Diving in a warming world: the thermal sensitivity and plasticity of diving performance in juvenile estuarine crocodiles (*Crocodylus porosus*)

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Air-breathing, diving ectotherms are a crucial component of the biodiversity and functioning of aquatic ecosystems, but these organisms may be particularly vulnerable to the effects of climate change on submergence times. Ectothermic dive capacity is thermally sensitive, with dive durations significantly reduced by acute increases in water temperature; it is unclear whether diving performance can acclimate/acclimatize in response to long-term exposure to elevated water temperatures. We assessed the thermal sensitivity and plasticity of 'fright-dive' capacity in juvenile estuarine crocodiles (*Crocodylus porosus*; n = 11). Crocodiles were exposed to one of three long-term thermal treatments, designed to emulate water temperatures under differing climate change scenarios (i.e. current summer, 28°C; 'moderate' climate warming, 31.5°C; 'high' climate warming, 35°C). Dive trials were conducted in a temperature-controlled tank across a range of water temperatures. Dive durations were independent of thermal acclimation treatment, indicating a lack of thermal acclimation response. Acute increases in water temperature resulted in significantly shorter dive durations, with mean submergence times effectively halving with every 3.5°C increase in water temperature (Q_{10} 0.17, $P < 0.001$). Maximal dive performances, however, were found to be thermally insensitive across the temperature range of 28–35°C. These results suggest that *C. porosus* have a limited or non-existent capacity to thermally acclimate sustained 'fright-dive' performance. If the findings here are applicable to other air-breathing, diving ectotherms, the functional capacity of these organisms will probably be compromised under climate warming.

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Introduction

Ectotherms face an ever-increasing risk of losing functional performance as ongoing climate change drives environmental temperatures beyond physiological limits (Pörtner and Farrell, 2008). The functional capacity of all organisms is optimized within a limited range of body temperatures (i.e. thermal window or envelope) as a result of molecular, cellular and metabolic processes being thermally sensitive (Pörtner and Farrell, 2008). Shifts in body temperatures outside this range are intrinsically linked to decrements in fitness-related traits, such as growth (Howe and Marshall, 2002), fecundity (Berger et al., 2008), immune competence (Yu et al., 2009), locomotor capacity (Wilson and Franklin, 2000) and survival (Berrigan, 2000).

The threat of overheating is particularly salient for ectothermic species (almost all fish, amphibians and reptiles), where body temperature is strongly tied to the thermal

environment. Ectotherms exposed to stressfully high temperatures experience compromised metabolic functioning, where maximal rates of oxygen consumption decrease but resting metabolic rates increase exponentially with increasing temperature, creating an imbalance between oxygen supply and demand (Pörtner, 2002). This mismatch in oxygen supply and demand constrains aerobic metabolism, depriving tissues of oxygen and subsequently causing a collapse in whole-organism functioning and/or mortality (Pörtner, 2001, 2002, 2010). Tropical ectotherms living close to their upper thermal limits are therefore predicted to be vulnerable to even very slight rises in temperature (Sunday *et al.*, 2014).

The deleterious effects of climate change on ectotherms may, however, be counteracted by compensatory responses. Thermal stress can be buffered by both behavioural and physiological strategies. Pockets of thermally favourable habitat can be sought out or shuttled between to maintain body temperature within a preferred thermal range (Seebacher, 2005; Seebacher and Franklin, 2005). Alternatively, long-term changes in thermal regimens (e.g. seasonal shifts in temperatures) can induce physiological changes, where the thermal effects on biochemical processes are blunted (Johnston and Dunn, 1987). The capacity to track thermal perturbations and responsively alter the sensitivity of underlying physiology allows ectotherms to maintain or optimize whole-animal function (Prosser, 1991) and is termed acclimatization when observed in the field in natural conditions, or alternatively termed acclimation, when observed in experimentally controlled conditions (Wilson and Franklin, 2002). Thermal acclimation responses operate at every level of organismal organization, from the up- or downregulation of particular genes (Dietz and Somero, 1992; Podrabsky and Somero, 2004) to the alteration of enzyme reaction rates (Somero, 1978) and cell membrane thickness (Hazel, 1995; Hulbert and Else, 1999) to changes in muscle contractility and cardiovascular control (Hicks and Farrell, 2000). These alterations often manifest into a functional cascade, with improved or maintained whole-animal performance resulting (Glanville and Seebacher, 2006).

