



NOTE

First case of male alloparental care in amphibians: tadpole stealing in Darwin's frogs

Oswaldo Cabeza-Alfaro¹, Andrés Valenzuela-Sánchez^{2,3,4}, Mario Alvarado-Rybak^{2,5}, José M. Serrano^{3,6}, Claudio Azat^{2,*}

¹Zoológico Nacional, Pío Nono 450, Recoleta, Santiago 8420541, Chile

²Sustainability Research Centre & PhD Programme in Conservation Medicine, Faculty of Life Sciences, Universidad Andres Bello, Republica 440, Santiago 8370251, Chile

³ONG Ranita de Darwin, Ruta T-340 s/n, Valdivia 5090000, Chile

⁴Instituto de Conservación, Biodiversidad y Territorio, Facultad de Ciencias Forestales y Recursos Naturales, Universidad Austral de Chile, Casilla 567, Valdivia 5110027, Chile

⁵Núcleo de Ciencias Aplicadas en Ciencias Veterinarias y Agronómicas, Universidad de las Américas, Echaurren 140, Santiago 8370065, Chile

⁶Museo de Zoología 'Alfonso L. Herrera', Departamento Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, Circuito Exterior s/n, Ciudad Universitaria, Coyoacán, Mexico City 04510, Mexico

ABSTRACT: Alloparental care, i.e. care directed at non-descendant offspring, has rarely been described in amphibians. *Rhinoderma darwinii* is an Endangered and endemic frog of the temperate forests of Chile and Argentina. This species has evolved a unique reproductive strategy whereby males brood their tadpoles within their vocal sacs (known as neomelia). Since 2009, the National Zoo of Chile has developed an *ex situ* conservation programme for *R. darwinii*, in which during reproduction, adults are kept in terraria in groups of 2 females with 2 males. In September 2018, one pair engaged in amplexus, with one of the males fertilizing the eggs. Twenty days later, the second male (the alloparent) ingested all of the 8 viable, recently hatched tadpoles, releasing 2 froglets 9 wk later. This unusual behaviour observed in captivity is complemented by observations in the field of male *R. darwinii* brooding tadpoles of markedly different sizes, possibly reflecting the brooding of tadpoles from different ovipositions. To our knowledge, this is the first documented case of male alloparental care in amphibians and an example of captive breeding programmes contributing important knowledge about the natural history of threatened species.

KEY WORDS: Allopaternal care · *Rhinoderma darwinii* · Neomelia · Cross-fostering · *Ex situ* conservation · Captive breeding

1. INTRODUCTION

Parental care is any parental behaviour or adaptation that increases offspring fitness, often at some cost to the care-giver (Riedman 1982). At least 10% of amphibian species exhibit some kind of parental care, from egg attendance to more complex behaviours such as food provisioning, viviparity and brood-

ing (Crump 2015, Schulte et al. 2020). An outstanding case among amphibians is the brooding of larvae within the vocal sac of the male (also known as neomelia), a type of parental care that is exclusively known in Darwin's frogs *Rhinoderma rufum* and *R. darwinii*. Darwin's frogs are endemic to the temperate forests of Chile and Argentina, and both species have recently experienced rapid and severe

*Corresponding author: claudio.azat@unab.cl

population declines (Soto-Azat et al. 2013, Azat et al. 2021). *R. rufum* has not been observed since 1981 and is categorized as Critically Endangered (Possibly Extinct) by the IUCN Red List of Threatened Species (Azat et al. 2021). Although *R. darwinii* survives across much of its historical range, its populations are small and severely isolated, and the species is currently classified as Endangered by the IUCN (Azat et al. 2021). Males of both species incorporate newly hatched tadpoles into their vocal sacs, leading to the appearance of being pregnant. Neomelia is completed upon metamorphosis in *R. darwinii* (Jorquera et al. 1972, Busse 2003, 2004).

R. darwinii is a fully terrestrial, diurnal and forest-specialist frog. During the reproductive season (September to March), females of this species approach calling males in their retreat sites to engage in amplexus (Busse 2003, 2004). Females lay a small clutch of eggs (typically 4–10 eggs, Busse 2004, Valenzuela-Sánchez et al. 2014; but up to 34 have been recorded, Wilhelm 1932). Observations from the field and captivity indicate that neither males nor females of *R. darwinii* attend egg clutches (Busse 2004, Valenzuela-Sánchez et al. 2014, Serrano et al. 2020, this study). Around 20 d after oviposition, the male parent returns to the egg clutch to collect the newly hatched larvae (Jorquera et al. 1972, Busse 2004). The movement of embryos within the eggs apparently serves as a stimulus for males to aid tadpole hatching by biting off the egg membrane and swallowing the tadpoles (Busse 2003). Males usually brood between 5 and 8 tadpoles at a time, although a maximum of 22 has been reported (Wilhelm 1927). After a period of generally 6 to 8 wk, metamorphosis is completed and froglets are released into the forest environment by the males (Busse 2004).

