

The costs of being a good dad: egg-carrying and clutch size impair locomotor performance in male midwife toads (*Alytes obstetricans*)

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Parental care is widespread across the animal kingdom. Parental behaviours are beneficial by increasing offspring survival but induce significant costs to the parents. Because parental care is far more common in females, the associated reproductive costs have been largely studied in this sex. Although male parental care is likely to involve significant costs, it has been markedly less well investigated. We studied the costs of egg-carrying on locomotor performance in an amphibian species (*Alytes obstetricans*) with male parental care. We examined complementary parameters including hopping performance, righting response, hindleg muscle response to egg burden, and homing time in males carrying or not carrying eggs. We found that carrying males showed altered locomotor performance for most traits. In addition, alteration of performance was closely related to relative clutch size. Clutch desertion occurred in smaller individuals carrying larger relative clutch mass, and performance after desertion was similar to that of non-reproductive individuals. Overall, our study demonstrates that carrying eggs significantly alters male mobility and that performance–clutch size trade-offs are relevant in understanding the evolution of paternal care.

ADDITIONAL KEYWORDS: egg transport – fecundity trade-offs – male parental care – sex role reversal.

INTRODUCTION

Parental care (PC) refers to all post-zygotic investments, and understanding the diversity of PC is a central focus in evolutionary biology (Clutton-Brock, 1991; Royle *et al.*, 2012). PC is predominantly delivered by females, sometimes by both parents and less frequently by males only (Clutton-Brock, 1991; Dulac *et al.*, 2014; Gilbert & Manica, 2015; Furness & Capellini, 2019). This asymmetry reflects sex-differences in allocations (anisogamy) and sex-specific fitness benefits from PC (Trivers, 1972; Westneat & Fox, 2010; Hayward & Gillooly, 2011). Notably, female reproductive success is maximized by increasing allocation of energy and resources to progeny, while males benefit from increasing mating opportunities (Trivers, 1972). Beside anisogamy, a diversity of traits can influence the sex of the parent involved in care

(Kokko & Jennions, 2008; Royle *et al.*, 2012). For example, mode of fertilization can influence which parent provides PC, male parental care (MPC) being more common in species with external fertilization (Dawkins & Carlisle, 1976; Gross & Shine, 1981; Beck, 1998; Vági *et al.*, 2019) or when mechanisms securing paternity exist (Kvarnemo, 2006; Kahn *et al.*, 2013). Overall, PC requires substantial time and energy investments which can compromise parental energy balance, increase exposure risk or induce parental–offspring conflict (Crespi & Semeniuk, 2004; Royle *et al.*, 2016). The magnitude of these costs is often related to the number of offspring but may vary among males and females, influencing the level of parental commitment (Alissa *et al.*, 2017).

Although exclusive MPC is relatively rare, it has been documented in both invertebrates and vertebrates (Ridley, 1978; Kahn *et al.*, 2013; Dulac *et al.*, 2014; Bleu *et al.*, 2016; Bukhari *et al.*, 2019; Quesada-Hidalgo *et al.*, 2019). MPC can include internal or external

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brooding, but also feeding, guarding and carrying the progeny (Ridley, 1978). Current concepts posit that exclusive MPC has emerged from a situation of no care (ancestral state, Gilbert & Manica, 2015) either by natural selection (e.g. ‘territoriality hypothesis’) or by female choice (e.g. ‘sexual selection hypothesis’; Nazareth & Machado, 2010). Multiple benefits of MPC have been highlighted for either the male, the female or both and include: (1) enhanced parental fitness, (2) increased male attractiveness, (3) secured paternity and (4) reduced physiological costs for the mother (Kvarnemo, 2006). Despite these benefits, it is essential to clarify the costs of MPC to better understand parental strategies (Alonso-Alvarez & Velando, 2012). Yet only a few studies have explicitly estimated these costs (but see Townsend, 1986; Svensson, 1988; Ahnesjö, 1995; Reguera & Gomendio, 1999). Important flexibility in the expression of MPC has been described in several taxa, and clutch abandonment can be observed in response to environmental constraints (Kight *et al.*, 2006). Examining species with exclusive MPC represents a powerful opportunity to assess the post-zygotic costs of reproduction and to address the evolutionary context leading to MPC.

Anuran amphibians display the largest diversity of reproductive and parental strategies among vertebrates (Duellman & Trueb, 1994; Crump, 2015). Multiple forms of PC exist in this group: egg attendance, egg transport, tadpole attendance, tadpole transport, tadpole feeding, and internal gestation whether in the oviduct, the stomach or a dorsal pouch (reviewed by Crump, 1996; Furness & Capellini, 2019). PC is found in 17 of the 27 extant anuran families. Among them, 47% of species with uniparental care demonstrate MPC (Crump, 1996; Dulac *et al.*, 2014). Male attendance has been widely described in different species, especially in the tropics (Townsend *et al.*, 1984; Downie *et al.*, 2005; Royan *et al.*, 2010; Delia *et al.*, 2013; Chuang *et al.*, 2017). Similarly to female PC, the benefits of MPC can involve the regulation of developmental conditions (protection against desiccation) or reduced predation risks for the offspring. However, progeny attendance or transport can influence predation risks for the parent (Dugas *et al.*, 2016; Valencia-Aguilar *et al.*, 2020). Interestingly, clutch desertion in species with uniparental care has been documented in different contexts, suggesting high levels of flexibility in parental commitment (Ringler *et al.*, 2015). Clutch desertion can be related to increased costs (predation risks, mating opportunities) or reduced benefits of care (when the number of offspring is low; Delia *et al.*, 2013; Chuang *et al.*, 2017).

The evolution of reproductive modes in amphibians is closely linked to climatic conditions and PC is particularly diverse under warm climates (da Silva *et al.*, 2012; Crump, 2015; Lion *et al.*, 2019). Species

showing PC and MPC can also be found under more constraining climates and deserve specific attention notably to study terrestrial reproduction (Crump, 1996; Úbeda & Nuñez, 2006; Vági *et al.*, 2019). In the Western Palearctic region, the genus *Alytes* comprises five species that all display terrestrial incubation and exclusive MPC (Marquez & Verrell, 1991; Verrell & Brown, 1993; Márquez *et al.*, 2011; Pinya & Pérez-Mellado, 2014; Salvador, 2015). Males carry the eggs during the whole period of embryonic development which lasts 3–6 weeks (Wells *et al.*, 2015). This genus provides an opportunity to explore the possible costs of paternal care. We investigated the influence of egg-carrying on locomotor performance and the possible interactions with clutch size in the common midwife toad (*Alytes obstetricans*). Our main hypothesis is that paternal care decreases male mobility and that locomotor constraints depend on reproductive effort. We considered locomotor traits that are ecologically relevant for a male midwife toad while carrying eggs. These traits included hopping performance, righting response, hindleg muscle response to egg burden, and homing time by comparing carrying and non-carrying males.

Specifically, we tested the following predictions:

- 1) Egg-carrying males should exhibit lower locomotor performance when compared to non-carrying males.
- 2) Clutch size should influence performance in carrying males. We expect a negative influence of egg burden (relative clutch size) on male locomotion.
- 3) Clutch desertion should occur in response to disturbance notably among males with lower reproductive effort.