Plasticity in functional traits is set to play a pivotal role in buffering the deleterious effects of anthropogenic climate warming on ectotherms (Chevin *et al.*, 2010; Somero, 2010; Huey *et al.*, 2012; Seebacher *et al.*, 2015), but its role is often overlooked when making inferences relating to how species will fare. Increases in global temperatures and thermal variability are theorized to create or strengthen selection pressure favouring plastic phenotypes, particularly in long-lived organisms (Kawecki, 2000). Genetic adaptation is also recognized as a crucial mechanism in predicting extinction risk; however, successful genetic adaptation is dependent on generation time being shorter than the rate of climate warming and on changes in environmental temperature being constant to facilitate directional selection (Kawecki, 2000). The persistence of long-lived ectothermic species (e.g. crocodilians, marine turtles and marine iguanas), is therefore likely to be determined by

changes occurring within a single lifetime (i.e. thermal acclimatization capacity).

Ectothermic, air-breathing divers (e.g. marine turtles, iguanas, marine or freshwater snakes and crocodilians) provide a good model for examining the potential buffering role of physiological acclimation, because diving capacity is optimized within a limited thermal window. The maximal duration an animal can remain submerged before oxygen debt is incurred is defined as the aerobic dive limit (ADL; Butler, 2006). The ADL of ectotherms is inversely related to water temperature (Herbert and Jackson, 1985; Fuster *et al.*, 1997, Prassack *et al.*, 2001; Priest and Franklin, 2002). As the body temperature of a diving ectotherm equilibrates with water temperature, oxygen depletion rates become directly related to water temperature [temperature quotient (Q_{10} effect); Pough, 1976; Jackson, 2007], with oxygen stores being consumed more rapidly at 'warm' compared with 'cool' temperatures.

Elevated temperatures in marine and freshwater habitats, set to come with climate change, pose a threat to ectothermic divers, with the duration of time performing obligate underwater activities potentially being greatly reduced (Kooyman *et al.*, 1980; Kramer, 1988; Costa *et al.*, 2004). The dive capacity of aerially respiring ectotherms may be compromised at elevated water temperatures in the following ways: (i) dives may be terminated sooner to maintain submergences within aerobic limits; or (ii) dive durations may remain unchanged, but animals increasingly rely on anaerobic metabolism, incurring the cost of longer post-dive surface intervals (PDSIs) to clear anaerobic debt (Kooyman *et al.*, 1980; Costa *et al.*, 2004). Both modifications to diving behaviour result in a reduction of total time available for underwater activities throughout a bout of continuous diving.

Although acute (i.e. short-term) increases in water temperature markedly reduce diving capacity in a number of ectothermic vertebrates (Priest and Franklin, 2002; Gordos *et al.*, 2003; Campbell *et al.*, 2010b; Pratt and Franklin, 2010), these findings are limited when estimating an organism's vulnerability to climate warming, as the capacity for thermal acclimation to act as a safeguard is rarely assessed (cf. Clark *et al.*, 2008; Bruton *et al.*, 2012). To remedy this, we assessed the capacity for thermal acclimation to mitigate the impact of elevated water temperatures on the diving performance of a long-lived, aerially respiring (Wright, 1986) ectotherm, the estuarine crocodile (*Crocodylus porosus*, Schneider, 1801). Crocodilians are primarily aquatic organisms, spending up to 11 h a day submerged (Campbell *et al.*, 2010a). Diving capacity is thought to be linked to the survival and reproductive success of crocodilians because predator avoidance, foraging, sleep/recovery and social interactions all occur underwater (Seebacher *et al.*, 2005; Campbell *et al.*, 2010b).

The estuarine crocodile is a physiologically plastic organism, with the capacity 'perfectly' to compensate swimming performance, muscle power output and lower level physiological function (i.e. mitochondrial oxygen consumption,

membrane fatty acid composition and regulatory enzymes activity) in response to cool temperatures approaching the lower end of its thermal window (Glanville and Seebacher, 2006; Seebacher and James, 2007). It is presently unclear whether the estuarine crocodile can undergo adequate physiological acclimation at the upper, 'hot' spectrum of its thermal window, but we predicted that the capacity to acclimate to low temperatures would translate to high temperatures. Specifically, we hypothesized dive performance of *C. porosus* to be thermally sensitive, exemplified by an inverse relationship between dive duration and water temperature, and/or a positive correlation between PDSI and water temperature (H_1). Further to this, we predicted *C. porosus* to demonstrate complete thermal acclimation in dive capacity following long-term exposure to elevated water temperatures, so that maximal diving performance shifts in parallel with treatment temperature (H_2). The assessment of thermal acclimation capacity was subsequently used to gauge how future climate warming may impact the diving capacity of estuarine crocodiles.

