ANIMAL SCIENCE

Key roles of paternal care and climate on offspring survival of an Amazonian poison frog

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Abstract: In poison frogs (Dendrobatoidea), usually the males are territorial, care for terrestrial nests and later transport their offspring to waterbodies where they complete larval development. In some species, mothers care for their offspring or may exhibit flexible care to compensate for father absence. We conducted a multi-season field experiment with the Amazonian species *Allobates paleovarzensis*, in which it was possible to study the joint impact of paternal care and the El Niño climatic anomaly on offspring survival. The experiment consisted of two treatments: non-removal, and removal of the father from their territories. We observed that parental care was performed exclusively by the father, and none of the mothers of the 21 monitored nests transported the tadpoles. We also observed that the severe drought in a year under the influence of the El Niño event caused such a high mortality in all pre-metamorphic stages, that the role of parental care became irrelevant for offspring survival during that season. We found that pre-metamorphic *Allobates paleovarzensis* are highly vulnerable to the loss of paternal care. In addition, we showed that paternal care, when present, does not prevent offspring death under these increasingly frequent climatic anomalies.

Key words: *Allobates paleovarzensis*, Aromobatidae, Dendrobatoidea, ENSO 2015-2016, nurse frogs, parental care.

INTRODUCTION

Parental care probably originated and continued to increase offspring survival in settings where early mortality was a frequent event (Klug et al. 2012). Parental care can be given by one (uniparental care) or both parents (biparental care), a pattern that is determined by the physiology and anatomy of each sex, and by environmental and behavioural conditions occurring over evolutionary and ecological time which, together, define how each sex is able to provide care to offspring (Webb et al. 1999, Klug et al. 2012, Royle et al. 2014).

Amongst the organisms that exhibit parental care, amphibians are interesting for displaying different forms of care, such as egg attendance, egg transport, egg brooding, tadpole attendance, tadpole transport, tadpole brooding, tadpole feeding, and froglet transport (Wells 2007). Strong evidence that parental care in amphibians is related to the conquest and occupation of the terrestrial environment was provided by Vági et al. (2019) who showed that terrestrial species present different forms of care when compared to aquatic species. Egg care is the most frequent form of parental care in these animals, and is one of the simplest care behaviours (Furness & Capellini 2019). Egg care can be performed by the father, mother or both, and is evolutionarily gained and lost faster than any other form of care (Furness & Capellini 2019). Egg care reduces or prevents the development of anomalies (e.g., Simon 1983),

predation (e.g., Townsend et al. 1984, Juncá 1996) and dehydration (e.g., Delia et al. 2013). In addition, attending to eggs can reduce fungal infestation in embryos, as parents can excrete antimicrobial substances in eggs through their cloaca (Salthe & Mecham 1974, Simon 1983, Wells 2007). The male's attendance to eggs appears to be the basal and most common form of parental care in anurans (Brown et al. 2010, Vági et al. 2019).

Amongst anuran species with parental care, Dendrobatoidea (a superfamily popularly known as poison frogs) is notable for semi-terrestrial frogs with uni- and bi-parental care systems (Lötters et al. 2007). The genus *Allobates* is the most species-rich within the family Aromobatidae (Grant et al. 2006, Frost 2020), which, together with Dendrobatidae family, forms the superfamily Dendrobatoidea (Grant et al. 2006, 2017). As with most members of the superfamily, *Allobates* is composed of species where territorial males act as caregivers (Pröhl 2005, Summers & Tumulty 2014). The main forms of offspring care consist of simple defence of the region where the eggs are deposited, clutch hydration and transportation of tadpoles to a waterbody, so that they can continue their development to the adult stage. The role of females in offspring care is still uncertain, even for the majority of species whose reproductive behaviour has been studied. However, there are some records of female transport of tadpoles in species with mostly paternal care (Pröhl 2005, Ringler et al. 2013, 2015). In addition, when artificially inducing tadpole transport, Pašukonis et al. (2017) showed that there was no difference between the sexes in transport capacity or in the spatial precision with which they located sites for tadpole deposition.