MATERIAL AND METHODS

STUDY SPECIES

The common midwife toad (*Alytes obstetricans*) is a small (4–5 cm) species from the family Alytidae that ranges across Western Europe (Rodríguez-Rodríguez *et al.*, 2020). *Alytes* have a unique type of reproduction with terrestrial egg development. During mating, the male collects the fertilized eggs and attaches them in strings around his hind legs. Eggs are carried until the end of embryonic development which lasts up to 32 days in the wild (Márquez, 1992). Once embryos have completed their development, the male reaches small water bodies (ponds, puddles, ditches) to deposit the tadpoles (Márquez, 1992). In *A. obstetricans*, clutch size ranges from 32 to 171 eggs (Márquez, 1996). Males can carry simultaneously several clutches from different females (Márquez, 1992; Pinya & Pérez-Mellado, 2014).

CAPTURE, HOUSING AND SEX IDENTIFICATION

The study site is located in western France (Chizé forest, 46.145°, -0.394°) and harbours a relatively large population. Adult toads were collected during the reproductive season (April to July 2018) (Márquez, 1990). Every week, up to ten individuals were captured by hand and brought to the laboratory where they were measured (snout–vent length, SVL, mm) and weighed (mg). They were individually housed in plastic boxes (32 × 18.5 cm and 10.5 cm high) with 2 litres of moistened vermiculite (4 cm thick) and allocated in a controlled temperature 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. Individuals were kept in the laboratory for 3 days before being released at the exact capture site.

Midwife toads show limited sexual dimorphism (Bosch & Márquez, 1996). Each individual was inspected to identify its sex using a combination of different methods. Individuals found carrying a clutch were straightforwardly sexed as males. Individuals found with visible eggs in their body cavity were identified as females. Otherwise the sex was set as undetermined unless the individuals were subsequently recaptured either carrying eggs or with eggs in the body cavity. We captured a total of 91 different males. Several individuals (18) were captured twice but we only used naive individuals in the analysis. A total of 63 males were found carrying eggs. Clutch size was recorded for a subset of 56 individuals for which adjusted clutch size was calculated. Among the 63 carrying individuals, 14 deserted their clutch during the captivity period. Due to technical constraints, performance measures were not collected for all individuals. Effective sample sizes for each parameter are specified.

CLUTCH SIZE OF CARRYING MALES

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). We validated this method with a subset of 11 individuals for which we compared estimated clutch size and actual clutch size [linear model (Lm), $F_{(1,9)} = 20.6$, $R^2 = 0.70$, $P = 0.001$]. A positive relationship between SVL and estimated clutch size was found (Fig. 1). We calculated the relative clutch size (residuals of the linear relationship between estimated clutch size and male SVL) to test the independent effects of body size and reproductive effort on locomotion.

LOCOMOTOR PERFORMANCE

We used an integrative approach to describe locomotor performance and address potential costs of

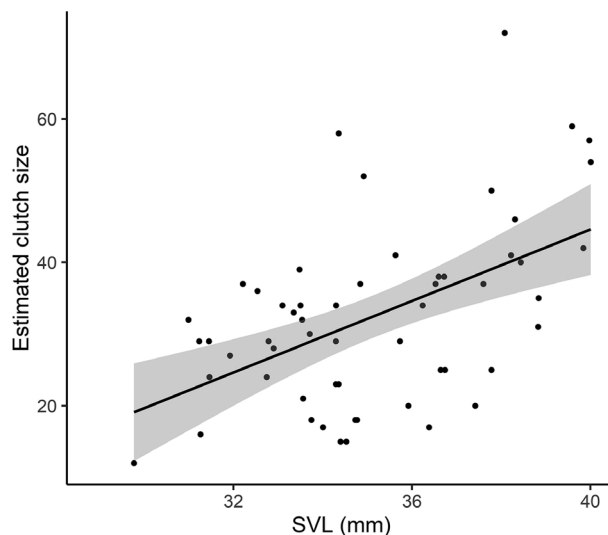


Figure 1. Relationship between estimated clutch size and snout–vent length (SVL). The line represents the fitted linear regression and the grey area the 95% confidence interval.

egg-carrying. Locomotor performance was measured after 1 day of acclimatization to laboratory conditions. We focused on four parameters described below.

Hopping performance

Hopping performance is essential for locomotion or escape in anurans. We used an anti-sliding support made of synthetic turf in a room set at 20 °C. During the trial, toads were stimulated by gently touching the end of the urostyle to induce a hop. Toads were stimulated until no reaction appeared after 15 touches in a row. For individuals that refused to jump, hop response was set as 0. For toads that jumped at least once, hop response was set as 1 and the number of hops before the response ceased was counted. Each trial was video-recorded (Handicam HDR-XR100; 30 FPS), so that we could measure hopping distances using ImageJ software (Rasband, 1997–2018). We measured hopping performance of 16 non-carrying males, 38 carrying males and eight deserting males (that had previously abandoned their clutch).

Righting response

This parameter provides a defence response when confronted by a potential predator. This parameter is a fitness index in terrestrial ectotherms, as a faster righting response is correlated with survival (Delmas *et al.*, 2007). Righting response was measured on a horizontal surface (plastic board) in a room set to 20 °C. A trial comprised turning the toads upside

down and measuring the duration needed for each toad to turn back upright. If handling induced catalepsy, data were discarded. Each trial was video-recorded (Handycam HDR-XR100; 30 FPS), so that we could measure the number of images, convert this to seconds using the frame rate setting of our camera, and therefore determine the duration of each righting response. We measured the righting response of 12 non-carrying males, 41 carrying-males and six deserting males.

Hindleg muscle response to egg burden

We designed a simple test to address the response to gravity, i.e. the burden of clutch mass on muscular performance. The hindleg muscles are key to locomotion including hopping and walking capacities. Each individual was held vertically by holding it between the thumb and the forefinger in the axillary region and leaving the hind limbs suspended in the air. We assessed if the animal was able to maintain its hind legs close to the body (typical response). None of the males dropped their clutch in the process. Hindleg muscle response to egg burden was measured in a room set at 20 °C. Each toad was measured on ten successive occasions. Each trial lasted for 1 min after which a new trial was started if the toad did not completely drop both hind legs indicating an inability to maintain muscular contraction. If handling induced struggling, toads were immediately released, before starting again a few seconds later and this procedure was repeated until ten trials had been completed. We determined for each trial if the individual failed (1) or succeeded (0) in maintaining its hind legs folded over 1 min. We also recorded the time at which the toad completely dropped both legs. We estimated the hindleg muscle response to egg burden of 15 non-carrying males, 37 carrying males and eight deserting males.

Homing time

We designed a simple test to examine homing time. We tested individuals in a room set to 20 °C with toads placed in a plastic corridor (73 × 11 cm and 17 cm high) the bottom of which was covered with synthetic turf and with a refuge, similar to those of the housing environment, positioned at one end. A trial started with the introduction of the toad, by hand, at the other end. Each trial lasted for 10 min and was video-recorded (Handycam HDR-XR100; 30 FPS). We compared homing times and locomotion modes (number of hops vs. walking). We collected homing time data for 16 non-carrying males, 35 carrying males and eight deserting males.