Materials and methods

Animal maintenance

Estuarine crocodile (*Crocodylus porosus*, Schneider 1801; $n = 11$) eggs were obtained from a single clutch at David Fleay Wildlife Park (Burleigh Heads, Queensland, Australia). Eggs were transported to The University of Queensland (St Lucia, Queensland, Australia), where they were incubated in an *R*-com 50 egg incubator (Auto Elex Co. Ltd, GimHae, Korea) for 88 days at $32 \pm 1°C$ and 85–90% humidity. Upon hatching [hatchling body mass (BM) = 81.7 ± 7.9 g mean ± SD], animals were maintained in an environment aimed at optimizing healthy growth for 8 months prior to experimentation (water temperature = 29°C; photoperiod = 12 h:12 h light:dark). Crocodiles were fed regularly (three times per week, totalling 15% of their body mass) a mixture of minced beef and chicken supplemented with powdered calcium and vitamin D (Vetafarm, Wagga Wagga, NSW, Australia), pilchards (*Clupeidae*) and freshwater prawns (*Macrobrachium australiense*). Enclosures were cleaned, with complete water changes after feeding. All experiments complied with The University of Queensland animal ethics requirements (permit no. SBS/018/14/ARC/AUST ZOO).

Experimental design and thermal acclimation treatments

Crocodiles were randomly assigned to one of three thermal acclimation treatments ($n = 3$ or 4 per treatment; 11 individuals in total). Thermal acclimation treatments were identical apart from water temperature. Animals were acclimated to one of three water temperatures, based on the following Intergovernmental Panel on Climate Change (Solomon *et al.*, 2007) climate change scenarios:

(i) Low rate of global warming (SRES B1 storyline; 10th percentile of IPPC global warming range)/current summer water temperature. This scenario is based on a low-emissions future, with a global shift towards clean and sustainable resource use. Summer water temperatures do not increase under this scenario and are representative of temperatures currently experienced by *C. porosus*. Experimental water temperature emulating this scenario was $28 \pm 0.5°C$.

(ii) Moderate rate of global warming (SRES A1B storyline; 50th percentile of IPCC global warming range). This scenario is based on a moderate rate of warming, resulting from a rapidly expanding economy and growing population. Summer water temperatures are predicted to average 32°C. Experimental water temperature emulating this scenario was $31.5 \pm 0.5°C$.

(iii) High rate of global warming (SRES A1F1 storyline; 90th percentile of IPCC global warming range). This scenario is centred on the intensive and continued use of fossil fuels, with unprecedented levels of carbon emissions, population growth and industrial expansion. Summer water temperatures are predicted to average 33°C, reaching highs of 35°C. Experimental water temperature emulating this scenario was $35 \pm 0.5°C$.

Thermal acclimation enclosures were large wooden tanks (3.35 m × 0.85 m × 0.75 m; length × width × height), designed to emulate thermally heterogeneous environments conducive to thermoregulatory behaviour. The tanks contained freshwater filled to a depth of 0.15 m (sufficient for full submersion of animals). Water temperature was maintained using 200 W submersible heaters (AquaOne; Ingleberg) attached to thermostats (HabiStat; Living Earth Electronics). Dry platforms were situated at each end of the tank, one being a relatively 'warm' platform situated underneath a ceramic heat lamp (250 W; OzWhite, Enfield, South Australia; suspended 26 cm above the platform) and a UV-B light (10%, 18 W; Sylvania ReptiStar) and the other a relatively 'cool' platform with no lamps. Basking opportunity (i.e. time heat lamp was switched on) was 8 h day^{-1} (08.00–16.00 h) for all treatments, with substrate temperature underneath the heat lamp averaging $29 \pm 7°C$ (mean ± SD). A summer photoperiod was used, with a constant 14 h:10 h light:dark regimen (05.00–19.00 h) for all treatments. Animals were left to acclimate to thermal treatments for 30 days prior to performance testing. To maximise sample size, three rounds of acclimation experiments took place, during which all crocodiles were run through each acclimation treatment [acclimation period 1, 28 May 2014 to 29 June 2014 [total length (TL), mean ± SD 52.9 ± 5.7 cm; BM, mean ± SD 479.2 ± 137.8 g); acclimation period 2, 4 September 2014 to 6 October 2014 (TL, mean ± SD 52.3 ± 9.9 cm; BM, mean ± SD 542.24 ± 210.3 g) and acclimation period 3, 9 December 2014 to 7 January 2015 (TL, mean ± SD 59.33 ± 9.6 cm; BM, mean ± SD 710.7 ± 267.5 g)]. Experimental round number was included in all statistical analyses.

