# FIELD OBSERVATIONS ON THE NATURAL HISTORY AND BREEDING BEHAVIOR OF THE ATACAMA TOAD *RHINELLA ATACAMENSIS*, (ANURA: BUFONIDAE) FROM CHILE

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## ABSTRACT

Amphibians have diversified predominantly across tropical environments where humidity, temperature and microhabitat availability facilitate demographic stability. However, a number of lineages have colonized extreme deserts, where their diversities are considerably lower. One species in particular, the Atacama toad (*Rhinella atacamensis*), has adapted to the Atacama Desert, the driest place on Earth. Despite the ecological uniqueness of this species, most aspects of its natural history, reproduction, patterns of activity and behavior remain unknown. Using camera traps and *in situ* field observations, we report a set of novel natural history findings in a population from the Llanos de Challe National Park. We show that *R. atacamensis* remains hidden from the sun in small pools under vegetation, where 100% of observed femalJes have a male permanently attached in amplexus at all times. The toads emerge to gather in small ponds after the sunset (~20:00h), where males engage in active contests over females, with up to four males competing for one female. They retreat into the covered pools after ~06:00h. Eggs laid during the night hatch within 24 hours. We discuss these observations in the context of the global diversity of bufonid toads in general, and in relation with the effects that selection emerging from the harsh conditions of Atacama may exert on the expression of these traits.

**Key words:** *Rhinella*, Atacama, Reproduction, Amphibians, Camera traps, Chile

## RESUMEN

**Observaciones en Terreno sobre la Historia Natural y Conducta Reproductiva del Sapo de Atacama *Rhinella atacamensis* (Anura: Bufonidae) de Chile.** Los anfibios han diversificado predominantemente en ambientes tropicales donde la humedad, temperatura y disponibilidad de microhábitats facilitan la estabilidad demográfica. Sin embargo, múltiples linajes han colonizado desiertos extremos, donde su diversidad es considerablemente menor. Una especie en particular, el sapo de Atacama (*Rhinella atacamensis*), se ha adaptado al Desierto de Atacama, la región más árida en la Tierra. Pese a la singularidad ecológica de esta especie, la mayor parte de los aspectos de su historia natural, reproducción, patrones de actividad y conducta permanecen desconocidos. Usando cámaras trampa y observaciones de campo *in situ*, documentamos una serie de nuevos hallazgos en una población del Parque Nacional Llanos de Challe. Nuestro estudio muestra que *R. atacamensis* permanence oculto del sol en pequeñas pozas cubiertas por vegetación, donde el 100% de las hembras observadas mantiene un macho permanentemente abrazado en amplexo a todas horas del día. Los sapos emergen para conglomerarse en pequeñas pozas expuestas después de la puesta de sol (~20:00h), donde los machos se enfrentan en activos combates por hembras. Hasta cuatro machos se enfrentan por una misma hembra. Los sapos se vuelven a ocultar en las pozas cubiertas después de las ~06:00h. Los huevos depositados durante la noche eclosionan dentro de 24 horas. Discutimos estas observaciones en el contexto de la diversidad global de los sapos bufónidos en general, y en relación con los efectos que la selección que emerge de las duras condiciones de Atacama puede ejercer sobre la expresión de estos caracteres.

**Palabras clave:** *Rhinella*, Atacama, Reproducción, Anfibios, Cámaras trampa, Chile

## INTRODUCTION

The radiation of modern amphibians (anurans, salamanders and caecilians) has successfully proliferated across most ecosystems on Earth (Wells 2007; Hof *et al*. 2011; Roll *et al*. 2017). Most hotspots of amphibian biodiversity concentrate around tropical latitudes, where environmental factors such as high humidity, high and stable temperatures, and dense vegetation coverage facilitate the 'water-dependent' demands of their life cycles (Wiens *et al*. 2006; Wiens 2007). However, multiple amphibian lineages have actively expanded into non-tropical ecosystems where extremely low humidity, high thermal variance and scarce vegetation have imposed strong natural selection on physiological, ecological and life history traits (Wells 2007). Among biogeographic regions sharing those conditions, desert ecosystems in particular have been observed to impose exceptionally strong ecological barriers for the radiation of modern amphibians. Global analyses of the distribution of amphibians consistently show that the species richness of these vertebrates declines steeply in the major desert regions on the planet (Wells 2007; Hof *et al*. 2011). One such example is the Atacama Desert in Chile, regarded as the driest place on Earth. In this region, isolated at the Western side of the Andes range, the harsh environmental conditions have been shown to even impose ecological limits to the radiation of reptiles (Pincheira-Donoso *et al*. 2013), which tend to be common across multiple desert regions in other parts of the world.