An alloparent is an individual, other than the genetic parent, that provides care for conspecific offspring (Riedman 1982). This type of care has been well studied in birds, mammals, fish and invertebrates (Riedman 1982, Eggert & Müller 1992, Largiadèr et al. 2001). Rarely reported in amphibians, alloparental care in this group has thus far been documented only in females; however, alloparental care is likely underestimated in amphibians (Schulte et al. 2020). In eastern North America, four-toed salamanders *Hemidactylium scutatum* and marbled salamanders *Ambystoma opacum* build communal nests in which a small proportion of females stay to protect the eggs (including non-related offspring) from predation and dehydration (Harris et al. 1995, Croshaw & Scott 2005). In Brazil, Rodrigues et al. (2011) reported an anecdotal case of tadpole brood mixing and female interspecific alloparental care between criolla frogs

Leptodactylus latrans and pointed belly frogs *L. podicipinus*, possibly as a result of failed recognition. The best documented case of alloparental care in amphibians is for the caecilian *Boulengerula taitana* from the Taita hills in Kenya, where female parents provide their own skin as a food source for their offspring, and young from different litters have shown genotypes not matching the guarding female (Kupfer et al. 2008).

The possibility of male alloparental care in *R. darwinii* has been raised before (Valenzuela-Sánchez et al. 2014), but evidence to support this has remained elusive until now. In the present study, based on observations made of frogs in captivity, we report the successful brooding of tadpoles by a foster male. We discuss hypotheses that could explain this observed behaviour.

2. MATERIALS AND METHODS

Since 2009, the National Zoo of Chile has developed a Darwin's frog *ex situ* conservation programme, aiming to safeguard the genetic diversity of *Rhinoderma darwinii*, with a plan to re-introduce the species using captive-bred animals in areas where this species has recently disappeared and to assist *R. rufum* conservation if this species is re-discovered.

Founder *R. darwinii* for this project were 16 adult individuals (8 collected in 2009 and 8 in 2012), and 86 offspring have been born since 2009. Adult *R. darwinii* were maintained in groups of 2 males and 2 females in glass terraria of 50 × 50 × 50 cm with a 4 cm layer of sphagnum moss. Supplied water was filtered in sediment carbon filters, treated in an inverse osmosis system and reconstituted. Water was provided through a spray pump system, simulating rain to keep the humidity at a constant ~90%. During most of the year (September to June), mean air temperature was maintained between 12 and 15°C. During the peak of the cold season (July and August) frogs were kept in a refrigerator at 5°C to induce hibernation. Food consisted of small invertebrates, including live fruit flies *Drosophila melanogaster*, crickets *Acheta domesticus*, woodlice *Porcellio scaber*, weevils *Sitophilus garnarius* and larvae of tenebrids *Tenebrio molitor*, given 3 times per week. Each frog was individually identified using its unique ventral colouration patterns (Soto-Azat et al. 2013), but different colouration on the flanks and gular area also allowed easy individual recognition from the outside of the terrarium (Fig. 1B,C). When frogs in a terrarium showed signals of reproductive activity, they were monitored daily between 09:00 and