Fright-dive trials

Fright-dive trials were held in a large experimental tank (1.8 m × 2.0 m × 1.9 m, length × width × height; volume = 6840 l) constructed from foam fibreglass. The dive tank was evenly partitioned into three sections with opaque plastic partitions to enable three dive trials to run concurrently. The dive tank contained filtered freshwater to a depth of 1.3 m. Water temperature was finely controlled using a spa heater (900 EVO; Elecro Engineering, Stevenage, UK). Previous thermal profiling of the dive tank using thermocron temperature loggers (iButtonLink Technology, Whitewater, WI, USA) confirmed uniformity of temperature throughout the water column. Each partitioned section of the dive tank contained a floating rest platform (0.6 m × 0.15 m × 0.05 m; length × width × height), where crocodiles could rest and breathe on the water's surface whilst their body remained submerged. Dive trials were video recorded (JVC Everio Inc., Mississauga, CA), and the dive tank was sectioned off in a quiet area, free from visual and auditory disturbances.

Diving performance was assessed in animals from all three thermal acclimation treatments (i.e. 'low', 'moderate' and 'high' climate warming scenarios) at three test temperatures (water temperatures = 28, 31.5 and 35°C). The order of test temperature and crocodile position in the dive tank (i.e. partition assignment) was randomized. Animals were allowed to habituate to tank conditions for 1 h before a dive trial commenced.

After the habituation period, a 'fright-dive' trial commenced, in which crocodiles were continuously 'threatened' by a loud auditory disturbance, created by the experimenter banging a plastic lid over the back end of each tank partition once each minute for an hour. Dive data were extracted from the video recordings, and each dive event was characterized by depth (in metres), duration (in minutes) and PDSI (i.e. time spent at the surface between dives; in minutes). The following three measures of dive performance were extracted from the data: maximal dive performance (i.e. longest submergence); mean dive performance (average dive duration); and sustained dive performance (cumulated time spent underwater throughout the 1 h bout of continuous 'fright-diving'). A dive event was defined as an animal being entirely submerged at a depth ≥0.3 m for longer than 30 s.

Statistical analyses

Analyses were performed using the statistical programming package R (version 3.1.2; R Core Development Team, 2010). The effects of acute and long-term thermal treatments on maximal, mean and sustained dive performance were analysed using linear mixed-effects and non-linear mixed-effects models (Pinheiro *et al*., 2012). Indicator variables were incorporated for each level of acclimation temperature and test temperature. Body mass, dive depth, dive frequency and partition number were included as fixed factors, and experimental round and crocodile identification number were included as a random-effect factors. Assumptions of homoscedasticity and normality of errors were graphically assessed, and dive duration was log_{10}-transformed to comply with model assumptions. A Wald *post hoc* test was used to discern statistical differences among thermal treatments, where necessary. The minimal adequate model was determined using maximum likelihood simplification. Statistical significance was accepted at $P \leq 0.05$. Results are presented as mean values ± SEM.

Results

Thermal sensitivity of dive performance

Sustained dive performance (i.e. total time spent submerged during the 60 min continual threat treatment) was thermally sensitive, with performance inversely related to water temperature (Fig. 1A). Crocodiles diving at 28°C spent an average of 36.8 ± 2.9 min underwater, whereas animals diving at 31.5 and 35°C spent an average of 23.5 ± 3.9 and 21.3 ± 2.7 min submerged, respectively (Q_{10} = 0.46, d.f. = 10, $P < 0.001$, non-linear mixed-effects model). Wald *post hoc* analyses showed that sustained dive performance was significantly greater at 28°C compared with other water temperatures, but no differences were observed between 31.5 and 35°C.

