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Short Communication

Reproductive biology of the emerald frog *Hylorina sylvatica* (Anura: Batrachylidae) in northwest Patagonia, Argentina

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Abstract: *Hylorina sylvatica* (Anura: Batrachyidae), or the emerald frog, is a pond-breeding anuran endemic to the austral temperate forests of Chile and Argentina. It is considered a vulnerable species in Argentina, where it has a narrow distribution; records and biological information relating to the frog in this area are scarce. In this study, conducted in 2016, the reproductive parameters of the emerald frog were investigated in detail in a semi-temporary wetland of Northwestern Patagonia, Argentina. During the spring, the wetland was visited weekly in search of calling males, adults and individuals in amplexus. When amplexus was observed, eggs and larvae were collected from among the aquatic vegetation using dip-nets. Egg size and larval stage at hatching were registered. Calling males were registered during the second week of October, and the first couples were observed on 26 October. Three pairs of emerald frogs were captured and placed in enclosures within the wetlands in the afternoon. The males were smaller than the females. After 24 h the number of eggs laid in each enclosure was counted, and the males and females were released. The eggs were kept in each enclosure until hatching. Hatching occurred after 10–14 days in the enclosures. This information contributes to our knowledge of the biology of the emerald frog in Patagonia, Argentina, and is the only information currently available on this species in Nahuel Huapi National Park, where the species breeds in various aquatic environments, from semi-temporary wet meadows to large permanent ponds.
Keywords: *Patagonian anurans; wetlands; amplexus; eggs; embryonic development.*

Biología reproductiva de la rana esmeralda *Hylorina sylvatica* (Anura: Batrachylidae) en el noroeste de la Patagonia Argentina

Resumen: *Hylorina sylvatica* (Anura: Batrachyidae) o rana esmeralda, es un anuro que se reproduce en estanques, endémico de los bosques templados australes de Chile y Argentina. Actualmente es considerada una especie vulnerable en Argentina. La distribución es estrecha en Argentina con pocos registros de la especie y muy limitada información sobre la biología de esta especie en la región. En este estudio realizado durante 2016, investigué en detalle los parámetros reproductivos de la rana esmeralda en un humedal semitemporal del Noroeste de la Patagonia Argentina. Durante la primavera de 2016, visité el humedal semanalmente en busca de coros, adultos y amplexos. También después de que se observaron amplexos, recolecté huevos y larvas entre la vegetación acuática utilizando un muestreo con redes de mano. Se registró el tamaño del huevo y el estado larvario al eclosionar. Los coros de machos se registraron durante la tercera semana de octubre y los primeros amplexos se observaron el 26 de octubre. Tres parejas de rana esmeralda fueron capturadas y colocadas en clausuras dentro de los humedales durante la tarde. Los machos eran más pequeños que las hembras. Después de 24 h conté el número de huevos puestos en cada recinto y luego se liberaron machos y hembras. Los huevos se mantuvieron en cada clausura hasta que eclosionaron. La eclosión se produjo entre 10–14 días en las clausuras. Esta información contribuye al conocimiento de la biología de la rana esmeralda que habita la Patagonia Argentina y es la única información disponible al momento de esta especie en el Parque Nacional Nahuel Huapi, donde la especie se reproduce en diferentes ambientes acuáticos, desde humedales semitemporales hasta lagos y lagunas permanentes.
Palabras claves: *anuros patagónicos; humedales; amplexo; huevos; desarrollo embrionario.*

