The emergence emergency: A mudskipper's response to temperatures

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\textbf{A B S T R A C T}

Temperature has a profound effect on all life and a particularly influential effect on ectotherms, such as fishes. Amphibious fishes have a variety of strategies, both physiological and/or behavioural, to cope with a broad range of thermal conditions. This study examined the relationship between prolonged (5 weeks) exposure to a range of temperatures (22, 25, 28, or 32 °C) on oxygen uptake rate and movement behaviours (i.e., thermoregulation and emergence) in a common amphibiown fish, the barred mudskipper (\textit{Periophthalmus argentilineatus}). At the highest temperature examined (32 °C, approximately 5 °C above their summer average temperatures), barred mudskippers exhibited 33.7–97.7% greater oxygen uptake rates at rest (\textit{MO_{rest}}), emerged at a higher temperature (\textit{CT_{e}}; i.e., a modified critical thermal maxima (\textit{CT_{max}}) methodology) of 41.3 ± 0.3 °C relative to those maintained at 28, 25, or 22 °C. The 32 °C-maintained fish also ceased movement activity at the highest holding temperature suggesting that prolonged submergence at elevated temperatures is physiologically and energetically stressful to the individual. Using exhaustive exercise protocols with and without air exposure to simulate a predatory chase, the time to recovery was examined for all individuals. When submerged, mudskippers required 2.5x longer recovery time to return to resting oxygen uptake from exhaustive exercise than those fully emerged in air. Oxygen uptake data revealed that air exposure did not accrue oxygen debt, thereby allowing faster return to resting oxygen consumption rates. If the option to emerge was not available, mudskippers preferentially sought more benign water temperatures (26.7 ± 2.1 °C), resembling those experienced by these fish during the Austral autumn, regardless of prolonged exposure higher or lower temperatures. These results add to our understanding of the strategies that amphibious fishes may use to mitigate extra costs associated with living in warm waters, and could be the key to understanding how such species will cope with increasing temperatures in the future.

\textbf{1. Introduction}

Temperature has a profound effect on all life, and a particularly influential effect on ectotherms, as the rate of their biochemical and physiological processes are largely governed by the temperature of their external environment (Tewksbury et al., 2008; Pörtner and Peck, 2010). For example, fishes have been shown to increase resting oxygen uptake rates when exposed to acute or chronic increases in water temperatures (e.g., Lefevre, 2016; Gillooly et al., 2001; Clarke and Johnston, 1999; Brown, 1989). This increased oxygen demand suggests that more energy will be required to maintain daily performance at higher temperatures, potentially reducing the energy available for key processes, such as growth and reproduction (Fry, 1947; Pörtner and Peck, 2010).

As global temperatures continue to increase, many tropical ectotherms are thought to be at risk as they are already experiencing temperatures close to their upper thermal limits (Rummer et al., 2014). Most of the fishes, as obligate aquatic ectotherms, can utilize strategies such as acclimation, adaptation, or behaviour to cope with changes in temperatures (Fry, 1947; Pörtner and Peck, 2010); whereas amphibious fishes (i.e., those who spend part of their life on land) are uniquely adapted to life in two different environments and may use a range of strategies (e.g., emergence) to cope with changing temperatures.

Mudskippers (family Gobiidae, subfamily Oxudercinae) are amphibiuous fishes that are common inhabitants of mudflat and mangrove environments, regularly emerging from the water (e.g., to forage, evade predation; Gordon et al., 1968). Mudskipper species have the ability to...
take in oxygen through gills, other brachial surfaces (i.e., buccal, pharyngeal, branchial, and opercular cavities; Graham, 1997), and cutaneous surfaces, and although all species have this ability, the ratio at which they utilize a particular oxygen uptake method differs between species (Graham, 1997). For example, Tamura and colleagues (1976) found that *Boleophthalmus chinnensis* relied more heavily on gills (59%) than skin (43%) for oxygen uptake. In contrast, *Periophthalmus cantonensis* relied more heavily on skin (76%) than gills (27%) for oxygen uptake. They concluded that the differences between oxygen uptake sources were related to the transition from aquatic to terrestrial air breathing. These adaptations in mudskipper fishes allow them to use several behaviours that can be used with changes in tide, temperature, light (day vs. night), and salinity (Baek et al., 2013; Clayton, 1993).