Likewise, increasing water temperature from 28 to 35°C resulted in a ~3.5-fold reduction in mean 'fright-dive' duration from 8.0 ± 0.8 to 2.3 ± 0.9 min (Fig. 1B; Q_{10} = 0.17, d.f. = 10 , $P < 0.0001$, linear mixed-effects model). Wald *post hoc* analyses showed that the mean 'fright-dive' duration was significantly different between all three test temperatures, with an approximate twofold reduction in dive duration for every 3.5°C increase in water temperature. In contrast, maximal dive performance showed a plateau of thermal independence across the temperature range assessed here (i.e. 28–35°C), with all dives lasting 8.8 ± 0.4 min (Fig. 2A; $P \geq 0.37$, d.f. = 10, linear mixed-effects model).

Post-dive surface intervals of both sustained and maximal dive performances were independent of water temperature ($P \geq 0.76$) and dive duration ($P \geq 0.7$), with crocodiles surfacing for an average of 3.2 ± 0.3 min between submergences at all water temperatures (Fig. 1C). Dive frequency was independent of water temperature, with animals performing 5.2 ± 1, 6.74 ± 1.1 and 6.2 ± 1.3 dives h⁻¹ at 28, 31.5 and 35°C, respectively ($P \geq 0.2$ Fig. 1D). Dive depth did not vary, with 99.98% of all recorded dives settling at the bottom of the dive tank (1.27 m).

Thermal plasticity of dive performance

