Journal of Thermal Biology 81 (2019) 170–177

Contents lists available at ScienceDirect

Journal of Thermal Biology

journal homepage: www.elsevier.com/locate/jtherbio

Thermal niche adaptations of common mudskipper (*Periophthalmus kalolo*) and barred mudskipper (*Periophthalmus argentilineatus*) in air and water

Theresa F. Dabruzzi[a,*], Nann A. Fangue[b], Nadiarti N. Kadir[c], Wayne A. Bennett[d]

[a] Department of Biology, Saint Anselm College, 100 Saint Anselm Drive, Manchester, NH 03102, USA
[b] Department of Wildlife, Fish, and Conservation Biology, University of California, 1088 Academic Surge, Davis, CA 95616, USA
[c] Department of Fisheries, Hasanuddin University, Makassar, Indonesia
[d] Department of Biology, University of West Florida, 11000 University Parkway, Pensacola, FL 32514, USA

ARTICLE INFO

Keywords:
Air-breathing fish
Thermal tolerance
Mangrove
Temperature tolerance polygon
Ectotherms

ABSTRACT

Thermal tolerance niche analyses have been used extensively to identify adaptive thermal tactics used by wholly aquatic fishes, however no study to date has quantified thermal niche characteristics of air-breathing fishes. We use standardized thermal methodologies to estimate temperature acclimation ranges, upper and lower acclimation response ratios, and thermal niche areas in common (*Periophthalmus kalolo*) and barred (*Periophthalmus argentilineatus*) mudskippers in air and water. Common and barred mudskippers had an upper chronic limit of 37.0 °C, and respective low chronic temperatures of 14.0 and 11.4 °C, resulting in acclimation scope values of 23.0 °C and 25.6 °C. Both fishes had moderately large thermal niches, with barred mudskipper expressing larger niche areas in both water and air than common mudskipper (676.6 and 704.2 °C^2 compared to 641.6 and 646.5 °C^2). Acclimation response ratios were relatively low, with fish gaining or losing between 0.10 and 0.43 °C of heat tolerance with each 1 °C change in acclimation temperature. Although intraspecific total niche areas remained largely unchanged between media (≤10%), both species showed a slight increase in heat tolerance but a notable upward shift in intrinsic tolerance when emerged. Media-dependent thermal niche adjustment is a unique, and thus far undescribed physiological adaptation that in combination with behavioral responses, allow mudskippers to thrive in some of the most austere thermal environments experienced by any fish.

1. Introduction

With over 34,100 valid species currently described (Eschmeyer et al., 2017), fishes account for more than one-half of all extant vertebrates. While the vast majority of fish are obligate water-breathers, at least 16 families include one or more amphibious, air-breathing species (Sayer and Davenport, 1991). Amphibious fishes are defined by their ability to remain fully terrestrial for some portion of the daily cycle as part of their normal life history (Gordon et al., 1969; Murdy, 1989; Sayer and Davenport, 1991). The subfamily Oxudercinae is the most terrestrial of the amphibious fish groups and includes 41 marine and brackish gobiids from nine genera — the largest being the genus *Periophthalmus* with 18 valid species (Eschmeyer et al., 2017). The term "mudskipper" is traditionally used to describe species within the four most terrestrial oxudurcine genera i.e., *Boleophthalmus*, *Periophthalmodon*, *Periophthalmus*, and *Scartelaos* (Graham and Lee, 2004; Polgar and Crosa, 2009; Agorreta et al., 2013). Additionally, the slender mudskipper, *Zappa confluentus*, may be included based on activity patterns and life history similarities (Polgar et al., 2010). Presently these five genera include 32 mudskipper species (Eschmeyer et al., 2017) from soft-bottom mudflat zones, mangal habitats, river deltas (Tamura et al., 1976; Colombini et al., 1995), and rocky coastlines (Gordon et al., 1968, 1969) throughout the Indo-Pacific to southern Japan and west to the Atlantic coast of Africa (Murdy, 1989).

Under favorable conditions, mudskippers emerge during ebb tide to feed, find mates, and defend or modify their territories (e.g., Clayton and Vaughan, 1988, Chen et al., 2007; Takeda et al., 2012). Some species are known to remain emerged for hours (Gordon et al., 1978), or even days (Takeda et al., 2012) waiting for high tide to return. Long emersion times in the group are supported by various key adaptations including multiple respiratory exchange surfaces (Bridges, 1988), unique biochemical and physiological modifications for avoiding ammonia toxicity (Ip et al., 2004), and significant cutaneous resistance to evaporative water loss (Dabruzzi et al., 2011). Shuttling between media

* Corresponding author.
E-mail addresses: tdabruzzi@anselm.edu (T.F. Dabruzzi), nafangue@ucdavis.edu (N.A. Fangue), nadiarti@unhas.ac.id (N.N. Kadir), wbennett@uwf.edu (W.A. Bennett).

https://doi.org/10.1016/j.jtherbio.2019.02.023
Received 6 October 2018; Received in revised form 2 February 2019; Accepted 27 February 2019
Available online 28 February 2019
0306-4565/

benefits mudskippers by reducing competition for food and mates, allowing access to a wider array of food resources, and providing a means to effectively evade both aerial and aquatic predators (Sayer and Davenport, 1991). In warm seasons, however; mudskippers can experience potentially dangerous increases in body temperature, as well as high rates of desiccation when moving from water to air (Gordon et al., 1969; Bennett, 2010). Even so, field studies have reported no less than seven periophthalmid mudskippers (see Burhanuddin, 1979; El-Ziady et al., 1979; Tytler and Vaughan, 1983; Gordon and Gabaldon, 1985; Clayton and Snowden, 2000; Takita et al., 2011; and Takeda et al., 2012) and two boleophthalmid mudskippers (see Clayton and Vaughan, 1988; Chen et al., 2007) that are active at air temperatures between 38 and 40 °C, temperatures only a few degrees below the fishes' lethal upper limit (Gordon et al., 1969). Cold temperatures may also exert a great deal of control over mudskipper activity. Most mudskippers avoid the harshest low temperatures by retreating to burrows where temperatures may be several degrees warmer than air and emerging only after air temperatures rise (El-Ziady et al., 1979; Chen et al., 2007). A few mudskipper species have been observed to spend the entire cold season in their burrows (Tytler and Vaughan, 1983; Ikebe and Oishi, 1997), presumably in an inactive brumation state, sometimes mistakenly referred to as hibernation (Park et al., 2008). Although populations in temperate regions may experience air temperatures as low as 1 °C (El-Ziady et al., 1979), exposure to cold temperature is seldom lethal. A known exception are juvenile great blue spotted mudskippers (*Boleophthalmus pectinirostris*) that sometimes choose mud habitats which reach low lethal levels of 0.8 °C (Takegaki et al., 2006).

In spite of temperature's obvious importance to mudskipper activity and survival, thermal relationships are seldom the main focus of field studies. Most field observations customarily list a range of air, water, substrate, and/or burrow temperatures measured during the study interval (e.g., Takegaki et al., 2006; Chen et al., 2007; Mahadevan and Ravi, 2015). A few studies report temperature ranges over which mudskippers are emerged and active (Stebbins and Kalk, 1961; Clayton and Vaughan, 1988; Colombini et al., 1995). Occasionally, spontaneous field experiments have been used to evaluate the effect of natural occurring thermal conditions on mudskipper behavior and survival (e.g., Gordon et al., 1969; Takeda et al., 2012). Unfortunately, most of these experiments lack controls or standardized methods, making data comparison difficult, and interpretations somewhat subjective. While empirical manipulation studies are rare, experimental measures of thermal preference (Chen et al., 2007; Gordon and Gabaldon, 1985), upper or lower temperature tolerance (Clayton and Snowden, 2000; Taylor et al., 2005; Bennett, 2010), incipient lethal temperatures (El-Ziady et al., 1979), and lethal exposure times (Takegaki et al., 2006) are available for a few species. Although the role of influencing factors such as previous acclimation history, life stage, and species or population differences on thermal tolerance remain largely unexplored (Gordon and Gabaldon, 1985), recent studies suggest increased oxygen concentrations may improve thermal tolerance of some air breathing fish and crabs (Graham and Lee, 2004; Taylor et al., 2005; Bennett, 2010; Giomi et al., 2014). These findings have important ramifications for mudskippers when choosing between tidal pools with high biological oxygen demand and low oxygen content, and oxygen-rich aerial environments with critically high air temperatures.