Introduction

The austral temperate forest of Chile and Argentina harbors around 62 anuran species, of which approximately 80% are endemic (Formas 1979). These species breed during spring or summer, and some species can extend the reproductive season until autumn (Formas 1979, Úbeda 1998). The endemic Batrachylidae family has a wide diversity of reproductive modes and life history traits (Jara et al. 2021, Grosso et al. 2022). Batrachylidae present a total of 12 species, belonging to four genera: *Chaltenobatrachus* and *Hylorina* with one species each and *Atelognathus* and *Batrachyla* with five species each (Formas 1997, Barrasso & Basso 2019). *Hylorina*, *Atelognathus* and *Chaltenobatrachus* are typical pond-breeding amphibians that breed during spring and summer in lakes, lagoons, ponds or small streams (Barrio 1967, Úbeda et al. 1999, Cisternas et al. 2013, Barrasso et al. 2020). In contrast, *Batrachyla* species have developed the strategy of laying their eggs in humid soils with embryonic development outside water (Cei 1980, Úbeda 1998, Lobos et al. 2013; Jara et al. 2019, 2021). The breeding phenology of this family is unknown, as well as other aspects of their biology and ecology. Since these aspects provide valuable information for conservation programs (Paton & Crouch 2002), more field studies on this batrachofauna should be carried out. For example, Paton & Crouch (2002) determined that the timing of wetland inundation and duration of the wetland can explain the success of several amphibian species in Rhode Island (USA); they recommend that biologists gather data on amphibian movement phenology in other regions to help regulators and managers develop legislation to protect the habitat of pond-breeding amphibians. The relationship between local variations in breeding phenology and climate conditions can also provide clues to various aspects of species conservation (Loman 2016).

One of the most interesting species of Batrachylidae is the emerald frog or golden frog, *Hylorina sylvatica* Bell 1843; its striking coloration is similar to that of tropical species. It also has arboreal habits, slender limbs with long, slender fingers, and opposable thumbs associated with its ability to climb (Cei 1962, Formas 1979, Rabanal & Ñuñez 2008, Charrier 2019). Endemic to the southern temperate forests of Chile and Argentina, this species is found in Chile from Ramadillas (Biobío Region) to Bernardo O'Higgins National Park on Wellington Island (Magallanes and Chilean Antarctic Region) (Donoso-Barros 1976, González et al. 2015), while in Argentina it has a more limited latitudinal distribution and is restricted to a narrow strip of forest on the eastern slope of the Andes: from Yuco, on the north shore of Lake Lácar, Lanín National Park, to the Middle Lake in Los Alerces National Park (Vellido & Úbeda 2001, Mut Coll et al. 2002).

In Argentina *H. sylvatica* has been found in some locations in Nahuel Huapi National Park (southern Neuquén Province and northwestern Río Negro Province) and in Los Alerces National Park (northern Chubut Province) (Úbeda 1998). Adults are diurnal, have arboreal habits and are found in dark areas under fallen trunks, hidden in leaf litter. During the breeding season males concentrate in open areas and call from the shoreline of the water bodies (Úbeda 1998). Larval development is prolonged (about 13 months according to Moncada 2011), hence adults breed in permanent and semi-permanent water bodies, including shallow lakes and large wet meadows (Figure 1). According to Vaira et al. (2012), this species is considered vulnerable because the number