Consistent with the global patterns of amphibian distribution, the diversity of these vertebrates declines sharply to only two frog species in the Atacama Desert (Cei 1962; Lobos *et al*. 2013). However, only one of these, the Atacama toad (*Rhinella atacamensis*) is considered a true inhabitant of the desert, while the other species (*Pleurodema thaul*) occupies peripheral areas with considerable irrigation from freshwater bodies, such as meadows and river basins (Cei 1962). *Rhinella atacamensis*, in contrast, is widespread across Atacama, being commonly observed in extremely arid regions with limited vegetation (Cei 1962; Sallaberry *et al*. 2007; Correa *et al*. 2008, 2012, 2013; Lobos *et al*. 2013). While this species can be found around major river bodies (*e.g.*, Huasco river; Cei 1962; Urra 2013), it is also commonly observed associated with predominantly arid areas irrigated by tiny streams and even in dry areas where only occasional rainfalls create sporadic bodies of freshwater (*e.g.*, during "flowering desert" episodes).

Despite the widespread distribution of *R. atacamensis* and its exceptional tolerance to extreme desert environments, most aspects of its natural history, ecology and life history remain unknown. A number of field observations have led to an accurate picture about the species' distribution (Sallaberry *et al*. 2007; Correa *et al*. 2008, 2012, 2013), coloration and sexual dichromatism (Cei 1962; Lobos *et al*. 2013; Urra 2013) and, to some extent, microhabitat preferences (Cei 1962; Lobos *et al*. 2013; Urra 2013). However, data on its reproduction, breeding habits and interactions with the environment remains very limited (*e.g.*, see Veloso and Nuñez 2003, 2004). In this article, we report a series of novel findings that will contribute to fill the gap on the breeding ecology of the Atacama toad, by providing field data collected with camera traps in the Llanos de Challe National Park (Atacama Region, Chile).

## METHODS

We conducted field observations in the area of Quebrada del Carrizo, Llanos de Challe National Park, in Atacama (28°06'57''S, 71°05'10''W; 150 masl), during the period 6th-13th September (2017). We aimed to identify the patterns of daily activity and breeding behavior of *Rhinella atacamensis*, by setting up a series of camera traps (model ABASK 8MP) around the edges of two small ponds, approximately 1-2 meters in diameter and approximately 40 meters apart. We set up the cameras in the morning (10:00-11:00h) and let them record pictures and films (which activate by sensors that perceive movements in front of the devices) for entire cycles of 24h, in order to establish a clear picture of their daily activities. These camera traps are equipped with microphones, thermometers, and clocks that automatically record sounds, air temperature and time of each visual record. These cameras, thus, allowed us to have a complete view of the ponds and their immediate surroundings.

In addition, we performed observations of the areas surrounding the ponds, in order to establish the environmental conditions associated with these exposed freshwater spots. Finally, we counted numbers

of males and females and measured their snout-vent length to establish magnitude of sexual dimorphism. We employed snout-vent length (SVL), the standard proxy for body size in amphibians (e.g., Wells, 2007), to quantify the extent of sexual size dimorphism (SSD). The degree of SSD was calculated with the formula $\ln(SVL_{Male}/SVL_{Female})$. This measure of dimorphism is intuitive and has been shown to perform with satisfactory statistical power (Smith 1999; Fairbairn 2007). No voucher specimens were collected, and after collection of measures, all individuals were returned to exactly the same spots in which they were found.

Finally, we contrasted the ecological and life history traits observed in *Rhinella atacamensis* with the range of variation in the same traits observed across the entire family Bufonidae globally. To perform these comparisons, we created a dataset from the primary literature (*e.g.*, Channing 2001; Channing and Howell 2006; Kohler 2012; Halliday 2016). These comparisons included the (*i*) directionality (female- or male-biased) and magnitude of sexual size dimorphism (see above for details); (*ii*) use of micro habitat type, for which we defined four main categories: aquatic (species that live permanently associated with water, which include species that occasionally dwell on the ground), fossorial (species that remain burrowing underground when they are not feeding or breeding), bush-dwellers (species that forage and perch on vegetation), and terrestrial (species that do not depend on permanent contact with water bodies, being often observed dwelling in dry substrates, but which are not fossorial); (*iii*) daily activity, which was defined in three categories: nocturnal (species that are active during the night only), diurnal (species that are active during the day only), and cathemeral (species that are active during both day and night hours); and (*iv*) egg diameter, a standard measure in amphibian life histories which is the diameter of the egg itself (*i.e.*, excluding the external jelly capsule).