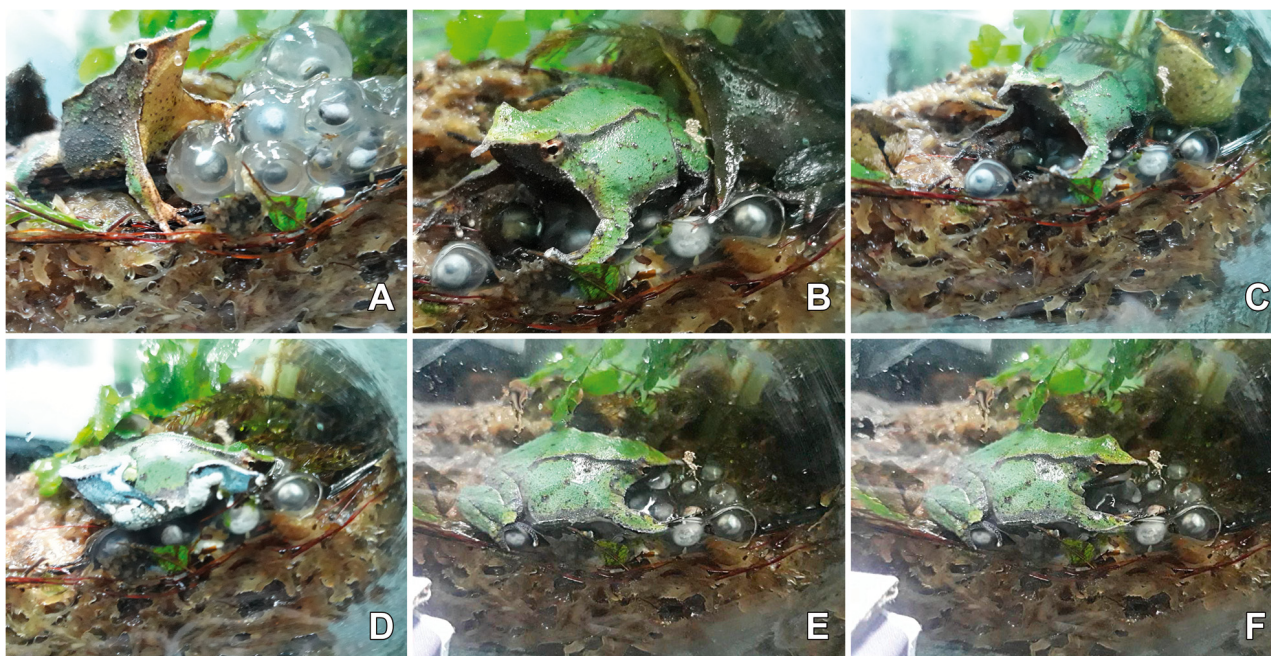


Fig. 1. Male alloparental care of Darwin's frogs *Rhinoderma darwinii* at the National Zoo of Chile. Post-oviposition (A) Day 19. Stimulated by the movements of embryos within the eggs, the male genetic parent stays close to the egg clutch, displaying a defensive posture. (B,C) Day 20. The alloparent male (on the left) positions itself over the eggs, and while the male genetic parent attempts to approach the eggs, the alloparent chases him away. (D–F) Day 21 (sequence of images). The alloparent initiates the brooding process by breaking the egg membranes and swallowing the tadpoles, incorporating them into his vocal sac

17:00 h by direct observation, with their activities and behaviours recorded on a standardized data sheet. The behaviour reported here is complemented by 12 yr of observations of *R. darwinii* in the field.

3. RESULTS

On 2 September 2018, we observed 2 males (M83 and M89) that started calling and competing to engage in amplexus with the same female (F86). The second female (F87) did not show reproductive behaviour during the reproductive season. On 27 September, we observed that amplexus took place between individuals F86 and M89 (hereafter the 'genetic parent'), while M83 (hereafter the 'alloparent') consistently attempted to interfere. These interruptions occurred on at least 5 occasions with variable intensity, and consisted of attempts to embrace the amplexant couple and perform jumps and/or calls right next to the couple. The egg clutch, fertilized by the genetic parent, consisted of 14 eggs that were deposited on one side of the terrarium into a hole within moss and under a fern (Fig. 1). After oviposition, the reproductive female (F86) retreated and was never observed near the egg clutch again. For the next 21 d post-oviposition, both

males were observed occasionally in proximity to the egg clutch: the alloparent made 5 approaches and the genetic parent made 18. Most egg approaches by males occurred between Days 18 and 21 (14 approaches), when visible embryos started moving within the eggs. On Day 20, the male alloparent positioned itself over the egg clutch, exhibiting defensive behaviour and preventing the genetic parent from approaching (Fig. 1B,C). On Day 21, the alloparent began to ingest the tadpoles into its vocal sac, a process that the genetic parent attempted to interrupt several times (Fig. 1D–F). At this point, we counted 8 viable embryos, all ingested by the alloparent within 6 h (see Video S1 in the Supplement at www.int-res.com/articles/suppl/n045p331_supp/). The genetic male parent then withdrew and started calling from a new retreat site. After 62 d, the male alloparent completed brooding and released 2 froglets.

4. DISCUSSION

Ex situ conservation programmes can contribute relevant biological knowledge that otherwise would be difficult to obtain from field studies (Harding et al. 2016). To our knowledge, our observations are the

first documented case of male alloparental care in an amphibian.

