



Benefits of paternal thermoregulation: male midwife toads select warmer temperature to shorten embryonic development

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Abstract

Parental thermoregulation can provide an efficient way to control embryonic temperature and optimize developmental durations and timing. To date, most studies on parental thermal effects have focused on maternal influences, because of the predominant role of females for parental care in most systems. Yet, paternal thermal effects are equally expected to occur in species that display paternal care to the progeny. We studied the midwife toad, a small amphibian with exclusive male parental care and terrestrial eggs transport. We examined the variations of body temperature of egg-carrying males and non-reproductive individuals in the field, and determined their thermal preferences in a laboratory thermal gradient. We also experimentally examined the influence of temperature on embryonic development durations across five treatments (16, 18, 20, 22, 24 °C). In support of our predictions, we detected a shift in thermal preferences with reproduction, with carrying males preferring higher temperatures than non-carrying ones. Field monitoring indicated that carrying males used diurnal shelters early in the season when thermal conditions were more constraining. Meanwhile, they minimized overheating risks by avoiding shelters with high substrate temperature. Finally, we detected a marked thermal dependence of embryonic development, suggesting potential phenological benefits of paternal thermoregulation in this species.

Significance statement

Paternal thermal care is well known among birds during incubation but male thermal care and thermal effects remain virtually unstudied among terrestrial ectotherms. In ectotherms, body temperature directly depends on the thermal environment and behavioral thermoregulation can influence developmental durations and offspring traits. We studied the midwife toad, a species that display exclusive male parental care with terrestrial egg transport. Our results demonstrate that carrying males selected preferred higher temperatures than non-reproductive individuals in a thermal gradient but also avoided thermal extreme in the field. The duration of development was considerably shortened at the temperature selected by reproductive male. Our study provides evidences of active paternal thermoregulation for an amphibian species with exclusive male parental care.

Keywords Thermal effect · Paternal care · Thermoregulation · Embryo · Development

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Introduction

Variations in thermal conditions can have major consequences on organismal physiology, performance, and ultimately fitness (Angilletta 2009). Early life stages are particularly sensitive to temperature (Farmer 2000; Gillooly et al. 2002; Angilletta et al. 2010) and thermal effects can have long lasting consequences on individual trajectories (Singh et al. 2020). Parental behavior has been highlighted as a key mechanism to buffer temperature effects on developing embryo (Shine 1995; Farmer 2000; Delia et al. 2013; Lorioux et al. 2013). In ectotherms, body temperature directly

depends on the thermal environment (Rubalcaba et al. 2019) and behavioral thermoregulation provides a robust way of reaching and maintaining optimal body temperatures (Blouin-Demers et al. 2003; Lorient et al. 2013). In such systems, parental thermoregulation can induce phenological effects by optimizing developmental durations and timing, but also phenotypical effects, such as modifications of offspring traits (Anduaga and Huerta 2001; Reed et al. 2009; Telemeco et al. 2010; Lorient et al. 2012, 2013).

The relationship between embryonic development and temperature has been a focus of research for decades (reviewed in Singh et al. 2020) and several competing hypotheses have emerged (Zamorano et al. 2017). For instance, the “warmer is better” hypothesis posits that higher temperatures are beneficial by enhancing physiological rates, and thus reducing development time (Treasure and Chown 2019). Alternatively, the “colder is better” hypothesis suggests that individuals developing at a lower temperature will reach larger body size (i.e., temperature size rule, see Zuo et al. 2012). Finally, the “optimal developmental temperature” hypothesis states that accessing an optimal, intermediate temperature during embryonic life is critical to optimize adult performance in diverse thermal environments (Huey et al. 1999). These contrasted hypotheses have received substantial support from empirical studies suggesting that various strategies of thermal parental care may improve offspring traits (Kingsolver and Huey 2008). As a consequence, the thermal sensitivity of embryonic development could be a driving force in the evolution of parental care and reproductive modes (Farmer 2000; Angilletta and Sears 2003).