The purpose of this study was to use standardized thermal tolerance methodologies to elucidate thermal niche requirements of common (*Periophthalmus kalolo*) and barred (*Periophthalmus argentilineatus*) mudskippers in air and water. Both species are widespread and abundant throughout their range and often occur together in the same habitat (Murdy, 1989). Mudskippers are important components of mudflat and mangal communities. Their excretions increase ammonium and nitrate sediment levels, while organic matter mixed into sediments during burrowing is oxidized to sulfates, making sediments more acidic (Ng et al., 2007). *Avicennia alba* seedlings growing in mudskipper-enhanced sediment exhibit higher leaf number, greater height, and increased dry mass over plants grown in sediments that have not been altered by mudskippers (Shenoy et al., 2012). Their close association with water, air, and sediment also make mudskippers excellent indicators of mangrove habitat health (Polgar, 2009; Ansari et al., 2014).

Specific research objectives of this study were to, 1) estimate thermal acclimation ranges for both mudskipper species, 2) quantify upper and lower thermal tolerance responses of mudskippers acclimated at selected temperatures across their acclimation range, 3) use the acclimation and tolerance values to describe thermal niche boundaries for both species in air and water, and 4) compare aerial and aquatic thermal niche components within and between species, and interpret the results relative to each species' activity within their natural habitat. While thermal tolerance niche analyses have been extremely useful in identifying adaptive thermal tactics used by wholly aquatic fishes from a variety of thermal environments (Fry et al., 1942; Bennett and Beitinger, 1997; Eme and Bennett, 2009; Elliott and Elliott, 2010; Dabruzzi et al., 2013), this study is the first to evaluate thermal tolerance niche characteristics for amphibious fish species in two media.

2. Materials and methods

2.1. Collection, site description, and laboratory holding conditions

Barred mudskipper (n = 74; wet mass 3.2 ± 1.89 g; standard length 5.8 ± 1.08 cm) and common mudskipper (n = 74; wet mass 5.8 ± 4.45 g; standard length 6.9 ± 1.91 cm) were captured at night during low tide using aquarium dip nets. Fishes were collected from the mangrove habitat on the northeast side of Hoga Island (05°27.53'S, 123°46.33'E) in the Wakatobi National Park, Sulawesi Indonesia. The collection site borders a mangrove forest overlaying the island's coral base, and is best characterized as a low slope, low exposure open mudflat (see Dyer et al., 2000). Maximum spring tidal amplitude at the site is approximately 1.2 m, with mudflat and mangle margins fully inundated at high tide and fully exposed at low tide. Common mudskippers were collected from the open mudflat zone, whereas barred mudskippers were collected from nearby vegetated regions at the mangrove margins (Stebbins and Kalk, 1961; Colombini et al., 1995). Captured fish were transported to the Hoga Island Research Laboratory where they were segregated by species and housed in 70-L insulated plastic holding tanks. Holding tanks were supplied with flow-through seawater (approximately 3 cm deep) at field temperatures (26 ± 1.0 °C), and holding environments were enhanced by addition of partly emerged coconut shell fragments that provided hiding space and allowed fish free movement between air and water. The constant turnover of seawater provided by the flow-through system provided tanks with clean seawater, a constant pH, high dissolved oxygen concentration, and no ammonia buildup.

Mudskippers were fed fresh chopped tuna daily and remained at holding conditions for at least one week before being assigned to experimental treatment groups. Mudskippers were used only once in an experiment, and all fish were released at their site of capture following trials.

2.2. Acclimation range experiments

Upper and lower acclimation temperatures defining the thermal acclimation ranges for barred and common mudskippers, were estimated using chronic thermal methodology (Beitinger et al., 2000; Dabruzzi et al., 2013). For chronic trials, 10 mudskippers of a given species were randomly sorted into one of three replicate plastic 10-L tanks with lids. Two tanks housed three mudskippers each, while the third contained four fish. Chronic tanks were not flow-through systems, and water quality was maintained by twice daily 30% water changes with clean seawater within 0.5 °C of current tank temperatures (Oakton, Model 300, Digital Thermometer). Each chronic tank was filled with 3 cm of seawater and included partly emerged coconut shell fragments, allowing the fish free access to aerial and aquatic portions of the tank. Air and water temperatures in chronic treatment experiments were held within 0.2 °C of each other and were regulated by placing treatment tanks in an insulated, temperature controlled, recirculating water bath (Azoo Micro-controller with a 300-W heater element and ¼ hp water chiller). Temperatures were increased during chronic maximum trials and decreased during chronic minimum trials by 1 °C per day until fish experienced loss of righting response (LRR). The rate of temperature change employed by chronic thermal methodology enables an accurate determination of a fish's acclimation range (Beitinger et al., 2000; Eme and Bennett, 2009; Dabruzzi et al., 2017). As LRR was observed, fish were removed from the treatment tank, and water temperature (± 0.1 °C), wet mass (± 0.1 g), and standard length (± 0.1 cm) recorded. Upper and lower mean LRR temperatures were determined for each replicate tank, and upper and lower chronic limits for the population were taken as the respective replicate tank mean values (Beitinger et al., 2000).

2.3. Constant temperature acclimation experiments

Critical Thermal Methodology (CTM) was used to quantify acclimation responses of barred and common mudskipper held at constant air and water temperatures (Cowles and Bogert, 1944; Paladino et al., 1980; Beitinger et al., 2000). Temperature treatments were established by randomly sorting each species (n = 64 per species) into one of eight 4-L acclimation treatment tanks with lids. Each acclimation tank was further divided into eight sections containing one fish each, with four fish in each tank used in heat tolerance trials, and the remaining four used in cold tolerance trials (Fig. 1). Two acclimation tanks were set up for each temperature treatment, and each acclimation tank was supplied with flow-through seawater either heated, or chilled to temperatures of 20.0, 26.0, 29.5, or 32.5 (± 0.3 °C). Similar to chronic trials, coconut fragments were placed in each tank section to provided fish access to air. Tank acclimation temperatures were measured three times daily (Oakton, Model 300, Digital Thermometer), and air and water temperatures varied by less than 0.3 °C from one another. Mudskippers were held at constant acclimation treatment temperatures for 14 days (Cox, 1974; Fangue et al., 2014) before undergoing CTM trials. It is widely accepted that fish acclimate to a new temperature between 1 (Brett, 1944) and 20 (Doudoroff, 1942) days, with the actual rate of acclimation differing across species. While acclimation rate data are limited in the literature, several fishes show complete acclimation by 12 days and acclimation rate data from rockskipper species on Hoga Island show complete acclimation by 7 days (Bennett et al., unpublished data). As a fish's thermal acclimation pattern is tied to the temperature profile of their habitat, fish such as mudskippers which experience large, rapid, and unpredictable temperature shifts require less acclimation time. Mudskippers were not fed 24 h prior to, or during CTM experiments.