An experimental study with the poison frog *A. femoralis* (Boulenger 1884), which is considered to have a uniparental male caregiver system,

showed that females are flexible and can show caring behaviour to compensate for the loss of a male under laboratory (Ringler et al. 2015) and natural conditions (Ringler et al. 2013). We therefore suggest that other species of poison frogs with similar natural histories will show caregiving strategies that parallel or resemble those found in *A. femoralis*. As an example, in *A. subfolionidificans* (Lima et al. 2007), which is considered as a paternal caregiver, females were occasionally registered performing care for eggs and transport of tadpoles (Souza et al. 2017).

Species of poison frogs use the terrestrial environment for oviposition and offspring care, at least during the initial stages of larval development, and strongly depend on humidity and/or waterbody availability during the reproductive cycle (Weygoldt 1987, Lötters et al. 2007, Wells 2007). They are tropical forest species (Lötters et al. 2007), whose reproductive period occurs in the rainy season. Given this relationship between reproduction and rainfall, as well as the presence of parental care, offspring mortality would be expected to be elevated in years of atypical rainfall. For example, a field study showed that, in years of low rainfall, the offspring of the caregiver treefrog *Hyalinobatrachium fleischmanni* (Boettger 1893; Centrolenidae) suffers increased mortality due to dehydration (Delia et al. 2013). In addition, a clear relationship between the environment and parental care was observed, as males frequently increased the amount of time spent on egg care in response to a decline in relative humidity. However, there is also a threshold at which reduced rainfall is accompanied by spawn abandonment by the male care (Delia et al. 2013). The study with *H. fleischmanni* stands out for being the only one to have tested, in the field, the effect of climatic changes on the reproduction of semi-terrestrial frogs. In addition, this investigation included both a dry year, influence

of El Niño Southern Oscillation (ENSO), and a year with high rainfall, influence of La Niña. Both greater- and less-than-normal annual rainfall reduced survival and altered parental care investment and offspring maturation rates. This suggests that climatic anomalies, as increased or reduced rainfall, are likely to negatively affect anuran reproduction. We can, therefore, expect that anomalous precipitation rates during the rainy season, as well changes in rainy season duration, will impact both the nature of parental care and offspring survival (Beever et al. 2017), and that such climatic anomalies could elicit flexible responses from females to avoid total offspring loss (Beever et al. 2017).

Here we studied the dendrobatoid *Allobates paleovarzensis* (Lima et al. 2010) experimentally in the field, a species known for paternal care and male territoriality (Rocha et al. 2018a, b). We analyzed the patterns of paternal care to see whether the *Allobates femoralis* system of flexible female care in the absence of males, is also present in the congeneric species, *A. paleovarzensis*. The two-year study, 2015-2016, occurred during a rainfall period influenced by an ENSO (El Niño Southern Oscillation), that caused one of the strongest climatic anomalies observed since 1950 (L'Heureux et al. 2017), and which delayed the rainy season in Amazonia (Jiménez-Muñoz et al. 2016). As a result, it became possible to observe the effect of this climatic anomaly on offspring survival in this caregiver amphibian.

Accordingly, we tested the following hypotheses: (H1) Care by the father will positively influence offspring survival during their development; (H2) The female will show flexible parental care in the absence (removal) of the male caregiver; (H3) Under the influence of a climatic anomaly, parental care will be extended to guarantee offspring survival. This is one of the few experimental, field-based studies

on parental care involving *Allobates*. In addition, it is the first to report the effect of an El Niño climatic anomaly on the survival of poison frogs.

MATERIALS AND METHODS

Reproductive biology of *Allobates paleovarzensis*

Allobates paleovarzensis is a diurnal species that inhabits the leaflitter of *paleovárzea* forests, occurring on the banks of the Amazon River and its tributaries (Kaefer & Lima 2012). *Paleovárzeas* are plains flooded periodically after the rainy season by freshwater rich in Andean sediments (Assis et al. 2014). Below we provide an overview on the reproductive biology and the determinants of male mating success based on two previous studies on this species (Rocha et al. 2018a, b).