The unusual case of alloparental tadpole brooding exhibited by male *Rhinoderma darwinii* differs from other types of alloparental care reported in nature. Conspecific brood parasitism consisting of parasitic laying of eggs in a conspecific nest without providing parental care is well described among birds, and less commonly in fish and arthropods (Riedman 1982, Largiadèr et al. 2001). Cooperative breeding with non-breeding helpers (usually females) has also been reported in many species of social birds and mammals (see Clutton-Brock 2002), and adoption of orphaned newborns has been described in birds and mammals (Riedman 1982).

The causes and consequences of alloparental care remain poorly understood. Although this behaviour has been claimed to be inconsistent with classic evolutionary theory (Riedman 1982), it has also been proposed that selective benefits, along with various environmental pressures or reproductive mistakes, may collectively promote the evolution of alloparental care and adoption (Wisenden 1999). What could drive this unusual behaviour in *R. darwinii* is unknown, but based on knowledge of alloparental care in other taxonomic groups, 2 hypotheses can be proposed: (1) fostering unrelated offspring could increase parental care experience in the alloparent male and hence promote its future breeding success (Riedman 1982, Largiadèr et al. 2001); and (2) because of their apparent breeding success and thus high individual quality, the alloparent male could also enhance its probability of finding a mate for the next breeding event (Clutton-Brock 2002). It is possible that *R. darwinii* females may exhibit a preference for brooding males for mating, thus potentially forcing non-brooding males to steal unrelated eggs to become 'pregnant', as has been reported in some fish species with male egg attendance that actively steal eggs to increase reproductive success (Largiadèr et al. 2001). However, female midwife toads *Alytes obstetricans* in captivity have shown no preference between egg-brooding and non-egg-brooding males (Bush 1996). Although 'pregnant' males continue to vocalize after the onset of brooding, calling ceases after several days later as tadpoles grow (Serrano et al. 2020). Our observations in captive *R. darwinii* have not shown sexual engagement of 'pregnant' males, despite being kept in groups with additional females.

Offspring stealing and adoption have sometimes been attributed to reproductive mistakes (Riedman 1982, Rodrigues et al. 2011). Male alloparental care in *R. darwinii*, as we witnessed in captivity, might

also occur in the wild. In almost all cases (captive and wild), 'pregnant' males brood tadpoles of similar size, but observations in the field have sporadically noted concurrently brooded tadpoles with marked differences in size (Busse 1970, Crump 2002, Valenzuela-Sánchez et al. 2014; Video S1). This might be the result of either different speeds of development among tadpoles of the same brood, or the ability of some males to swallow newly hatched tadpoles from different oviposition events. Even when they are not exhibiting territorial behaviour, such as egg defence, the high home range overlap between adult males (84%; Valenzuela-Sánchez et al. 2014) allows males to be in contact with non-related conspecific eggs, providing an opportunity for tadpole stealing. Also, the low number of successfully brooded tadpoles reported herein has been observed previously in captivity (O. Cabeza-Alfaro pers. obs.). Only 2 of the 8 ingested tadpoles completed metamorphosis. The fate of the other 6 tadpoles is unknown, but possibilities are: (1) tadpoles passed to the gastrointestinal tract where they were digested, and (2) dead tadpoles in the vocal sac served as food for the brooding tadpoles. However, the second option is less likely, as the oral structures of *R. darwinii* tadpoles are not adapted for adelphophagy. These issues should be addressed in future research.

Dramatic population declines have recently been observed in both species of Darwin's frogs (Soto-Azat et al. 2013). Similarly, the gastric-brooding frogs *Rheobatrachus vitellinus* and *R. silus* from eastern Australia are considered to have been extinct since the 1980s. As with gastric-brooding frogs, the potential extinction of Darwin's frogs would imply the loss of a unique reproductive behaviour before it is fully understood. Our study provides important information about the reproductive biology of *Rhinoderma*, which is highly valuable for efforts to conserve these Endangered amphibians.

Acknowledgements. We are grateful to Loreto Peña, animal keeper of herpetology at the National Zoo. C.A. is funded by Fondecyt Regular N° 1211587. We extend our gratitude to 4 reviewers and the editor for their valuable comments that helped to improve the manuscript. We would like to dedicate this work to the memory of Dr. Alberto Veloso, a conservationist and passionate pioneer of Darwin's frog research.

