**Introduction**

Diving ectotherms are crucial to the functioning of aquatic ecosystems and represent a taxonomically diverse group (Butler and Jones, 1982; Costa, 2007). The evolution of air-breathing organs made terrestrial environs habitable, but many species returned to aquatic habitats, leading to the reinvasion of air-breathing species (Butler and Jones, 1982; Seymour, 1982). This transition occurred in all groups of tetrapod vertebrates across multiple points in evolutionary history, leading to a diverse array of diving ectotherms (turtles, snakes, crocodiles, iguanas, frogs and salamanders) living in freshwater and marine habitats (Butler and Jones, 1982). The ecology of these species in closely tied to the underwater environment and submergences are necessary to fulfill a range of critical tasks. For example, the viperine water snake (*Natrix maura*) dives to hunt fish by actively chasing them or by using a sit-and-wait strategy (Aubret et al., 2015). In contrast, juvenile estuarine crocodiles (*Crocodylus porosus*) dive to avoid both aerial and subsurface predators, whilst in adult crocodiles, diving is more important for social interactions, facilitating sit-and-wait hunting and avoiding rapid surface water currents (Campbell et al., 2010b; Grigg and Kirshner, 2015). Time available for these underwater tasks is, however, constrained by the need to surface to replenish oxygen stores (Butler and Jones, 1982).

The maximum amount of time an air-breathing animal can remain underwater prior to the switch to anaerobic metabolism and concomitant lactate accumulation is termed the aerobic dive limit (ADL; Butler, 2006). A diver’s ADL can be calculated by dividing its total body oxygen stores (stored in the lungs, blood and tissue) by the rate at which oxygen is consumed (i.e. metabolic rate). The ADL of ectotherms is inversely related to water temperature, due to the thermal sensitivity of metabolism (Rodgers and Franklin, 2017). As the body temperature of a diving ectotherm equilibrates with water temperature, the rate at which body oxygen stores are consumed becomes directly related to water temperature, with oxygen stores consumed more rapidly as temperatures rise. For example, ectotherm metabolic rates typically double (or even triples) with every 10°C increase in body temperature, meaning that an ectotherm diving at 30°C is predicted to dive for only half as long compared to an ectotherm diving at 20°C. For example, the ADL of a freshwater turtle (*Mauremys caspica leprosa*) fell from 221 min at 15°C to 58 min at 25°C (Fuster et al., 1997). Anthropogenically forced warming in marine and freshwater habitats therefore poses a threat to ectothermic divers, with the duration of time performing critical underwater tasks potentially being greatly reduced.

The threat of climate warming to diving ectotherms has been largely overlooked, despite many studies showing substantial decreases in dive durations as temperatures rise. Dive durations in the Arafura file snake (*Acrochordus arafurae)*, for example, decreased by 63% in response to an acute temperature increase from 20°C to 32°C (Pratt and Franklin, 2010). Similarly, during a one-hour continual predator threat juvenile *C. porosus* were able to remain submerged for 36.8 min at 28°C and only 21.3 min at 35°C (Rodgers et al., 2015). Dive durations are also influenced by seasonal temperature increases. Field studies have shown marked declines in dive durations during summer months compared to winter months in both turtles (Bentivegna et al., 2003; Bradshaw et al., 2007; Gordos et al., 2003) and crocodilians (Campbell et al., 2010a). Warmer waters, brought about by climate change, could have deleterious consequences for diving ectotherms. Elevated temperatures may cut short time available for critical underwater activities (e.g. foraging, predator-avoidance and social interactions) and force animals to spend more time at the water’s surface where predation risk is highest.

The deleterious effects of climate change on ectotherms diving behaviour may, however, be counteracted by thermal acclimatization responses (Seebacher et al., 2015). Increased oxygen demands at elevated temperatures could be offset by thermal acclimation (under experimentally controlled conditions) or acclimatization (in a natural field setting) (Johnston and Dunn, 1987; Wilson and Franklin, 2002). Thermal acclimation/acclimatization involves physiological remodelling across multiple levels of organismal organisation- from the up- or downregulation of particular genes (Podrabsky and Somero, 2004), to the alteration of enzyme reaction rates and changes to cell membrane thickness (Glanville and Seebacher, 2006), to alterations to muscle contractility (Shuman and J., 2018) and cardiovascular functioning (Keen et al., 2017). In the context of diving in a warming world, thermal acclimation/acclimatization would involve a dampening of thermodynamic effects on metabolism at elevated temperatures, so that total body oxygen stores are consumed at a slow rate **(and/or increase body oxygen stores).**