Following acclimation, CTM was used to estimate critical thermal maximum (CTmax), and critical thermal minimum (CTmin) temperatures in air and water for both mudskipper species (Becker and Genoway, 1979; Paladino et al., 1980; Beitinger et al., 2000). Mora and Maya (2006) evaluated rates of temperature change for critical thermal trials and determined rates that are too fast or slow can cause an over or under estimated CTmax. Mudskipper CTM values were determined by increasing or decreasing air or water temperature by 0.3 °C/min until loss of equilibrium (LOE) or loss of righting response (LRR) was reached. The rate was fast enough to prevent acclimation, but slow enough to allow fish body temperature to track water temperature (Cox, 1974; Taylor et al., 2005; Fangue et al., 2014). This rate is also a reasonable temperature ramp similar to what mudskippers would might experience in a tide pool habitat during summer. In aquatic trials, heating or cooling continued until mudskippers exhibited LOE, with LOE defined as the inability to maintain dorso-ventral orientation for one minute. Mudskippers in aerial trials were heated or cooled until they displayed LRR, or the inability to right themselves within one minute of being overturned with a glass rod (Beitinger et al., 2000). Both endpoints were near, but non-lethal and all fish survived CTM trials when returned to their previous acclimation temperature. Coutant (1969, 1970) has effectively argued, loss of locomotor control in nature is ecologically equivalent to death, either due to predation, or the continued exposure to a subsequently lethal temperature.

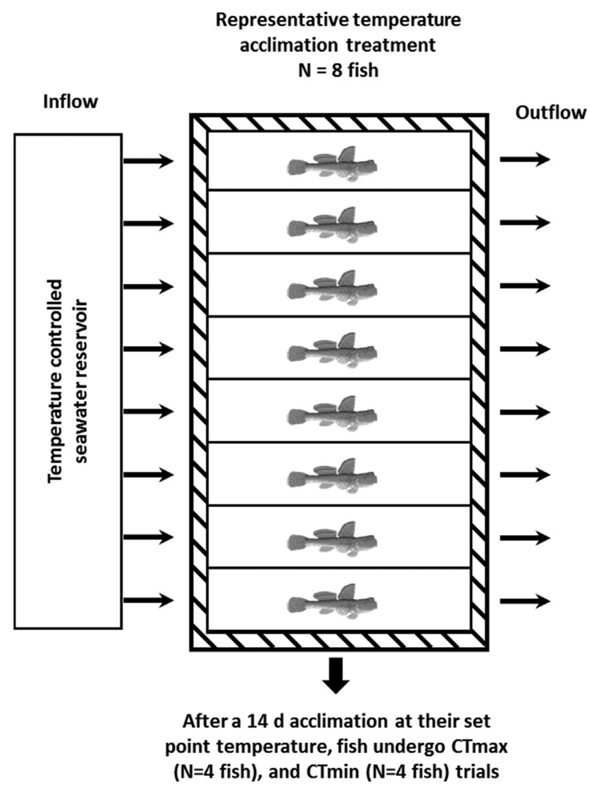

Fig. 1. Insulated, flow-through, temperature acclimation system for mudskippers used in aerial and aquatic Critical Thermal Maximum (CTmax) and Critical Thermal Minimum (CTmin) experiments. A total of 16 treatment systems were used (2 species × 2 media × 4 treatment temperatures). Within each system, mudskippers were individually separated into one of eight cells supplied with flow-through seawater and fish had access to both water and air during the acclimation period (see text for details).

Testing in two media required two CTM chamber designs. In aquatic CTM trials, mudskippers were individually placed into 250-mL glass beakers filled with clean seawater within 1 °C of the fishes' acclimation temperature. A screen secured to the top of the beaker prevented escape and assured that fish remained submerged during trials. Moderate aeration was provided to each beaker to promote oxygen saturation and prevent thermal stratification. For aerial trials, mudskippers were placed singly into insulated, plastic, 2-L CTM chambers. A fiberglass screen ~2 cm above the chamber floor prevented fish from directly contacting the chamber bottom, and 20 mL of water added to the space assured that chamber air remained fully saturated during trials (Heatwole et al., 1965; Dunlap, 1968; Krakauer, 1970). Temperatures were monitored using an Oakton, Model 300, Digital Thermometer. A recirculating water bath (insulated plastic container 9 × 28 × 43 cm containing an Azoo Powerhead model 1200) was used to heat or cool test chambers for both aquatic and aerial trials. For aquatic trials, water temperature next to the fish was recorded as LOE was observed (Hutchison, 1961; Kaufmann and Bennett, 1989). In aerial experiments, fish were quickly removed from the aerial CTM chamber as LRR occurred, and an ultra-thin thermocouple wire (Omega 5LSC-KK-K-24–36) was inserted into the vent to measure body temperature.

Table 1
Niche temperature summary data for barred (*Periophthalamus argentilineatus*) and common (*Periophthalamus kalolo*) mudskippers. Treatment group CTmaxima or CTminima with similar superscripts are statistically indistinguishable. Tolerance values for fish acclimated at 32.4 °C are given in the table but were not used in thermal niche development. Data are mean ± SE; p ≤ 0.05.

Critical thermal maxima trials		High acclimation response ratio model			Critical thermal minima trials		Low acclimation response ratio model			Chronic temperatures	
Acclimation (°C)	CTmax (°C)	n	Slope	Y-intercept	Acclimation (°C)	CTmin (°C)	n	Slope	Y-intercept	High Limit (°C)	Low Limit (°C)
Aquatic Trials for Barred Mudskipper, *Periophthalamus argentilineatus*											
20.1	38.7(± 0.28)^{A}	4	0.22	34.26	20.1	11.0(± 0.50)^{A}	4	0.25	6.02	37.0	11.4
26.0	39.9(± 0.24)^{A}	4			26.0	12.7(± 0.51)^{A}	4				
29.6	40.8(± 0.38)^{B}	4			29.6	13.3 (± 0.26)^{B}	4				
32.4	38.3	1			32.4	16.7	1				
Aerial Trials for Barred Mudskipper, *Periophthalamus argentilineatus*											
20.1	39.8(± 0.82)^{A}	4	0.15	36.30	20.1	13.0(± 0.44)^{A}	4	0.25	6.03		
26.0	38.9(± 0.79)^{A}	4			26.0	13.0(± 1.20)^{A}	4				
29.6	41.7(± 0.48)^{A}	4			29.6	15.5(± 0.86)^{A}	4				
32.4	42.4(± 1.00)	2			32.4	15.9	1				
Aquatic Trials for Common Mudskipper, *Periophthalamus kalolo*											
20.1	39.6(± 0.42)^{A}	4	0.20	35.49	20.1	11.3(± 0.)^{A}	4	0.23	6.65	37.0	14.0
26.0	40.9(± 0.21)^{AB}	4			26.0	12.9(± 051.)^{A}	4				
29.6	41.5(± 0.37)^{B}	4			29.6	13.5(± 0.31)^{B}	4				
32.4	39.3(± 0.52)	3			32.4	17.5(± 0.25)	2				
Aerial Trials for Common Mudskipper, *Periophthalamus kalolo*											
20.1	40.7(± 0.20)^{A}	4	0.10	39.00	20.1	11.7(± 1.54)^{A}	4	0.43	2.58		
26.0	42.2(± 0.39)^{A}	4			26.0	13.0(± 1.20)^{A}	4				
29.6	41.6(± 0.59)^{A}	4			29.6	16.1(± 1.3)^{A}	3				
32.4	41.1(± 0.86)	3			32.4	13.3(± 1.3)	2				

Following trials all fish were weighed (wet mass ± 0.1 g), measured (standard length ± 0.1 cm), and returned to their previous acclimation temperature to recover.