To date, most studies on thermal effects have focused on maternal influences (Du et al. 2005; Dvořák and Gvoždík 2009; da Silva et al. 2012; Toufarova and Gvozdik 2016; Li et al. 2018; Foucart et al. 2018). It is partly due to the predominant role of females for parental care in most systems (Royle et al. 2012). In ectotherms, the maternal shift in thermoregulation during reproduction has sparked debates as to whether this corresponds to a maternal effort to optimize the fitness of her offspring (i.e., the maternal manipulation hypothesis), or her own (Gvozdik 2005; Kurdikova et al. 2011; DeNardo et al. 2012; Shine 2012; Schwarzkopf and Andrews 2012). However, male parental care (MPC) has been described in multiple taxa and males can regulate multiple facets of the abiotic environment. For instance, among nest-guarding fishes, males will attend the nest and actively ventilate the eggs to avoid hypoxia (Lissåker and Kvarnemo 2006). Pouch-breeding species can also manipulate salinity to optimize development for the embryos (Monteiro et al. 2005). Similarly, in many amphibians, MPC is involved in the regulation of the hydric environment to avoid egg desiccation (Townsend et al. 1984; Chen et al. 2007; Poo and Bickford 2013; Gururaja et al. 2014; Lehtinen et al. 2014; Vargas-Salinas

et al. 2014; Consolmagno et al. 2016; Chuang et al. 2017). Paternal thermal care to the eggs is well known among birds during incubation and this trait likely emerged through sexual selection (Hanley 2013). Yet, male thermal care and thermal effects remain virtually unstudied among terrestrial ectotherms (but see Cook et al. 2001).

In many amphibians, males are actively involved in the regulation of developmental conditions (Eens and Pinxten 2000; Royle et al. 2012). MPC is often exclusive, which permits to study paternal effect on development in a simplified context (low or absent prezygotic costs, see Hayward and Gillooly 2011). Thermal constraints on embryonic development are particularly relevant under thermally constraining environments, such as temperate climates (Le Henanff et al. 2013). In this context, the genus *Alytes* (midwife toads) is composed of six species living in the Western Palearctic region and characterized by terrestrial incubation and exclusive MPC. In these species, males carry the clutch attached to their hind legs during the whole embryonic development, and recent evidences suggest that males carrying eggs select their microhabitat to buffer thermal conditions and that such paternal care can be costly (Lange et al. 2020, 2021). The hatching date can induce drastic changes in ontogenetic trajectories and early hatching has been shown to positively influence the fitness (Rodríguez-Díaz et al. 2010; Le Henanff et al. 2013; Lorient et al. 2013). Conversely, late hatching can induce overwintering in a larval stage in these species and thus significantly lengthens larval development duration (Wells et al. 2015). Due to the high levels of predation risks and competition in breeding ponds (Pearman 1995), this is likely to influence larval survival.

Herein, we studied the common midwife toad, *Alytes obstetricans*, which is the northernmost species of the genus and which has an extended reproductive period lasting from early spring to late summer (Márquez 1992). Under constraining (cold) ambient temperatures in spring, male midwife toads are expected to optimize thermoregulation in order to hasten embryonic development and generate early hatching as demonstrated in other ectotherms (Wapstra et al. 2010; Le Henanff et al. 2013). In this context, we tested the following predictions:

1. In order to enhance embryonic developmental rate, egg-carrying males should have higher preferred temperature (T_{pref}) than non-carrying ones
2. Because midwife toads thermoregulate under diurnal shelter (Lange et al. 2020), field body temperature (T_b) should be influenced by reproductive status and by shelter temperature over the reproductive season
3. The duration of terrestrial embryonic development should be dependent of temperature and shorter at preferred body temperature of carrying males

Material and methods

Study species

The common midwife toad is a small (4–5 cm) species ranging across Western Europe (Bosch and Márquez 1996). These toads often shelter under refuges such as rocks and slabs and they can frequently be found in anthropic habitats (Pellet and Schmidt 2005; Lange et al. 2020). Because sexual dimorphism is poorly marked in this species (Bosch and Márquez 1996), we relied on an indirect method for sexing adult individuals. If toads were carrying eggs on their back, they were confidently sexed as males, because during mating, males collect the fertilized eggs and attach the string of eggs around their hind legs. Otherwise, individuals were sexed as non-determined until further recaptures. Eggs are carried for up to 32 days in the wild (Wells et al. 2015), and once embryos have completed their development, the male reaches small water bodies (ponds) to deposit the hatching tadpoles (Márquez 1992).