## RESULTS AND DISCUSSION

### Ecology

Our field observations reveal a strictly nocturnal, aquatic, gregarious and moderately female-biased sexually size dimorphic species ($SVL_{males}$ = 79.8±6.9mm, range: 70.3-88.5, $SVL_{females}$ = 88.8±2.2mm, range: 86.4-91.2; Figure 1a, c, d). These observations are compatible with the predominantly nocturnal habits of the majority of bufonid species globally (Figure 1d), while it contrasts with the predominantly terrestrial use of microhabitats observed within this family globally (Figure 1c). Both nocturnality and the use of aquatic microhabitats are compatible with the occupation of a harsh desert environment, in which solar radiation is expected to have created strong natural selection against a range of ecological and life history components of this species' phenotype. Therefore, these two traits are likely to have been shaped to avoid desiccation. In fact, the nocturnal habits of this species were strongly confirmed by both camera traps and field observations. During day hours, this species remains hiding in small permanent ponds entirely covered by a layer of 20-40 cm of grassy vegetation, and no specimens were recorded before 20:14h in the evening, or after 06:18h in the morning (Figures 1e, 2 and 3). During the coldest night hours (when temperatures fluctuate between 1-4°C), we observed a tendency for fewer individuals to be dwelling around the pond, while their numbers remained remarkably constant across temperatures ranging between 5-18°C (Figure 1f). It remains unknown the role that pressures such as male-male competition over females (i.e., sexual selection; see Andersson 1994) or the need to maximize transient fecundity (*i.e.*, clutch size via fecundity selection on females; see Pincheira-Donoso and Hunt 2017) may play on the moderate magnitude of sexual size dimorphism in this species (especially relative to *Rhinella* species in particular; Figure 1a-inset plot). However, it is possible that both factors may interact via sex-specific selection favouring larger body size in each sex in parallel (*e.g.*, see Zamudio 1998; Pincheira-Donoso and Tregenza 2011). While male fitness is likely to be enhanced by higher success during contests as a function of larger body size, transient fecundity may be promoted to increase via larger female size in an environment where access to ponds for breeding can be unpredictable and sporadic. These are possibilities that warrant future quantitative testing.