STATISTICS

All statistical analyses were run in R (v.3.5.0; [R Core Team, 2013](#)) and R studio (v.1.2.5001). We examined SVL differences between carrying and non-carrying males using an ANOVA. We investigated the effects of SVL and reproductive status (carrying/non-carrying) on hopping response and on number of hops using a general linear model (Glm) with respectively a binomial and Poisson distribution. Two individuals had an abnormally elevated number of hops and therefore were not included in the analysis. We used a linear mixed-effects model (with Gaussian distribution) to evaluate the effects of SVL and reproductive status on hop distances and SVL, reproductive status, and trials on righting response. Individual identity was set as a random factor. Similarly, we used generalized linear mixed-effects models to test the effect of SVL, reproductive status and trial on hindleg muscle response (binomial function) and hindleg muscle response time (logarithmic distribution). Finally, we used a linear model and a Glm (Poisson distribution) to explore effect of reproductive status and SVL on homing time and number of hops in locomotion during the homing time experiment (see [Table 1](#) for a summary). In all cases the effect of SVL was entered first in the models and we considered the interaction term between SVL and reproductive status to test for allometric differences among groups. However, the interaction was never significant and therefore we only report main effects.

We tested the effects of relative clutch size (RCS) and SVL on hop success and number of hops using a Glm with respectively a binomial and Poisson distribution. The effects of RCS and SVL on hop distances and righting response were analysed using linear mixed-effects models with Gaussian distribution with individual identity sets as a random factor. We used linear mixed-effects models with a logarithmic distribution to investigate the effects of RCS, SVL and trial on hindleg muscle response time with individual identity sets as a random factor (see [Table 2](#) for a summary). Finally, we used binomial logistic regressions to test the influence of SVL and relative clutch size on desertion probability. We examined the influence of desertion on locomotor performance in a separate set of analysis considering only response variables that were influenced by egg-carrying. We applied the same models (type and family) described above to compare carrying and non-carrying males, with the third reproductive status 'deserting male' and with reproductive status as the only variable tested (i.e. without SVL or trial effects). Tukey tests were then used for post-hoc analysis. Values presented are means ± standard deviation (SD).

Table 1. Statistical output of the models used to test for the influence of SVL, reproductive status (deserting males not included), and trial on midwife toad locomotor performance

Response variable	Lm type – Family	Random effect	Explanatory variables	Estimate	SE	<i>z/t</i> value	<i>P</i> -value
Hop response (0/1)	Glm – Binomial	None	(Intercept)	-0.32	4.22	-0.08	0.94
			SVL	-5.37e-3	0.12	-0.05	0.96
Number of hops	Glm – Poisson	None	Status	1.68	0.64	2.61	0.009
			(Intercept)	-0.97	0.89	-1.08	0.27
Hop distances	Lmer – Gaussian	(1 Individual)	SVL	0.07	0.02	2.89	0.003
			Status	0.62	0.22	2.81	0.005
Righting response (time)	Lmer – Gaussian	(1 Individual)	(Intercept)	16.78	14.99	1.12	0.27
			SVL	0.12	0.43	0.27	0.79
Hindleg response (0/1)	Glm – Binomial	(1 Individual)	Status	-8.74	2.72	-3.22	0.003
			(Intercept)	0.18	0.29	0.63	0.53
Homing time	Lmer – Logarithm	(1 Individual)	SVL	9.47e-03	7.88e-03	1.20	0.24
			Status	0.19	0.05	3.92	<0.001
Number of hops (Voluntary loc.)	Glm – Poisson	None	Trial	0.02	4.19e-03	5.50	<0.0001
			(Intercept)	-20.76	5.89	-3.53	<0.001
			SVL	0.59	0.17	3.52	<0.001
			Status	7.44	1.38	5.41	<0.0001
			Trial	0.03	0.08	0.34	0.73
			(Intercept)	2.30	2.64	0.87	0.39
			SVL	-0.05	0.07	-0.73	0.47
			Status	-0.07	0.45	-0.15	0.88
			Trial	-0.18	0.04	-4.91	<0.0001
			(Intercept)	114.18	268.90	0.42	0.67
			SVL	3.05	7.67	0.40	0.69
			Status	-1.84	41.42	-0.04	0.97
			(Intercept)	11.85	1.85	6.40	<0.0001
			SVL	-0.33	0.06	-5.77	<0.0001
			Status	-0.62	0.24	-2.54	0.01

Loc., locomotion; SE, standard error; SVL, snout to vent length.

Table 2. Statistical output of the models used to test for the influence of SVL, RCS and trial on locomotor performance in carrying males

Response variable	Lm type – Family	Random effect	Explanatory variables	Estimate	SE	<i>z</i> / <i>t</i> value	<i>P</i> -value
Hop response (0/1)	Glm – Binomial	None	(Intercept)	-2.01	5.77	-0.35	0.73
			SVL	0.09	0.16	0.55	0.58
			RCS	0.02	0.04	0.46	0.65
Number of hops	Glm – Poisson	None	(Intercept)	-2.24	0.86	-2.61	0.009
			SVL	0.13	0.02	5.45	<0.0001
			RCS	0.01	0.01	1.38	0.17
Hop distances	Lmer – Gaussian	(1 Individual)	(Intercept)	13.99	11.90	1.18	0.25
			SVL	-0.05	0.33	-0.14	0.89
			RCS	-0.26	0.08	-3.27	0.003
Righting response (time)	Lmer – Gaussian	(1 Individual)	(Intercept)	0.34	0.33	1.05	0.30
			SVL	0.01	9.28e-3	1.15	0.26
			RCS	7.21e-3	2.56e-3	2.82	0.008
			Trial	0.02	5.03e-3	4.46	<0.0001
Hindleg response (time)	Lmer – Logarithm	(1 Individual)	(Intercept)	2.01	2.37	0.85	0.40
			SVL	-0.04	0.07	-0.63	0.53
			RCS	0.03	0.02	1.53	0.14
			Trial	-0.19	0.04	-5.05	<0.0001

SE, standard error; RCS, relative clutch size; SVL, snout to vent length.

RESULTS

MALE MORPHOLOGY AND CLUTCH SIZE

SVL was on average 35.01 ± 2.74 mm with carrying and non-carrying males having similar body size (ANOVA, $F_{(1,89)} = 0.33$, $P = 0.57$). For carrying males, estimated clutch size ranged from 12 to 72 eggs (32.43 ± 12.66), and estimated clutch size was significantly correlated with SVL (Fig. 1, Lm, $F_{(1,54)} = 18.97$, $R^2 = 0.25$, $P < 0.0001$).