Study site and captures

The study site is situated in Western France (Chizé forest, 46.1459 N, 0.3949 W) in a sawmill where a large population is established and currently monitored. To detect toads, we used different artificial refuges: rubber covers, tarpaulin covers, and wood covers. All artificial refuges had a sawdust substrate underneath since it increases detection (Lange et al. 2020). We placed eight line transects of the three types of artificial refuges, for a total of 24 artificial refuges. Each transect was visited during the reproductive season (between April and July). Artificial refuges were then gently lifted to capture individuals. All toads captured and brought to the lab were weighted (mg) and measured for Snout-Vent Length (SVL, mm). They were individually marked with a RFID nanotag (Trovan), placed in the dorsal region, just before the release.

A total of 92 adult toads were collected in spring 2018 to assess temperature preference (see below). Among these individuals, 62 were identified as males because they were carrying a clutch of eggs at the time of capture or later during the year. Seventeen males were captured twice with different statuses: eight were firstly captured as non-carrying and then as carrying eggs and nine were initially captured as carrying and then as non-carrying eggs. It was therefore possible to test for changes in preferred temperature within the same individuals. For 23 individuals, it was not possible to assign a sex because they were not captured or recaptured with eggs. Finally, seven reproductive females were identified with growing ova visible

through the belly. We also captured 45 males carrying eggs (16 males in 2018 and 29 in 2019) to examine the thermal dependence of development. We selected males carrying recently laid clutches (e.g., < 3 days, eggs characterized by a bright yellow colour). Toads were individually housed in plastic boxes (32 × 18.5 cm and 10.5 cm high) with 2 L of moistened vermiculite (4 cm thick) and maintained in a temperature-controlled room (20 °C). Two shelters (halved PVC tubes) and a small water bowl (7 cm diameter and 4 cm high) were placed inside each box.

Variables measured

Preferred body temperature in laboratory conditions

We used a thermal gradient ranging from 15 to 45 °C to assess individual preferred body temperature (T_{pref}). The experimental unit consisted of a PVC box (102 cm [L] × 72 cm [W] × 25.5 cm [H]). The warm side was heated by a heating cord set on 45 °C, the middle by a heating cord set on 30 °C and the other side was cooled by copper pipes, traversed by a stream of water and antifreeze solution, cooled by a cold unit, set on 5 °C. Six compartments (68 cm l × 16 cm L) in 2-mm-thick aluminum (chosen for its thermic conductance) were placed above the temperature gradient. Each compartment was filled with a mixture of 100 g of vermiculite and 250 g of water and provided with a long shelter (half PVC tubes of 66 cm L × 11 cm w × 4.5 cm h) that covered the entire length of the compartment. The gradient was placed in a temperature-controlled room (mean air temperature 15 °C) to maintain thermal gradient stability over time. The room was under an LD 12:12 h photoperiod.

Individuals were acclimated to lab conditions for 24 h after captures before testing. They were placed in the thermal gradient in the evening of the day preceding measures. In small ectotherms, skin surface temperature is highly correlated to the internal body temperature (T_b). Skin surface temperature was taken every hour from 9:00 to 18:00 h using an infrared thermometer (Fluke 572 Infrared Thermometer, resolution 0.1 °C, Accuracy ± 0.75 °C). The skin surface temperature was collected in the dorsal area without handling to minimize perturbations. Eight carrying males, for which clutch desertion occurred after capture, were discarded from the analysis. The seven reproductive females were also excluded. We analyzed variations in T_{pref} comparing the three statuses (carrying males, non-carrying males and unsexed adults). We also analyzed T_{pref} on the subset of 17 males sequentially found either carrying or non-carrying eggs. A minimum period of two weeks was applied between recapture and repetition of the experiment.

Temperature measurements in the field

Skin surface temperatures (°C) were collected through 42 field sessions carried in 2018 and 2019. For each toad, the dorsal temperature was measured before any handling using a Fluke 572 Infrared Thermometer. A total of 586 temperature measurements were obtained, with 342 on carrying males, 21 on non-carrying males, 123 on undetermined (unsexed) adults. Substrate temperature under each shelter was measured on three different points with the IR thermometer and the average value was used for analyses. For each field session, we also collected air temperature (1.5 m high in the shade) at the beginning and the end of the session, and we used the average value to estimate air temperature. We also calculated daily temperature average (24 h) from hourly air temperatures (www.meteociel.fr) recorded in Niort, the closest station from our study site (~ 19 km straight line).