Critical thermal maxima and minima of barred and common mudskippers at each acclimation temperature treatment were calculated as the arithmetic mean (± one standard deviation) of the collective replicate endpoints (Cox, 1974; Becker and Genoway, 1979; Beitinger et al., 2000). Statistics analysis was performed (Statistical Analysis Software, SAS 9.4) by one-way analysis of variance (ANOVA) followed by the post-hoc Tukey's test to find mean differences using an alpha level of 0.05 (Table 1). Relationships between acclimation temperature and mudskipper CTmax and CTmin values in air and water were modeled for both species via general linearized model (GLM) regression analysis. The resulting slope values are reported as acclimation response ratios (ARR) which quantify heat or cold tolerance gained or lost with every 1 °C change in acclimation temperature (Table 1) and is a convenient means of comparing acclimation responses in different species or populations (Claussen, 1977; Cuculescu et al., 1998). A test for interaction between the treatment factor and the independent variable was used to test for equal ARR slope values within species.

2.4. Thermal niche determinations in air and water

Critical and chronic temperature tolerance data were used to define the ecological thermal niche in both air and water for each mudskipper species. Data are graphically expressed as quadrilateral polygons representing each fishes' ecological thermal niche (Bennett and Beitinger, 1997; Fangue and Bennett, 2003; Dabruzzi et al., 2013). Lower and upper chronic tolerance limits were used to define the lateral polygon boundaries, whereas respective top and bottom polygon boundaries were estimated from a general linearized model (GLM) regression of CTmax or CTmin values on acclimation temperature. The resulting polygons are expressed quantitatively using areal units of $°C^2$ and define the total niche area for each mudskipper species in each media. Total polygonal areas were further divided into intrinsic tolerance zones (i.e., thermal tolerance independent of previous thermal acclimation) as well as upper and lower acquired tolerance zones (i.e., thermal tolerance gained through acclimation) by dividing the polygon with horizontal lines originating from the intersection of the CTmin and CTmax regressions at their respective upper and lower chronic limits.

3. Results

3.1. Mudskipper acclimation ranges

Mudskippers exposed to chronic temperature change remained active and continued feeding across a wide temperature range. While the upper chronic temperature limit for both species was 37.0 °C, low chronic temperatures differed between species, with common mudskipper and barred mudskipper exhibiting low chronic limits of 14.0 and 11.4 °C, respectively. The resulting acclimation ranges (difference between maximum and minimum chronic temperatures) differed by 2.6 °C between species with barred mudskipper exhibiting an acclimation range approximately 11% larger than that estimated for the common mudskipper. While both mudskippers tolerated temperatures up to 37.0 °C during high chronic trials (temperature increase of 1 °C per day over approximately seven days), longer-term exposure to a constant temperature of 32.5 °C (i.e., the high temperature treatment acclimation group) resulted in high mudskipper mortality after 10 days to two weeks. Barred mudskippers were most sensitive to prolonged high temperature exposure and experienced 69% mortality (11 of 16 fish) at 32.5 °C. Over the same period, mortality of common mudskipper was 37% (6 of 16 fish), or roughly half that of barred mudskipper. Surviving mudskippers of both species in 32.5 °C acclimation groups demonstrated markedly diminished CTmax or CTmin performance compared to fish from the other three constant treatment temperatures. Common and barred mudskippers demonstrated inherently greater individual variability in aerial temperature tolerance trials than in aquatic trials, with both fishes exhibiting significant GLM regression relationships between acclimation temperature and high and low thermal tolerance values when measured in water ($p = 0.0001–0.006$), but not in air ($p = 0.13–0.22$). Data from fish in the 32.5 °C treatments are reported in Table 1 but were not used in determining thermal niche areas.

3.2. Responses to constant temperature acclimation

Both mudskipper species showed a rise in heat tolerance and a reduction in cold tolerance as acclimation temperatures increased (Table 1). On average, barred mudskipper gained approximately 2.1 °C of heat tolerance and lost about 2.4 °C in cold tolerance as acclimation temperatures increased from 20.0 and 29.5 °C. General linearize model regressions estimated low (CTmin) and high (CTmax) acclimation response ratios of 0.25 and 0.15 in air, and 0.25 and 0.22 in water for barred mudskippers acclimated between 20.0 and 29.5 °C. Common mudskipper exposed to 20.0 and 29.5 °C constant temperatures, lost 2.2 °C of cold tolerance in water, but exhibited a markedly higher cold tolerance loss of 5.3 °C in air. Average heat tolerance gain between 20.0 and 29.5 °C was 1.9 °C in water, and 0.9 °C in air. Common mudskipper showed similar CTmin and CTmax acclimation response ratios in water of 0.23 and 0.20, respectively. In air, however, the fish exhibited a relatively low CTmax response ratio of 0.10, but a markedly higher CTmin acclimation response ratio of 0.43 in air. Comparisons of upper and lower acclimation response ratios (i.e., slopes) within species reveled statistically similar slope values in air and water for barred mudskipper ($p = 0.17$ and 0.07, respectively). Conversely, between media comparisons for common mudskipper found significant differences in response ratios ($P = 0.0001$).

3.3. Thermal niche responses

While total niche area (measured as °C^2) remained largely unchanged for barred and common mudskippers between air and water, notable shifts in the fishes' zones of intrinsic tolerance were apparent (Table 2). Critical thermal maximum values of both mudskippers in air were approximately 1 °C higher than in water. Interestingly, the increase in high temperature tolerance did not substantially change total niche dimensions for barred or common mudskippers, but did result in an upward migration of the species' intrinsic tolerance zones (Fig. 2). The effect was most pronounced in the common mudskipper which exhibited a 3.8 °C upward shift in its intrinsic tolerance zone. Barred mudskipper experienced less upward movement of 1.6 °C. The shift did not alter the intrinsic zone size, as both species showed only a slight 1–5% decrease in intrinsic area, but rather reduced the upper acquired tolerance zone area in barred and common mudskipper by 26% and 48%, respectively. In addition, the intrinsic zone migration produced a 54% increase in the lower acquired tolerance zone of common mudskipper tested in air, but did not affect the lower acquired tolerance of barred mudskipper. Overall, barred mudskipper generally express larger thermal niche areas in both air and water than common mudskipper (Table 2), although total niche area varied between species by no more than 65 °C^2 regardless of media.

4. Discussion

Relatively few studies have examined heat tolerance of air-breathing fishes moved between water and air (Taylor et al., 2005; Barreda, 2010; also see Fusi et al., 2016), and with exception of the present work, none have documented effects of media-dependent heat tolerance shifts across an animals' entire thermal niche. The mechanism by which tolerance is altered by media is a topic of some debate, but it is thought that increased oxygen availability in aerial environments delays the onset of anaerobic metabolism, allowing the animal to reach higher critical temperatures. There is some evidence to suggest that the effect is manifested at the mitochondrial level (Pörtner, 2001; Abele et al., 2002), however the nearly ubiquitous expression of muscle spasms and loss of nervous control seen in heat tolerance trials of various fishes suggest that changes in membrane permeability, leading to uncontrolled ion-exchange, should also be considered (Lutterschmidt and Hutchison, 1997). At the organismal level, differential efficiency of the gill, or air breathing complex may influence changes in oxygen uptake rate between media, such that the same heat treatment might result in an increase in heat tolerance for one species but produce a decrease in another. Among mudskippers, for example, Shuttle's hoppfish, *Periophthalmus modestus*, (Tamura et al., 1976) and Boddart's goggle-eyed goby, *Boleophthalmus boddarti*, (Kok et al., 1998) exhibit better oxygen extraction in water than in air. One might reasonably expect these mudskippers to exhibit lower heat tolerance in air than in water—an emergent property that is the product of oxygen adaptations and not oxygen concentrations.