For instance, juvenile mudskippers in Kuwait Bay have been observed to regulate their body temperature by ‘basking’, specifically by orientating their body at a right angle to the sun to increase body temperature (Clayton and Vaughan, 1988; Tyler and Vaughan, 1983). Conversely, several amphibious fishes, *Pseudogobius sp.* and *Favonigobius exquisitus* (Ford et al., 2004), and *Kryptolebias marmoratus* (Gibson et al., 2015) are known to leave the water in response to elevated temperatures. This emergent behaviour has been linked to the use of evaporative cooling (Tyler and Vaughan, 1983). Although mudskippers may be well equipped to cope with their environmental conditions, predicted changes in sea surface temperatures, including those in marginal habitats (i.e., habitats supporting relatively few species because of limited environmental conditions) may challenge the mudskippers’ present adaptations.

Physiological responses and behavioural strategies can differ greatly over the temporal scale at which fish can utilize them. Physiological responses can last from a couple of minutes to acclimation or adaptation processes over months, years, and across generations (Atkins and Travis, 2010; Buckley et al., 2015; Donelson et al., 2012). Behavioural strategies can support more immediate thermoregulation over short time scales. By moving away (e.g., jumping, ‘skipping’, or ‘crutching’ in amphibious species, see Harris, 1960) from thermally stressful conditions into more benign conditions closer to preferred temperatures, species can mitigate some of the physiological costs that sub-optimal temperature present, such as increased maintenance costs. To date, the use of movement to select a preferred temperature has been studied across many taxa (Buckley et al., 2015; Coggan et al., 2011; Killen, 2014; Medvick and Miller, 1979; Speed et al., 2012); however, this has yet to be examined for amphibious fishes. Previous studies have suggested the use behaviour to occupy a thermal preference (Tyler and Vaughan, 1983); however, such preferences have not been established in the laboratory.

Determining oxygen uptake rates and understanding how movement (i.e., emergence and thermoregulation) is used to mitigate the effects of suboptimal temperatures could help explain how amphibious fishes, such as mudskippers, are able to live under a range of thermal conditions. The objective of this study was to establish how exposure to different thermal conditions influences oxygen uptake rates, preferred water temperatures and emergence behaviours of a common amphibious fish, the barred mudskipper, *Periophthalmus argentinaeactis*. Additionally, as temperatures can vary greatly between day and night-time periods, we aimed to investigate the differences in thermal preferences between day and night-time periods. As mudskipper species exhibit emergence behaviours, we also aimed to establish the upper thermal emergence limits to gain a better understanding of other behavioural strategies this and perhaps other amphibious fish species may utilize to mitigate future increases in temperature.

2. Materials and methods

2.1. Animal care

Adult barred mudskipper (*Periophthalmus argentinaeactis*) of similar size (mean ± SE: mass: 6.12 ± 1.43 g, standard length: 6.85 ± 0.55 cm) were collected in March 2015 from a mangrove forest in Cockle Bay on Magnetic Island, Queensland, Australia (19°10’32.4”S 146°49’45.8”E) using hand nets. To quantify temporal variation in environmental temperature, a HOBO Pendant® Temperature/Light Data Logger (Onetop Australia) was deployed in March 2015 at low tide in a 10 cm deep pool at the site of collection, and it was retrieved in January 2016. Following collection, animals were transported in aerated bags partially filled with seawater to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University, Townsville, Queensland, Australia. All individuals were placed into two 68 L (64.5 × 41.3 × 39.7 cm) aquarium with flow-through filtered 28 °C seawater. Each aquarium contained 10 L of water and submerged PVC shelters and brick platforms. Following a two-week habitation period to ensure that fish were feeding appropriately, each individual was randomly assigned to one of five temperature treatments (22, 25, 28, or 32 °C) and tagged with a unique subcutaneous visible elastomer (Northwest Marine Technology, Washington, USA) to allow individuals to be identified. Each treatment consisted of four 8.5 L aquarium (22 × 23 × 22 cm), each housing 2–3 fish of similar body size (weight: one way ANOVA $F_{3,26} = 1.137, P = 0.36$; length: one way ANOVA $F_{3,26} = 0.08, P = 0.97$). Each aquarium consisted of submerged PVC shelters; however, these did not allow for emergence. Animals were isolated in the aquatic environment during acclimation to ensure that the acclimation conditions were constant, given that emergence would create inconsistent temperature, humidity, and osmotic conditions. Throughout the study, a 12:12 h photoperiod was maintained. Fish were fed daily commercial pellets and marine green frozen fish food (Fish Fuel Co., South Australia, Australia). Prior to experiments, fish were fasted for 24 h to ensure post-absorptive state that would maximize energy available for performance (Niimi and Beamish, 1974).