### Breeding behaviour and life history

Our observations showed an active breeding activity throughout the day in *R. atacamensis*. Indeed, Cei (1962) reported that the Atacama toad remains reproductively active continuously throughout the year po-
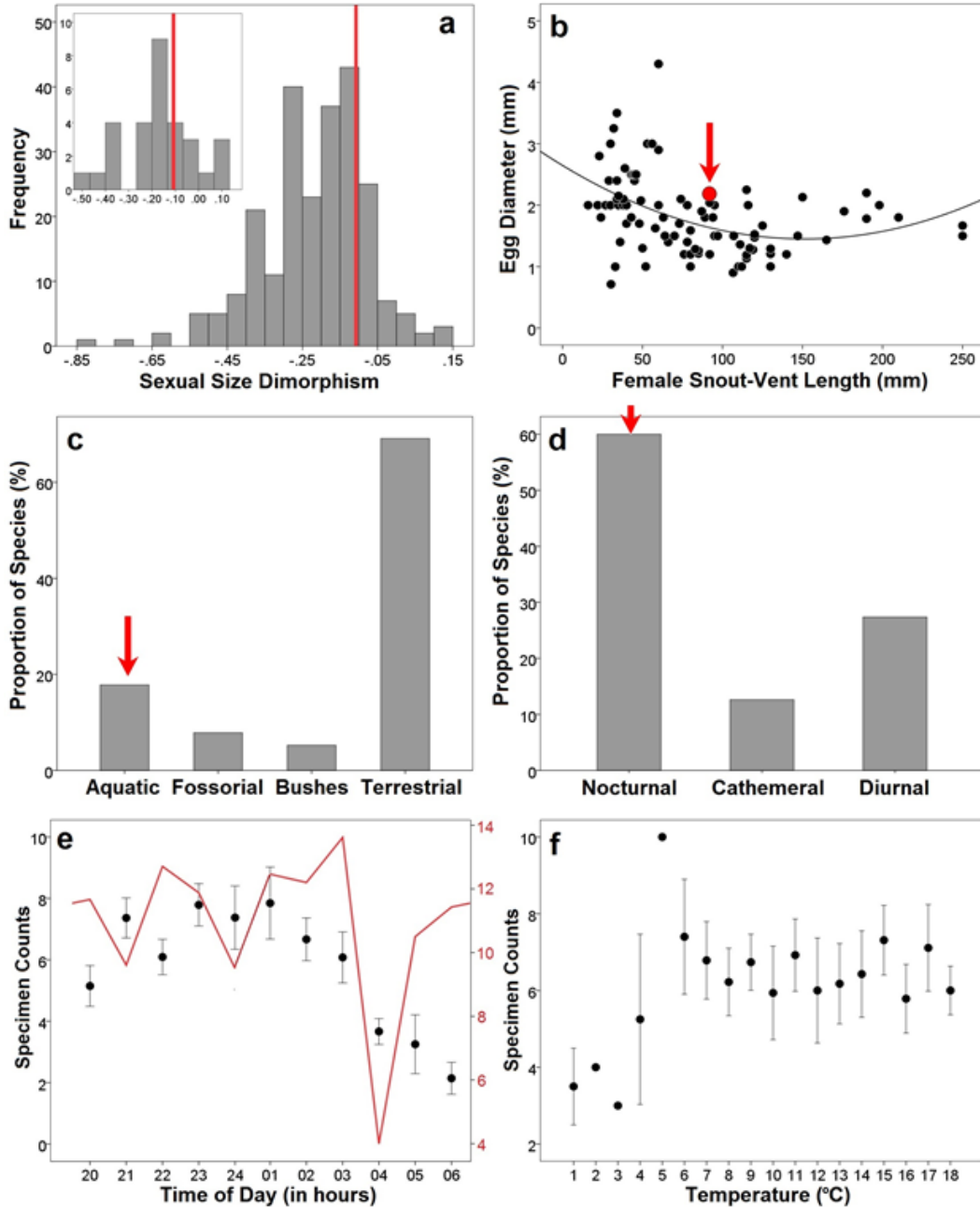
FIGURE 1. Synthesis of observed features in *Rhinella atacamensis*. The plots show (a) the magnitude of sexual size dimorphism (where negative values indicate that females are larger than males) measured in this species (red line) relative to the same trait measured across all Bufonidae globally (main graph) and relative to *Rhinella* species only (inset graph); (b) egg diameter relative to female size (red dot indicates the egg of *R. atacamensis*); (c) the proportion (in %) of species of Bufonidae globally specialized in the use of different microhabitats, and (d) in their daily activity times (red arrow shows the position of *R. atacamensis* in both graphs); (e) the number of specimens