LITERATURE CITED

- ✦ Azat C, Valenzuela-Sánchez A, Delgado S, Cunningham AA and others (2021) A flagship for Austral temperate forest conservation: an action plan for Darwin's frogs bringing together key stakeholders. *Oryx* 55:356–363

- Bush SL (1996) Why is double clutching rare in the Majorcan midwife toad? *Anim Behav* 52:913–922
- Busse K (1970) Care of the young by male *Rhinoderma darwinii*. *Copeia* 1970:395
- Busse K (2003) Fortpflanzungsbiologie von *Rhinoderma darwinii* (Anura: Rhinodermatidae) und die stammesgeschichtliche und funktionelle Verkettung der einzelnen Verhaltensabläufe. *Bonn Zool Beitr* 51:3–34
- Busse K (2004) Biología de la reproducción del Sapito de Darwin (*Rhinoderma darwinii*) y su cría en cautividad. In: Iriarte A, Tala C, González B, Zapata B, González G, Maino M (eds) Cría en cautividad de fauna Chilena. Servicio Agrícola y Ganadero, Santiago de Chile, p 139–146
- Clutton-Brock T (2002) Breeding together: kin selection and mutualism in cooperative vertebrates. *Science* 296:69–72
- Croshaw DA, Scott DE (2005) Experimental evidence that nest attendance benefits female marbled salamanders (*Ambystoma opacum*) by reducing egg mortality. *Am Midl Nat* 154:398–411
- Crump ML (2002) Natural history of Darwin's frog, *Rhinoderma darwinii*. *Herpetol Nat Hist* 9:21–30
- Crump ML (2015) Anuran reproductive modes: evolving perspectives. *J Herpetol* 49:1–16
- Eggert AK, Müller JK (1992) Joint breeding in female burying beetles. *Behav Ecol Sociobiol* 31:237–242
- Harding G, Griffiths RA, Pavajeau L (2016) Developments in amphibian captive breeding and reintroduction programs. *Conserv Biol* 30:340–349
- Harris RN, Hames WW, Knight IT, Carreno CA, Vess TJ (1995) An experimental analysis of joint nesting in the salamander *Hemidactylum scutatum* (Caudata: Plethodontidae): the effects of population density. *Anim Behav* 50:1309–1316
- Jorquera B, Pugin E, Goicoechea O (1972) Tabla de desarrollo normal de *Rhinoderma darwini*. *Arch Med Vet* 4: 5–18
- Kupfer A, Wilkinson M, Gower DJ, Müller H, Jehle R (2008) Care and parentage in a skin-feeding caecilian amphibian. *J Exp Zool A Ecol Integr Physiol* 309:460–467
- Largiadèr CR, Fries V, Bakker TCM (2001) Genetic analysis of sneaking and egg-thievery in a natural population of the three-spined stickleback (*Gasterosteus aculeatus* L.). *Heredity* 86:459–468
- Riedman ML (1982) The evolution of alloparental care and adoption in mammals and birds. *Q Rev Biol* 57:405–435
- Rodrigues AP, Giaretta AA, da Silva DR, Facure KG (2011) Reproductive features of three maternal-caring species of *Leptodactylus* (Anura: Leptodactylidae) with a report on alloparental care in frogs. *J Nat Hist* 45: 2037–2047
- Schulte LM, Ringler E, Rojas B, Stynoski JL (2020) Developments in amphibian parental care research: history, present advances, and future perspectives. *Herpetol Monogr* 34:71–97
- Serrano JM, Penna M, Valenzuela-Sánchez A, Méndez MA, Azat C (2020) Monomorphic call structure and dimorphic vocal phenology in a sex-role reversed frog. *Behav Ecol Sociobiol* 74:127
- Soto-Azat C, Valenzuela-Sánchez A, Collen B, Rowcliffe MC, Veloso A, Cunningham AA (2013) The population decline and extinction of Darwin's frogs. *PLOS ONE* 8: e66957
- Valenzuela-Sánchez A, Harding G, Cunningham AA, Chirgwin C, Soto-Azat C (2014) Home range and social analyses in a mouth brooding frog: testing the co-existence of paternal care and male territoriality. *J Zool (Lond)* 294:215–223
- Wilhelm O (1927) La *Rhinoderma darwini* D. y B. *Bol Soc Biol Concepc* 1:11–39
- Wilhelm O (1932) Nuevas observaciones acerca de la neomelia de la *Rhinoderma darwini*. *Rev Chil Hist Nat* 36: 166–170
- Wisenden BD (1999) Alloparental care in fishes. *Rev Fish Biol Fish* 9:45–70

Editorial responsibility: Michael Mahony,
Callaghan, New South Wales, Australia

Reviewed by: A. Kupfer, K. Busse, M. L. Crump
and 1 anonymous referee

Submitted: January 14, 2021

Accepted: May 31, 2021

Proofs received from author(s): August 11, 2021