2.2. Temperature treatments

The treatment temperatures were chosen to reflect a range of ecologically relevant temperatures experienced throughout the year in an adjacent intertidal seagrass meadows in Cockle Bay, Magnetic Island; winter minimum (22 °C), temperatures experienced throughout the year (25 °C, and 28 °C, respectively), and summer average (32 °C) (Collier and Waycott, 2014). Treatment temperatures were reached either by decreasing the ambient water temperature (28 °C) by 0.5 °C per day or by increasing it by 0.3 °C per day until the target temperature was attained. Rates of temperature increase were slower than rates of decrease, as pilot trials showed that daily increases of 0.5 °C over several days resulted in prolonged erratic behaviours (e.g., continuous rapid, burst swimming behaviours) until mudskippers became unresponsive. These behaviours were not observed with a daily increase of 0.3 °C. Upon reaching treatment temperature, fish were maintained at treatment temperatures for five-weeks (prior to any experiments). This acclimation time was chosen as it is follows best practices and is thought long enough for critical acclimation processes to be complete (Nilsson et al., 2010).

2.3. Oxygen uptake rates

Fish were placed individually into 0.46 L intermittent-flow respirometry chambers inside a temperature-controlled water bath (following Svendsen et al., 2016). Each chamber was connected to a flush pump and recirculating pump to maintain water circulation and homogenous oxygen levels within the system throughout the trial. A digital relay timer controlled the flush pumps so that the water in the respirometry chambers was flushed with well-aerated, filtered seawater for 5 mins every 10 min throughout the 20-h trial. The flush cycle was determined such that O2 levels did not fall below 80% air saturation (Clark et al., 2013). Temperature-compensated oxygen concentrations were recorded every 2 s using contactless spots (2 mm) with O2–
sensitive REDFLASH dye attached to the inside of glass tubes in line with each recirculating pump. The spots were linked to a Firingsting Optical Oxygen Meter (Pyro Science e. K., Aachen, Germany) via 2 m fibre-optic cables.

The established method incorporating a 3-min chase followed by 1-min air exposure (see Clark et al., 2012, 2013; Roche et al., 2013) was used to determine maximum oxygen uptake rates ($\dot{M}O_{\text{O2Max}}$) for fish from all temperature treatments (22 °C: n = 6, 25 °C: n = 7, 28 °C: n = 4, and 32 °C: n = 4). Immediately following the exercise protocol, fish were placed into respirometry chambers. Each chamber was sealed, and the measurement period began within 10 s. Following each trial, all equipment was rinsed in a 10% bleach solution, rinsed in freshwater, and then sun-dried to reduce microbial background oxygen uptake.

Given mudskippers are able to withstand extended periods of air exposure (Gordon et al., 1968), the standard chase protocol consisting of 3 min chase and 1 min air exposure (see Clark et al., 2012, 2013; Roche et al., 2013) might not elicit maximal exhaustion typically seen in species less resilient to air exposure. Therefore, we assessed the utility of the standard protocol by comparing it to maximum oxygen uptake rate elicited by three complimentary protocols adjusted for their unique physiology. Individuals within the 25 °C treatment group were exposed to either: (i) 3-min chase at a high water level without any air exposure, (ii) 3-min chase at a low water level to induce a jumping behaviour followed by a 1-min air exposure, and (iii) 3-min air exposure. In total, these four different chase protocols allowed for direct comparison between the standard protocol and different ‘exhaustive treatments’ such as jumping, swimming or complete removal from water. Following each protocol, individuals were immediately placed into respirometry chambers in the aquatic environment to establish recovery time. Each individual from the 25 °C treatment was given 48 h to recover from experimental trials before tested under alternate protocols, and the sequence of chase protocols were randomised among individuals.