Mean, maximal and sustained dive performances were independent of thermal acclimation treatment, with no observed differences in dive duration between C. *porosus* acclimated at 28, 31.5 and 35°C ($P \geq 0.51$, d.f. = 10, linear mixed-effects model; Fig. 2A and B). Thermal acclimation treatment had no effect on PDSI ($P \geq 0.26$) or dive frequency ($P \geq 0.61$). Covariate interactions among body mass ($P \geq 0.23$), partition number ($P \geq 0.33$) and experimental round ($P \geq 0.46$) were not significant in all analyses, and were excluded from final models.
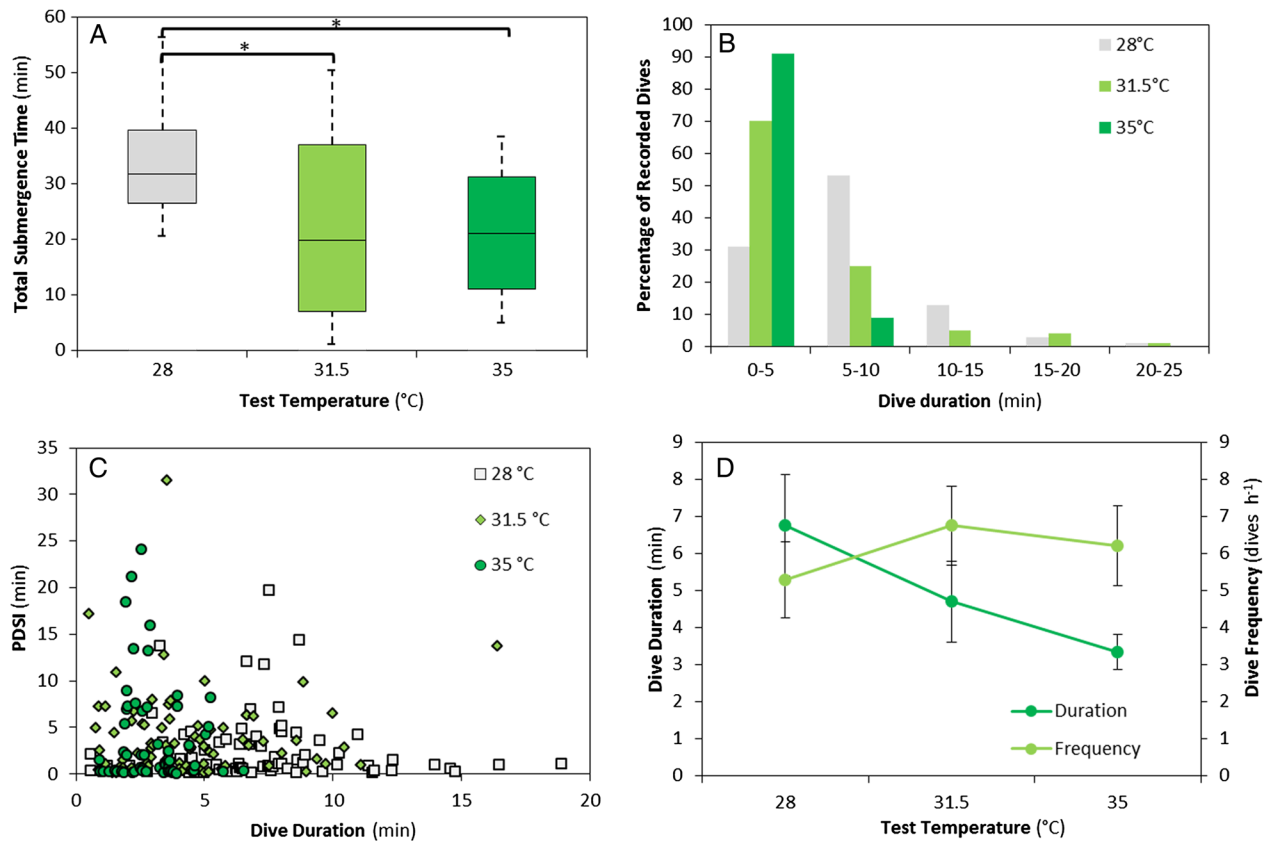
Figure 1: Thermal sensitivity of dive performance in juvenile estuarine crocodiles (*Crocodylus porosus*). Pooled data showing the effect of water temperature on the following: (**A**) total submergence time (throughout the 60 min continual threat treatment); (**B**) frequency histogram of 'fright-dive' durations; (**C**) post-dive surface interval (PDSI); and (**D**) frequency and duration of 'fright-dives'. 'Fright-dive' duration was inversely related to water temperature, with 91% of fright-dives at 35°C lasting <5 min, whereas the majority (70%) of submergences performed at 28°C exceeded 5 min. Total submergence time was significantly greater at 28°C compared with the warmer water temperatures ($P < 0.001$, d.f. = 10, non-linear mixed-effects model). Post-dive surface intervals were independent of water temperature, with crocodiles surfacing for 2–3 min between dives at all water temperatures. Dive frequency was independent of water temperature and dive duration. *$P \leq 0.05$.

