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ORIGINAL ARTICLE

Diving behavior in semi-aquatic *Anolis* lizards results in heat loss with sex-specific cooling tolerance

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Abstract

Males and females often differ in use of antipredator behaviors, particularly when antipredator behavior comes at the cost of missed mating opportunities or territory defense. When using thermally suboptimal refugia, ectotherms are especially vulnerable to these costs, as their performance is linked to body temperature. To flee from predators, semi-aquatic *Anolis* lizards dive underwater for long periods and rebreathe from a bubble of air. We hypothesized that using aquatic refugia would result in body heat loss, that dive duration is influenced by sex, and that oxygen consumption when diving would help explain sex differences. We tested these hypotheses by measuring dive length and body temperatures in *A. aquaticus*, and by recording oxygen consumption and final oxygen partial pressure during controlled dives in several semi-aquatic *Anolis* species. Not only was there a significant thermal cost to diving, but *A. aquaticus* males and females appeared to tolerate different levels of this cost: males re-emerged from water more quickly and at higher body temperatures than did females. Body temperature decreased according to an exponential decay function, dropping up to 6 °C in 5 min. Oxygen consumption rates in semi-aquatic anoles were primarily explained by the expected allometric scaling relationship with mass and, therefore, are unlikely to lead to sex differences in physiological limits to dive times. Instead, shorter male dives may help them maintain physiological performance, mating opportunities or territory defense. Antipredator diving behavior is physiologically costly but undoubtedly beneficial to both sexes, highlighting the need for further study of sex-based antipredator optimization.

Significance statement

To avoid predators, semi-aquatic *Anolis* lizards can dive underwater and remain there for an extended time by rebreathing a bubble of air over their heads. In this study, we reveal that diving to escape predators also comes with a cost: submersion in water reduces lizard body temperatures. Reduced body temperature can impair a lizard's ability to move quickly and defend mates or territories, suggesting that there may be divergent diving behaviors in males and females. Our findings confirm that males do indeed spend less time underwater than females. We measured oxygen consumption during dives, and our data suggest that sex differences in diving behavior are unrelated to oxygen use. This study sheds light on the sex-specific balance of antipredator behaviors and the maintenance of optimal body temperatures, and more broadly contributes insight into adaptive responses to environmental challenges.

Keywords *Anolis aquaticus* · Lizard · Respiration · Sex differences · Thermal biology · Tradeoff

Introduction

Antipredator behavior is often at odds with other behaviors important to an animal's survival and fitness (Ydenberg and Dill 1986). When predators are present, foraging efficiency may decrease as antipredator responses become more pronounced (e.g., Milinski and Heller 1978; Sih 1980; Mateo 2007) and reproductive behavior is often reduced (Herczeg et al. 2008; Brunel-Pons et al. 2011; Lea and Blumstein 2011; Martín et al. 2003). The balance between accepting risk to gain long-term fitness benefits versus prioritizing immediate survival can vary among species, individuals, and the sexes (Ydenberg and Dill 1986; Samia et al. 2016). Though variation exists, females often respond more strongly to predator cues than do males, across invertebrate (e.g., Xu et al. 2014; Tanis et al. 2018) and vertebrate taxa

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(e.g., Blanchard et al. 1992; Swierk et al. 2014; Curlis et al. 2016). Such trends suggest that males weigh tradeoffs differently than females, often placing more investment into activities outside of antipredator responses. By doing so, males may increase their risk of predation but may also increase reproductive opportunities and thereby lifetime fitness (Cooper 1999; Cooper and Wilson 2007; Polis et al. 1998). In this respect, it is often the more optimal strategy for males to forego extended predator avoidance in favor of an opportunity to reproduce, despite the potential risk (Trivers 1972).

For ectotherms, which rely on the external environment to regulate their body temperatures, antipredator behavior frequently results in animals fleeing to areas where achieving thermal optima is not possible, such as under rocks or inside logs (DeWitt 1967; Huey and Slatkin 1976; Sannolo et al. 2019). The costs of retreating to thermally suboptimal microhabitats have been well documented and can include reduced growth rates (Webb and Whiting 2005; Lelièvre et al. 2013) and reduced time spent in safety (*Lacerta monticola*, Polo et al. 2005; *Gopherus polyphemus*, Radzio and O'Connor 2017). Lower than optimal temperatures result in reduction of locomotor capacity (Avery et al. 1982; Huey 1983; Weatherhead and Robertson 1992; Lailvaux and Irschick 2007) and reduced bite force (Segall et al. 2013), which puts individuals at greater predation risk and can impede competitive ability (Lailvaux 2007).