Oxygen uptake rates (as a proxy for energetic costs) during the measurement period (i.e., non-flushing) were calculated using linear least square regression of oxygen concentration over time in LabChart v.6.1.3 (AD Instruments, Colorado Springs, CO, USA). The highest rate of change for every 30-sec period, during the initial three closed respirometry phases following the placement of individuals inside the chamber, were used to determine the maximum $O_2$ uptake (i.e., $\dot{M}O_{\text{O2Max}}$). This method was deemed appropriate as R2 values for the closed respirometry phases were above 0.95. Resting oxygen uptake rate (i.e., $\dot{M}O_{\text{O2Rest}}$) was calculated as the ‘mean of the lowest normal distribution’ method (MLND) as described by Chabot et al. (2016). Recovery time was defined as the time difference between $\dot{M}O_{2\text{Max}}$ until the first oxygen uptake rate equivalent to $\dot{M}O_{2\text{Rest}}$. Aerobic scope was defined as the absolute difference between $\dot{M}O_{2\text{Max}}$ and $\dot{M}O_{2\text{Rest}}$. Background $O_2$ uptake in the system was determined prior to the fish being placed into the chambers and again completing the trial after fish were removed. The background $O_2$ uptake was assumed linear (Clark et al., 2013) and was determined to be less than 5% of mudskipper oxygen uptake rates. Given that this value was established as low, background $O_2$ uptake was incrementally subtracted from each slope.

2.4. Critical thermal emergence ($C_T$)

The critical emergence temperature ($C_T$) for barred mudskippers was estimated using a modified critical thermal maxima ($C_{T\text{Max}}$) methodology (Beitinger and Bennett, 2000) in which fish were allowed to emerge from the water (22 °C: n = 4, 25 °C: n = 7, 28 °C: n = 5, and 32 °C: n = 5). A 125 L (64.5 × 64.5 × 30.15 cm) square, glass tank was fitted with a 71.2 cm x 64.5 cm PVC ramp with evenly spaced holes (10 mm wide diameter and separate by approximately 5 cm). The ramp was positioned at a 30° angle from the base of the tank to the far top corner. A 2000 W heater (Omega 2000 W, Full gauge TIC-17RGT Thermostat) and submersible mixing pump (WH-500, Weipro®, Guangdong, China) were placed below the ramp away from the experimental compartment to ensure homogeneous temperature throughout the entire aquarium. An air stone was placed inside the experimental compartment to ensure sufficient aeration. First, water was heated or chilled to the fish’s treatment temperature. Then, the fish was placed inside the experimental compartment, and a plastic sheet was placed on the surface of the water to ensure the fish could not emerge prematurely. Fish were observed inside the compartment for 2 min, and the time spent actively moving (i.e., henceforth ‘activity’) was recorded. Following the initial 2-min observation period, the plastic sheet was moved along the water’s surface towards the back of the tank opening a 5 cm gap that allowed for emergence. The water temperature was then increased at a rate of $0.27 \pm 0.01$ °C min$^{-1}$ until the fish emerged onto the ramp. Emergence temperatures were established as the temperatures at which the fish first emerged from the water (i.e., the temperature at which the eyes were completely above the water) and at which the entire body emerged from the water (i.e., the temperature at which the caudal peduncle was out of the water). These endpoints were selected to represent the point at which the mudskipper may evaluate the aerial environment prior to full emergence. Air temperatures were maintained at 25 °C, and humidity was constant between 40% and 60% throughout all trials.