Thermal dependence of embryonic development

The 45 egg-carrying males were distributed in five thermal treatments (16, 18, 20, 22, and 24 °C) using climatic chamber (Vötsch Industrietechnik, VP 600, Balingen, Germany). They were individually housed in plastic boxes (32 × 18.5 cm and 10.5 cm high) with 2 L of moistened vermiculite (4 cm thick). Two shelters (halved PVC tubes) and a small water bowl (7 cm diameter and 4 cm high) were placed inside each box. Air humidity in individual boxes was close to saturation (> 95%) to avoid evaporative cooling and egg desiccation. Three thermal treatments were used per year, 18, 20, and 22 °C in 2018 and 16, 20, and 24 °C in 2019. Each year, males were randomly distributed in the three thermal treatments to avoid differences in capture date between groups. Over the 2 years, development durations were obtained for 45 clutches allocated in five temperatures as follow 16 °C (10), 18 °C (6), 20 °C (13), 22 °C (6), and 24 °C (10). Date of birth (hatching) was recorded, and the development time (days) was calculated as date of birth minus date of capture. We estimated clutch size using top-side photographs to count the number of eggs of each carrying male with the multi-point tool of ImageJ (Rasband, 1997–2018).

Statistics

All statistical analyses were carried on R (version 4.0.2) and R studio (version 1.3.959) using packages “nlme,” “AICcmodavg,” “emmeans,” “lsmeans,” and segmented packages. We used the anova() function set to a “marginal” type to obtain an equivalence of type III sums of square. The relation between daily temperature average and the number carrying males and adult observations was tested using

a generalized linear model (GLM) model with a Poisson distribution.

We analyzed T_{pref} in the gradient using a linear mixed model (LMM) to test the influence of the reproductive status, the SVL, and the time of day. Individual identity was set as a random factor. We first conducted this analysis on the entire data set and then to the subset of 17 males that were measured sequentially under the two statuses.

Variations in substrate temperature were analyzed in a LMM to evaluate the effects of shelter type, air temperature, and the interaction term, with shelter identity as a random factor. We investigated the effects of substrate temperature and midwife toad occurrence in a generalized linear mixed-effects model (GLMM) with a binomial distribution and treating shelter identity as a random factor. Field T_b were first analyzed in a LMM to test the effects of individual status (egg carrying or not), mean substrate air, and the interaction term. Shelter identity was set as a random factor. We tested three functions (i.e., linear, logarithmic and quadratic) and used Akaike’s information criterion (AIC) to select the best model. When two models differed by less than two AIC, they were considered equivalent. Then, we applied separately for each status a segmented regression model (broken-stick model) from the R package “segmented.” The significance of this model was tested using a Davies test on the linear relation between males T_b and substrate temperature.

Development durations were log transformed to meet normality assumption and were first analyzed in a linear model (LM) with Gaussian distribution with thermal treatment as a fixed factor and addressing the additive effects of estimated clutch size and male size. In a second analysis, treatment temperatures were treated as a continuous variable and we tested three functions (i.e., linear, logarithmic and quadratic). It was not possible to record data blind because our study involved focal animals. Tukey tests were then used as post-hoc analysis. Values presented in the results are means ± standard error (SE).

Results

Phenology of carrying male observations

The total number of carrying males found per session was higher early in the season (Fig. 1). It was negatively correlated to the mean daily air temperature ($F_{(1,40)} = 57.71$, $p < 0.001$, $\beta \pm SE = -0.39 \pm 0.05$). The total number of adults (irrespective their sex or reproductive status) was also negatively correlated to the mean daily air temperature although the relation was weaker ($F_{(1,40)} = 24.30$, $p < 0.001$, $\beta \pm SE = -0.14 \pm 0.03$).

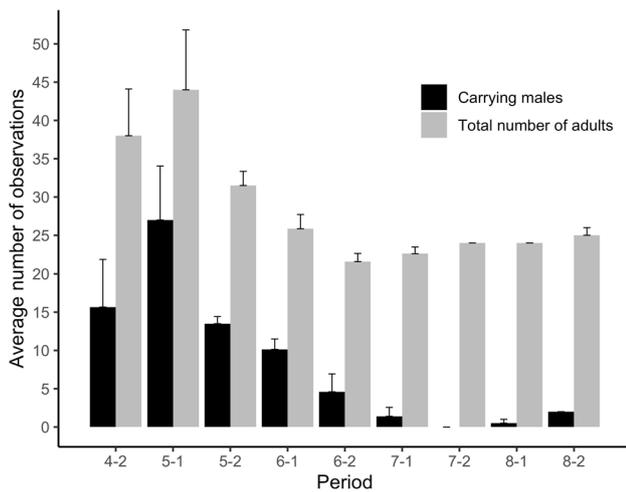


Fig. 1 Variations in the mean number of observations of carrying males and total number of adults over the field season. Number of contacts per visit were averaged over successive fortnight periods (1 or 2) from April to August (4 to 8). The whiskers represent the standard errors

