breathing Gobiids inhabiting mangrove tidepools on Palau Hoga, Indonesia. *Copeia* 4:886-893.
- Teal, J. M. and F. G. Carey. 1967. Skin respiration and oxygen debt in the mudskipper *Periophthalmus sobrinus*. *Copeia* 1967: 677-679.
- Truchot, J. P. and A. Duhamel-Jouve. 1980. Oxygen and carbon dioxide in the marine intertidal environment: diurnal and tidal changes in rockpools. *Respiratory Physiology* 39:241-254.
- Tytler, P. and T. Vaughn. 1983. Thermal ecology of the mudskippers, *Periophthalmus koelreuteri* (Pallas) and *Boleophthalmus boddarti* (Pallas) of Kuwait Bay. *Journal of Fish Biology* 23:327-337.
- Wannamaker, C. M. and J. A. Rice. 2000. Effects of hypoxia on movements and behavior of selected estuarine organisms from the southeastern United States. *Journal of Experimental Marine Biology and Ecology* 249:145-163.
- Wygoda, M. L. 1984. Low cutaneous evaporative water loss in arboreal frogs. *Physiological Zoology* 57:329-337.
- Young, J. E., K. A. Christian, S. Donnellan, C. R. Tracy, and D. Parry. 2005. Comparative analysis of cutaneous evaporative water loss in frogs demonstrates correlation with ecological habits. *Physiological and Biochemical Zoology* 78:839-846.

- Young, J. E., C. R. Tracy, K. A. Christian, and L. J. McArthur. 2006. Rates of cutaneous water loss of native Fijian frogs. *Copeia* 1:83-88.
- Zander, C. D. 1972. Beziehungen zwischen Körperbau und Lebensweise bei Blenniidae (Pisces) aus dem Roten Meer. I. Äußere Morphologie. *Marine Biology* 13: 238-246.
- Zander, C. D., J. Nieder, and K. L. M. Martin. 1999. Respiration in water and air, p. 54-74. *In*: Intertidal Fishes: Life in Two Worlds. M. H. Horn, K. L. M. Martin, and M. A. Chotkowski (eds.). Academic Press, San Diego, California.

APPENDIX

Appendix A

Animal Care and Use Committee Approval

Memorandum

Date: June 28, 2006

To: Ms. Joni Barreda
Biology
0058

From: Dr. Christine Cavanaugh, Interim Director
Research and Sponsored Programs

*CCA
for*

Subject: ACUC Proposal Approval

RE: Proposal Number 2006-004

1. The Animal Care and Use Committee has completed its review of your proposal entitled, **Comparative Rates of Water Loss in Air-Breathing Fishes on Hoga Island, Indonesia**, and has granted approval for you to proceed with your study. Please note the following:

- Prior ACUC approval is required for significant changes to your protocol.
- The maximum approved period is three years. Should your project continue beyond the three year period, you must request ACUC approval prior to the end of the approval period.
- Annual status reports may be required by the ACUC. These reports must include a complete description of any and all changes to your project.

2. Please take time to review our ACUC web page at:

<http://www.research.uwf.edu/boards-committees/acuc.htm>

which includes the ACUC Policies and Procedures, the PHS Policy on Human Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and other pertinent ACUC documents.

3. The University of West Florida wishes you the best in your research endeavors.

CC: Dr. Wayne Bennett Jr.
Biology

Phone 850.474.2824 Fax 850.474.2082
Web research.uwf.edu
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Research and Sponsored Programs

Building 11, Room 109
11000 University Parkway
Pensacola, FL 32514-5750

December 3, 2007

Ms. Joni Barreda
Biology Department
211 Tooke St.
Apt. B
Fort Walton Beach, FL 32547

Dear Ms. Barreda:

The Institutional Animal Care and Use Committee has completed its review of your proposal titled "Thermal and Hypoxia Tolerance in Air-Breathing Fishes" and has granted approval for you to proceed with your study. Please note the following:

- Prior ACUC approval is required for significant changes to your protocol.
- The maximum approval period is three years. Should your project continue beyond the three year period, you must request ACUC approval prior to the end of the approval period, you must request ACUC approval prior to the end of the approval period.
- Annual status reports may be required by the ACUC. These reports must include a complete description of any and all changes in your project.

Please take time to review our ACUC web page at: <http://research.uwf.edu/boards-committees/acuc.htm> which includes the ACUC Policies and Procedures, the PHS Policy on Humane Care and Use of Laboratory Animals, the Guide for the Care and Use of Laboratory Animals, and other pertinent ACUC documents.

Good luck in your research endeavors.

Sincerely,

Richard S. Podemski, Assoc. Vice President for Research
and Dean of Graduate Studies

CC: Dr. Wayne A. Bennett, PhD
Dr. George Stewart, Chair

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