INFLUENCE OF REPRODUCTIVE STATUS ON PERFORMANCE

Hopping performance

Carrying males hopped more frequently than non-carrying males (76.3%, $N = 29/38$ vs. 37.5%, $N = 6/16$; Table 1). Restricting the analysis to individuals that responded at least once, we found that the total number of hops recorded was higher in carrying-males than in non-carrying males (9.61 ± 6.44 vs. 4.60 ± 4.83 ; Table 1). Body size positively influenced the total number of hops independently of status (Table 1). Hopping distance was shorter for carrying males than for non-carrying males (12.53 ± 5.16 cm vs. 20.67 ± 6.68 cm; Fig. 2; Table 1) but was not correlated with SVL. Within carrying males, relative clutch size had a negative influence on hopping distance (Fig. 3; Table 2), but not on responsiveness to stimulations or on number of hops (Table 2).

Righting response

Righting response was significantly influenced by reproductive status and trials (Table 1). Carrying males required more time to flip back than non-carrying males (0.83 ± 0.32 s vs. 0.64 ± 0.23 s; Fig. 4; Table 1). Righting response was longer across trials (Table 1), independently of reproductive status. Within reproductive males, relative clutch size positively influenced righting response duration (Fig. 5; Table 2).

Hindleg muscle response to egg burden

Body size had a significant influence, with smaller individual being more prone to keep their legs folded (Table 1). Carrying males were less able to retain their legs (0.27%) than non-carrying males (50.67%; Fig. 6; Table 1). We found no influence of SVL, reproductive status or relative clutch size on leg dropping time (Tables 1 and 2) but males dropped faster as trials progressed (Table 1).

Homing time

When placed in the arena, all individuals reach the refuge in an average time of 219.61 ± 129.50 s. We found no effect of SVL or status on homing time (Table 1). However, carrying males used fewer hops to reach the refuge than non-carrying males (1.06 ± 2.63 vs. 2.40 ± 3.36 ; Table 1). Finally, larger individuals hopped less and relied more on walking to reach the refuge (Table 1).

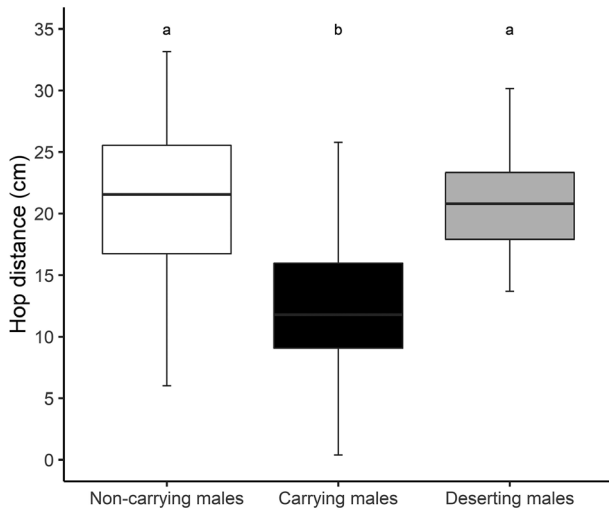


Figure 2. Effect of reproductive status on hopping distance. Boxes represent the intervals between the 25% and 75% quartiles and the whiskers represent the ranges. The middle horizontal line in each box plot represents the median. Different letters indicate significant differences between groups.

CLUTCH DESERTION

Determinant of desertion

Fourteen individuals (12.77%) abandoned their clutch during our study. Deserting males were significantly smaller than non-deserting males (33.64 ± 3.27 mm vs. 35.54 ± 2.54 mm, ANOVA, $F_{(1,61)} = 5.36$, $P = 0.023$) and SVL negatively influenced the probability of desertion (logistic regression, $\chi^2_1 = 5.21$, $N = 63$, $P = 0.02$, $\beta \pm SE = -0.26 \pm 0.12$). Relative clutch size was higher in deserting males than in non-deserting males (7.20 ± 13.99 vs. -1.76 ± 9.37 , ANOVA, $F_{(1,54)} = 6.59$, $P = 0.013$) and positively influenced the probability of desertion (logistic regression, $\chi^2_1 = 6.03$, $N = 56$, $p = 0.01$, $\beta \pm SE = +0.08 \pm 0.03$).

Influence on performance

Hopping probability of deserting males did not differ significantly from carrying or non-carrying males (75.00%, $N = 6/8$; all Tukey's tests > 0.21). Deserting males performed a lower number of hops than carrying and non-carrying males (5.33 ± 3.08 cm; Tukey's tests, carrying males – deserting males: $P < 0.001$, non-carrying males – deserting males: $P < 0.001$). Their hopping distance did not differ from non-carrying males (Tukey's test, $P = 0.99$) and was therefore higher than hopping distances of carrying males (20.71 ± 5.21 cm; Fig. 2; Tukey's test, $P < 0.001$). Righting response times of deserting males were similar to those of both non-carrying males (0.70 ± 0.31 s; Fig. 4; Tukey's

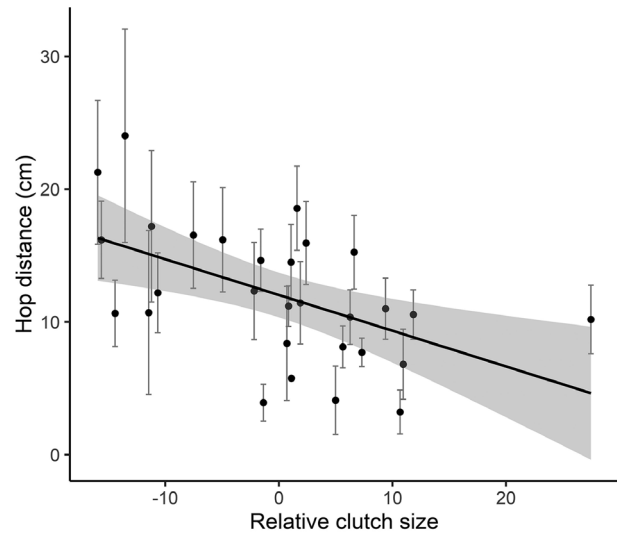


Figure 3. Influence of relative clutch size on hopping distance. Relative clutch size was determined from the residuals of the linear regression between estimated clutch size against SVL (see text for details). The line represents the fitted linear regression and the grey area the 95% confidence interval. Closed circles represent mean individual values and the whiskers the standard deviations.

test, $P = 0.77$) and carrying males (Tukey's test, $P = 0.10$). Deserting males did not differ from non-carrying males in their ability to maintain their hind legs folded (Tukey's test, $P = 0.61$) but retained their legs more often than carrying males (36.25%; Fig. 6; Tukey's test, $P < 0.001$). Finally, in deserting males, the number of hops to reach the refuge (4.29 ± 4.35) was similar to non-carrying males (Tukey's test, $P = 0.049$) and therefore higher than carrying males (Tukey's test, $P < 0.001$).

DISCUSSION

The costs of PC have been widely studied but remain largely overlooked in species with reversed sex roles (Eens & Pinxten, 2000). We examined the influence of egg-carrying on locomotion and behaviour in an anuran with exclusive MPC. In support of our predictions, we found that carrying males exhibited altered locomotor performance and that this comparatively poor performance was linked to their reproductive burden (relative clutch size). We also addressed the determinant of clutch desertion. We discuss our results below.