Despite its prevalence across taxa, the potential thermal costs of antipredator behavior that specifically includes water as a refuge are not well addressed in the literature. Although land-to-water escape is widely documented, from early studies (e.g., birds: *Alcedo atthis*, Johnson 1925; mammals: *Sorex vagrans*, *Microtus californicus*, Johnston 1957) to more recent (e.g., spiders: *Dolomedes triton*, Johnson and Sih 2007; arboreal crabs: *Aratus pisonii*, Yeager et al. 2016), an understanding of the thermal fitness tradeoffs associated with such antipredator behavior is generally lacking. For ectotherms that rely upon maintaining body temperatures that are warmer than water temperatures, thermal costs of aquatic escape would be especially high (Huey 1982; Angilletta et al. 2002), including, but not limited to, reduced performance (John-Alder et al. 1989; Weatherhead and Robertson 1992) or physiological function (Martín and López 1999).

The recently discovered diving and rebreathing behavior of semi-aquatic *Anolis* lizards (Swierk 2019; Boccia et al. 2021) provides an ideal system in which to study sex differences in a potentially costly antipredator tactic. One semi-aquatic anole, *Anolis aquaticus*, inhabits rocky areas in and around streams in lowland and premontane wet forests in Costa Rica and Panama. Observed predators of this small species (ca. 57 to 71 mm adult *A. aquaticus* snout-vent length; Savage 2002) include snakes (specifically *Chironius grandisquamis* and *Ptychophalus tristanis*), motmots (*Momotus lessonii*; Márquez and Márquez 2009), and other birds such as sunbitterns (*Eurypyga helias*), kingbirds (*Tyrannus* spp.), and kingfishers (the Alcedinidae family) (R. Quirós Flores, personal communication). *Anolis aquaticus* attempts to avoid predation either by color change (Boyer and Swierk 2017; Wuthrich et al. 2022), sprinting and seeking refuge, or by diving underwater for long durations (longest recorded dive: 16 min; Swierk 2019), a feat likely facilitated by rebreathing a bubble of air that forms above a lizard's nares (Fig. 1). Within this bubble, oxygen concentration decreases over time, suggesting that it is continuous with the lizard's oxygen supply and may enhance dive duration (through multiple mechanisms discussed in Boccia et al. 2021).

We hypothesized that, as an ectotherm, *A. aquaticus* incurs a thermal cost due to this land-to-water antipredator behavior, and that characteristics of this antipredator behavior (i.e., dive duration) would consequently be influenced by sex. Male *A. aquaticus* defend territory and court females through pushup displays and dewlap extensions (Márquez and Márquez 2009), which increase male conspicuousness (Swierk et al. 2021; Talavera et al. 2021)

Fig. 1 The exhalation of a rebreathed bubble in *Anolis aquaticus*, time progression from left to right. Hydrophobic scales allow the bubble to cling to body surface and be re-inhaled. Photographs by L. Swierk

but are energetically expensive (e.g., Leal 1999). From a functional perspective, we predict that male *A. aquaticus* will perform land-to-water antipredator behavior (diving) for shorter durations than females, not only due to the lost opportunity time for mate/territory defense but also because of the negative effects of body heat loss on locomotor and competitive performance. From a mechanistic perspective, we compare male and female oxygen consumption during dives as a complementary possible explanation for dive duration sex differences, using a total of three additional tropical semi-aquatic rebreathing species (*Anolis barkeri*, *Anolis maculigula*, and *Anolis oxylophus*) with similar ecologies and behaviors to *A. aquaticus* (Boccia et al. 2021). To test these predictions, we performed field and laboratory trials of dive durations and temperature differences after controlled dives in *A. aquaticus* and measured sex differences in oxygen consumption in multiple semi-aquatic *Anolis* species. A summary of the objectives and sample sizes in the study can be found in Supplementary Table 1.

Materials and methods

Field temperature trials

Field temperature trials and dive duration trials (described in *Laboratory Dive Trials*) were conducted at Las Cruces Biological Station, Puntarenas Province, Costa Rica (elevation of ca. 1200 m a.s.l.) from 2019 to 2022. The study site consisted of a relatively narrow river (about 3.5 to 5 m wide), bordered by large rocks and boulders, steep riverbanks, vegetation, and overhanging trees. The river is mostly under full canopy, with occasional gaps. We caught *A. aquaticus* by hand or fishing pole with a lasso attached to its end between 9:00 AM and 1:00 PM (hours in which lizards are most active at this site).

