Herpetological Conservation and Biology 12(2):410–421.
Submitted: 15 August 2016; Accepted: 20 May 2017; Published: 31 August 2017.

Habitat Associations for the Endangered Frog *Atelognathus patagonicus* Within the Aquatic Environment: Key Microhabitats for Conservation

María E. Cuello[2], Carmen A. Úbeda, and María T. Bello[1]

Centro Regional Bariloche, Universidad Nacional del Comahue, 8400 San Carlos de Bariloche, Argentina
[1]Deceased
[2]Corresponding author e-mail: melenacuello@gmail.com

Abstract.—**The Laguna Blanca Aquatic Frog, *Atelognathus patagonicus* (Batrachylidae), is an endangered species endemic to a system of endorheic (i.e., no outlet) ponds in northwest Argentine Patagonia and is threatened mainly by habitat alteration. This study assesses the microspatial distribution of the seven ontogenetic classes of *A. patagonicus* in a structurally complex pond to determine the relative importance of the different aquatic microhabitats. We recognized 10 aquatic microhabitats with different substrate types and presence of macrophytes. We sampled the *A. patagonicus* population in summer using aquatic funnel traps, and quantified the relative abundance of each ontogenetic class per microhabitat. Ontogenetic classes differed in their distributions among the microhabitat types. We collected the greatest number of individuals from vegetated and rocky microhabitats in the peripheral zone; early tadpole stages were most abundant in the vegetated microhabitat, whereas metamorphosing tadpoles, metamorphs and post-metamorphs were most abundant in the rocky microhabitat. In the central zone of the pond, we collected most tadpoles from vegetated microhabitats at mid-depth and bottom levels, and very few post-metamorphs from the bottom, either with or without vegetation. We found no metamorphosing tadpoles or metamorphs in the central zone. *Atelognathus patagonicus* thus appears to have complex habitat requirements reflecting the distinct demands of each stage in its ontogeny. The three-dimensionally complex macrophyte *Myriophyllum quitense* and rocky pond bottoms are key components for the survival of *A. patagonicus*, which rarely uses simple, largely exposed, microhabitats. Thus, vegetated and rocky microhabitats should be protected to contribute to the conservation of this species.**

Key Words.—Anura; conservation; endangered species; microhabitat complexity; Patagonia; stages distribution; threatened habitat

Introduction

The use of specific microhabitats by a species is one of the mechanisms that enable the maintenance of high population density and optimum exploitation of available resources (Schoener 1974; Toft 1985). Some habitats are physically complex and provide qualitatively different living space to that available in physically simple habitats. Such diversity of living space should increase the number of niches and consequently increase species richness (Douglas and Lake 1994; Downes et al. 1998).

In aquatic environments, the presence of macrophytes increases habitat complexity and creates a wide range of niches that support more diverse and abundant communities than do non-vegetated areas (Grenouillet et al. 2002; Tarr and Babbitt 2002; Phiri 2010; Thomaz and Cunha 2010). The structural complexity created by macrophytes provides shelter, feeding sites, and physical structure for breeding and/or nesting for aquatic animals, as well as a substrate for periphyton and small associated animals (Cronin et al. 2006; Menone 2015). Rocky bottoms and surface irregularities such as pits, crevices, moss, and other projections create structural complexity that may affect food supply and provide shelter, and have been recognized to exert significant influence on the abundance and diversity of benthic invertebrates (Taniguchi and Tokeshi 2004).

Amphibians may display distribution patterns among microhabitats according to the requirements of their different developmental stages (Alford 1986; Holomuzki 1986; Warkentin 1992). The use of different microhabitats has been widely documented for tadpoles and post-metamorphic individuals (Alford and Crump 1982; Parris 2001; Hamer et al. 2002; Nájera-Hillman et al. 2009; Genova 2011). *Atelognathus patagonicus* (Batrachylidae) is an endemic frog from north-western Argentinean Patagonia. It is classified as Endangered by the International Union for the Conservation of Nature (IUCN 2010). It lives in permanent and temporary water bodies in a system of endorheic (i.e., no outlet) ponds located on the arid basalt plateaus of Laguna Blanca National Park (LBNP) and surrounding areas in mid-western Neuquén Province (38°55´S to 39°08´S; 70°05´W to 70°30´W; Cuello et al. 2006, 2009b), Argentina.