Discussion

Thermal acclimation responses are considered to be one of the most important defences that ectotherms have against the deleterious effects of climate change (Chevin *et al.* 2010; Somero, 2010). In the present study, we found that estuarine crocodiles were unable to acclimate dive capacity to long-term increases in water temperatures, with mean submergence times almost halving with every 3.5°C increment in temperature, regardless of thermal acclimation treatment. Together, these findings indicate that sustained diving performance is thermally sensitive but not plastic.

Dissimilar thermal sensitivities of sustained and maximal dive performance

Water temperature has long been identified as an influential factor constraining dive times in ectotherms (Herbert and Jackson, 1985; Fuster *et al.*, 1997), but the separate effects of water temperature on maximal once-off performance compared with continuous, sustained diving have not been previously assessed. Here, maximal dive performance was found to be thermally insensitive, with all dives lasting between 7.39 and 9.5 min, whereas sustained diving performance was markedly reduced at high temperatures. It is likely that the differences in thermal sensitivity between sustained and maximal dive performances reflect the extent to which each is supported by aerobic vs. anaerobic metabolism. Sustained diving requires continuous descents and ascents between the water surface and substratum and is likely to be supported aerobically if extended recovery times (i.e. PDSIs) are to be avoided. Optimizing sustained diving performance can, in theory, be achieved by performing a series of shorter dives within aerobic limits, thereby minimizing recovery times and maximizing total time underwater. Alternatively, maximal once-off dives are likely to involve submergences exceeding ADLs, reliant on anaerobiosis. Both 'strategies' can explain our observed results, with sustained dive performance seeing animals terminate dives sooner at elevated temperatures to maintain dives within aerobic limits, and maximal dive times
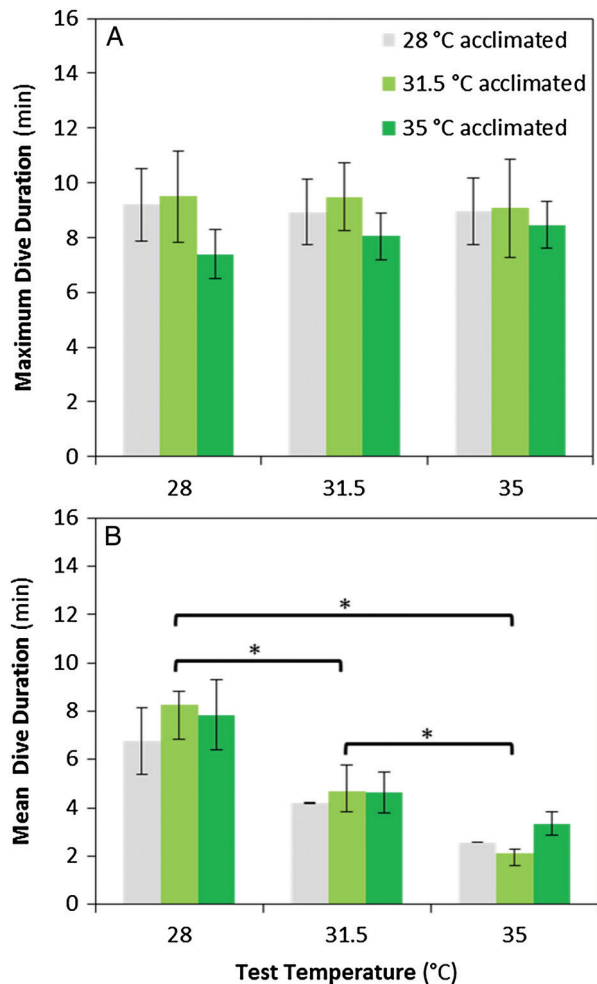
Figure 2: Thermal plasticity of dive performance in juvenile estuarine crocodiles (*C. porosus*). Effect of test water temperature on 'fright-dive' performance (i.e. dive duration, expressed as minutes submerged) in *C. porosus* acclimated to 28, 31.5 or 35°C (*n* = 11). (**A**) Maximal submergence times (±SEM) were independent of both test temperature ($P ≥ 0.37$, d.f. = 10, linear mixed-effects model) and thermal acclimation treatment ($P ≥ 0.51$, d.f. = 10, linear mixed-effects model). (**B**) Mean (±SEM) submergence times were inversely related to water temperature, with shorter dives performed at 35 compared with 28°C, regardless of thermal acclimation treatment ($P < 0.001$, d.f. = 10, linear mixed-effects model). *$P ≤ 0.05$.

remaining unchanged, with respect to temperature, as animals may increasingly rely on anaerobic metabolism to extend dive times beyond ADLs.

Indications of anaerobic debt accumulated during a dive can be drawn from post-dive recovery times (i.e. PDSI). Recovery times remained constant for both sustained and maximal dives, suggesting that all dives were performed aerobically, but anaerobic debt incurred during maximal performances might be masked by recovery processes occurring more rapidly at elevated temperatures (Galloway and Kieffer, 2003). Direct measurements of post-dive physiology, such as

blood lactate levels and post-dive oxygen consumption, are recommended for future endeavours.

The plateau of thermal independence observed for maximal dives may play an adaptive role where once-off maximal performances are integral to survival or in situations where extended recovery times incur a minimal fitness cost. Free-ranging crocodilians, however, spend the majority of their time in the water, performing between 50 and 70 dives per day (Campbell *et al.*, 2010a), so it is likely that sustained diving performance or endurance is of greater ecological relevance than maximal performance. Estuarine crocodiles have previously demonstrated a plateau of thermal independence in prolonged swimming performance, across a similar thermal range (23–33°C; Elsworth *et al.*, 2003), and this came at the cost of post-exercise recovery times tripling to clear anaerobic debt (Campbell *et al.*, 2013). Maintaining performance across a breadth of temperatures may come at the cost of increasingly relying on anaerobic metabolism, but our results suggest that this cost may be minimal at very high temperatures because PDSIs remained constant. Investigation into the possible trade-offs between reduced dive durations and faster recovery rates at elevated temperatures could provide a better understanding of the diving behaviour observed here.