The reproductive activity occurs during the rainy season, usually between November and April and ceases in the driest months, when total rainfall falls below 100 mm. Males vocalize exclusively during the day, and call activity is more intense during the morning. All events of oviposition and tadpole transportation were observed on rainy days. Males and females are similar in size, but differ visually by colour: yellow throat in females, and violet throat in males (Lima et al. 2010). Male territory size varies between 0.31 and 44.62 m² and females have home ranges varying from 5.14 to 15.31 m², which can overlap the territory of multiple males. Mating is polygamous and courtship begins with male attraction of females mainly through advertisement call. During the courtship march, the female jumps following the male throughout the territory. When accepting the male, the female chooses a dry leaf on the forest floor as a nest and enter into amplexus with the male, stimulating oocyte release (15 to 50 eggs). Following oviposition, the male sits on

the oocyte mass, pressing the eggs against the leaf with his belly and moving over the spawn, probably for egg fertilization and hydration. The male cares for the offspring and continues vocal activity to attract additional females and repel invading males. Because males mate with various females, male territories may contain multiple spawns at different stages of development, and there is a positive relation between territory size and number of spawns. Courtship and oviposition occur within male territories and eggs remain in the leaf nests until the tadpole phase (ca. 21 days), when they must reach an aquatic environment to continue development. When tadpoles are close to stage 25 (sensu Gosner 1960), the male transports them by adhering the tadpoles to his back (3 to 60 in a single event) and depositing them in small permanent or semi-permanent ponds.

Study area

The study was carried out at the *Allobates paleovarzensis* type locality, located in the municipality of Careiro da Várzea (03°22'26.3"S, 59°52'06.4"W), Amazonas State, Brazil. Data collection was conducted on a sample area of 1200 m², in a closed-canopy *paleovárzea* forest fragment containing a network of *igarapés* (small streams) where males of *A. paleovarzensis* occupy territories during the breeding season. Average annual rainfall varies between 2,000 and 2,400 mm (Sombroek 2001). The area has an annual cycle of seasonal flooding (Cintra et al. 2013), beginning in mid-March and April when the region's water bodies overflow and mix. The study was conducted in two consecutive rainy seasons (December 2015 to April 2016, and December 2016 to February 2017). The first sampling period (2015-2016) was impacted by the ENSO (El Niño Southern Oscillation) climatic anomaly (Jiménez-Muñoz et al. 2016, Kogan & Guo 2017). Data from previous years (2008-2015,

season 360000; Hydrological Information System – HidroWeb) showed October as the first month of the rainy season. In 2015, rainfall increased only in December, and daily rainfall patterns stabilized only in February 2016. The second sample period (2016-2017) had daily rain in the manner expected for the local rainy season. Thus, the results of two years were grouped experimentally as: "2015-2016" (ENSO season) and "2016-2017" (standard season).

Observational and experimental field procedures

We started the field activities by geographically marking the points of vocalization and spawns of the males to calculate the size of their territories and by identifying the individuals using body colour patterns recorded by photographs (Rocha et al. 2018a). Sampling was performed daily between 0530 h and 1800 h (GMT-0400), the period of activity for the species.

The experiment consisted of two treatments: non-removal of the father (paternally-cared or 'fathered' spawns, control), and artificial removal of the father from their territories ('non-fathered' spawns). Study area males were selected for inclusion in the experiment if they had at least one developing spawn in their territories. Males assigned for each treatment were selected randomly from the males present at the study site. The number of males involved in the study was limited by the number of breeding males present at the study site during the sampling periods, thus restricting our sample size and minimizing potential offspring loss due to absence of the father. We did not remove females, since our aim was to test whether they would show post-oviposition offspring care. We monitored the territories of 17 non-removed fathers (ENSO season: seven males, eight spawns; standard season: 10 males, 24 spawns) and 11 removed fathers: seven

males removed in the first sampling period (12 spawns) and four males removed in the second period (nine spawns). Of the 11 males removed, four were removed from their territories after oviposition (N = 11 spawns; oviposition day), four were removed in the embryo stage (N = 6 spawns; 7 days after oviposition), and three males removed in the tadpole phase at Gosner (1960) stages 20-25 (N = 4 spawns; 15 days after oviposition). We removed males at different stages of offspring development to test the effect of father absence during this process. Removed males were released at least 400 meters away from their territories so that they were unable to return to their points of origin (Pašukonis et al. 2013, 2014).