To examine how diving affects lizard body temperatures, we used a field diving arena in which we could subject lizards to controlled dives. These trials were conducted in June and July 2019. The arena (74 × 45 × 34 cm; L x W x H) was made of clear plastic and outfitted with rocks found along the field site, including a single large, flat stone to serve as a foothold for the lizards (Supplementary Fig. 1). The configuration of the rocks within the arena was consistent between trials. We used river water to fill the arena halfway at the start of each trial and checked water temperature (Digi-Sense Single-Input Thermocouple Thermometer with NIST-Traceable Calibration, Cole-Parmer Instrument Company, LLC., Vernon Hills, IL, United States) prior to each trial to ensure that it was the same temperature as river water at our study site. We placed an underwater action camera (EK7000Pro, AKASO, Frederick, MD, United States) in the arena in front of the foothold so that field trials could be recorded and reviewed later for further analysis.

We recorded the internal (cloacal) body temperatures (° C) of each lizard ($n = 18$, 8 male and 10 female) using the thermocouple's probe immediately before the start of the trial (within 30 min of initial capture). To begin a trial, we submerged a lizard in the arena and released it on the underwater foothold. We started a timer upon initial submersion of the lizard, observers retreated behind a blind, and the arena was undisturbed for the duration of the trial. If the individual swam to the surface at any point before grasping the foothold autonomously, the trial was excluded from analysis. Lizard behavior during the trial was observed from a distance behind the blind and confirmed by later cross-referencing the underwater video recordings. Once the lizard resurfaced, the timer was stopped, and body temperature was immediately remeasured. Body mass, sex, and snout-vent length (SVL) were also measured post-trial.

To measure the rate of internal temperature loss, we collected a separate set of lizards ($n = 21$) in 2021 and continuously monitored their cloacal temperature (°C) during submersion. Lizards were loosely secured to a thin wooden stake using self-adhering tape (Nexcare No Hurt Wrap, 3 M, Maplewood, MN, USA) with the thermocouple placed in the cloaca. Lizards were then submerged in the stream next to their location of capture, and temperature was recorded every 10 s over a 5-min dive. During this time, lizards were monitored to ensure that they were rebreathing regularly; lizards did not fail to rebreathe in any of the trials.

At the conclusion of each trial, lizard body mass, sex, and SVL were recorded, and the lizard was given an identifying marking and was released at its site of capture. We recorded stream temperature again immediately following each trial.

Laboratory dive trials

To complement the temperature loss field study, we conducted additional trials in the laboratory to quantify dive duration. In 2019, a subset ($n = 12$) of lizards from the field temperature trials was transported to the laboratory at Las Cruces Biological Station for laboratory dive duration trials, with additional lizards collected for laboratory dive duration trials in June and July 2021 and 2022, for 82 dive duration trials in total (49 male, 33 female). In laboratory dive duration trials, each lizard was individually submerged and placed on the paper substrate of a small aquarium (17.8 × 8.3 × 15.2 cm; L x W x H) filled with water. A digital camera (Olympus Tough TG4, Olympus Corporation of the Americas, Center Valley, PA) was used to record underwater behaviors for a separate study. As in the field trials, lizards resurfaced on their own, and the duration of each dive was recorded. These trials were not used in the temperature loss analyses. Lizards were released at their respective points of capture within 24 h of initial collection. Each lizard was given an identifying marking (a single drop of nail polish at the base of its tail) to prevent resampling during the field season.

Oxygen consumption measurements

We measured oxygen partial pressure (pO_2) within the rebreathed bubbles of males and females of three species of semi-aquatic anoles (*Anolis barkeri*, *Anolis maculigula*, and *Anolis oxylophus*) between 2017 and 2019 (see Supplementary Table 2 for study site locations). We also measured pO_2 in *Anolis aquaticus*, though only males were available for this analysis and so no sex comparisons for this species could be made. Full details can be found in Boccia et al. (2021) but, in brief, we inserted a bare fiber oxygen microsensor (PyroScience OXB50 or OXR50 probe and FireSting GO2 m) into the rebreathed bubble of air above the nares of a lizard that we submerged in a tank of water. In these trials, the lizard was held by the pelvic girdle while underwater but could easily swim free to the surface, if desired. The sensor recorded the pO_2 in hectopascals (hPa) once per second within the rebreathed bubbles for the length of the trial. Trials were concluded when lizards broke free of the hold and swam to the surface. Trials that had fewer than 10 pO_2 reads or with poor quality oxygen sensor reads ($R^2 < 0.75$ in a linear regression of pO_2 hPa vs. dive duration) were excluded from analyses, and lizards participated in multiple trials to better ensure at least one trial was usable. Two variables of interest were extracted from these trials for analysis: oxygen consumption rate (nmols/s) and final oxygen read immediately prior to surfacing ($pO_{2\ \mathrm{Final}}$; potentially indicative of an individual's lowest tolerance point for anoxia).