2.5. Preferred temperature ($T_{\text{pref}}$)

A shuttlebox system, designed by Schurmann and Steffensen (1991) and Petersen and Steffensen (2003), was used to determine the preferred temperature ($T_{\text{pref}}$) for each fish from each treatment temperature (22 °C: n = 6, 25 °C: n = 7, 28 °C: n = 4, and 32 °C: n = 4). A detailed description of the shuttlebox system is provided in Nay et al. (2015). Briefly, the system is composed of two chambers joined by a 50 mm wide opening allowing the fish to travel freely between the two chambers. One chamber was established as the ‘warm’ chamber and the other as a ‘cool’ chamber, with a 1 °C difference maintained between the chambers throughout the trial (Kullen, 2014). When the fish entered the ‘warm’ chamber, the temperature of the entire system would increase at a rate of 6 °C h$^{-1}$. Conversely, when the fish entered the ‘cool’ chamber, the temperature of the system would decrease at a rate of 6 °C h$^{-1}$. By moving between chambers the fish can control their thermal environment and therefore their internal body temperature. The ‘warm’ and ‘cool’ chamber assignments were switched halfway through trials to ensure there was no bias toward a particular chamber.

An individual fish was placed inside the chamber set to the individual’s treatment temperature, and a plexiglass cover was placed on the surface of the water to prevent the fish from emerging. Fish were allowed a 1.5 h adjustment time prior to turning on the system. This time period was used as fish were observed entering both chambers of the shuttlebox system. Water within one chamber flowed clockwise while water in the other chamber flowed counter-clockwise to prevent the water from mixing and allow for the 1 °C temperature difference to be maintained.

A custom program was written using Labtech Notebook Pro (Laboratories Technology Corp., Andover MA) to track the position of the fish and automate the activation/deactivation of the appropriate pumps based on the position of the fish. From each trial we recorded preferred temperature, number of chamber movements, and the selected temperature range. The preferred temperature ($T_{\text{pref}}$) was defined as the temperature at which the fish spent the largest proportion of time (i.e., modal temperature), while the chamber movements were the number of movements made between the chambers during diurnal and nocturnal periods. Selected temperature ranges were represented as the difference between maximum and minimum temperatures chosen by each fish.
2.6. Data analyses

To test the effect of treatment temperature on minimum and maximum oxygen uptake rates as well as initial and final emergence temperatures, one-way ANOVAs were used. To test the effect of chase protocols for fish maintained at 25 °C, \( \dot{M}O_2_{\text{Max}}, \dot{M}O_2_{\text{Rest}}, \) and recovery time were analysed using one-way ANOVAs. The \( \dot{M}O_2_{\text{Max}}, \dot{M}O_2_{\text{Rest}}, \) and recovery time data were log10 transformed prior to analysis to conform to requirements for normality and homogeneity of variance. Activity was analysed with Kruskal-Wallis test given the data were not normally distributed. Temperature preference, chamber movements, and temperature range were analysed using a two-way ANOVA (treatment temperature and diel period [night-time/day-time]). All values were log transformed to meet assumptions of analysis (homogeneous variance and normal distribution). Holm-Sidak post-hoc tests were performed when significant differences were observed. To account for running multiple tests on the same individuals within experiments, an FDR correction was used post-hoc (Benjamini and Hochberg, 1995). All values are presented as mean ± SE.

3. Results

The field data logger revealed the average temperatures within the tide pool at Cockle Bay, Magnetic Island to be 25.48 ± 3.23 °C during 2015 (mean ± SD; Fig. 1). Temperatures ranged from 23.29 °C to 39.50 °C during Austral summer (i.e., November to January) and from 12.40 °C to 32.60 °C during Austral winter (i.e., June to August).