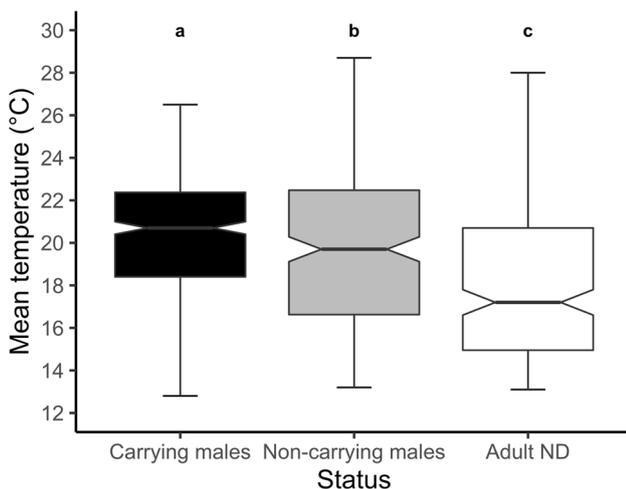


Fig. 2 Preferred body temperatures (T_{pref} , °C) measured in carrying males, non-carrying males and unsexed adults. The boxes represent the lower and upper quartiles, and the whiskers represent the range. The middle horizontal line in the box plot represents the median. Different letters mean a significant difference between groups with a p value < 0.05

Thermal preference of carrying males and non-carrying individuals

When considering the complete data set (76 individuals), we found that T_{pref} was influenced by status ($F_{(2,74)} = 10.96$, $p = 0.006$, Fig. 2). The mean T_{pref} of carrying males (20.8 ± 0.31 °C) was higher when compared to non-carrying ones (19.4 ± 0.5 °C, Tukey post hoc test $p = 0.001$) and unsexed adults (17.6 ± 0.7 °C; Tukey

post-hoc test, $p < 0.001$). The time of day had no influence on T_{pref} ($F_{(9,843)} = 0.77$, $p = 0.64$) nor the SVL ($F_{(1,74)} = 0.06$, $p = 0.94$). Similar results were found when restricting the analysis to the subset of 17 males measured two times, carrying eggs or not. We found a significant effect of status ($F_{(1,15)} = 7.18$, $p = 0.007$) with males having higher T_{pref} when they were carrying eggs (20.0 ± 0.3 °C) than when they were not (19.3 ± 0.3 °C). Male size did not influence T_{pref} ($F_{(1,15)} = 0.40$, $p = 0.53$), nor the time of day ($F_{(9,313)} = 0.81$, $p = 0.6$).

Determinant of field body temperatures

Variations in substrate temperature were explained by air temperature ($F_{(1,1287)} = 1039.43$, $p < 0.001$), shelter type ($F_{(2,21)} = 5.88$, $p = 0.009$), and the interaction between the two ($F_{(2,1287)} = 22.71$, $p < 0.001$). Substrate temperature was different between all shelter types (Tukey post-hoc test, $p < 0.001$) and was the highest under rubber cover and the lowest under wood cover. Midwife toad occurrence was negatively related to substrate temperature ($\chi^2 = 14.84$, $df = 1$, $p < 0.001$, $\beta \pm SE = -0.58 \pm 0.15$) with no effect of shelter type ($\chi^2 = 1.44$, $df = 2$, $p = 0.48$) and no interaction ($\chi^2 = 4.3$, $df = 1$, $p = 0.11$). Substrate temperature was lower under refuges where midwife toad occurred (24.4 ± 0.5 °C) than where they were not contacted (26.8 ± 0.5 °C, Tukey post-hoc test, $p < 0.001$).