For all of these procedures, it was not possible to record data blind because our study involved focal animals in the field.

Statistical methods

We used the “lmer” function in the *lme4* package (Bates et al. 2015) in R to test how *A. aquaticus* body temperature differed before and after a dive in the field trials. We used a linear mixed model, in which log-transformed body temperature was the response variable, and period (before or after the dive), sex, body mass, and trial length were predictors. To account for the repeated measures of individual temperatures before and after a dive, lizard ID was included as a random effect. The *emmeans* package (Lenth 2022) was used to test posthoc pairwise comparisons in mixed models.

We modeled the relationship of temperature loss over time in *A. aquaticus* using the function *SSasymp* within the nonlinear regression function *nlm* (*nlme* package; Pinheiro et al. 2021). Prior to the nonlinear regression, we subtracted water temperature from internal lizard body temperature to standardize trials by correcting for local and/or daily variability in river water temperature.

We ran a linear mixed model to test the effects of *A. aquaticus* sex and body mass on dive duration. Lizard ID was included as a random effect, and trial location (field or lab) and year were additional factors. We then also used a Cox proportional hazards regression to analyze how sex and body mass influenced sex-specific patterns of time to emergence (i.e., the duration of a dive), using the *coxme* package (Therneau 2020), with lizard ID as a random effect and trial location and year as additional factors.

We tested what affected oxygen consumption rate and $pO_{2\ \mathrm{Final}}$ in three semi-aquatic species using two linear mixed effect models, with sex, body mass, species (*A. barkeri, A. maculigula,* and *A. oxylophus*), and trial length as predictors and lizard ID as the random effect. Oxygen consumption rate and $pO_{2\ \mathrm{Final}}$ were log-transformed prior to analysis. To test for any differences in the scaling relationship of oxygen consumption and body between the sexes, we used ordinary least squares (OLS) regression and the *smatr* package (Warton et al. 2012). We note that, due to sampling constraints, all *A. aquaticus* individuals used in the oxygen trace experiment were male, whereas both sexes were represented in *A. barkeri*, *A. maculigula*, and *A. oxylophus*; models run with and without *A. aquaticus* data produced qualitatively identical results.

Model assumptions were met and diagnostic plots of residuals verified model appropriateness (Zuur et al. 2010). Body mass was log-transformed in all analyses. P-values in all mixed models (fit by maximum likelihood, REML = false) were obtained by likelihood ratio tests of the full model against a null model without the variable of interest. All statistical tests maintained an alpha level at 0.05, were two-tailed, and were performed in R (version 3.6.3; R Core Team 2020).

Results

Diving reduced *A. aquaticus* body temperature ($\chi^2_1 = 12.708$, $P < 0.001$; Fig. 2). Body temperature was also affected by sex ($\chi^2_1 = 6.694$, $P = 0.010$), such that males overall maintained higher internal temperatures (Fig. 2). Body temperature was not influenced by body mass ($\chi^2_1 = 0.008$, $P = 0.928$) or dive duration ($\chi^2_1 = 1.973$, $P = 0.160$). Lizard body heat loss over time was successfully modeled using an exponential decay function ($R = 0.824$; rate constant = -3.94; $y_0 = 4.43$; $y_f = 0.16$) (Fig. 3).

Dive duration in *A. aquaticus* was affected by sex ($\chi^2_1 = 6.167$, $P = 0.013$), such that males had shorter dives (Fig. 4a). The Cox proportional hazards model likewise identified that males surfaced more quickly than females