3.1. Oxygen uptake rates

The resting oxygen uptake rate (\( \dot{M}O_{2\text{Rest}} \)) of \( P. \) argentilineatus differed among temperature treatments (\( F_{3,17} = 3.40, P = 0.04 \)), with individuals maintained at 32 °C having higher \( \dot{M}O_{2\text{Rest}} \) (225.88 ± 23.44 mg O2 kg\(^{-1}\) h\(^{-1}\)) than those maintained at 22 °C (119.06 ± 23.76 mg O2 kg\(^{-1}\) h\(^{-1}\)), 25 °C (114.21 ± 16.19 mg O2 kg\(^{-1}\) h\(^{-1}\)), and 28 °C (169.00 ± 41.68 mg O2 kg\(^{-1}\) h\(^{-1}\); Fig. 2). In contrast, there was no difference in maximum oxygen uptake rates (\( \dot{M}O_{2\text{Max}} \)) following standard chase protocols among fish from each temperature treatment group (22 °C: 739.61 ± 142.07 mg O2 kg\(^{-1}\) h\(^{-1}\), 25 °C: 631.42 ± 90.00 mg O2 kg\(^{-1}\) h\(^{-1}\), 28 °C: 522.92 ± 66.70 mg O2 kg\(^{-1}\) h\(^{-1}\), 32 °C: 534.85 ± 80.45 mg O2 kg\(^{-1}\) h\(^{-1}\); \( F_{3,17} = 0.53, P = 0.67 \)). Although aerobic scope appears to be decreasing across treatments, it did not differ significantly across temperature treatments (22 °C: 649.91 ± 142.71 mg O2 kg\(^{-1}\) h\(^{-1}\), 25 °C: 548.17 ± 89.87 mg O2 kg\(^{-1}\) h\(^{-1}\), 28 °C: 444.94 ± 70.29 mg O2 kg\(^{-1}\) h\(^{-1}\), 32 °C: 377.74 ± 82.12 mg O2 kg\(^{-1}\) h\(^{-1}\); \( F_{3,17} = 0.91, P = 0.46 \)). Comparing chase protocols among fish maintained at 25 °C revealed that recovery time was significantly shorter when fish were exposed to air and then allowed to recover in the aquatic environment (1.53 ± 0.41 h) compared to fish exposed to the standard chase protocol (3.93 ± 0.71 h) or modified chase protocol (3.37 ± 0.67 h) and then placed into water for recovery. Neither maximum oxygen uptake rates (\( F_{3,32} = 0.64, P = 0.59 \)), resting oxygen uptake rates (\( F_{3,32} = 2.07, P = 0.12 \)), nor aerobic scope (\( F_{3,32} = 1.14, P = 0.35 \)) differed among protocols.

![Temperature data collected with HOBO data loggers in Cockle Bay, Magnetic Island, Queensland, Australia from March 2015-January 2016. The data logger was placed within a 10 cm deep tide pool at low tide at the base of mangrove, areas commonly frequented by the barred mudskipper, Periophthalmus argentilineatus.](image1)

![Maximum oxygen uptake rates (\( \dot{M}O_{2\text{Max}} \)) and resting oxygen uptake (\( \dot{M}O_{2\text{Rest}} \)) of fish from each treatment temperature (22 °C: \( n = 6 \), 25 °C: \( n = 7 \), 28 °C: \( n = 4 \), and 32 °C: \( n = 4 \)). Maximum oxygen uptake rates (\( \dot{M}O_{2\text{Max}} \)) were established using a 3 min chase and a 1 min air exposure. B) Recovery time required for each protocol within the 25 °C treatment group. All points are represented as means with standard error of the mean. Significant values are indicated as an asterisk with \( \alpha = 0.05 \).](image2)
3.2. Critical thermal emergence (CTe)

The fish’s initial and final emergence temperatures as well as the duration of activity prior to emergence differed among temperature treatments (Fig. 3). The temperature at which individuals initially emerged from the water (i.e., eyes are fully above the water) was higher for fish that had been maintained at 32 °C (37.79 ± 1.16 °C) when compared to those maintained at 22 °C (28.34 ± 2.33 °C; F3,19 = 6.69, P = 0.003). Fish that were maintained at 32 °C and 25 °C completely emerged themselves (i.e., withdrew caudal peduncle fully above the water) at significantly warmer temperatures (41.31 ± 0.36 °C and 40.89 ± 0.78 °C respectively) than fish maintained at 28 °C (37.94 ± 1.96 °C; F3,17 = 4.15, P = 0.02). The time spent actively moving was greatest for fish that had been maintained at 28 °C (19.40 ± 7.10 s of the 2-min observation period), and decreased for fish from both lower (25 °C: 11.25 ± 5.93 s; 22 °C: 6.33 ± 2.03 s) and higher (32 °C: 0 ± 0 s) temperatures (F3,10 = 14.32, P < 0.05).