Aerial and aquatic thermal niche characteristics of common and barred mudskipper explain in large part the success of these species in exploiting a variety of thermal environments from southern Japan to the Indo-Pacific and East Africa (Eschmeyer et al., 2017). The thermal acclimation range of both fishes spans more than a 20 °C interval. (23 °C for common and 25.6 °C for barred mudskipper), and both have thermal scope values (difference between CTmax and CTmin values) of approximately 30 °C (Table 1). As a result, common and barred mudskippers display moderately large total niche areas between approximately 642 °C and 704 °C^2 (Table 2). Comparable niche values have been reported for wholly aquatic thermophilic gobies living in or near common and barred mudskipper habitats (Taylor et al., 2005; Eme and Bennett, 2009). Mudskipper niche areas are similar to those reported for the sandflat goby, *Bathygobius* sp., value of 639 °C^2, but somewhat lower than the thermal niche of 829 °C^2 reported for the common goby, *Bathygobius fuscus*, (Eme and Bennett, 2009). For contrast, it is worth noting that the current range of niche area values for thermophilic fishes is between approximately 140 °C^2 for neon cleaner gobies (family Gobiidae; Di Santo and Lobel, 2017), and 1500 °C^2 for desert pupfishes (family Cyprinodntidae; Bennett and Beitinger, 1997).

Giomi and coworkers (2014) postulated that amphibious fish and crabs may widen their thermal niche when breathing air. While barred and common mudskippers did demonstrate a moderate increase in aerial heat tolerance of about 1 °C, overall acclimation range and thermal scope measures changed very little between air and water trials (Fig. 2). What was apparent however, was that the slight increase seen in heat tolerance was accompanied by a marked upward shift in the

Table 2
Between-species comparisons of niche zone areas in air and water for barred (*Periophthalamus argentilineatus*) and common mudskipper (*Periophthalamus kalolo*). Niche values are given in areal units of °C^2, with changes in niche component area expressed as percentage increase or decrease.

Medium	Zone	Niche area values by species (°C^2)		Relative % difference
		Barred Mudskipper *Periophthalamus argentilineatus*	Common Mudskipper *Periophthalamus kalolo*	
Water	Total	704.2	646.5	8.9
	Upper Acquired	71.4	53.3	34.0
	Lower Acquired	81.6	60.9	33.9
	Intrinsic	551.2	532.3	3.6
Air	Total	676.6	641.6	5.4
	Upper Acquired	50.1	27.6	81.4
	Lower Acquired	72.3	110.2	34.4
	Intrinsic	544.2	503.7	10.0

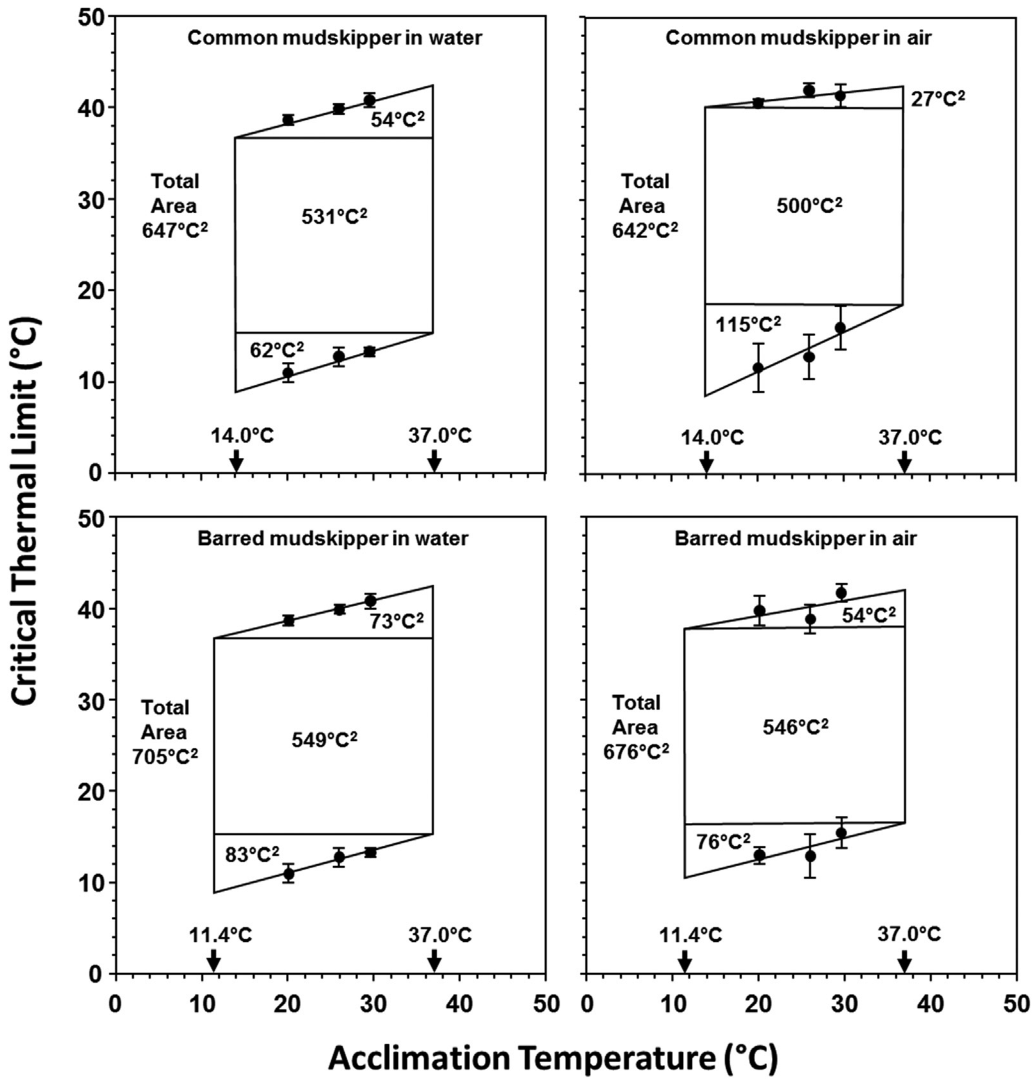

Fig. 2. Thermal niche areas for common mudskipper, *Periophthalamus kalolo*, and barred mudskipper, *Periophthalamus argentilineatus*, in air and water. Total species' niche area for each media is divided into upper acquired tolerance (upper triangular area), lower acquired tolerance (lower triangular area), and intrinsic tolerance (square, central area). Lower and upper chronic temperatures in each graph are indicated by arrows on the X-axis. Critical thermal maxima and minima are represented as filled circles with error bars (± one standard deviation). All values are reported as °C^2 and rounded to the nearest whole unit.

intrinsic tolerance zone (1.6 °C in barred mudskipper and 3.8 °C in common mudskipper). The intrinsic zone represents the range and scope of temperatures to which a fish is always acclimated (Beitinger and Bennett, 2000), and circumscribes the single largest component of a species thermal niche (Table 2). Fishes may move freely within the bounds of their intrinsic temperature zone without the need to establish new tolerance limits through the process of thermal acclimation. The upward migration of the intrinsic zone during emergence would be of considerable benefit to mudskippers in that it immediately improves the fish's ability to tolerate higher air temperatures.

Media-dependent shifts in intrinsic temperatures also had some influence on mudskipper cold tolerance. Barred mudskipper showed a modest decrease in acquired cold tolerance in air. In general, low temperature exposure is much less dangerous to fishes than high temperature exposure (Brett, 1956), and given time, barred mudskippers are capable of achieving CTmin values near or below 10 °C, levels that would provide adequate protection in cooler environments within their geographic range. Conversely, common mudskipper tested in air (Table 1) demonstrated a moderately high increase in the lower acquired thermal tolerance zone. Although it seems odd that common mudskipper would exhibit a strong capacity for cold acclimation, the species does have a wide distribution reaching as far north as the Ryukyu Islands in Japan (Eschmeyer et al., 2017), and may rely more heavily on cold tolerance in cooler regions of its range. Exaggerated cold tolerance responses have also been reported for blue spotted mudskippers, *Boleophthalmus pectinirostris*, from the Rokkaku River, Japan, where the fish may experience winter air temperatures as low as 3 °C. Takegaki et al. (2006) report field acclimatized mudskippers acutely exposed to 3, 5, and 7 °C show survival times from ~24 h to 15 days — the lowest cold tolerance values measured to date for a mudskipper.