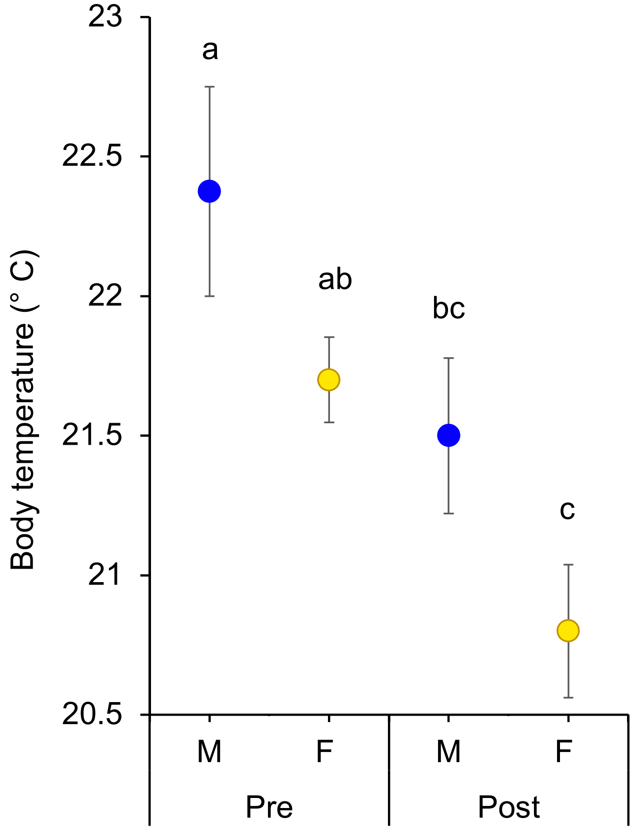

Fig. 2 Mean body temperatures (°C) of male (M) and female (F) *Anolis aquaticus* pre- and post-dive. Different letters indicate significant differences among groups. Errors bars represent ± 1 standard error

(hazard ratio = 0.708, $z = 2.02$, $P = 0.044$; Fig. 4b). Dive duration was not affected by body mass ($\chi^2_1 = 0.244$, $P = 0.622$), trial location ($\chi^2_1 = 0.558$, $P = 0.455$), or year ($\chi^2_2 = 3.345$, $P = 0.188$).

In the oxygen trace experiment, oxygen consumption rate did not differ by sex ($\chi^2_1 < 0.001$, $P = 0.993$) or species ($\chi^2_3 = 6.548$, $P = 0.088$), but it did increase with body mass ($\chi^2_1 = 28.827$, $P < 0.001$). The slope of the oxygen consumption-mass relationship (0.855; ln-ln scale) did not significantly differ ($r = 0.158$, df = 37, $P = 0.336$) from the basal metabolic and field metabolic scaling coefficient (0.75) observed by previous studies in other lizard species (Vleck and Bartholomew 1979; Savage et al. 2004; Nagy 2005); the slopes of male and female oxygen consumption-mass likewise did not independently differ from the expected scaling coefficient (male: $r = 0.021$, df = 24, $P = 0.920$; female: $r = 0.134$, df = 11, $P = 0.662$). Neither sex ($\chi^2_1 = 0.530$, $P = 0.4664$) nor body mass ($\chi^2_1 = 0.080$, $P = 0.777$) affected $pO_{2\ Final}$, although $pO_{2\ Final}$ varied among species ($\chi^2_3 = 20.953$, $P < 0.001$). Both oxygen consumption rate ($\chi^2_1 = 29.171$, $P < 0.001$) and $pO_{2\ Final}$ ($\chi^2_1 = 18.544$, $P < 0.001$) decreased with increasing trial length. The body mass variable was responsible for moderate multicollinearity in the two oxygen models, with variance inflation factors of 4.73 and 4.37 (for the oxygen consumption rate and $pO_{2\ Final}$ models, respectively; Zuur et al. 2010).

Discussion

Males and females are known to differ in how they prioritize the tradeoffs of antipredator behaviors, especially those antipredator behaviors that are significant risks to territory or mate defense. Land-to-water escape from threats is predicted to be an especially costly antipredator behavior for ectotherms, with expected sex-specific differences. Our results suggest that diving behavior in *A. aquaticus* was associated with costly body temperature reduction, conservatively a loss of up to 2 °C in our field trial arenas (Fig. 2) but up