LOCOMOTOR COSTS OF EGG-CARRYING

Reproductive status had a major impact on locomotor performance. We found that carrying males had a

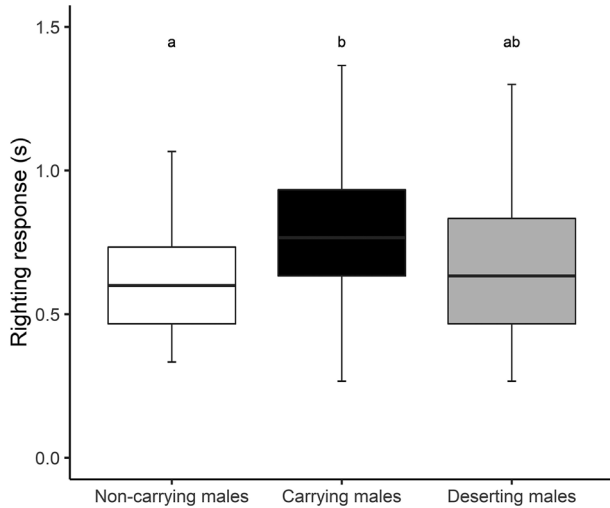


Figure 4. Effect of reproductive status on righting response. Boxes represent the intervals between the 25% and 75% quartiles and the whiskers represent the ranges. The middle horizontal line in each box plot represents the median. Different letters indicate significant differences between groups.

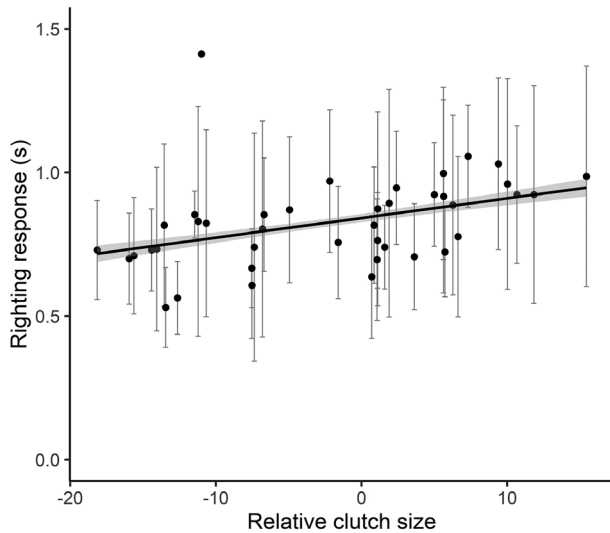


Figure 5. Influence of relative clutch size on righting response. The line represents the fitted linear regression and the grey area the 95% confidence interval. Closed circles represent mean individual values and the whiskers the standard deviations.

higher probability of hopping, had shorter hopping distance, needed more time for righting response and were unable to maintain hind leg muscular contraction. Carrying males mainly walked for homing and were more responsive to stimulations across trials. Overall, all the locomotor traits we

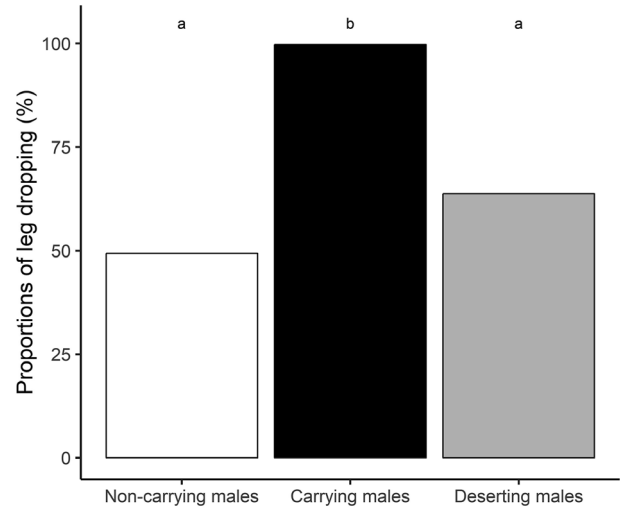


Figure 6. Effect of reproductive status on hindleg muscle response (proportion of leg dropping). Bars represent the proportions for each category. Different letters indicate significant differences between groups.

studied were negatively influenced by relative clutch size. Locomotor performance directly affects crucial ecological traits, such as foraging efficiency and anti-predation behaviour (Webb, 1986). For instance, it has been shown in arthropods that egg-carrying decreases mobility, decreases food intake and increases predator attacks (Burris, 2011; Ruhland *et al.*, 2016). Therefore altered locomotor performance, as reported in this study, may well have significant fitness implications (Webb, 1986; Burris, 2011; Munguía-Steyer *et al.*, 2019). Our findings are similar to the locomotor costs demonstrated in females in numerous other taxa (Ruhland *et al.*, 2016). Therefore, despite reduced prezygotic investment, MPC is likely to induce constraints similar to those typically observed when female parental care occurs (Ruhland *et al.*, 2016; Williams *et al.*, 2016; Saweck *et al.*, 2019).

The contrasted response to stimulations between carrying and non-carrying males (e.g. hopping vs. immobility) could also indicate a shift in anti-predation strategies (van Bergen & Beldade, 2019). Indeed, carrying males may be more susceptible to predation than non-carrying males for two complementary reasons. First, as highlighted above, the locomotor performance of carrying males was strongly hindered by the presence of eggs. Carrying males may benefit from early flight response to enhance their survival (Domínguez-López *et al.*, 2018). In addition, the presence of large egg masses visible from above is likely to make carrying males more conspicuous to an approaching predator. As a consequence, carrying males

are expected to rely less on crypsis (as observed in non-carrying males; [Polo-Cavia *et al.*, 2016](#)). Such a shift in anti-predation strategy would suggest the existence of trade-offs between egg-carrying and survival costs. As a consequence, these modified behavioural responses in carrying males may alleviate potential risks and limit survival costs linked to MPC and its associated reproductive burden ([Requena *et al.*, 2012](#)).

CLUTCH SIZE-DEPENDENCE OF LOCOMOTOR COSTS

According to life history theory, clutch size is a central parameter to consider when addressing potential costs of reproduction ([Stearns, 1992](#)). Such costs can be clutch size-independent or tightly related to reproductive effort ([Ladyman *et al.*, 2003](#); [Foucart *et al.*, 2018](#)). Addressing clutch size-dependence is essential because the proximate nature of the relationship should drive evolutionary trade-offs and adaptations ([Ratikainen *et al.*, 2018](#)). We found that larger males tended to carry larger clutches, reflecting assortative mating and/or female choice ([Márquez, 1993](#); [Böll & Linsenmair, 1998](#)). When factoring out this allometric relationship and considering relative clutch size, we found that reproductive burden negatively influenced hopping distance and righting response time. While males may derive direct fitness benefits from carrying larger clutches relative to their size (e.g. producing comparatively more offspring), potential impacts on locomotion may influence their reproductive decision and mate selection. Interestingly, other parameters such as response to stimulations and number of hops were more tightly related to reproductive status than clutch size. Therefore, egg-carrying may induce both clutch size-dependent and independent constraints.