Field T_b ranged from 12.0 to 33.6 °C and were highly related to substrate temperature (Fig. 3A, B). Variations were best explained by the influence of log transformed substrate temperature, status, and interaction between substrate temp and status (Table 1). Carrying males maintained lower temperature T_b than undetermined adult and the difference was more pronounced as substrate temperature increased. The broken stick model identified a threshold temperature of 21.16 ± 2.1 °C for carrying males. Below this threshold, T_b were positively correlated with substrate temperature with a slope of 0.78 ± 0.05 (Fig. 3A; $F_{(1,191)} = 83.16$, $p < 0.001$). Above this threshold, T_b were more weakly correlated to substrate temperature, with a slope of 0.17 ± 0.02 (Fig. 3A; $F_{(1,176)} = 244.85$, $p < 0.001$). The same pattern was found for non-carrying individuals but with a higher threshold temperature (25.8 ± 2.8 °C). Below this threshold, T_b were positively correlated with substrate temperature with a slope of 0.69 ± 0.10 (Fig. 3B; $F_{(1,65)} = 69.08$, $p < 0.001$). Above this threshold, T_b were marginally correlated to substrate temperature, with a slope of 0.18 ± 0.05 (Fig. 3B; $F_{(1,43)} = 3.42$, $p = 0.07$).

Influence of temperature on development duration

The duration of the embryonic development was significantly different between thermal treatments (Fig. 4; $F_{(4,40)} = 43.02$,

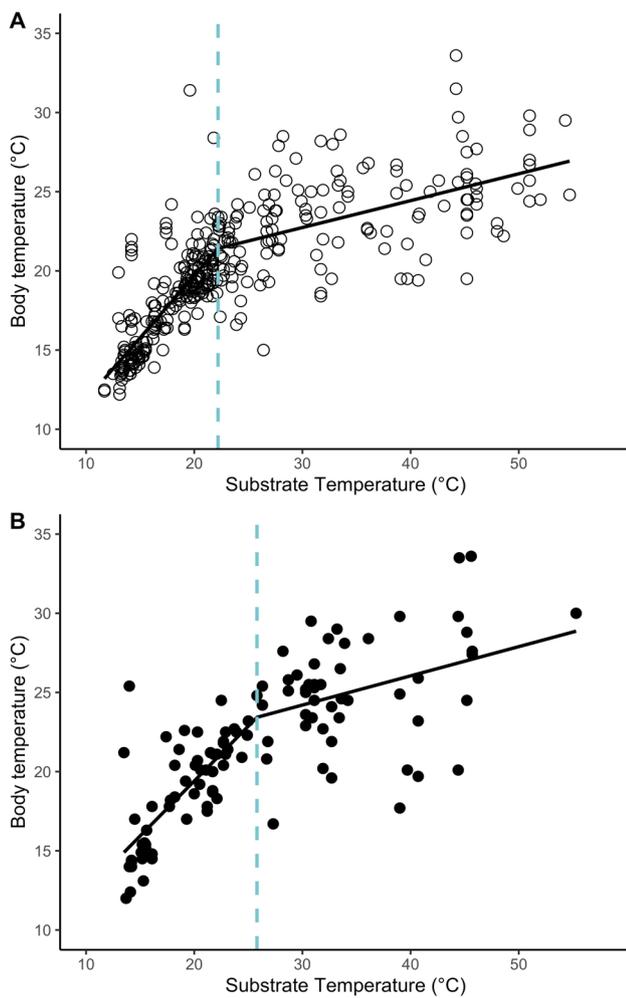


Fig. 3 Relation between body temperatures T_b (°C) and substrate temperatures T_{sub} in the field in **A** carrying males and **B** unsexed individuals. The black lines represent the regressions of the segmented relationship. The vertical dashed line (blue) represents the estimated breakpoint of the segmented relationship

$p < 0.001$) with no influence of clutch size ($F_{(1,38)} = 0.39$, $p = 0.53$) or male size ($F_{(1,39)} = 1.13$, $p = 0.29$). The average duration ranged from 40.7 ± 1.9 days (16 °C) to 18.3 ± 1.0 days (22 °C). All groups differed significantly (Tukey tests, $p < 0.04$) except duration at the 24 °C treatment that were similar to the one at 22 °C ($p = 0.30$) and marginally different from the one at 20 °C ($p = 0.058$). The relation between chamber temperature and development duration (untransformed) was best explained by a quadratic function when compared to logarithmic or linear link (respective AICc: 264.1, 267.1, and 282.64).