Gas-exchange systems may also play an important role in mediating the effects of elevated temperatures on diving behaviour. Many divers rely solely on pulmonary gas exchange and have a negligible capacity for aquatic respiration (Wright, 1986). In contrast, bimodal breathers can supplement aerial respiration with aquatic respiration. Aquatic gas exchange typically occurs via diffusion across the skin (i.e. cutaneous respiration; Feder and Burggren, 1985), but some divers also have specialised organs (e.g. buccopharyngeal cavity, cloacal bursae) to facilitate the exchange of respiratory gases with the aquatic environment (Maina, 2002). For example, several freshwater turtles (e.g. *Elseya albagula, Elusor macrurus* and *Rheodytes leukops)* have cloacal bursae which are highly vascularised, sac-like evaginations of the cloaca, that are ventilated with water by the contraction of cloacal muscles (FitzGibbon and Franklin, 2010; Gordos and Franklin, 2002; Storey et al., 2008). The ability to respire aquatically allows bimodal breathers to prolong dive durations and it has been suggested that supplemental aquatic respiration may reduce the energetic cost of diving, prolong time available for underwater tasks and facilitate the exploitation of oxygen-rich riffle zones (Mathie and Franklin, 2006; Storey et al., 2008; Tucker et al., 2001). Due to these advantages, it has been hypothesised that bimodal breathers may be less sensitive to the effects of temperature on dive duration compared to divers solely reliant on pulmonary gas exchange. Moreover, bimodal breathers are predicted to upregulate their reliance on aquatic respiration at high temperatures to meet increased metabolic demands and defend dive durations. Studies testing this prediction have returned mixed findings, with some studies suggesting reliance on aquatic respiration is independent of temperature (Bruton et al., 2012; Prassack et al., 2001; Pratt and Franklin, 2010; Udyawer et al., 2016).

Although the profound effects of temperature on ectotherm metabolism, life-history and fitness are well-established, the effects of elevated temperature on diving has been overlooked. Two reviews show that dive duration tend to decrease as temperatures rise in ectotherms (Hayward et al., 2016; Jackson, 2007), but a systematic review has not yet been conducted. Here, we test whether diving is thermally sensitive in ectotherms using meta-analytic methods. Specifically, we tested the following predictions: (i) acute increases in temperature decrease mean dive durations, (ii) larger increases in temperature will exert a stronger effect on mean dive durations, (iii) temperature effects on mean dive durations will be more pronounced under laboratory compared to field conditions, (iv) the effects of temperature on mean dive durations will be weaker in bimodal breathers compared to aerial breathers, (v) the effects of temperature on mean dive durations will be weaker in larger (in terms of body mass) divers compared to smaller divers, and (vi) chronic exposure to elevated temperature will blunt (i.e. lessen) the effects of temperature on mean dive durations. (**include hypothesis about variability)**

These finding were used to predict how much underwater time could be lost to climate warming and how diving, ectothermic vertebrates may fare in a warmer world.

**Methods**

*Availability of data and code*

*Eligibility criteria*

*Study design*

We included experimental studies which measured dive durations (i.e. minutes submerged) in diving vertebrates at two more water temperatures. When dive durations were measured at multiple temperatures, we took each control-treatment pairwise comparisons, where the cooler temperature was considered the control and the warmer temperature was considered the treatment. The difference between control-treatment pairwise comparisons was calculated (i.e. ΔT) to test whether the magnitude of temperature increase mattered. **[acute vs. chronic temperature treatments]** We also included field studies where dive durations were measured in free-ranging animals across one or more seasons using biotelemetry tags (e.g. **list examples**). Field studies needed to couple dive duration recordings with either direct body temperature measures or water temperature measurements (assuming animal body temperature equaled water temperature). Studies on leatherback turtles (*Dermochelys coriacea*) were excluded because they are functionally endothermic (N = 24) (Bostrom and Jones, 2007; Southwood et al., 2005).

*Dive duration measurements*

Studies were only included if they measured voluntary diving behaviour under laboratory or field conditions. We excluded studies where animals were forced to dive due to atypical physiological alterations induced by forced submergence (refs). Where both mean and maximum dive durations were presented, we extracted mean dive durations because maximum dive duration were rarely reported and likely have anaerobic contributions. Studies needed to present means, variance (standard errors, standard deviations or confidence intervals) and sample sizes. Authors were contacted to request missing summary statistics where necessary. The minimum sample size to be included was three animals per treatment group or field recording.

*Literature search and data extraction*

We conducted a literature search using Scopus and Web of Science's (WoS) core collection on 25 February 2020, to identify studies seeking to test the thermal sensitivity of diving behaviour. We used the following search strings: TOPIC: (“dive” OR “diving”) AND (“temperature” OR “thermal” OR “season\*”) AND (“ectotherm\*” OR “reptile\*” OR “\*snake\*” OR “turtle\*” OR “crocod\*” OR “newt\*”) in WoS and TITLE-ABS-KEY (“dive” OR “diving”) AND (“temperature” OR “thermal” OR “season\*”) AND (“ectotherm\*” OR “reptile\*” OR “\*snake\*” OR “turtle\*” OR “crocod\*” OR “newt\*”) in Scopus.

We identified 225 and 222 studies meeting the search criteria in Scopus and WoS, respectively. A total of 149 duplicates (115 identified in EndNote, 32 identified in Rayyan) were removed leaving us with 298 papers for title and abstract screening. We cross-referenced this search with three major reviews: Jackson (2007), Brischoux et al. (2008) and Hayward et al. (2016), and included any papers missed in our initial search (N = x). Title and abstract screening was conducted in Rayyan (Ouzzani et al., 2016) and we excluded studies that: (1) were reviews or commentaries (N = 28), (2) were not on air-breathing, diving ectotherms (N = 33) or (3) did not measure voluntary dive durations across two or more experimental temperatures or across two or more field seasons (N =115).