Another unusual mudskipper niche feature was their low (0.10–0.22 °C) heat tolerance acclimation response ratios (Table 1). Fishes exposed to reduced heat tolerance selective pressures generally express weak acclimation responses. For example, Antarctic black rockcod, *Notothenia coriiceps*, and emerald rockcod, *Trematomus bernacchii*, living in perpetually cold polar waters exhibit low acclimation response ratios of 0.21 and 0.24 (Bilyk and DeVries, 2011). Differing environmental thermal conditions have resulted in a similar reduction in heat tolerance selective pressures for mudskippers. Mudskippers experience rapid and extreme temperature shifts as they move between water and air (Clayton and Snowden, 2000; Taylor et al., 2005), but the time required to bring traditional acclimation processes online are too long to be an effective defense against these rapid temperature changes

(Beitinger and McCauley, 1990). Eme and Bennett (2010) suggest that thermophilic gobies may mitigate the problem of sudden exposure to potentially damaging temperatures by evolving metabolic pathways that include suites of allozymes that operate sequentially across a range environmental temperatures (Somero, 1969). Temperature effects may be further ameliorated by the addition of protective protein components such as constitutive heat shock proteins (Feder and Hofmann, 1999). This hypothesis is consistent with acclimation responses seen in the mudskipper niche data and would also explain the sudden shift in the fishes' intrinsic tolerance zone between water and air.

While laboratory temperature responses have generated useful estimates of thermal niche requirements for many fully aquatic fish species (Beitinger et al., 2000), experimental results alone may not accurately reflect the full extent of adaptations used by air-breathing mudskipper fishes living in harsh thermal environments. Indeed, mudskippers exploit a number of behavioral thermoregulation tactics while emerged that may expand their realized thermal tolerance limits. Behavioral responses can be implemented within a few minutes to a few hours and are an organism's first line of defense against thermal stress (Beitinger and McCauley, 1990). One of the most ubiquitous temperature-related behaviors in mudskipper fishes is to retreat to a burrow as temperatures approach stressful levels (El-Ziady et al., 1979; Chen et al., 2007, 2008; Tytler and Vaughan, 1983). Burrows play an important role in mudskipper thermal ecology by protecting fish, as well as their developing eggs, from high and low temperature extremes. Common and barred mudskippers are active between approximately 24 and 38 °C (Gordon et al., 1969; Taylor et al., 2005; Takeda et al., 2012), but at temperatures outside their active range, fish may take refuge in burrows where temperatures are more moderate. El-Ziady and coworkers (1979) report that at summer air temperatures of 31–40 °C, burrows of gold-spotted mudskipper, *Periophthalmus chrysospilos*, exhibit lower-than-air temperatures of 17.5–22.5 °C. Alternatively, in Funing Bay, China, great blue spotted mudskipper burrows remained between 10.0 and 12.7 °C, at winter air temperatures as low as 4.0 °C (Chen et al., 2007). In addition to burrowing, it is widely believed that mudskippers can mitigate high temperature stress by exploiting evaporative cooling. Common (Taylor et al., 2005) and barred mudskippers (Gordon et al., 1968; Taylor et al., 2005) routinely shuttle between pools and sun-exposed mudflat or sand habitat and may utilize moist depressions when moving across relatively dry stretches (Nursall, 1981). Some species (including the barred mudskipper) have been observed moistening the skin by rapidly rolling side-to-side in damp mud, or by pectoral fin slapping (Stebbins and Kalk, 1961; Brillet, 1975; Nursall, 1981). Tytler and Vaughan (1983) found that evaporative cooling in the Atlantic mudskipper, *Periophthalmus barbarous*, resulted in body temperatures 4–7 °C cooler than substrate temperatures. Exploitation of evaporative cooling during high temperatures, and utilization of burrow microhabitats during both high and low temperatures would effectively broaden mudskipper acclimation zones by shifting the fishes' upper and lower chronic temperatures. Niche metrics of common and barred mudskipper suggest that a change in acclimation range of only 1 °C would expand their thermal niche by nearly 4%.

Barred and common mudskippers are the most widely distributed of the air-breathing periopthalmids, and occur together (i.e., are sympatric) across much of their range (Eschmeyer et al., 2017). A comparison of native ranges finds barred mudskipper having the more northerly distribution (Froese and Pauly, 2017), reaching as far north as the Ryukyu Islands in Japan (Latitude: 34.5809° N, 105.7250°E) where January to February low temperatures may reach 11.0 °C (Jensen, 2018) — temperatures similar the species' empirically derived low chronic value of 11.4 °C. Accordingly, mudskipper niche analysis reveals that barred mudskippers are more tolerant of cool conditions, having a chronic minimum temperature more than 2.5 °C lower than common mudskippers. In locales where they occur together, including Hoga Island, the two species exhibit some level of habitat segregation, with barred mudskippers generally occupying cooler, shaded upper mangal environments among mangrove prop roots, and common mudskippers residing in warmer, exposed mudflat zones (Frith et al., 1979). Colombini and coworkers (1995) concluded that barred mudskipper activity patterns and distributions are primarily influenced by local air temperature and relative humidity. Given the large degree of thermal niche overlap between the two species it seems unlikely that thermal niche constraints would play a large role in dictating local mangle distributions, although subtle differences in thermal niche requirements may help reinforce local distribution boundaries.

Nearly all fish species currently described are ideal ectotherms that are unable to maintain body temperatures different from the water they swim in. Mudskippers however, deviate from the "typical" fish design. Their ability to leave the water and breathe air has not only opened an entirely new niche for exploitation but has also opened new opportunities for regulating body temperatures independent of environmental temperature. The capacity to adjust their thermal niche relative to the media they occupy is certainly a unique adaptation, and one that compliments a suite of other unique processes that has allowed the group to move freely between terrestrial and aquatic mangal environments.

Acknowledgements

We thank Operation Wallacea, the Ernest Hill Fellowship, and the University of California Agricultural Experiment Station (grant 2098-H to N.A.F.) for providing funding for this project. All animals in this study were treated in accordance with guidelines approved by the University of West Florida Animal Care and Use Committee (Protocol 2017-007). We also thank David Kracov for funding and inspiration.

References

Abele, D., Heise, K., Pörtner, H.O., Puntarulo, S., 2002. Temperature-dependence of mitochondrial function and production of reactive oxygen species in the intertidal mud clam *Mya arenaria*. J. Exp. Biol. 205, 1831–1841.

Agorreta, A., San Mauro, D., Schliewen, U., Van Tassell, J.L., Kovačić, M., Zardoya, R., Rüber, L., 2013. Molecular phylogenetics of Gobioidei and phylogenetic placement of European gobies. Mol. Phylogenet. Evol. 69, 619–633.

Ansari, A.A., Subrata, T.I., Shalini, S., Hasibur, R., 2014. Mudskipper: a biological indicator for environmental monitoring and assessment of coastal waters. J. Entomol. Zool. 2, 22–33.

Barreda, J.E., 2010. A Comparative Study of Thermal Tolerance, Hypoxia Tolerance, and Water Loss Resistance in Five Species of Indo-pacific Amphibious Fishes (Master's Thesis). (Proquest Dissertations and Theses).

Becker, C.D., Genoway, R.G., 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. Environ. Biol. Fish. 4, 245–256.

Beitinger, T.L., Bennett, W.A., 2000. Quantification of the role of acclimation temperature in temperature tolerance of fishes. Environ. Biol. Fish. 58, 237–275.