3.3. Preferred temperature (Tpref)

Despite five weeks of continuous exposure to either 22, 25, 28, or 32 °C, *Periopthalmus argentilineatus* preferred approximately the same temperature (26.7 ± 2.1 °C; F3,43 = 2.20, P = 0.10), which also did not differ between time periods (i.e., nocturnal vs. diurnal: F1,43 = 1.62, P = 0.21; Fig. 4a). There was, however, considerable variation in preferred temperature among all individuals, as evidenced by the variation around the mean. Additionally, fish from some groups made more chamber movements to maintain their preferred temperature than others. For example, fish maintained at 28 °C moved more frequently to maintain their preferred temperature than 25 °C-maintained fish (F4,43 = 3.11, P = 0.02). During nighttime periods, fish moved more frequently to maintain their preferred temperatures than they did during the daytime periods (F1,43 = 18.99, P < 0.001). However, during daytime hours, fish selected a wider range of temperatures regardless of treatment temperature (F1,43 = 22.50, P < 0.05). There were no interactions between treatment, time period, preferred temperature, movements, and selected temperature ranges (F3,43 = 0.91, P = 0.45; F3,43 = 2.67, P = 0.06; F3,43 = 0.90, P = 0.45).

4. Discussion

Amphibious fishes may use behavioural strategies to minimise their exposure to unfavourable environmental temperatures. Maintaining barred mudskippers, *Periopthalmus argentilineatus*, for 5 weeks at constant temperatures that they naturally experience (22–32 °C) in the wild had no effect on their preferred water temperatures (26.7 °C). Fishes that had been maintained at 28 °C were also the most active compared to those maintained at either lower (22 and 25 °C) or higher water temperatures (32 °C). Despite the similarities in preferred water temperatures, fish that had been maintained at 32 °C emerged from the water at higher water temperatures, exhibited an increase in their resting oxygen uptake rates, and selected a wider range of water temperatures than their counterparts that had been maintained at cooler temperatures. Exposure to air prior to submersion provided the shortest recovery time of any of the exercise protocols (1.53 ± 0.41 h) likely due to their ability to uptake oxygen in air. The longest recovery time was required by those who were exercised for 3 min in water and allowed a 1 min air exposure (3.93 ± 0.71 h) followed by submersion. Although we recognize that the barred mudskipper occupies naturally thermally variable environments (12.40–39.50 °C), when they are maintained long-term at constant water temperatures closer to the upper limits of what they currently experience (32 °C), their oxygen uptake rates may increase and behaviour altered. Therefore, prolonged exposure to elevated temperatures (e.g., 32 °C) may force mudskipper species to either move or risk elevated energetic costs.

Mudskippers, similar to many intertidal species, have the ability to leave the water when water conditions become suboptimal, and several factors can alter this emergence temperature threshold. For example, *Favonigobius exquisitus* and *Pseudogobius* sp., two gobid fish species common to the intertidal area of Moreton Bay, Australia, emerge from the water when exposed to high thermal stress (Ford et al., 2004). Similarly, *Kryptolebias marmoratus* emerge when exposed to elevated temperatures (Gibson et al., 2015). Local air temperatures experienced across daily and seasonal cycles, together with maximum daily water temperatures appear to play an important role in initiating emergence of amphibious fishes. Along the coast of central Chile, *Sicyases sanguineus* emerged at 17.8 °C when water temperatures mimicked maximum daily air temperatures (Ebeling et al., 1970). Similarly, barred mudskippers in this study maintained at cooler temperatures exhibited initial emergence at 32.63 °C reflecting average maximum daily air temperatures recorded at Magnetic Island (~31.4 °C). Humidity, along with temperature, can also play an important role in emergence temperatures. Gibson et al. (2015) found that *K. marmoratus* individuals lost more body heat in low humidity environments than those in high humidity scenarios. As relative humidity increased, the amount of body heat lost by *K. marmoratus* decreased. Given that relative humidity in
A thoroughly published research has shown that temperature changes can lead to benefits and risks, as well as potential mechanisms such as anaerobic physiological processes. In our study, we examined the specific physiological mechanisms underlying emergence behaviours that occur at higher temperatures. Our findings suggest that emergence may be a valid strategy to alleviate negative effects of high temperatures (e.g., evaporative cooling and/or oxygen uptake). Indeed, some mudskipper species are thought to have the ability to adjust their temperature in order to avoid predation. This study was between 40% and 60%, emergence behaviours could have led to beneficial evaporative cooling mechanisms. Although we did not examine the specific physiological mechanisms underpinning emergence behaviours that occur at higher temperatures, previous studies have suggested potential mechanisms such as anaerobic metabolic pathways, antioxidative mechanisms, or heat-shock protein responses. These mechanisms may allow more time to be spent at elevated temperatures during a period of time where temperatures are increasing. A thorough investigation into these mechanisms is an avenue for future studies. Emergence behaviour can be a useful response for mudskipper species to escape extreme conditions; however, such behaviours come with associated trade-offs.