DETERMINANT OF CLUTCH DESERTION

Parental desertion is frequently observed in species with MPC, often reflecting a trade-off between current and future reproductive effort ([Delia *et al.*, 2013, 2014](#); [Consolmagno *et al.*, 2016](#); [Chuang *et al.*, 2017](#)). We found that deserting males were smaller and carried relatively larger clutches. Previous studies in nest-guarding amphibians have demonstrated that clutch abandonment is more likely when environmental constraints are high ([Consolmagno *et al.*, 2016](#)) and reproductive benefits (number of eggs) are low ([Chuang *et al.*, 2017](#)). However, egg attendance is a flexible behaviour and clutch desertion is often temporary ([Chen *et al.*, 2007](#); [Delia *et al.*, 2013](#); [Consolmagno *et al.*, 2016](#)). Herein, the type of care (egg-carrying) imposes very different proximate constraints for the male and clutch abandonment is permanent, resulting in entire clutch mortality. This relationship between desertion risk and reproductive burden suggests a

causal link with locomotor impairment. Importantly, clutch desertion occurs in the wild (e.g. four abandoned clutches were found on the study site during the study period). Desertion may relate to inexperienced (i.e. smaller, as age and body size are generally positively correlated in amphibians; [Halliday & Verrell, 1988](#)) or exhausted individuals (i.e. carrying a heavy clutch). Deserting toads showed similar performance to non-carrying males regarding hopping distance, righting response duration, hindleg muscle response to egg burden and homing time. Therefore, the costs we detected here are more likely to be due to physical restriction of the legs by the clutch mass (e.g. hindering effect) than physiological costs of MPC.

ECOLOGICAL AND EVOLUTIONARY IMPLICATIONS

PC often induces significant energy constraints by limiting movements or because reproductive activities conflict with feeding ([Lourdais *et al.*, 2002](#)). However, in many taxa, foraging activities are critical for survival and must be maintained during reproduction ([Marconato *et al.*, 1993](#)). This is the case in the common midwife toad which continues feeding while carrying eggs (our personal observations). Egg-carrying probably induces additional costs of transport in relation to feeding activities. Potential costs will also depend on the quality of the microhabitat and climatic conditions. Indeed, beside food intake, males may also provide direct hydric care to their eggs by immersion in water during drier nights ([Boulenger, 1897](#)). Such active PC probably increases mobility (if the water body is relatively far from the diurnal refuge) and could add significant energy and/or survival costs. Further studies are required to address the potential costs of feeding and hydric care during reproduction in this species.

The evolution of PC is based on proximate trade-offs between possible energy/survival costs and fitness benefits. Sex role reversal also has major implications with regard to this relationship. MPC can be beneficial for females, which are thus freed from egg care and have more opportunities to accumulate reserves to fuel future reproductive episodes ([Westneat & Fox, 2010](#)). This is typically the case for midwife toads, for which the reproductive season is prolonged in the study area (from late March to late September) and includes multiple reproductive episodes for both females and males. Such a situation probably offers the opportunity for females to lay more than one clutch during a breeding season ([Tallamy & Brown, 1999](#)). In addition, males can both carry different clutches at the same time ([Márquez, 1993, 1996](#)) and multiple sequential clutches across the breeding season. In contrast to many other temperate amphibian species that mate communally in aquatic environments

during a restricted time period (Wells, 1977; Heyer *et al.*, 2014), mating probabilities of midwife toads are linked to spatial and temporal variations of mate availability in the terrestrial microhabitats selected by individuals (Márquez, 1990). In this context, MPC may secure mating opportunities if female presence and reproductive status are hard to predict. As a consequence, the ability of a male to sustain repeated periods of MPC during the prolonged reproductive season is likely to be strongly linked to its physical condition.

CONCLUSION

Terrestrial reproduction in amphibians has been intensively studied (Lion *et al.*, 2019; Vági *et al.*, 2019) and the midwife toad constitutes a valuable model for studying the determinants of exclusive MPC in this context. We have highlighted clear proximate trade-offs between locomotor performance and relative clutch size, underlining that egg-carrying alters male performance. Such costs are likely to shape (1) the level of male commitment and (2) female mate choice depending on individual quality. This reproductive strategy (egg-carrying) is unique in the Palearctic region and observed in all members of the genus *Alytes* covering rather constraining climates (dry and warm to cold) from Western Europe to North Africa. Important evolutionary benefits are likely to exist to overcome potential costs. For instance, male midwife toads actively collect the eggs from the female and fertilize them on land, which provides a robust way to secure paternity for the males (Westneat & Fox, 2010). Predation risks and competition are major drivers of reproductive strategies in amphibians and MPC might have emerged to improve the survival of offspring by extracting the eggs from the breeding ponds (Márquez, 1990; Pearman, 1995). Recent work suggests that males actively select their microhabitats based on the thermal and hydric properties of the microhabitats (Lange *et al.*, 2020). Further studies are required to explore the fitness benefits of exclusive MPC and to better understand the determinants of male egg-carrying in this group.

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REFERENCES

- Ahnesjö I. 1995. Temperature affects male and female potential reproductive rates differently in the sex-role reversed pipefish, *Syngnathus typhle*. *Behavioral Ecology* **6**: 229–233.
- Alissa LM, Muniz DG, Machado G. 2017. Devoted fathers or selfish lovers? Conflict between mating effort and parental care in a harem-defending arachnid. *Journal of Evolutionary Biology* **30**: 191–201.
- Alonso-Alvarez C, Velando A. 2012. Benefits and costs of parental care. In: Royle NJ, Smiseth PT, Kölliker M, eds. *The evolution of parental care*. New York: Oxford University Press, 40–61.
- Beck CW. 1998. Mode of fertilization and parental care in anurans. *Animal Behaviour* **55**: 439–449.
- van Bergen E, Beldade P. 2019. Seasonal plasticity in anti-predatory strategies: Matching of color and color preference for effective crypsis. *Evolution Letters* **3**: 313–320.
- Bleu J, Gamelon M, Sæther BE. 2016. Reproductive costs in terrestrial male vertebrates: insights from bird studies. *Proceedings of the Royal Society B: Biological Sciences* **283**: 20152600.
- Böll S, Linsenmair K. 1998. Size-dependent male reproductive success and size-assortative mating in the midwife toad *Alytes obstetricans*. *Amphibia-Reptilia* **19**: 75–89.
- Bosch J, Márquez R. 1996. Discriminant functions for sex identification in two midwife toads (*Alytes obstetricans* and *A. cisternasii*). *Herpetological Journal* **6**: 105–110.
- Boulenger G. 1897. *The tailless batrachians of Europe*. London: Printed for the Ray Society.