Beitinger, T.L., McCauley, R.W., 1990. Whole-animal physiological processes for the assessment of stress in fishes. J. Gt. Lakes. Res. 16, 542–575.

Beitinger, T.L., Bennett, W.A., McCauley, R.W., 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ. Biol. Fish. 58, 237–275.

Bennett, W.A., 2010. Extreme physiology of intertidal fishes of the Wakatobi. In: Clifton, J., Unsworth, R.K.F., Smith, D.J. (Eds.), Marine Research and Conservation in the Coral Triangle: The Wakatobi National Park. Nova Science Publishers, Hauppauge, pp. 111–128.

Bennett, W.A., Beitinger, T.L., 1997. Temperature tolerance of the sheepshead minnow, *Cyprinodon variegatus*. Copeia 1997, 77–87.

Bilyk, K.T., DeVries, A.L., 2011. Heat tolerance and its plasticity in Antarctic fishes. Comp. Biochem. Physiol. A Physiol. 158, 382–390.

Brett, J.R., 1944. Some lethal temperatures of Algonquin Park fishes. Publ Ont Fish Res Lab 63. University of Toronto Studies Biological Series 52.

Brett, J.R., 1956. Some principles in the thermal requirements of fishes. Q. Rev. Biol. 31, 75–87.

Bridges, C.R., 1988. Respiratory adaptations in intertidal fish. Am. Zool. 28, 79–96.

Brillet, C., 1975. Relations entre territoire et comportément agressif chez Periophthalmus sobrinus Eggert (Pisces, Periophthalmidae) au laboratoire et en milieu naturel. Z. Tierpsychol. 39, 283–331.

Burhanuddin, Martosewojo, S., 1979. Observations on the natural history of ikan gelodok *Periophthalmus koelreuteri* in Par Island, In: Soemodihardjo S., Nortji, A., Djamali, A. (Eds.), Proceedings of the Seminar on Mangrove Forest Ecosystems. Jakarta, pp. 86–92.

Chen, S., Hong, W., Zhang, Q., Su, Y., 2007. Why does the mudskipper *Boleophthalmus pectinirostris* form territories in farming ponds? J. Mar. Biol. Assoc. U.K. 87, 615–619.

Chen, S.X., Hong, W.S., Su, Y.Q., Zhang, Q.Y., 2008. Microhabitat selection in the early