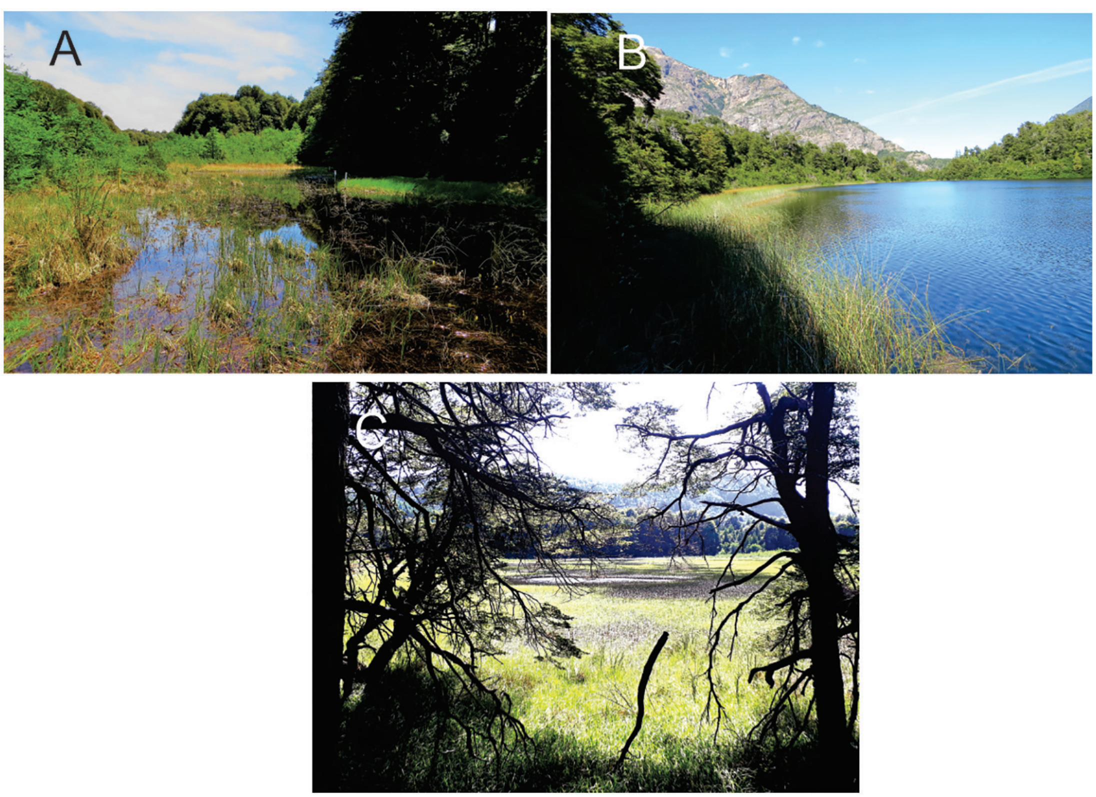

Figure 1. Aquatic environments inhabited by *H. sylvatica*: A) Llao Llao, the wet meadow where the study was conducted, B) Lake Escondido, showing the littoral zone where males call during November, and C) Mallin Goye, a large semi-temporary wetland where males call during December.

of sites they inhabit is relatively lower than in Chile, and information on the biology and ecology of Argentinian *H. sylvatica* populations is scarce in comparison with that of Chilean populations. Our aim in this work is to gather data on the reproduction and egg development of *H. sylvatica* in a population located in a protected area of Northwest Patagonia, Argentina, and then compare the life history traits of *H. sylvatica* with other syntopic species in the area.

Materials and Methods

1. Study area

This research was conducted in Llao Llao Nature Reserve (–41.0500 latitude and –71.500 longitude, 821 m elevation) in Northwestern Patagonia, Argentina. The area includes 1226 ha of a well preserved Andean Patagonian forest that receives approximately 1800 mm rainfall each year, primarily during fall and winter (May–August) (Jara 2016). Another four species of anuran amphibians have been recorded in the area: *Batrachyla taeniata*, *B. leptopus*, (Batrachylidae), *P. thaul* (Leptodactylidae), and *Rhinella spinulosa* (Bofonidae) (Moncada 2011, Jara et al. 2021). The calling activity of *Hylorina sylvatica* males goes from October to December (Moncada 2011). Data on their reproductive phenology was collected from the Llao Llao wetland, a wet meadow of around 0.16 ha with 0.38 m maximum water depth (Figure 1A). This wetland may undergo several years with permanent waters, then have years with a dry phase in summer, depending on annual precipitation. The bottom of the pond is completely covered by dense mats of *Schoenoplectus californicus*, *Carex niderdenliana* (Cyperaceae) and *Agrostis leptotricha* (Poaceae), with patches of rushes such as *Juncus balticus* and *J. invulucratus* in the center of the pond (Jara 2016).

2. Collected field data

The wetland was visited once a week from September 2016 to December 2016. Throughout the study, daily precipitation (mm) and air temperature (maximum and minimum temperature) were obtained from AIC (Autoridad Interjurisdiccional de las Cuencas de los ríos Limay, Neuquén y Negro) meteorological station in Bahía López (41° 4' 27.88" S latitude; 71° 34' 6.85" O longitude), which is located 2.75 km from the wetland.