Table 1 Determinants of field body temperature (T_b) in the midwife toad. We tested the effects of “status” (i.e., egg-carrying males or unsexed individuals), substrate temperature (T_{sub}) and the interaction term between substrate temperature and status. We tested three functions (i.e., linear, logarithmic and quadratic) for substrate temperature. Shelter identity was treated as a random factor to account for repeated measures

Model	k	AICc	Δ AICc	wi	LogLike
Statut * $\log(T_{sub})$	6	2267.14	0	0.73	-1127.48
Statut + $\log(T_{sub})$	5	2269.53	2.38	0.22	-1129.7
$\log(T_{sub})$	4	2272.83	5.69	0.04	-1132.37
Statut * T_{sub}	6	2358.22	91.08	0	-1173.02
Statut + T_{sub}	5	2361.3	94.15	0	-1175.59
T_{sub}	4	2366.84	99.7	0	-1179.38
Statut * $(T_{sub})^2$	6	2453.15	186	0	-1220.49
Statut + $(T_{sub})^2$	5	2456.61	189.47	0	-1223.24
T_{sub}	4	2464.82	197.68	0	-1228.37
Statut	4	2770.44	503.29	0	-1381.18
Null	3	2779.91	512.77	0	-1386.93

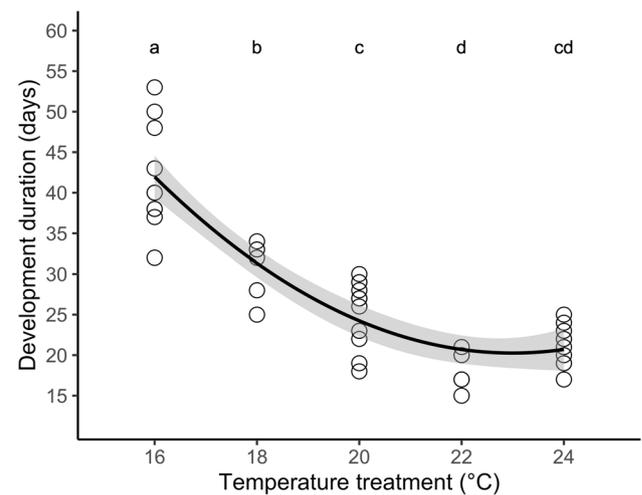


Fig. 4 Embryonic development durations (days) in relation to thermal treatment. The black line represents the fit of a quadratic polynomial regression, and the gray surface the standard error. Different letters indicate a significant difference between groups with a p value < 0.05

Discussion

Paternal thermal care has received limited scientific attention notably in species with exclusive male parental care (MPC; Cook et al. 2001). We hypothesized that by adjusting thermoregulatory behavior, midwife toads accelerate embryonic development. Using a thermal gradient, we found that males carrying eggs displayed higher T_{pref} , and this result was supported either considering all individuals or restricting the analysis to the 17 males with sequential

measures with the two statuses. Shifts in thermal preferences during reproduction are widespread in oviparous or viviparous ectotherms (Dvořák and Gvoždík 2009; DeNardo et al. 2012; Toufarova and Gvozdik 2016). Parental thermal influences on development combine both phenological and phenotypical effects (Lorioux et al. 2013). Under temperate climate, birth phenology is a key driver of reproductive success (Dickerson et al. 2005) and early hatching is often associated with a better fitness for the juveniles (Warner and Shine 2007; Le Henanff et al. 2013). Parental manipulation of embryonic temperature is likely adaptive (Abbey-Lee and Dingemans 2019) in shortening development time and possibly provides significant advantage at birth (Anduaga and Huerta 2001; Reed et al. 2009; Telemeco et al. 2010; Lorioux et al. 2012, 2013). Developmental temperature can also influence important larval traits and body size at metamorphosis (Álvarez and Nicieza 2002; Ruthsatz et al. 2018). Further studies are required to address long term consequences on fitness-related traits.