Under scenarios where emergence behaviours are too risky and/or energetically costly, it may be necessary to regulate body temperature while submerged. Barred mudskippers in this study showed a preference for relocating to thermal conditions similar to those experienced in Austral autumn months. The mangrove habitat on Magnetic Island experiences temperatures between 24.6 and 28.8 °C (i.e., the range of preferred temperatures determined for mudskippers in this study) for nearly 5 months of the year during both high and low tidal cycles. These temperatures are also suggested as the preferred temperature ranges for other amphibious species, although selected using alternative thermoregulatory behaviours such as body placement next to cooler tide pools (e.g., *Mniepes microcephalus*; *Graham*, 1973). Furthermore, the temperature range throughout the year between low and high tides is much greater, fluctuating by 10–18 °C, than has been previously noted (e.g., 12 °C) in other intertidal habitats (*Potts and Swart*, 1984). Unlike fishes that are fully aquatic, amphibious fishes, such as mudskippers, may require more frequent movements to occupy preferred temperatures, given the dynamic nature of temperature within their habitats; however, such movements may expose these fish to new threats, including aerial or aquatic predation (*Sayer and Davenport*, 1991).

Ultimately using behaviours to regulate temperature can have several associated risks including but not limited to attracting predation, missing opportunities, and the energy trade-offs required for movement (*Sayer and Davenport*, 1991). For example, movement may draw the attention of predators and/or scare off potential prey (i.e., missed opportunity for foraging) (*Angilletta*, 2009). Furthermore, the energy conserved as part of moving to a preferred temperature needs to be greater than the energy required to relocate (*Angilletta*, 2009). Here, barred mudskippers increased movements during nocturnal periods, which may reflect a reduced predation risk at night. However, as temperatures approach the upper thermal limits of resident species, occupying thermally beneficial areas could become essential in order to reduce energetic costs associated with elevated temperatures.

Elevated temperatures, above an organism’s natural range, can be associated with increased energetic costs, which in turn can affect a species’ ecological patterns. Many fully aquatic/marine fish species have exhibited elevated resting oxygen uptake rates at higher temperatures causing declines in traits such as aerobic scope and swimming performance (*Johansen and Jones*, 2011; *Munday et al.*, 2008; *Rummer et al.*, 2014). These declines can, although not always, coincide with declines in growth and reproductive output, as energy may be allocated to more essential life-sustaining processes (*Lefevre*, 2016; *Pörtner and Peck*, 2010). In contrast, recent studies have suggested that the decline in aerobic scope seen in some species may not be universal and may not align with other performance traits such as growth (*Pörtner and Peck*, 2010). Aerobic scope in barred mudskippers in the present study did not decline as exposure temperatures were increased or decreased, which suggests that this metric may not align with all performance traits of this, and other amphibious fish, species. For example, unlike aerobic scope, emergence temperatures did follow the trend of increasing as exposure temperature increased. A previous study on *Blennius pholis* suggests that emergence depends on the oxygen demand of the fish (*Davenport and Woolmington*, 1981) and therefore, as oxygen demand increases upon exposure to elevated temperatures, emergence may be a valid strategy to alleviate negative effects of temperatures (e.g., evaporative cooling and/or oxygen uptake). Indeed, some mudskipper species are thought to have the ability to uptake enough oxygen in air to satisfy resting metabolic demands (*Teal and...*)