Males had between one and four spawns developing simultaneously in their territories. Spawns were identified and monitored to quantify the daily mortality of offspring, and to record their development stages. The visual appearance of eggs, presence of fungi and signs of predation were recorded. We monitored tadpole transport, timing it from the moment just before transport, with tadpoles still in the nest, to their arrival at the water body release site. To avoid artificially influencing behaviour, the observer maintained a minimum distance of two meters from focal individuals. The observer moved in a grid that never crossed the territory of the experimental males, and had no apparent effect on the behaviour of individuals. The grid consisted of marked trails in the study area (Rocha et al. 2018a, b). In both sampling periods, at the endpoint of non-fathered experimental units when the colloidal gel of the spawn melted, we considered that there would be no more transport by the females and then rescued tadpoles to avoid immediate death. In these cases, we transported the tadpoles in plastic recipients to nearby water bodies commonly chosen by parents to deposit their tadpoles. The
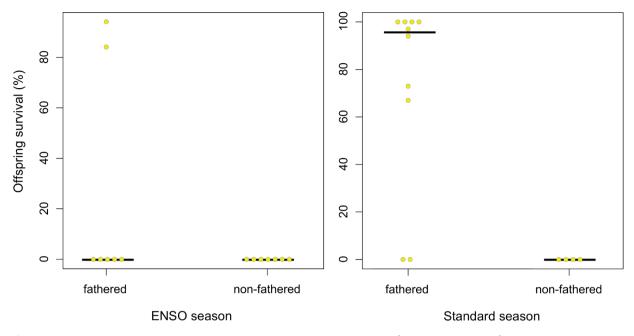
study was approved by the Ethics Committee on the Use of Animals (CEUA) of the National Institute of Amazonian Research (INPA, process 0142016), and by the Biodiversity Information and Authorization System (SISBIO) of the Chico Mendes Institute for Biodiversity Conservation (process 51412).

Analysis

The difference between the percentage of offspring survival for each father (territory) in both treatments and years was assayed using a binomial two-way ANOVA (% offspring survival ~ years + treatments). Preliminary analyses indicated that the number of sister spawns (developing simultaneously in the father's territory) does not affect the survival of the offspring (N = 10 fathers in standard season; Pearson's correlation coefficient = - 0.093; Supplementary Material - Figure S1). Therefore, we decided not to include the number of simultaneous spawns as covariates. When males had more than one spawn simultaneously, only one spawn was picked at random to enter the analyses. The difference between the offspring survival in both years for fathered treatment was assayed using a Mann-Whitney-Wilcoxon U test. All analyses were performed in the R computational environment (R Core Team 2020).

RESULTS

The percentage of offspring survival per father (Figure 1) differed between treatments (ANOVA: df = 1, F = 3.30, p < 0.01), but did not differ between ENSO and standard seasons (ANOVA: df = 1, F = 14.69, p = 0.082, grouped data). This is explained because in the two seasons, mortality was higher in non-fathered treatment. The offspring survival was different between ENSO and standard seasons when considering only

Figure 1. Percentage survivorship of *Allobates paleovarzensis* offspring (spawns per father), up to the point when they were transported to a waterbody by their male caregiver, for the: ENSO season (2015-2016; fathered: *N* = 7, non-fathered: *N* = 7) and standard season (2016-2017; fathered: *N* = 10; non-fathered: *N* = 4). A black horizontal bar indicates the median value. In the ENSO season, only 8.6% of tadpoles survived to the transport phase, all on fathered treatment.

the fathered treatment (Mann-Whitney U test: *W* = 15, *p* = 0.04). Figure 2 shows the main causes of offspring mortality in both treatments and years.

All observed incidences of tadpole transport were performed by male caregivers. We did not record any of the 21 females (mothers of 21 spawns, treatment non-fathered) carrying tadpoles or having any kind of post-oviposition contact with the offspring. In one instance, five tadpoles at Gosner stage 25 of a non-care receiving spawn disappeared from the nest in one day, although we did not observe if they suffered predation or were transported. In this single case, the male caregiver had been removed with offspring at the embryo stage during the ENSO season. We considered this spawn as lost and removed it from the analyses.

Pre-metamorphic individuals in both care treatments and both sampling periods suffered mortality during development (Figure 3). However, the percentage curve of surviving offspring in the non-fathered treatment had a sharper decline than that observed in the fathered treatment.