The presence of calling males and mating pairs were considered indicators of breeding activity. The number of male calls was recorded on each sampling day (male calls were registered on 8 days). Using the Pearson product-moment correlation coefficient, this variable was then tested to determine whether it was associated with air temperature. Active searches were conducted during daylight hours (12:00–17:00 pm), and water depth, water temperature, pH and conductivity were recorded at each visit. Water temperature was measured using i-Button temperature loggers (one logger per enclosure, temperature range –40/+80 °C, precision 0.5 °C). Once the pairs were observed, sampling was carried out with a hand net in search of eggs and tadpoles. The shoreline and the deepest area of the wetland were each swept 10 times.

To study egg oviposition and the number of eggs laid by each female, three pairs were captured. Each pair was kept in an enclosure of 41 × 29 × 18 cm, consisting of a plastic basket covered with a mesh (1 mm) that allowed water flow. Enclosures were placed 1 m apart. To prevent frogs from escaping or being predated by birds, the top of the enclosure was covered with a cotton mesh. After 24 h (spontaneous separation of the breeding pairs), individuals were measured (snout-vent length) with a caliper to the nearest 0.1 mm, then released at the capture sites. All the eggs were counted in situ, and a subsample of eggs (10 per enclosure) was taken to the laboratory where their diameter, with and without jelly, was measured (to the nearest 0.01 mm) using a stereoscopic microscope with an ocular micrometer. The eggs were then returned to the field (to each enclosure) and kept there until they had all hatched. The newly hatched larvae were released into the pond.

Results

There were two distinct rainfall peaks during September and November (Figure 2). Reproductive activity started in the second week of October, with calling males registered after precipitation on sunny days. The males were observed calling from the shoreline of the wetland at noon, when the air temperature was relatively high (Figure 3A). The number of male calls increased with air temperature (Pearson correlation = 0.891, p = 0.003, n = 8); no calling was recorded on cold days (air temperature below 10 °C). The first breeding pairs were observed on October 26, and five more the following week. The *H. sylvatica* pairs (in axillary amplexus, Figure 3D) were detected easily due to their conspicuous coloration, whether partially concealed in the

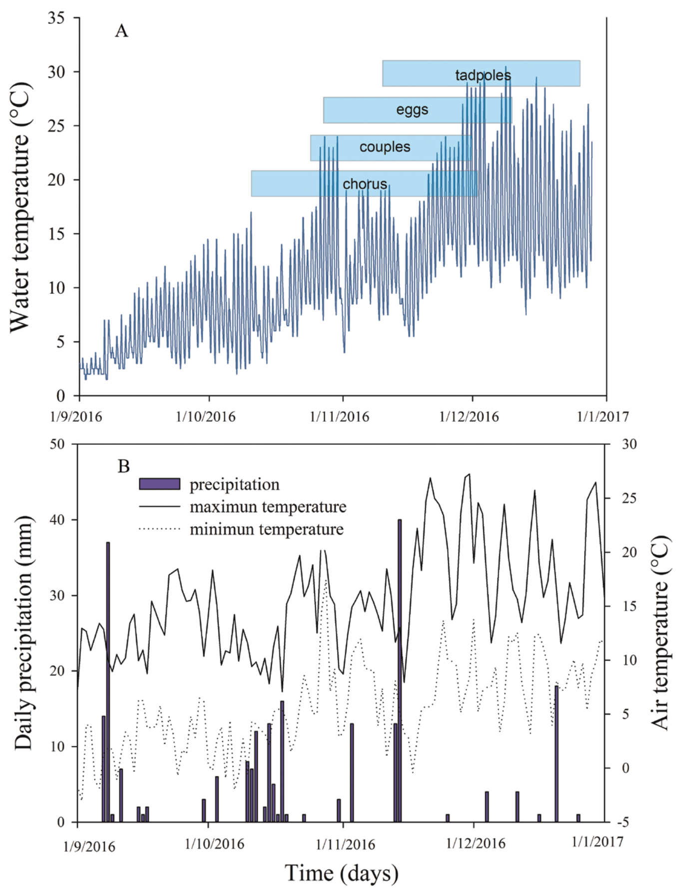

Figure 2. Breeding phenology of *H. sylvatica* in relation to climatic conditions and water temperature of the wetland. A) shows the duration of each phenological event with horizontal bars, and water temperature in spring 2016, and B) shows accumulated precipitation per day and air temperature during the study period.