Following these exclusion steps, we identified 100 papers with extractable data (Fig. 2). From these papers we extracted means, variance (standard errors, standard deviations or confidence intervals) and sample sizes. Data presented in figures were extracted using metaDigitise (Pick et al., 2018). Studies were excluded when missing summary statistics could not be obtained by contacting authors (N=x). The following moderator variables were also extracted from each study: respiration mode (i.e. aerial or bimodal), body mass (g) and magnitude of temperature change (i.e. ΔT). When body mass data were not presented, we obtained body mass estimates for the same species and life-stage from published literature (N=x, supplementary info.)

*Data Analysis*

*Effect size calculation*

To test the effect of temperature increases on dive duration, we calculated two effect sizes for each pairwise comparison (e.g. control temperature – treatment temperature); the log response ratio (*ln*RR; Hedges, ref) and the log coefficient of variation ratio (*ln*CVR; ref). To assess mean dive duration differences we used *ln*RR, which is the natural logarithm of the ratio between mean dive durations at control and treatment temperatures. To assess differences in dive duration variance we used *ln*CVR, where the ratio reflects the difference between the coefficients of variation for both control and treatment temperatures. Effect sizes were calculated in *R (ref)* using the *escal* function in the *metaphor* package (version, ref). For both logged ratios, we specified the control temperature (i.e. lower temperature) as the numerator and the treatment temperature (i.e. warmer temperature) as the denominator, so that negative values indicate a decrease in dive duration at the treatment temperature, whereas positive values indicate an increase in dive duration at the treatment temperature.

*Meta-analysis*

We fit meta-analytic and meta-regression multilevel mixed-effects models using the *rma.mv* function in the *metaphor* package in *R.* When the 95% confidence intervals of model estimates did not cross zero, they were considered statistically significant. Respiration mode (i.e. aerial or bimodal), logged body mass (g) and magnitude of temperature change (i.e. ΔT) were included as moderator variables. To visualize the impact of moderator variables, effect sizes were calculated for aerial versus bimodal breathers, for divers of various body mass (**insert bins**), and for different magnitudes of temperature increases (i.e. + 3°C, + 5-7°C, + 8-9°C and + ≥ 10°C). Our data contained several levels of non-independence and we accounted for this by including random-effects and sampling variance co-variance matrices. Specifically, we generated a phylogeny by searching for species names in the TimeTree database (timetree.org; Hedges et al., 2006), which uses published molecular data to build a phylogenetic tree with branch lengths (Fig. x). This phylogeny was then converted into a phylogenetic correlation matrix. Study ID and observation number were included as random effects. Where multiple pairwise comparisons were extracted from a single study, some of these comparisons shared the same control value, and sampling variance for these observations were non-independent. To account for shared controls, we specified sampling variance as a variance-covariance matrix, with sampling variance for each effect size on the diagonal and covariance between these measures as off-diagonal elements.

In addition to calculating dive duration differences between control and treatment temperatures using pairwise contrasts, we also estimated the effect of temperature on mean dive durations using an arm-based, Bayesian approach, using the *MCMCglmm* package (ref). This approach allowed for comparisons across multiple temperatures within each study. For each observation, water temperature was centered within species (i.e. Tw; subtracting the mean test temperature for each species from each value of the input variable), which standardized any large temperature differences among species (e.g. tropical compared to temperature species). The same moderator variables, random effects and variance co-variance matrices were included as the contrast-based model, however there was no need to account for shared controls as the arm-based approach has the capacity to make comparisons across more than two groups. We also included a random slope for each set of dive durations across two or more test temperatures (within each study). To evaluate the effect of temperature on variability in dive duration, we modelled the logged standard deviation (*lnSD*) of each observation as a function of logged mean dive duration and Tw as fixed effects, and logged body mass and respiration mode as moderator variables. Logged mean dive duration was included as a fixed effect to account for mean-variance relationships. The phylogenetic correlation matrix, random effects (study ID, observation number) and a random slope for each set of dive durations across two or more test temperatures (within each study) were also included. MCMC chains were run for 130 000 iterations, with a 30 000 burn and a 50 thinning interval (effective sample size = 2000), and we visually checked if chains were mixing well.

*Publication bias*

**Results**

*Description of data set*

* No. species and orders, no. of effect sizes, temperature range, mean delta t. No. bimodal breathers, body mass range.

1. *Did acute increases in temperature reduce dive duration means and variability?*

(i) acute increases in temperature decrease mean dive durations, (ii) larger increases in temperature will exert a stronger effect on mean dive durations, (iii) temperature effects on mean dive durations will be more pronounced under laboratory compared to field conditions, (iv) the effects of temperature on mean dive durations will be weaker in bimodal breathers compared to aerial breathers, (v) the effects of temperature on mean dive durations will be weaker in larger (in terms of body mass) divers compared to smaller divers, and (vi) chronic exposure to elevated temperature will blunt (i.e. lessen) the effects of temperature on mean dive durations.

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