A collection of the offspring mortality data in the two sampling periods, ENSO and standard season, is presented in Figure 4. This graph shows longitudinal survival data, illustrating the effect of male caregiver removal at three different stages of offspring development (egg, embryo and tadpole). Offspring not receiving paternal care died at all stages of the developmental process, so that the survivorship curve reached zero due to the high mortality following male removal.
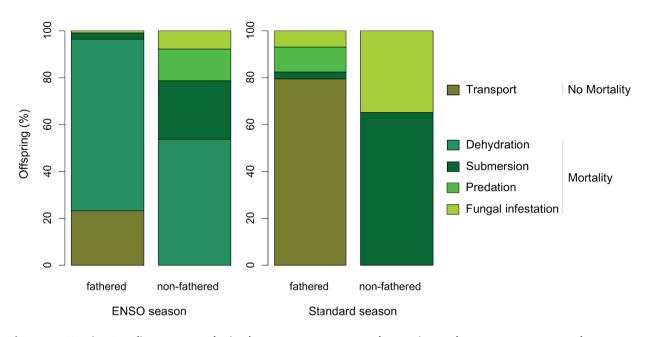
Figure 2. Offspring fate (in percentage) with (*N* = 561 eggs, 24 spawns) and without (*N* = 187 eggs, 9 spawns) male care for *Allobates paleovarzensis* during the ENSO and standard seasons.

DISCUSSION

We found that parental care in *Allobates paleovarzensis* is performed exclusively by the father. While mortality may impact offspring in both the absence and presence of parental care, mortality is higher, at all stages of development, when the male caregiver is absent. During the study, we observed physical interactions between fathers and spawns in two moments: a) after oviposition, when the male presses the belly on the spawn humidifying the colloidal substance; and b) during the transport of the tadpoles, when the father sits besides the spawn and tadpoles climb onto its back. No physical contact between males and spawns during egg development was observed. We do not know whether the father provides moisture or another substance during the development of egg to tadpole, although this has been suggested for other poison frogs (e.g., Juncá 1996, Souza et al. 2017).

Anuran embryo mortality may be caused by a variety of biotic and abiotic factors (Vockenhuber et al. 2009, Lehtinen et al. 2014), many of which were observed in this study. Predation of offspring by heterospecifics occurred in both experimental groups, in both the egg and embryonic phases. In the group receiving paternal care, a single predation event occurred: two spawns were eaten by a Gray-cowled Wood-Rail *Aramides cajaneus* (Statius Muller 1776), a bird that forages in litter near water and in flooded environments (Sick 1997). The father was not present during predation and, in any case, would not have been able to prevent the event without also being at risk of predation itself. The other observed predation events were all carried out by ants, and occurred on spawns from the non-fathered group.

Fungal infestation of *A. paleovarzensis* eggs occurred frequently, mainly in those that had died due to developmental problems. However, in the presence of a male caregiver the fungal infection was restricted to single eggs.
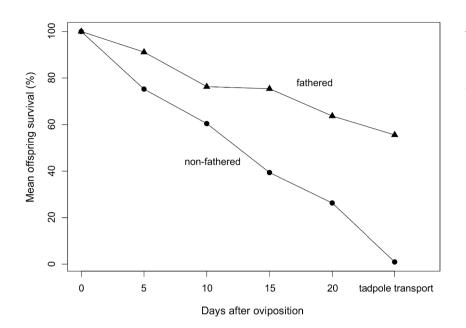
Figure 3. Survival of *Allobates paleovarzensis* offspring in the presence (triangle, *N*= 561 eggs, 24 spawns) and absence of the male caregiver (circle, *N* = 187 eggs, 9 spawns) during the ENSO and standard seasons. Points represent mean survival percentages as a function of time.
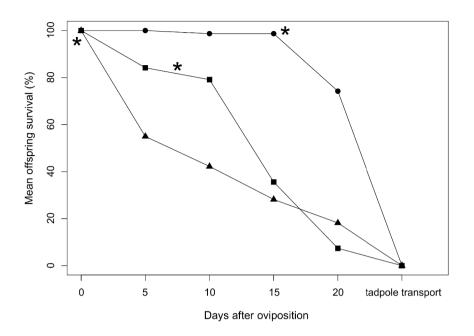
This contrasted with the situation for spawns receiving no paternal care, in which all eggs were eventually infected by fungi. We suggest that, as reported for other anuran species (including dendrobatoids: Juncá 1996), males may hydrate spawns by positioning themselves above them (Vockenhuber et al. 2009, Lehtinen et al. 2014).

In *A. paleovarzensis* spawn dehydration was strongly aggravated by climatic variations influenced by the ENSO event. Parental care has evolved to increase the survival of offspring in adverse environmental conditions, and among abiotic environmental factors, the influence of climate can be crucial. Unfavourable variations in climate can alter the ideal reproductive period of species and result in an increase or decrease in paternal care (see Vági et al. 2020) or in the evolution of alternative care strategies (Schulte & Lötters 2013).