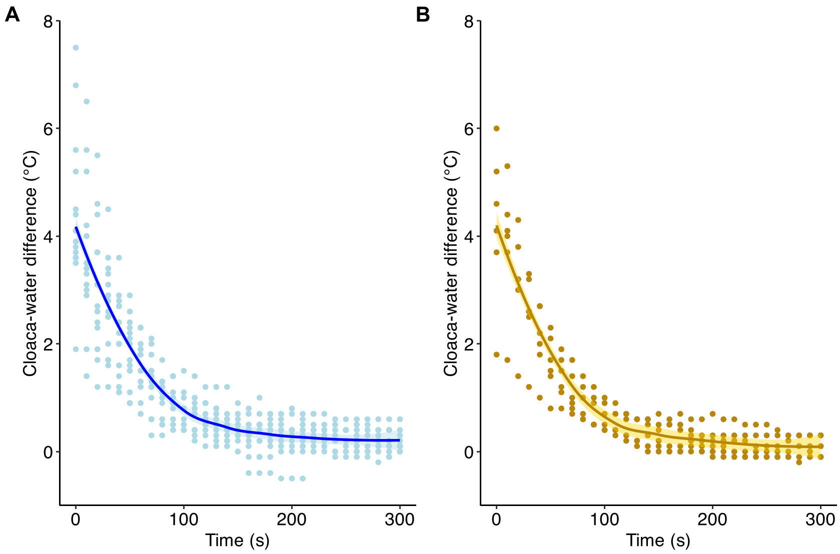

Fig. 3 Internal body temperature loss of **A**) male and **B**) female *Anolis aquaticus* over time. Body (cloacal) temperature is shown as degrees (°C) above water temperature to standardize measurement in variable water temperatures (between 20 and 22 °C). Temperature loss over time is represented using a loess smoothing function with shaded areas indicating ± 1 standard error

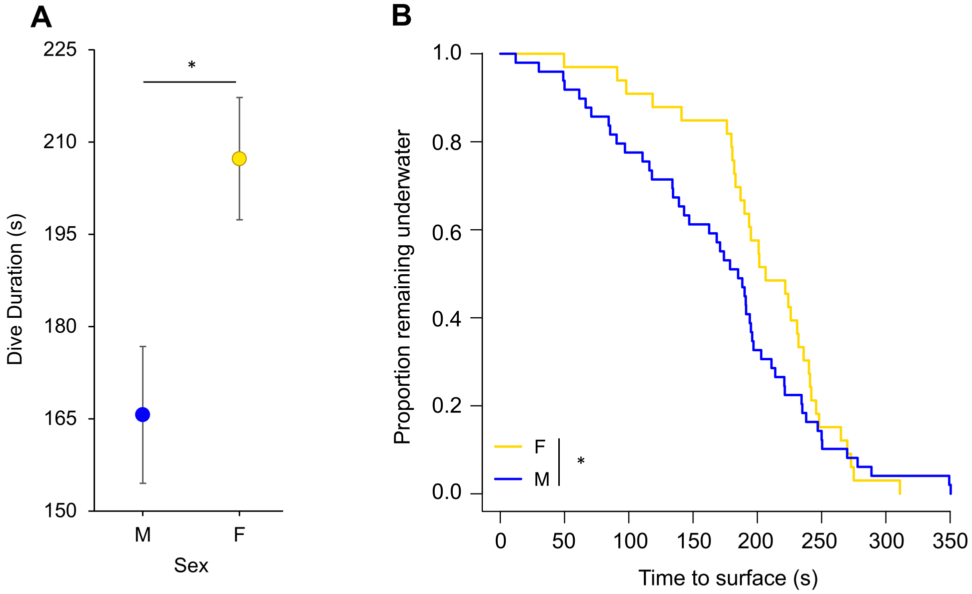

Fig. 4 **A** Mean dive durations (s) of male (M) and female (F) *Anolis aquaticus* during dive trials. Errors bars represent ± 1 standard error. **B** Survival curves depicting male (blue) and female (gold) *Anolis aquaticus* times-to-surface during dive trials; survival curves are based on a Cox proportional hazards regression. Asterisks indicate significant differences between groups

to 6 °C in the natural conditions of flowing stream water (Fig. 3). The sexes differed such that males maintained higher body temperatures and on average had shorter dives, although temperature was not linearly related to dive duration (discussed below).

The thermal costs of land-to-water refuge in small ectotherms may be more severe than the thermal costs of other types of refugia. Underwater refugia can reduce *A. aquaticus* body temperature by several degrees in less than 90 s (Fig. 3). Among reptiles, such reductions in temperature reduce sprint speed (van Berkum 1986), swimming speed (Weatherhead and Robertson 1992), jump performance (Lailvaux and Irschick 2007), bite force (Segall et al. 2013), and the ability to defend territory and mates (Lailvaux 2007), thus directly impacting fitness. Although the use of terrestrial refugia by terrestrial animals may also result in thermal costs (e.g., Daniels et al. 1987; Polo et al. 2005; Sannolo et al. 2019), costs accrue rapidly in terrestrial-to-water escape routes due to the high thermal conductivity of water (Grigg and Alchin 1976; Daniels et al. 1987; Weatherhead and Robertson 1992). Similar thermal observations have been documented with water snakes (*Nerodia sipedon*; Weatherhead and Robertson 1992), in which snakes fleeing in colder water experienced a decrease in swimming speed relative to those in warmer water. In contrast, *A. aquaticus*'s stationary underwater refuge seeking likely does not generate as much metabolic heat as the active fleeing behavior of water snakes (Weatherhead and Robertson 1992). In combination with the potentially long durations spent underwater during natural dives (> 15 min), *A. aquaticus*'s thermal tradeoff is a fascinating optimization question.