Ecological implications for a lack of thermal plasticity

Aquatic and semi-aquatic ectotherms have long been revered for their capacity to acclimate to altered thermal regimens (Hazel and Prosser, 1974; Johnston and Temple, 2002), but the bulk of this research has examined organismal responses to decreases in ambient temperature (Aho and Vornanen, 2001; Glanville and Seebacher, 2006; Seebacher and James, 2007; Guderley and Seebacher, 2011). Our study here complements this body of work by examining thermal compensation at the other, 'hot', end of the spectrum. The results from the present study reveal that *C. porosus* have a limited capacity to acclimate sustained diving performance to elevated water temperatures, as chronic exposure to high water temperatures does not elicit improved performance. This finding is unusual, because thermal acclimation in the form of altered cardiovascular control (Jayasundara and Healy, 2013), muscle contractility rates (Johnston and Temple, 2002) and locomotor performance (Johnson and Bennett, 1995) has been extensively documented in ectotherms. Further to this, estuarine crocodiles have a remarkable capacity to compensate during cold acclimation, exemplified by musculature, metabolic and biochemical alterations leading to 'perfect' maintenance of swimming performance (Glanville and Seebacher, 2006; Seebacher and James, 2007). Taken together, these findings are consistent with the idea that tropical ectotherms, such as *C. porosus*, have greater flexibility in adjusting to decreases in temperatures compared with increases, as they are assumed to inhabit microclimates nearing their upper thermal limits (Janzen, 1967; Somero, 2010; Sunday *et al.*, 2014). Our findings therefore emphasize the need to assess acclimation capacity

across a range of temperatures, because complete acclimation to 'cool' temperatures may only translate into partial or non-existent acclimation capacity at elevated temperatures.

Crocodilian diving offers an ideal study system to test the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (Pörtner, 2001, 2002) in air-breathing vertebrates, because performance is directly related to oxygen supply and demand. A lack of compensation in diving performance at elevated temperatures may be a reflection of an inability to adjust resting/diving metabolic rates. Physiological acclimation to 'low' temperatures typically involves increasing maximal metabolic rates (MMR, $M_{O2,max}$), whereas acclimation to 'high' temperatures involves decreasing resting metabolic rates (RMR, $M_{O2,min}$; Pörtner, 2001, 2002, 2010). The estuarine crocodile, and perhaps other tropical ectotherms, may have greater flexibility in adjusting MMRs than RMRs, potentially creating a reduction in aerobic scope (i.e. the difference between $M_{O2,max}$ and $M_{O2,min}$) at 'high' but not 'low' thermal extremes. The OCLTT hypothesis suggests that the decrement in diving performance observed here is linked to a reduction in aerobic scope at elevated temperatures, but this needs to be tested directly because evidence supporting the OCLTT is mixed (Ern *et al*., 2014; Norin *et al*., 2014).

Partial or non-existent acclimation capacity in diving performance at elevated temperatures appears to be a shared trait among ectothermic divers, although comparative data are scant. Diving performance in the Mary River turtle (*Elusor macrurus*), for instance, only partly acclimates to elevated water temperatures of 28°C, but this is linked to differential reliance on aquatic respiration at low temperatures (Clark *et al*., 2008). Likewise, submergence times of free-ranging crocodilians and turtles are reduced in summer months compared with winter months (Carr *et al*.,1980; Bentivegna *et al*., 2003; Gordos *et al*., 2003; Hochscheid *et al*., 2005; Bradshaw *et al*., 2007; Campbell *et al*., 2010a), suggesting that these ectotherms are not fully compensating for present-day seasonal changes. Our results suggest that sustained dive capacity of juvenile estuarine crocodiles is likely to be further compromised as summer water temperatures continue to rise under climate change. Reduced dive durations may see cumulated time available for underwater activities cut short, forcing animals to spend a greater amount of time at the water's surface and potentially making them more conspicuous to aerial predators.

Organisms have a finite number of responses to climate change; they can change their thermal tolerance limits by genetic adaptation (requiring multiple generations in a short space of time), acclimatize to the altered thermal regime (requiring within-lifetime physiological changes), behaviourally compensate, or shift their geographical range to thermally preferable habitat (typically towards the poles or higher altitudes). Our findings reveal that thermal acclimation/acclimatization is unlikely to buffer estuarine crocodiles from the negative consequences of elevated temperatures on dive capacity. This, together with the long-lived life-history of

estuarine crocodiles, suggests that this species may be reliant on behavioural strategies to buffer extreme temperatures. Crocodiles may be able to seek refuge in deep, cool water pockets, thereby defending dive times, or shift their geographic range to cooler, southerly waters. Before we can fully understand the constraints that elevated temperatures place on the ecological functioning of *C. porosus*, an entire suite of performance traits (e.g. swimming capacity, bite force, aerobic scope) needs to be assessed (Kearney and Porter, 2009). Nonetheless, elevated water temperatures accompanying climate change will be likely to cause serious disruption to the diving behaviour of estuarine crocodiles.

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