counted during the course of the nights, in which hours outside those displayed on *x* have been excluded as the number of specimens was consistently zero (red line shows variations in air temperature in °C, and red column of numbers to the right the temperature); and (f) numbers of specimens in relation with air temperature in (°C). See text for details.
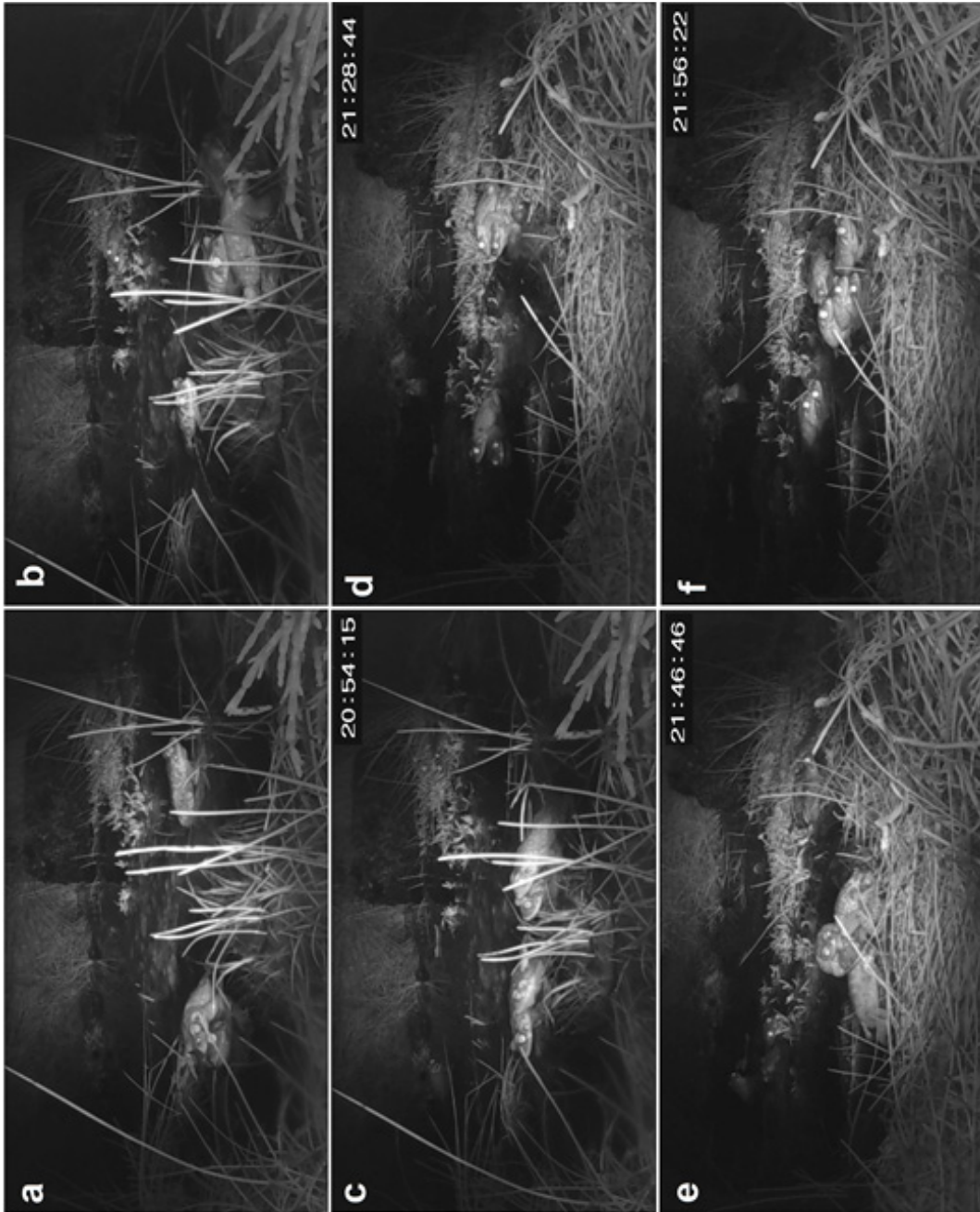
FIGURE 2. Breeding activity of *R. atacamensis* during the early hours of the night (a-f). Note some intense competition over one single female shown in some of the pictures (e, f).
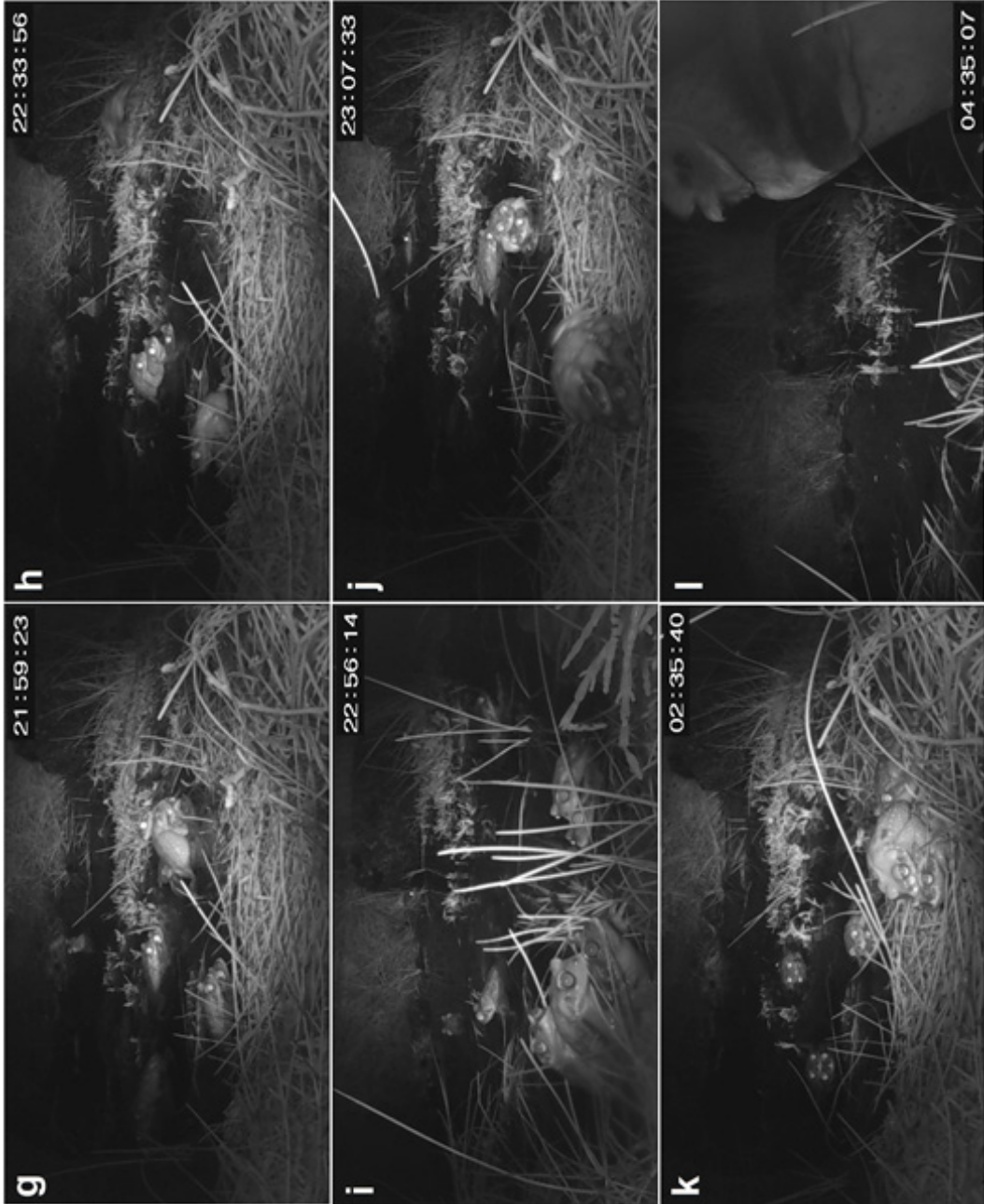
FIGURE 3. Breeding activity of *R. atacamensis* during the later hours of the night (g-l). Note some intense competition over single females shown in some of the pictures (g, i, j). Also, note that the presence of a barn owl (*Tyto alba*) cause an immediate decline of specimens to zero (l). Toad activity was restored after the owl flew away six minutes later.