In a study that related parental care and climate in anurans (971 species that represent 45 families; Vági et al. 2020), a negative relationship was observed between parental care and the average annual temperature. In addition, there was a positive relationship between male territoriality and paternal care and a negative relationship between male territoriality and maternal care. As observed for *A. paleovarzensis* in the present study, when males are protecting a territorial space, the costs for parental care are lower and the female does not show care to offspring (Vági et al. 2020). Regarding the climate change scenario, the female's cooperation could prevent the offspring's mortality, but this does not seem to be the case in anurans (Vincze et al. 2017). The chances of mothers presenting compensatory care seem to decrease in cases of climate change. Male care seems to have evolved with territoriality and this behaviour related to space and reproductive period may be more dependent on environmental conditions (this study), when compared to females that are less territorial (Delia et al. 2017, Pröhl 2005, Wells 2007).

Studies have addressed the consequences of climatic anomalies on a variety of wildlife (Beever et al. 2017). A study quantified climatic extremes at a spatial scale commensurate with the seasonal pools used by *Pseudophryne pengilleyi* Wells & Wellington 1985 for reproduction (Scheele et al.

Figure 4. Survival of *Allobates paleovarzensis* offspring at each stage of male caregiver removal in the two sampling periods, ENSO and standard seasons. For young lacking parental care from the egg stage onwards, the father was removed on the day of oviposition (triangle, *N* = 11 spawns, 304 eggs); for those lacking care from the embryo stage onwards, the male caregiver was removed at seven days following oviposition (square, *N* = 6 spawns, 180 embryos); In the tadpole phase the male caregiver was removed 15 days following oviposition (circle, *N* = 4 spawns, 88 tadpoles). Points represent mean survival percentages as a function of time. The symbol (*) represents the date after the oviposition on which the male caregiver was removed.

2012), and found: a) positive relation between local population extinction and periods of extended drought; b) reduction in reproductive success, with diminished recruitment exhibited by the reduced number of sexually mature individuals present during years of very low rainfall; c) the negative impact of El Niño 2002-2003 on the reproduction and offspring survival. As observed in *A. paleovarzensis*, local declines in humidity have been recorded as suppressing reproduction in *Pseudophryne pengilleyi*, as well as resulting in egg desiccation (Pengilley 1973) and tadpole mortality resulting from early dessication (Osborne 1989). All of these aspects can negatively affect species recruitment.

Changes in reproduction are the most common and quantifiable response to various rates of climate change (Beever et al. 2017). Thus, the present study provides evidence of

the powerful impacts that a delayed Amazonian rainy season has on the reproduction and survival of an amphibian species since, in the period under the influence of ENSO, only 8.6% of *Allobates paleovarzensis* tadpoles survived to the transport phase. This shows the importance of studies on climatic anomalies and the status of tropical anuran populations, as climatic extremes, largely caused by local deforestation, are occurring with increasing frequency in the Amazonian region (Lovejoy & Nobre 2018). ENSO is the main driver behind interannual climatic extremes in the Amazon and other tropical regions, and in the last decades there have been five events of extreme drought (1982-1983, 1997-1998, 2005, 2010 and 2015-2016), aggravated by increasing global warming (Jiménez-Muñoz et al. 2016). The ENSO event covered by our study was stronger than past events, probably being

the warmest and driest year recorded in the Amazon, and with the highest soil temperature (Jiménez-Muñoz et al. 2016). According to Cai et al. (2014, 2020), continuous global warming and a projected increase in the frequency of ENSO events, will lead to more frequent climatic extremes in the Amazon in the coming decades (Jiménez-Muñoz et al. 2016).

Climatically anomalous events can result in strategies that prioritize individual short-term self-maintenance, at the expense of immediate reproductive success (Schreiber 2002, Kiere & Drummond 2016). For *A. paleovarzensis*, the delay in the onset of the rainy season caused by the 2015-2016 ENSO led to the death by dehydration of the spawns under male care. A combination of a lack of air humidity and water available for hydration, high temperatures throughout the day, and strong solar incidence may have caused the observed high offspring mortality, even in the presence of the parental care. In our study, males were unable to protect their offspring from dehydration, and were also unable to protect them from the heavy rains and floods that naturally occur once the breeding season had begun. Offspring deposited in territories under open canopy, in areas of low relief or on steep slopes were generally washed into waterbodies by rain or were submerged when the region flooded.