- Bukhari SA, Saul MC, James N, Bensky MK, Stein LR, Trapp R, Bell AM. 2019.** Neurogenomic insights into paternal care and its relation to territorial aggression. *Nature Communications* **10**: 4437.
- Burris ZP. 2011.** Costs of exclusive male parental care in the sea spider *Achelia simplissima* (Arthropoda: Pycnogonida). *Marine Biology* **158**: 381–390.
- Chen YH, Yu HT, Kam YC. 2007.** The ecology of male egg attendance in an arboreal breeding frog, *Chirixalus eiffingeri* (Anura: Rhacophoridae), from Taiwan. *Zoological Science* **24**: 434–440.
- Chuang MF, Lee WH, Sun JS, You CH, Kam YC, Poo S. 2017.** Predation risk and breeding site value determine male behavior and indirectly affect survivorship of their offspring. *Behavioral Ecology and Sociobiology* **71**: 122.
- Clutton-Brock TH. 1991.** *The evolution of parental care*. Princeton: Princeton University Press.
- Consolmagnò RC, Requena GS, Machado G, Brasileiro CA. 2016.** Costs and benefits of temporary egg desertion in a rocky shore frog with male-only care. *Behavioral Ecology and Sociobiology* **70**: 785–795.
- Crespi B, Semeniuk C. 2004.** Parent–offspring conflict in the evolution of vertebrate reproductive mode. *American Naturalist* **163**: 635–653.
- Crump ML. 1996.** Parental care among the amphibia. *Advances in the Study of Behavior* **25**: 109–144.
- Crump ML. 2015.** Anuran reproductive modes: evolving perspectives. *Journal of Herpetology* **49**: 1–16.
- Dawkins R, Carlisle TR. 1976.** Parental investment, mate desertion and a fallacy. *Nature* **262**: 131–133.
- Delia JRJ, Ramirez-Bautista A, Summers K. 2013.** Parents adjust care in response to weather conditions and egg dehydration in a Neotropical glassfrog. *Behavioral Ecology and Sociobiology* **67**: 557–569.
- Delia JRJ, Ramirez-Bautista A, Summers K. 2014.** Glassfrog embryos hatch early after parental desertion. *Proceedings of the Royal Society B: Biological Sciences* **281**: 20133237.
- Delmas V, Baudry E, Girondot M, Prevot-Julliard AC. 2007.** The righting response as a fitness index in freshwater turtles. *Biological Journal of the Linnean Society* **91**: 99–109.
- Domínguez-López ME, Kacolis FP, Simoy MV. 2018.** Escape behavior in gravid and non-gravid females of *Gonatodes albogularis* (Squamata: Sphaerodactylidae). *Phyllomedusa* **17**: 73–81.
- Downie JR, Robinson E, Linklater-McLennan RJ, Somerville E, Kamenos N. 2005.** Are there costs to extended larval transport in the Trinidadian stream frog, *Mannophryne trinitatis* (Dendrobatidae)? *Journal of Natural History* **39**: 2023–2034.
- Duellman WE, Trueb L. 1994.** *Biology of amphibians*. Baltimore: Johns Hopkins University Press.
- Dugas MB, Wamelinck CN, Killius AM, Richards-Zawacki CL. 2016.** Parental care is beneficial for offspring, costly for mothers, and limited by family size in an egg-feeding frog. *Behavioral Ecology* **27**: 476–483.
- Dulac C, O’Connell LA, Wu Z. 2014.** Neural control of maternal and paternal behaviors. *Science* **345**: 765–770.
- Eens M, Pinxten R. 2000.** Sex-role reversal in vertebrates: behavioural and endocrinological accounts. *Behavioural Processes* **51**: 135–147.
- Foucart T, Heulin B, Lourdaís O. 2018.** Small changes, big benefits: testing the significance of maternal thermoregulation in a lizard with extended egg retention. *Biological Journal of the Linnean Society* **125**: 280–291.
- Furness AI, Capellini I. 2019.** The evolution of parental care diversity in amphibians. *Nature Communications* **10**: 4709.
- Gilbert JDJ, Manica A. 2015.** The evolution of parental care in insects: a test of current hypotheses. *Evolution* **69**: 1255–1270.
- Gross MR, Shine R. 1981.** Parental care and mode of fertilization in ectothermic vertebrates. *Evolution* **35**: 775.
- Halliday TR, Verrell PA. 1988.** Body size and age in amphibians and reptiles. *Journal of Herpetology* **22**: 253.
- Hayward A, Gillooly JF. 2011.** The cost of sex: quantifying energetic investment in gamete production by males and females. *PLoS ONE* **6**: e16557.
- Heyer R, Donnelly MA, Foster M, McDiarmid R. 2014.** *Measuring and monitoring biological diversity: standard methods for amphibians*. Washington: Smithsonian Institution Press.
- Kahn AT, Schwanz LE, Kokko H. 2013.** Paternity protection can provide a kick-start for the evolution of male-only parental care. *Evolution* **67**: 2207–2217.
- Kight SL, Batino M, Zhang Z. 2006.** Temperature-dependent parental investment in the giant waterbug *Belostoma flumineum* (Heteroptera: Belostomatidae). *Annals of the Entomological Society of America* **93**: 340–342.
- Kokko H, Jennions MD. 2008.** Parental investment, sexual selection and sex ratios. *Journal of Evolutionary Biology* **21**: 919–948.
- Kvarnemo C. 2006.** Evolution and maintenance of male care: is increased paternity a neglected benefit of care? *Behavioral Ecology* **17**: 144–148.
- Ladyman M, Bonnet X, Lourdaís O, Bradshaw D, Naulleau G. 2003.** Gestation, thermoregulation, and metabolism in a viviparous snake, *Vipera aspis*: evidence for fecundity-independent costs. *Physiological and Biochemical Zoology* **76**: 497–510.
- Lange L, Brischoux F, Lourdaís O. 2020.** Midwife toads (*Alytes obstetricans*) select their diurnal refuges based on hydric and thermal properties. *Amphibia-Reptilia* **41**: 275–280.
- Lion MB, Mazzochini GG, Garda AA, Lee TM, Bickford D, Costa GC, Fonseca CR. 2019.** Global patterns of terrestriality in amphibian reproduction. *Global Ecology and Biogeography* **28**: 744–756.
- Lourdaís O, Bonnet X, Doughty P. 2002.** Costs of anorexia during pregnancy in a viviparous snake (*Vipera aspis*). *Journal of Experimental Zoology* **292**: 487–493.
- Marconato A, Bisazza A, Fabris M. 1993.** The cost of parental care and egg cannibalism in the river bullhead, *Cottus gobio L.* (Pisces, Cottidae). *Behavioral Ecology and Sociobiology* **32**: 229–237.