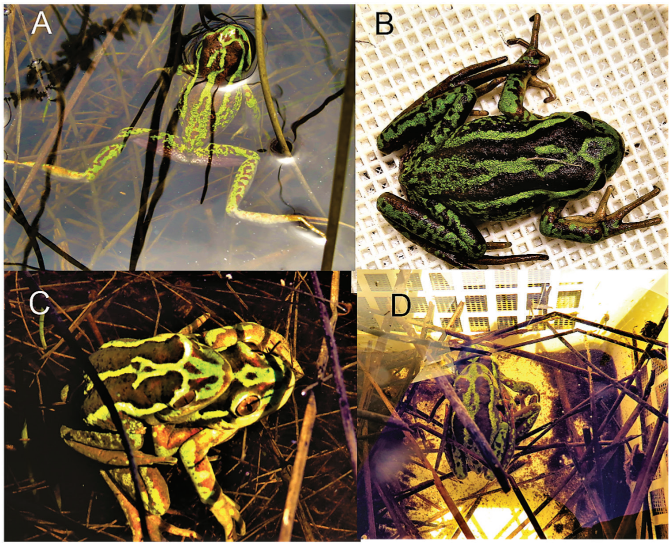

Figure 3. *Hylorina sylvatica*: A) adult calling from the littoral zone of the wetland, B) captured female, C) axillary amplexus (note differences in size between male and female) and D) breeding pair captured and placed in enclosure in the field.

vegetation or moving actively in deep areas. One pair was observed over a period of one hour (14:00 pm–15:00 pm, air temperature 22 °C ± 1) on October 26. I followed the pair with the camera zoom lens and recorded the time. The female swam actively while the male only clung to the female throughout the entire observation time. The female made wavy paths in the center of the pond as she swam. She stopped swimming frequently and submerged for a few minutes (3 minutes ± 2), then she surfaced again, apparently to breathe, before continuing to swim.

The *H. sylvatica* males observed were smaller than the females (Figure 3A, B) (male size 53.32 mm ± 3 mm (n = 5) and female 60.6 mm ± 0.9 mm (n = 4); t = –4.5, p = 0.003). From the fourth week of October and the second week of November I collected 118 eggs and captured 41 tadpoles. The eggs collected were adhered to submerged vegetation at depths of between 18 and 22 cm, whereas the tadpoles were captured in densely vegetated patches at depths of between 20 and 35 cm.

Three breeding pairs of *H. sylvatica* were captured and kept in the enclosures (Figure 3C), one on the 5th of November and two on the 18th. The average number of eggs per enclosure was 74 ± 11, and they were attached to vegetation stems or lying in the bottom of the enclosure. The diameter of the eggs without jelly was 2.35 ± 0.45 mm (n = 30) and the diameter with jelly was 3.92 ± 0.36 mm (n = 30). The eggs inside the enclosure developed in neutral pH (mean = 6.88 ± 0.37, range 6–7.5), low conductivity (mean = 69.2 ± 14.8 SD μs.cm^{-1}) and well oxygenated water (mean = 6.8 range 5–8 mg/l). The water temperature during egg development was 13.1 ± 9.1 °C; the minimum temperature was recorded during the mornings (5 °C) and the maximum in the afternoons (28 °C). Time to hatching was 10–14 days inside the enclosure and approximately 16 days in the pond. In the enclosures, approximately 76 % of the eggs hatched. Neither eggs parasitism by fungus nor predation by invertebrate was observed in the enclosures.