Allobates paleovarzensis is a riverine species, living according to the annual flood pulse as well as the human populations that occupy the banks of the Amazonian rivers. Therefore, this frog has a restricted climate window for its reproduction, starting its activity in the beginning of the rains (Rocha et al. 2018b) until the region is taken by the water that comes from the Andes to the Amazon River and floods the *paleovárzea* region. This means that the rain delay caused by ENSO 2015-2016 reduced the reproductive window that season and when

the rains started (February-March) the region was quickly flooded. This flood hardly depends on the local rains and caused the drowning of the offspring produced by the males that still managed to reproduce after the drought.

Our results also suggest that transport to a body of water is the key factor for tadpole survival, and that this depends directly on the presence of the male caregiver, corroborating other studies that indicate the importance of a parental caregiver at this crucial moment in offspring development (Downie et al. 2005, Ringler et al. 2013, 2015, Killius & Dugas 2014). In *A. femoralis*, females can replace paternal care in the transport of tadpoles if the male is absent. Such compensatory care by *A. femoralis* females was recorded in 7.8% of transport events in a natural population, and most cases of female flexibility occurred when the father had not been recorded as present for several days prior to transport by the mother (Ringler et al. 2013). Captive studies confirmed this behaviour (Ringler et al. 2015). In the current field-based study, no *A. paleovarzensis* females were recorded transporting tadpoles to water bodies, and all observed transportation events were performed by males. Although *A. paleovarzensis* and *A. femoralis* are similar model study animals, captive studies have reported *A. femoralis* behaviours, such as spawn cannibalism by males (Ringler et al. 2017), that have not been recorded in natural populations of *A. paleovarzensis*.

It is probable that females of *A. paleovarzensis* gain greatest fitness benefits with a strategy involving complete desertion of offspring and, after oviposition, investment in individual short-term self-maintenance and selection of new partners. Such a strategy in females would allow production of the greatest number of descendants within a reproductive life-span, and avoid all costs associated with

parental care (Downie et al. 2005). There is no record of multiple mating events involving the same pair in *A. paleovarzensis* (Rocha et al. 2018a, b). In addition, we think each spawn represents a different mother and certainly none of the spawns monitored in our study received any maternal care during its development. We suggest that male desertion is not common enough for female *A. paleovarzensis* to return to spawns to verify male efficiency in providing offspring care.

The developmental stages between the egg and tadpole represent particularly vulnerable stages in the anuran life cycle (Gosner 1960). Both egg and embryo are immobile units highly vulnerable to moisture loss (e.g., Taigen et al. 1984, Townsend et al. 1984, Juncá 1996, Lehtinen et al. 2014), predation (e.g., Juncá 1996, Burrowes 2000, Lehtinen et al. 2014) and infestation by parasites and diseases (e.g., Juncá 1996, Bourne 1998, Green 1999). The tadpole phase is mobile and, in addition to the threats mentioned in the previous stages, requires an aquatic environment for effective respiration, feeding, locomotion and for developmental continuity (Gosner 1960, Weygoldt 1987). Any behaviour that leads to reduced offspring mortality in any of these stages has a strong potential to increase the reproductive success of the parental caregiver involved (Vockenhuber et al. 2009). In our study, father removal directly affected offspring survival, with the main drop in the percentage survivorship occurring soon after the removal of the male caregiver, irrespective of the phase in development at which this manipulation occurred. This result shows the extent to which *Allobates paleovarzensis* pre-metamorphs are vulnerable to the loss of paternal care, because the mother does not care for the young in this species. In addition, we have shown that, even when present, parental care is ineffective in

preventing offspring death under the conditions generated by extreme climatic anomalies.

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SUPPLEMENTARY MATERIAL

Figure S1. Percentage offspring survival per male caregiver with different numbers of simultaneous spawns (1 to 4 spawns). Survival data for all offspring within the male territory of the control treatment, in the standard season, were considered (N = 10).

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SMCR contributed to acquisition, analysis and discussion of the data, conception of the idea and writing of the manuscript. APL and ILK contributed to orientation, conception of the idea and writing of the manuscript. All authors reviewed and contributed to the final manuscript.