The maximum number of carrying males was found under the diurnal refuges in early spring, when air temperatures were lower. Midwife toads select their diurnal refuges because of thermal and hydric properties (Lange et al. 2020). These refuges provide significant thermal benefits when thermal conditions are constraining. Midwife toads avoided shelters with high substrate temperature, thereby limiting risks of heat exposure. While we did not control for body size in field records, artificial refuges are mainly used by adults with limited variation in size (Lange et al. 2020). We showed that T_b was closely dependent of substrate temperature and that carrying males had lower T_b than non-carrying individuals notably when substrate temperature increased. Under a temperature threshold (~ 21 °C), carrying males appear to thermoconform, while above this threshold, active avoidance of high temperatures occurred. A similar pattern was observed for non-carrying individuals, but the temperature threshold was higher (~ 26 °C). These findings suggest that egg-carrying males may behaviorally avoid temperature increase when their T_b exceed 22 °C. Overall, our results dovetail well with our field observations of egg-carrying males shifting their position and exploiting the vertical dimension of the substrate to thermoregulate, as demonstrated in invertebrates (Penick and Tschinkel 2008). The reproductive phenology of the midwife toad is variable in space and time (Márquez 1992) and often spans across spring and summer. Our results indicate that egg-carrying males likely select warmer conditions to optimize embryonic development under cold conditions but, as temperature increase, they minimize heat stress and potentially lethal temperatures for the embryos. These requirements likely explain the desertion of diurnal refuges during summer as this helps avoid overheating and dehydration for both

carrying males and their embryos. It is worth noting that using substrate temperature to infer behavioral thermoregulation can be limited (Heath 1964) and our findings call for more detailed study of body temperatures in relation to available operative temperatures.

Our experimental incubation underlined a quadratic relation between temperature and embryonic development duration that provides support to the optimal developmental temperature hypothesis (Huey et al. 1999). At low temperatures, a small increment in temperature had a strong influence on the duration of embryonic development while these effects were reduced or canceled at higher temperatures (24 °C, see Fig. 4). These findings are consistent with thermal preferences of carrying males obtained in the gradient test, as well as body temperature recorded in the field. Previous studies in ectotherms have demonstrated that small changes in parental temperature considerably shorten development and influence hatching phenology (Smith et al. 2015; Foucart et al. 2018). Exposure to a temperature above 22 °C does not seem beneficial in shortening development. High developmental temperatures may induce significant risks for the embryos notably because larval stages of temperate amphibians have low upper critical thermal limits (Duarte et al. 2012). Field records suggest that carrying males can reach high body temperatures (> 25 °C). However, short-term exposure to high temperatures may not be problematic since males can face important diurnal variations while the thermal treatments applied were constant. Parental thermal manipulations have already been documented in amphibians, with thermal selection of oviposition sites in newts (Dvořák and Gvoždík 2009) or breeding sites in frogs (Cook et al. 2001). However, to our knowledge, our study is the first to report adjustments in male's thermoregulatory behavior associated with reproductive activity in an anuran amphibian species with terrestrial egg-transport. The emergence of MPC often leads to a trade-off between parental and offspring requirements (Stiver and Alonzo 2009; Kurdíková et al. 2011). Future studies are needed to assess the fitness benefits of parental thermoregulation during terrestrial development and interactions between thermal and water demand from the embryos.

In the midwife toad, early hatching may provide phenological benefits to offspring by reducing competition and predation risk during their aquatic larval period (Pearman 1995; Abbey-Lee and Dingemans 2019). Egg carrying is only displayed by males and thermal effects may benefit males through sexual selection (Royle et al. 2012). A shorter developmental duration may increase the probability of obtaining additional mating opportunities, since male midwife toads can sequentially carry multiple clutches throughout the breeding season. Parental care is associated with significant costs (Alonso-Alvarez and Velando 2012). Because energy demands increase with temperature, parental thermal care in the midwife toad can cause a temperature-induced increment

in metabolic rates and maintenance costs. In addition, egg desiccation in dry terrestrial environments is an important risk that can compromise reproduction (Taigen et al. 1984). The concept thermo-hydreregulation have recently been described for ectotherms (Rozen-Rechels et al. 2019) and the water-based trade-offs likely apply to amphibians species with terrestrial egg transport. Accessing warm temperatures, in a wet environment, is likely essential to promote embryonic development while avoiding dehydration risks.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00265-022-03155-z>.

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Author contribution LL carried out the field work and lab experiments, participated in the statistical analyses, and drafted the manuscript; OL and FB conceived and coordinated the study, participated in data analysis, and critically revised the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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Data availability The datasets generated during and/or analyzed during the current study are available as supplementary material.

Code availability Not applicable.

Declarations

Ethics approval All applicable national, and/or institutional guidelines for the use of animals were followed. This study was performed in accordance with French laws relative to capture, transport, and experiments on *Alytes obstetricans* (DREAL permit #04022016) and all procedures were approved by an independent ethical committee (Apafis #14193–2018032114365130 v3).

Consent for publication All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests The authors declare no competing interests.

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