juvenile mudskipper *Boleophthalmus pectinirostris* (L.). J. Fish Biol. 72, 585–593.
Claussen, D.L., 1977. Thermal acclimation in ambystomatid salamanders. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 58, 333–340.
Clayton, D.A., Snowden, R., 2000. Surface activity in the mudskipper, *Periophthalmus waltoni* Koumans 1941 in relation to prey activity and environmental factors. Trop. Zool. 13, 239–249.
Clayton, D.A., Vaughan, T.C., 1988. Ethogram of *Boleophthalmus boddarti* (Pallas) (Teleostei, Gobiidae), a mudskipper found on the mudflats of Kuwait. J. Univ. Kuwait (Sci.) 15, 115–140.
Colombini, I., Berti, R., Ercolini, A., Nocita, A., Chelazzi, L., 1995. Environmental factors influencing the zonation and activity patterns of a population of *Periophthalmus sobrinus* Eggert in a Kenyan mangrove. J. Exp. Mar. Biol. Ecol. 190, 135–149.
Coutant, C.C., 1969. Temperature, reproduction and behavior. Chesap. Sci. 10, 261–274.
Coutant, C.C., 1970. Biological aspects of thermal pollution I. Entrainment and discharge canal effects. Crit. Rev. Environ. Sci. Tec. 1, 341–381.
Cowles, R.B., Bogert, C.M., 1944. A preliminary study of the thermal requirements of desert reptiles. Bull. Am. Mus. Nat. Hist. 83, 265–296.
Cox, D.K., 1974. Effects of three heating rates on the critical thermal maximum of bluegill. In: Gibbons, J.W., Sharitz, R.R. (Eds.), Thermal Ecology. National Technical Information Service, Virginia, pp. 158–163.
Cuculescu, M., Hyde, D., Bowler, K., 1998. Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. J. Therm. Biol. 23, 107–110.
Dabruzzi, T.F., Wygoda, M.L., Wright, J.E., Eme, J., Bennett, W.A., 2011. Direct evidence of cutaneous resistance to evaporative water loss in amphibious mudskipper (family Gobiidae) and rockskipper (family Blenniidae) fishes from Pulau Hoga, southeast Sulawesi, Indonesia. J. Exp. Mar. Biol. Ecol. 406, 125–129.
Dabruzzi, T.F., Bennett, W.A., Rummer, J.L., Fangue, N.A., 2013. Juvenile ribbontail stingray, *Taeniura lymma* (Forsskål, 1775) (Chondrichthyes, Dasyatidae), demonstrate a unique suite of physiological adaptations to survive hyperthermic nursery conditions. Hydrobiologia 701, 37–49.
Dabruzzi, T.F., Bennett, W.A., Fangue, N.A., 2017. Thermal ecology of red lionfish *Pterois volitans* from southeast Sulawesi, Indonesia, with comparisons to other Scorpaenidae. Aquat. Biol. 26, 1–14.
Di Santo, V., Lobel, P.S., 2017. Body size and thermal tolerance in tropical gobies. J. Exp. Mar. Biol. Ecol. 487, 11–17.
Doudoroff, P., 1942. The resistance and acclimatization of marine fishes to temperature changes. I. Experiments with *Girella nigricans* (Ayres). Biol. Bull. 83, 219–244.
Dunlap, D.G., 1968. Critical thermal maximum as a function of temperature of acclimation in two hylid frogs. Physiol. Zool. 41, 432–439.
Dyer, K.R., Christie, M.C., Wright, E.W., 2000. The classification of intertidal mudflats. Cont. Shelf. Res. 20, 1039–1060.
Elliott, J.M., Elliott, J.A., 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: predicting the effects of climate change. J. Fish. Biol. 77, 1793–1817.
El-Ziady, S., Eissa, S.M., Al-Naqi, Z., 1979. Ecological studies on the mud-skipper fish *Periophthalmus chrysospilos* (Bleeker) in Kuwait. Bull. Fac. Sci. Cairo Univ. 48, 113–134.
Eme, J., Bennett, W.A., 2009. Critical thermal tolerance polygons of tropical marine fishes from Sulawesi, Indonesia. J. Therm. Biol. 34, 220–225.
Eme, J., Bennett, W.A., 2010. Acute temperature quotient responses of fishes reflect their divergent thermal habitats in the Banda Sea, Sulawesi, Indonesia. Aust. J. Zool. 57, 357–362.
Eschmeyer, W.N., Fricke, R., van der Laan, R., (Eds). 2017. Catalog of Fishes: genera, species, references. 〈http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp/〉 (Accessed 1 January 2017).
Fangue, N.A., Bennett, W.A., 2003. Thermal tolerance responses of laboratory-acclimated and seasonally acclimatized Atlantic stingray, *Dasyatis sabina*. Copeia 2003, 315–325.
Fangue, N.A., Wunderly, M.A., Dabruzzi, T.F., Bennett, W.A., 2014. Asymmetric thermal acclimation responses allow sheepshead minnow *Cyprinodon variegatus* to Cope with rapidly changing temperatures. Physiol. Biochem. Zool. 87, 805–816.
Feder, M.E., Hofmann, G.E., 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu. Rev. Physiol. 61, 43–282.
Frith, D.W., Tantanasiriwong, R., Bhatia, O., 1979. Zonation and abundance of macrofauna on a mangrove shore, Phuket Island. Phuket Mar. Biol. Cent. Res. Bull. 10, 1–37.
Froese, R,. Pauly, D., Editors, 2017. FishBase. World Wide Web electronic publication. 〈www.fishbase.org〉, (10/2017).
Fry, F.E.J., Brett, J.R., Clawson, G.H., 1942. Lethal limits of temperature for young goldfish. Rev. Can. Biol. 1, 50–56.
Fusi, M., Cannicci, S., Daffonchio, D., Mostert, B., Pörtner, H.O., Giomi, F., 2016. The trade-off between heat tolerance and metabolic cost drives the bimodal life strategy at the air-water interface. Sci. Rep. 6, 19158.
Giomi, F., Fusi, M., Barausse, A., Mostert, B., Pörtner, H.O., Cannicci, S., 2014. Improved heat tolerance in air drives the recurrent evolution of air-breathing. Proc. R. Soc. Lond. Biol. Sci. 281, 20132927.
Gordon, M.S., Gabaldon, D.J., 1985. Exploratory observations on microhabitat selection within the intertidal zone by the Chinese mudskipper fish *Periophthalmus cantonensis*. Mar. Biol. 85, 209–215.
Gordon, M.S., Boëtius, J., Evans, D.H., Oglesby, L.C., 1968. Additional observations on the natural history of the mudskipper, *Periophthalmus sobrinus*. Copeia 1968, 853–857.
Gordon, M.S., Boëtius, I., Evans, D.H., McCarthy, R., Oglesby, L.C., 1969. Aspects of the physiology of terrestrial life in amphibious fishes I. the mudskipper, *Periophthalmus sobrinus*. J. Exp. Biol. 50, 141–149.
Gordon, M.S., Ng, W.S., Yip, A.W., 1978. Aspects of the physiology of terrestrial life in amphibious fishes. III. The Chinese mudskipper *Periophthalmus cantonensis*. J. Exp. Biol. 72, 57–75.
Graham, J.B., Lee, H.J., 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? Physiol. Biochem. Zool. 77, 720–731.
Heatwole, H., Mercado, N., Ortiz, E., 1965. Comparison of critical thermal maxima of two species of Puerto Rican frogs of the genus *Eleutherodactylus*. Physiol. Zool. 38, 1–8.
Hutchison, V.H., 1961. Critical thermal maxima in salamanders. Physiol. Zool. 34, 92–125.
Ikebe, Y., Oishi, T., 1997. Relationships between environmental factors and diel and annual changes of the behaviors during low tides in *Periophthalmus modestus*. Zool. Sci. 14, 49–55.
Ip, Y.K., Chew, S.F., Randall, D.J., 2004. Five tropical air-breathing fishes, six different strategies to defend against ammonia toxicity on land. Physiol. Biochem. Zool. 77, 768–782.
Jensen, I.S. Editor., 2018. Weather statistics for Ryukyu Islands. Norwegian Meteorological Institute, Retrieved from URL. 〈https://www.yr.no/place/japan/Okinawa/Ryukyu_Islands/statistics.html#menu〉.
Kaufmann, J.S., Bennett, A.F., 1989. The effect of temperature and thermal acclimation on locomotor performance in *Xantusia vigilis*, the desert night lizard. Physiol. Zool. 62, 1047–1058.
Kok, T.W.K., Lim, C.B., Lam, T.J., Ip, Y.K., 1998. The mudskipper *Periophthalmodon schlosseri* respires more efficiently on land than in water and vice versa for *Boleophthalmus boddaerti*. J. Exp. Zool. 280, 86–90.
Krakauer, T., 1970. Tolerance limits of the toad, *Bufo* marinus, in south Florida. Comp. Biochem. Physiol. 33, 15–26.
Lutterschmidt, W.I., Hutchison, V.H., 1997. The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can. J. Zool. 75, 1553–1560.
Mahadevan, G., Ravi, V., 2015. Distribution of mudskippers in the mudflats of Muthupet, Southeast coast of India. Int. J. Fish. Aquat. Stud. 3, 268–272.
Mora, C., Maya, M.F., 2006. Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. J. Therm. Biol. 31, 337–341.
Murdy, E.O., 1989. A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: oxudercinae). Rec. Aust. Mus.(Supplement 11) (ISBN 7305 6374 X).
Ng, P.K., Wang, L.K., Lim, K.K., 2007. Private Lives: an Exposé of Singapore's Mangroves (Vol. 2). Raffles Museum of Biodiversity Research. National University of Singapore.
Nursall, J.R., 1981. Behavior and habitat affecting the distribution of five species of sympatric mudskippers in Queensland. Bull. Mar. Sci. 31, 730–735.
Paladino, R.V., Spotila, J.R., Schubauer, J.P., Kowalski, K.T., 1980. The critical thermal maximum: a technique used to elucidate physiological stress and adaptation in fishes. Rev. Can. Biol. 39, 115–122.
Park, K.D., Kim, J.K., Chang, D.S., Kim, J.I., Oh, C.W., 2008. Age and growth of the Mudskipper, *Scartelaos gigas* (Perciformes, Gobiidae) from Korea. Anim. Cells Syst. 12, 305–311.
Polgar, G., 2009. Species-area relationship and potential role as a biomonitor of mangrove communities of Malayan mudskippers. Wetl. Ecol. Manag. 17, 157–164.
Polgar, G., Crosa, G., 2009. Multivariate characterisation of the habitats of seven species of Malayan mudskippers (Gobiidae: oxudercinae). Mar. Biol. 156, 1475–1486.
Polgar, G., Sacchetti, A., Galli, P., 2010. Differentiation and adaptive radiation of amphibious gobies (Gobiidae: oxudercinae) in semi-terrestrial habitats. J. Fish Biol. 77, 1645–1664.
Pörtner, H.O., 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften 88, 137–146.
Sayer, M.D.J., Davenport, J., 1991. Amphibious fish: why do they leave water? Rev. Fish. Biol. Fish. 1, 159–181.
Shenoy, L., Patro, V.K., Ying, X., 2012. Effects of Mudskippers on the Soil Composition and Texture in Mangrove Forests, and Hence the Growth of Mangroves (*Avicennia alba*). Little Green Dot Research Project submitted to Singapore Nature Society.
Somero, G.N., 1969. Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. Am. Nat. 103, 517–530.
Stebbins, R.C., Kalk, M., 1961. Observations on the natural history of the mud-skipper, *Periophthalmus sobrinus*. Copeia 1961, 18–27.
Takeda, T., Hayashi, M., Toba, A., Soyano, K., Ishimatsu, A., 2012. Ecology of the Australian mudskipper *Periophthalmus minutus*, an amphibious fish inhabiting a mudflat in the highest intertidal zone. Aust. J. Zool. 59, 312–320.
Takegaki, T., Fujii, T., Ishimatsu, A., 2006. Overwintering habitat and low-temperature tolerance of the young mudskipper Boleophthalmus pectinirostris. Bull. Jpn. Soc. Sci. Fish. 72, 880–885.
Takita, T., Larson, H.K., Ishimatsu, A., 2011. The natural history of mudskippers in northern Australia, with field identification characters. Beagle 27, 189–204.
Tamura, S.O., Morii, H., Yuzuriha, M., 1976. Respiration of the amphibious fishes *Periophthalmus cantonensis* and *Boleophthalmus chinensis* in water and on land. J. Exp. Biol. 65, 97–107.
Taylor, J.R., Cook, M.M., Kirkpatrick, A.L., Galleher, S.N., Eme, J., Bennett, W.A., 2005. Thermal tactics of air-breathing and non air-breathing gobiids inhabiting mangrove tidepools on Pulau Hoga, Indonesia. Copeia 2005, 886–889.
Tytler, P., Vaughan, T., 1983. Thermal ecology of the mudskippers, *Periophthalmus koelreuteri* (Pallas) and *Boleophthalmus boddarti* (Pallas) of Kuwait Bay. J. Fish Biol. 23, 327–337.