Our results suggest that males have shorter dive durations and maintain higher post-dive internal body temperatures than females. Current literature on the effects of sex on antipredator behavior in lizards is equivocal: some studies report differences between males and females (Lailvaux et al. 2003; Cooper and Wilson 2007; Lailvaux and Irschick 2007; Herczeg et al. 2008; Curlis et al. 2016), whereas others have found no differences (Cooper 2011; Sannolo et al. 2019). Of these, the more risk-averse sex differed depending on environmental constraints. *Zootoca vivipara* females thermoregulate closer to preferred temperatures than males due to their greater risk-taking behavior, which may be due to the greater energetic needs of viviparous females in their high-altitude habitats (Herczeg et al. 2008). By contrast, male *Lacerta monticola* emerge from refugia sooner than females (Martín et al. 2003), and male *Sceloporus virgatus* have lower flight initiation distances than females in the presence of a potential predator (Cooper and Wilson 2007). Our own findings suggest that the thermal costs accrued by male *A. aquaticus* may limit their reproductive potential more severely than that of females. Male *A. aquaticus* spend considerable effort defending territories and mates (Márquez et al. 2005; Márquez and Márquez 2009), and boldness is honestly advertised by male sexual signals (Putman et al. 2018). *A. aquaticus* are thermoconformers (Savage 2002) and do not bask, which may make it difficult for them to regain lost body heat (Herczeg et al. 2008). Among anoles, low body temperatures can result in decreased physiological capacity for important locomotor (van Berkum 1986; Weatherhead and Robertson 1992; Lailvaux and Irschick 2007) and defensive (Lailvaux 2007; Segall et al. 2013) behaviors. As such, male *A. aquaticus* thus may be less willing than females to risk heat loss in extended dives due to corresponding potential loss of reproductive opportunities. Additionally, there may be other, yet unquantified, differences in antipredator behaviors between the sexes, for example, if males rely on locomotion rather than crypsis to avoid predators immediately following dives.

Sex differences were apparent in *A. aquaticus* dive duration, but no effect of sex was detected when examining oxygen consumption rates in other similar species of semi-aquatic anole. This may suggest that, within size classes, the sexes may have the same physiological capacity for diving, and thermal or behavioral tradeoffs are responsible for the sex differences we observed in *A. aquaticus* dive durations. Unfortunately, we were unable to collect female *A. aquaticus* at the time that oxygen consumption measurements were taken (see Boccia et al. 2021), but male *A. aquaticus* have oxygen consumption patterns that correspond well to that of males in the other three semi-aquatic *Anolis* species we tested (Fig. 5). As a result, and with full disclosure that there may still be unexpected species differences, we conservatively propose that due to ecological and phylogenetic similarity *A. aquaticus* may also lack sex differences in oxygen consumption.

As an alternative explanation, we acknowledge that body mass demonstrated moderate multicollinearity with sex and species in the oxygen models; if sex differences in physiological diving capacities do exist, they may be masked as a result, as males are on average larger than females in semi-aquatic anoles. Although *Anolis* underwater oxygen consumption rates are related to body mass, neither sex nor body mass affected $pO_{2\ Final}$, suggesting that the sexes share a common lower limit to the pO_2 they can tolerate within a rebreathed bubble. An experimental approach to testing oxygen consumption and lower limits of tolerance could offer a complementary mechanistic explanation of observed sex differences in diving behaviors.

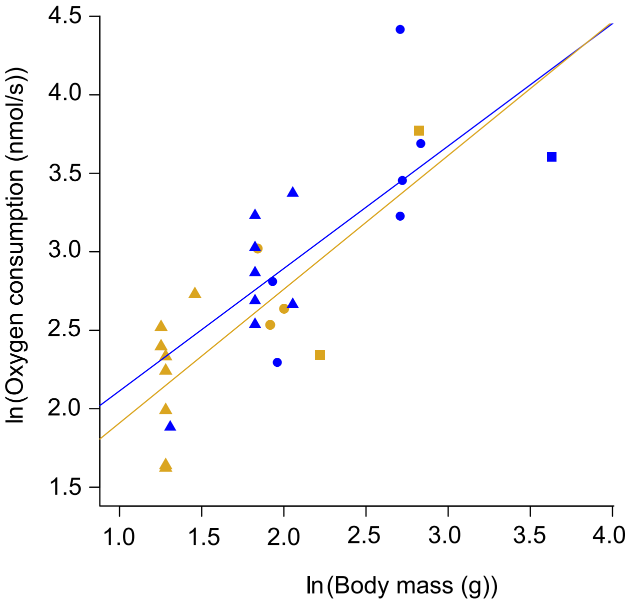

Fig. 5 Oxygen consumption rate (nmols/s) while diving in relation to body mass (g) of four rebreathing semiaquatic *Anolis* species (*A. aquaticus* = triangles, *A. barkeri* = circles, *A. maculigula* = squares, *A. oxylophus* = open diamonds). Males are represented in blue, and females are in gold