- Márquez R. 1990.** *Male parental care, sexual selection and the mating system of the midwife toads *Alytes obstetricans* and *Alytes cisternasii**. PhD Thesis, University of Chicago.
- Márquez R. 1992.** Terrestrial paternal care and short breeding seasons: reproductive phenology of the midwife toads *Alytes obstetricans* and *A. cisternasii*. *Ecography* **15**: 279–288.
- Márquez R. 1993.** Male reproductive success in two midwife toads, *Alytes obstetricans* and *A. cisternasii*. *Behavioral Ecology and Sociobiology* **32**: 283–291.
- Márquez R. 1996.** Egg mass and size of tadpoles at hatching in the midwife toads *Alytes obstetricans* and *Alytes cisternasii*: implications for female choice. *Copeia* **1996**: 824.
- Márquez R, Beltrán JF, Slimani T, Radi M, Llusia D, El Mouden EH. 2011.** Description of the advertisement call of the Moroccan midwife toad (*Alytes maurus* Pasteur & Bons, 1962). *Alytes* **27**: 142–150.
- Marquez R, Verrell P. 1991.** The courtship and mating of the Iberian midwife toad *Alytes cisternasii* (Amphibia: Anura: Discoglossidae). *Journal of Zoology* **225**: 125–139.
- Munguía-Steyer R, González-García E, Castaños CE, Córdoba-Aguilar A. 2019.** Costly parenting: physiological condition over time and season in males of the giant waterbug *Abedus dilatatus*. *Physiological Entomology* **44**: 236–244.
- Nazareth TM, Machado G. 2010.** Mating system and exclusive postzygotic paternal care in a Neotropical harvestman (Arachnida: Opiliones). *Animal Behaviour* **79**: 547–554.
- Pearman PB. 1995.** Effects of pond size and consequent predator density on two species of tadpoles. *Oecologia* **102**: 1–8.
- Pinya S, Pérez-Mellado V. 2014.** Clutch size in wild populations of *Alytes muletensis*. *Acta Herpetologica* **9**: 115–117.
- Polo-Cavia N, Oliveira JM, Redondo Villa AJ, Márquez R. 2016.** Background colour matching in a wild population of *Alytes obstetricans*. *Amphibia-Reptilia* **37**: 1–8.
- Quesada-Hidalgo R, Solano-Brenes D, Requena GS, Machado G. 2019.** The good fathers: efficiency of male care and the protective role of foster parents in a Neotropical arachnid. *Animal Behaviour* **150**: 147–155.
- R Core Team. 2013.** *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: <http://www.R-project.org/>.
- Rasband WS. 1997–2018.** *ImageJ*. Bethesda: U.S. National Institutes of Health. Available at: <https://imagej.nih.gov/ij/>.
- Ratikainen II, Haaland TR, Wright J. 2018.** Differential allocation of parental investment and the trade-off between size and number of offspring. *Proceedings of the Royal Society B: Biological Sciences* **285**: 20181074.
- Reguera P, Gomendio M. 1999.** Predation costs associated with parental care in the golden egg bug *Phyllomorpha laciniata* (Heteroptera: Coreidae). *Behavioral Ecology* **10**: 541–544.
- Requena GS, Buzatto BA, Martins EG, Machado G. 2012.** Paternal care decreases foraging activity and body condition, but does not impose survival costs to caring males in a neotropical arachnid. *PLoS ONE* **7**: e46701.
- Ridley M. 1978.** Paternal care. *Animal Behaviour* **26**: 904–932.
- Ringler E, Pašukonis A, Fitch WT, Huber L, Hödl W, Ringler M. 2015.** Flexible compensation of uniparental care: female poison frogs take over when males disappear. *Behavioral Ecology* **26**: 1219–1225.
- Rodríguez-Rodríguez EJ, Beltrán JF, Tejedo M, Nicieza AG, Llusia D, Márquez R, Aragón P. 2020.** Niche models at inter- and intraspecific levels reveal hierarchical niche differentiation in midwife toads. *Scientific Reports* **10**: 10942.
- Royan A, Muir AP, Downie JR. 2010.** Variability in escape trajectory in the Trinidadian stream frog and two treefrogs at different life-history stages. *Canadian Journal of Zoology* **88**: 922–934.
- Royle NJ, Alonzo SH, Moore AJ. 2016.** Co-evolution, conflict and complexity: what have we learned about the evolution of parental care behaviours? *Current Opinion in Behavioral Sciences* **12**: 30–36.
- Royle NJ, Smiseth PT, Kölliker M. 2012.** *The evolution of parental care*. Princeton: Princeton University Press.
- Ruhland F, Chiara V, Trabalon M. 2016.** Age and egg-sac loss determine maternal behaviour and locomotor activity of wolf spiders (Araneae, Lycosidae). *Behavioural Processes* **132**: 57–65.
- Salvador A. 2015.** Sapó partero bético - *Alytes dickhilleni*. En: Salvador A, Martínez-Solano I, eds. *Enciclopedia Virtual de los Vertebrados Españoles* Madrid: Museo Nacional de Ciencias Naturales. <http://www.vertebradosibericos.org/>.
- Sawecki J, Miros E, Border SE, Dijkstra PD. 2019.** Reproduction and maternal care increase oxidative stress in a mouthbrooding cichlid fish. *Behavioral Ecology* **30**: 1662–1671.
- da Silva FR, Almeida-Neto M, do Prado VHM, Haddad CFB, de Cerqueira Rossa-Feres D. 2012.** Humidity levels drive reproductive modes and phylogenetic diversity of amphibians in the Brazilian Atlantic Forest. *Journal of Biogeography* **39**: 1720–1732.
- Stearns SC. 1992.** *The evolution of life histories*. New York: Oxford University Press.
- Svensson I. 1988.** Reproductive costs in two sex-role reversed Pipefish species (Syngnathidae). *Journal of Animal Ecology* **57**: 929–942.
- Tallamy DW, Brown W. 1999.** Semelparity and the evolution of maternal care in insects. *Animal Behaviour* **57**: 727–730.
- Townsend DS. 1986.** The costs of male parental care and its evolution in a neotropical frog. *Behavioral Ecology and Sociobiology* **19**: 187–195.
- Townsend DS, Stewart MM, Pough FH. 1984.** Male parental care and its adaptive significance in a neotropical frog. *Animal Behaviour* **32**: 421–431.
- Trivers RL. 1972.** Parental investment and sexual selection. In: Campbell B, ed. *Sexual selection & the descent of man*. New York: Aldine Publishing Company, 136–179.
- Úbeda CA, Nuñez J. 2006.** New parental care behaviours in two telmatobiine genera from temperate Patagonian forests: *Batrachyla* and *Eupsophus* (Anura: Leptodactylidae). *Amphibia Reptilia* **27**: 441–444.
- Vági B, Végvári Z, Liker A, Frckleton RP, Székely T. 2019.** Parental care and the evolution of terrestriality in frogs. *Proceedings of the Royal Society B: Biological Sciences* **286**: 20182737.

- Valencia-Aguilar A, de Jesus Rodrigues D, Prado CPA. 2020.** Male care status influences the risk-taking decisions in a glassfrog. *Behavioral Ecology and Sociobiology* **74**: 84.
- Verrell PA, Brown LE. 1993.** Competition among females for mates in a species with male parental care, the midwife toad *Alytes obstetricans*. *Ethology* **93**: 247–257.
- Webb PW. 1986.** Locomotion and predator-prey relationships. In: Feder ME, Lauder GV, eds. *Predator-prey relationships*. Chicago: University of Chicago Press, 24–41.
- Wells E, Garcia-Alonso D, Rosa GM, Tapley B. 2015.** Husbandry guidelines for Alytes.
- Wells KD. 1977.** The social behaviour of anuran amphibians. *Animal Behaviour* **25**: 666–693.
- Westneat DF, Fox CW. 2010.** *Evolutionary behavioral ecology*. New York: Oxford University Press.
- Williams KL, Navins KC, Lewis SE. 2016.** Behavioral responses to predation risk in brooding female amphipods (*Gammarus pseudolimnaeus*). *Journal of Freshwater Ecology* **31**: 571–581.