tentially as a response to compensate for the extreme pressures that the harsh arid conditions may impose on the opportunities for reproduction. Consistent with these findings, it has been suggested that breeding activity is expected to intensify in climates where reproduction is compromised by fluctuating environmental conditions (which have particularly more severe effects on ectotherms) or by short breeding seasons (Pincheira-Donoso and Hunt 2017). Our observations carried out both directly during the day and with camera traps during the night reveal that females are constantly engaged in amplexus. During the day, when these toads remain under their vegetation shelters, every observed female had a male attached, and remain strongly grasped to the female even when they are manipulated for collection of measurements. During the night, competition over females intensifies considerably. Our camera trap records show that males engage in active physical confrontation over females (Figures 2 and 3). We observed up to four males attached to a single female. This competition is active, involving multiple females and males in small ponds of 1-2 meters of diameter, and males display limited fidelity, moving from one female to another. Towards the end of the night, the overall number of individuals declines steeply (Figure 1e), and as females retreat, presumably seeking for shelter to avoid solar radiation during the day (the presence of a barn owl perched by the pond showed the vegetation is also used for predator avoidance; Figure 3l), a single male will attach to each female and will remain there during the course of the day.

Finally, our field observations revealed rapid hatching time for a species with fairly average egg sizes relative to female body size (Figure 1b). Although we failed to obtain precise data on hatching time measured in hours, we observed that this process takes less than 24 hours: eggs laid during the course of the night had hatched by 16:00h on the following day (given the number of egg strings found in the pond, it was impossible for us to determine transient fecundity per female). Consistent with other ecological traits described above, it is possible that rapid hatching times are favored in this harsh desert environment to compensate for the risk of rapid desiccation of the small ponds used (at least in this isolated area of the desert) by this species to breed. More direct observations on the life history of *R. atacamensis* may reveal interesting adaptations to the life in Atacama, and are therefore encouraged for future field studies.

## ACKNOWLEDGEMENTS

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