Interestingly, body temperature was not significantly affected by dive duration. We propose that the lack of significance between body temperature and dive duration may be due to a slowing of the rate of cooling as body temperature approached equilibrium with the temperature of the water (see Fig. 3). This may conceal the effect of disparate cooling rates between dives of different durations. Similarly, although smaller ectotherms tend to have faster cooling rates in general (Stevenson 1985; Carrascal et al. 1992; Polo et al. 2005), we detected no effect of mass on body temperature, perhaps again due to a reduced rate of cooling as body temperature approaches equilibrium with the water temperature. A similar pattern is observed in Iberian rock lizards (*Lacerta monticola*) during terrestrial refuge use (Polo et al. 2005).

It is likely that when lizards are in natural flowing streams (such as those where temperature loss data were collected; Fig. 3), they may choose to have even shorter dives than when measured in our dive duration trial tanks (Fig. 4). Although dive duration tanks were kept at the same temperature as average stream water, the rate of cooling should be quicker in flowing water due to the additional cooling effect of boundary layer disruption at a lizard's body surface. Identifying if stream flow affects dive duration is an avenue for future research. We highlight this conservative aspect of our methodology to illustrate why males may terminate dives more quickly than females in nature; by doing so, they may yet avoid equilibrating with stream water temperature (asymptote of curves in Fig. 3).

It is possible that the decrease in body temperature that results from diving, while resulting in a performance cost, may also provide some benefits. A decrease in body temperature may slow an ectotherm's metabolism and therefore O_2 consumption (Bennett 1988; Dorcas et al. 2004; McConnachie 2014; Hayward et al. 2016), which could allow it to remain underwater longer. This has been documented in freshwater turtles (e.g., *Trionyx spiniferus*, *Sternotherus odoratus*, *Chrymsemys picta bellii*), in which cold temperatures contribute to a hypometabolic state, thereby conserving energy (Ultsch 1989). Here, we document an exponential decay in the relationship of body temperature and dive duration when semi-aquatic anoles dive in the natural conditions of a flowing stream. Previously, we noted a similar pattern of exponential decay in bubble pO_2 with anole submersion duration in more than half of the trials conducted (Fig. 4a–e in Boccia et al. 2021). If there are no other inputs of O_2 into the rebreathed bubble, then the exponential decay in pO_2 could suggest that O_2 consumption drops as dive time increases. O_2 consumption and temperature would then appear to follow the same exponential decay pattern with dive duration, a correlation which warrants further investigation.

When submerged, a semi-aquatic anole forms a thin layer of air around its body, which facilitates rebreathing behavior. This "plastron" of air may also confer a thermal benefit by acting similarly to a dry suit worn by human divers (Giesbrecht et al. 2005; Power et al. 2015). By keeping the surrounding water from directly touching the lizard's skin, the plastron may help mitigate rapid loss of body heat, particularly around a lizard's head.

This study provides evidence that, in a semi-aquatic ectotherm, land-to-water escape behavior carries potential costs, and that males and females differ in their tolerance of the costs of this antipredator behavior, which may explain sex differences in dive durations. We highlight a need for further studies in this area to better identify how ectothermic species balance mitigating predator risk with potentially extreme physiological costs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-024-03448-5.

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Data availability All data are freely available from the Binghamton ORB repository, https://orb.binghamton.edu/bio_fac/27

Declarations

Ethical approval All applicable national and institutional guidelines for the use of animals were followed.

This study was approved by animal ethics committees at Binghamton University (IACUC protocols #817-19 and #874-22) and University of Toronto (LACC protocol #20011469). Research permits were obtained from the Ministry of the Environment and Energy, Costa Rica (SINAC-CUS-PI-R-049-2017, R-SINAC-PNI-ACLAP-022-2019, SINAC-ACC-PI-R-064-2019, R-SINAC-PNI-ACLAP-022-2021, and R-SINAC-PNI-ACLAP-003-2022) and Autorización de Recolección en Parques Nacionales Naturales, Colombia (permit #009 de 2017). Mexican fieldwork was conducted on private property with the permission of the Universidad Nacional Autónoma de México.

Conflict of interest The authors declare no competing interests.

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