Discussion

The results shown here indicate that some aspects of the life history of the frog *H. sylvatica* reflect local variations in reproductive phenology and egg oviposition. Indeed, breeding activity has been observed during December and January in Chilean populations (Barrio 1967, Formas 1979); however, in this study males were observed calling in the Llao Llao wetland from the middle of October until December. The beginning of the breeding season varies from year to year in the area, and this variation is probably related to temperature and the amount of precipitation. The number of eggs laid was registered from late October until the end of November, when the wetland was drying more quickly. The eggs in the enclosures were found on the bottom or adhered to the stems of plants (*Carex* spp.), either individually or in groups of two or four eggs. The behavior observed in the *H. sylvatica* pairs indicates that during mating the eggs are dispersed throughout the pond, into both shallow and deep areas. This observation was also supported by the location of the eggs collected; they were observed individually at the bottom or attached to the vegetation, as in the enclosures. These results differ from those obtained by Barrio (1967), who describes the eggs of *H. sylvatica* as a gelatinous mass. Barrio (1967) observed *H. sylvatica* eggs laid in the water near the shore of a wetland in Chile, reported that the eggs were initially placed independently, but he noticed that after the first few days they tended to stick together. The number of eggs laid by each female (70–90) was lower than the number reported by other authors (400–600 eggs; Formas 1979, Charrier 2019) It is possible that the number of eggs was underestimated in this study. The enclosures were small and amplexant pairs observed in the study wetland swam long distance (around 9 meters in one hour) to disperse the eggs into the pond; this may have affected their behavior and consequently the number of eggs laid. The number of reproductive pairs analyzed was also low. Some aspects of reproduction in *H. sylvatica* require further investigation, to determine the real number of eggs that one female can produce.

During incubation of the eggs in the enclosures, no fungus infection was observed. Although two leech species (*Helobdella fantasmae* n. sp. and *H. nahuelhuapensis*) and a high density of aquatic beetles (*Rhantus antacticus nahuelis*, *R. signatus*, *Lancetes flavipes*) were observed during the sampling period, none of these predators were seen to feed on *H. sylvatica* eggs. However, recent investigation has shown that *H. sylvatica* tadpoles are predated by some aquatic insects, such as diving beetle larvae, dragonfly naiads and water bugs (Úbeda et al. 2021), and this is supported here by the tail damage shown by several captured tadpoles. Dispersion of the eggs in the pond by amplexant pairs during could be interpreted as a strategy to avoid predation by invertebrates and to prevent eggs from being colonized by fungus. Because the eggs are placed individually and are relatively small, they are difficult for predators to detect, which also reduces the impact of fungus contamination. In the years 2005–2006 and 2009–2010, *H. sylvatica* was able to complete its life cycle in this pond: as a result of heavy rains the pond did not dry out for two consecutive years (Moncada 2011, Jara et al. 2021). This meant that in 15 years only two breeding seasons were successful for this species in this wetland (Moncada 2011, Jara & Úbeda pers. obs.). It is important to know how species like *H. sylvatica* respond to an altered hydroperiod so as to predict how this species and other amphibians that breed in diverse

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Table 1. Summary of life history traits of sympatric and syntopic anuran species found in the study area (from Moncada 2011, Moncada & Úbeda 2020, Jara et al. 2018 and 2021 and Jara unpublished data*).

	Life history traits							
Anuran species	**Egg diameter (mm)**	**Clutch size**	**Type of egg**	**Parental care**	**Breeding season**	**Overwintering tadpoles**	**Larval period (months)**	**Metamorphosis weight (gr)**
Hylorina sylvatica	2.35 ± 0.45*	74 ± 11*	aquatic eggs laid individually*	no	Spring*	yes	12–13	no data available
Batrachyla taeniata	2.75 ± 0.32	108 ± 64	terrestrial eggs laid together in humid soil	yes	Summer–Fall	yes	8–9*	0.46 ± 0.05*
Batrachyla leptopus	no data available	70 ± 56*	terrestrial eggs laid together in humid soil	yes	Summer–Fall	yes	8–9*	0.49 ± 0.1*
Pleurodema thaul	1.58 ± 0.12	452 ± 172	aquatic eggs in a gelatinous mass attached to submerged vegetation	no	Spring	no	3–4*	0.62 ± 0.10*

aquatic habitats may respond to the influence of climate change on aquatic ecosystems.

Hylorina sylvatica sometimes shares the habitat with other species of the Batrachylidae family, such as *Batrachyla leptopus* and *B. taeniata,* and also with the most abundant lepdodactylid frog in the region, *Pleurodema thaul*. In Chilean populations, tadpoles of *H. sylvatica* have been seen to coexist with *Bufo variegatus* (*Nannophryne variegata*), *Batrachyla taeniata*, *Batrachyla leptopus*, and *Batrachyla antartandica* (Formas & Pugin 1978). Also in Chile, *H. sylvatica* was observed in lotic water bodies and temporary ponds (Formas & Pugin 1978, Veloso & Nuñez 2003). This shows that the species can use a great variety of habitats for breeding, and can therefore be considered a versatile species. Tadpole habitat can vary greatly in environmental conditions; for example, Formas & Pugin (1978) reported that in Chile, tadpoles of this species were found in acidic waters (pH 5.2).

The breeding phenology and reproductive strategy of *H. sylvatica* differ considerably from those of other species of the same family (Table 1). For example, the breeding cycle of *Batrachyla taeniata* and *B. leptopus* lasts from mid-January to April; the eggs are terrestrial and larger than those of *H. sylvatica* (Table 1). Moreover, clutches are cared for by the males in *Batrachyla*. Clutch size in *H. sylvatica* (around 500 eggs, Formas (1979) is larger than that of *Batrachyla* species (between 70 to 108 eggs per clutch, Table 1), which is not surprising considering that *H. sylvatica* do not take care of their eggs. However, both genera have overwintering tadpoles and long larval development (Table 1), (Moncada 2011). *Batrachyla* do not present overlapping of tadpole cohorts, whereas in *H. sylvatica* large premetamorphic tadpoles from one season frequently coexist with early-hatching tadpoles of the following season (Moncada 2011). Although *P. thaul* is a pond-breeding anuran like *H. sylvatica, P. thaul* eggs are smaller (Table 1) and are oviposited in a gelatinous string that can curl up in the aquatic vegetation, secondarily adopting a globular shape (Moncada & Úbeda 2020). For a short period of time tadpoles of four species coexist in the same wetland, therefore determining the diet of each species is essential to evaluate whether the species compete for resources. In addition, the differences in phenology between these species generates body-size asymmetries in the tadpole community (i.e., tadpoles of different sizes and stages coexist), which probably leads to interference between tadpoles of different sizes or predation of large tadpoles on smaller ones, as occurs in other amphibian communities (Heyer et al. 1975, Griffiths et al. 1991, Faragher & Jaeger 1998).

Finally, *H. sylvatica* was also observed in other aquatic habitats in the study area. For example, a high abundance of calling males was detected in the littoral zone of lake Escondido and in a semi-permanent wet meadow (Mallin Goye); both these water bodies are larger and deeper than Llao Llao wetland. The breeding phenology and dynamics of these larval populations may differ from those of the Llao Llao populations, since lake Escondido has fish predators (the native *Percichthys trucha* and exotic salmonid species *Oncorhynchus mykiss*, both voracious predators, Macchi et al. 2007) and large odonate naiad predators in littoral areas (Jara unpublished data), while Mallin Goye has many predatory invertebrates (Jara unpublished data). Predation therefore appears to be the main constraint regulating the survival rate of *H. sylvatica* in these habitats. More studies are necessary to analyze how hydroperiod and predation drive the larval population of this frog in local populations.

Acknowledgements

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Associate Editor

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Conflicts of Interest

I have no conflict of interest related to the publication of this manuscript.

Ethics

All applicable institutional and/or national guidelines for the care and use of animals were followed.

Data Availability

Supporting data are available at <http://rdi.uncoma.edu.ar/handle/uncomaid/16689>

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