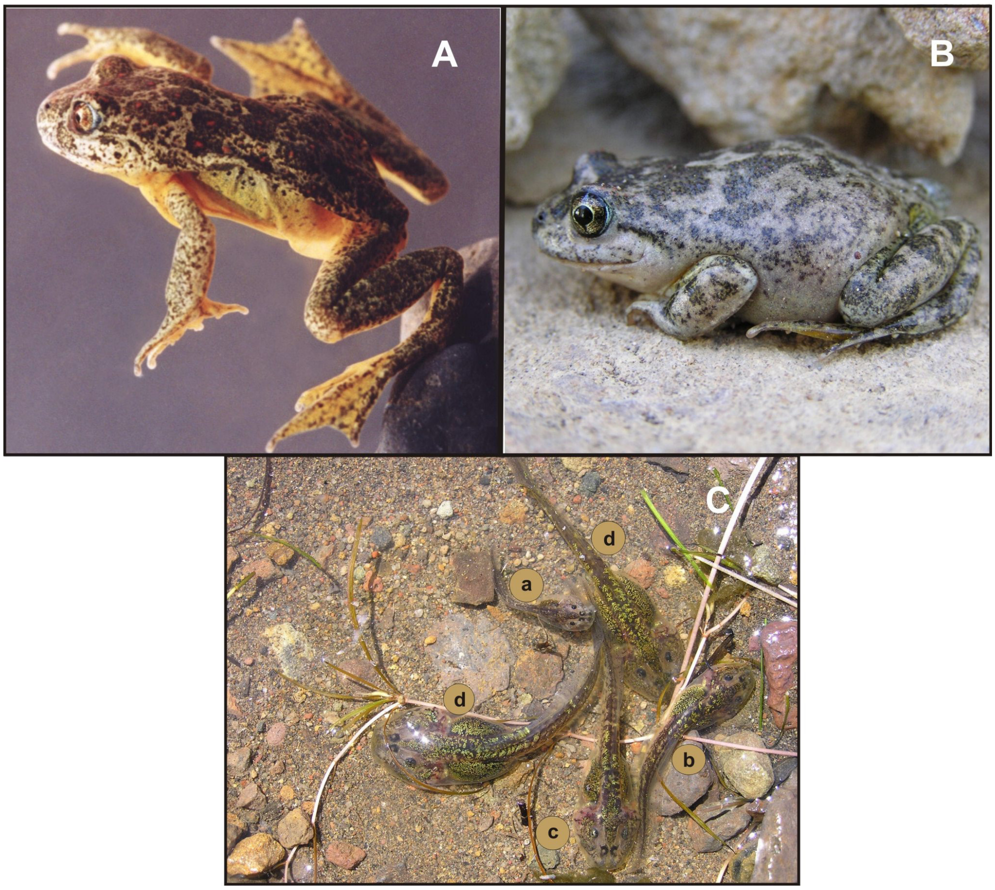

Figure 1. A) Adult *Atelognathus patagonicus* of aquatic morphotype; notice the well-developed cutaneous folds, extensive interdigital membranes, and orange-yellow ventral surface. B) Adult of littoral morphotype; notice the small cutaneous folds, little developed interdigital membranes, and a grayish ventral surface. C) Tadpoles belonging to different classes of development: a = Class I, b = Class II, c = Class III, d = Class IV . (A, photographed by Carmen Úbeda; B, photographed by Manuela Calvo; and C, photographed by Gonzalo Ignazi).

Atelognathus patagonicus has two morphotypes (Cei and Roig 1968). The aquatic form (Fig. 1A) has well developed cutaneous folds, extensive interdigital membranes, and an orange-yellow ventral surface, whereas the littoral form has small cutaneous folds, less-developed interdigital membranes, and a grayish ventral surface (Fig. 1B). Cuello et al. (2008) discovered that the two *A. patagonicus* morphotypes described above are reversible, and that individuals are able to change morphology to both aquatic and terrestrial environments in response to environmental changes. When the aquatic morphotype is expressed, the entire life cycle of *A. patagonicus* can take place in the water, which means that all ontogenetic stages can coexist in the same environment, and only metamorphs leave the water for a short time. The aquatic morphotype predominates in the population when there is water in the ponds (Cuello et al. 2008). In permanent ponds, tadpoles present two strategies (Fig. 1C): seasonal tadpoles (short larval period, metamorphs in the same growing season) and overwintering tadpoles (long larval period, tadpoles undergoing metamorphosis the following spring; Cuello et al. 2014).

On the shores of ponds inhabited by *A. patagonicus*, there are columnar basalt cliffs alternating with areas of medium to large overlapping volcanic rocks and gently sloping areas with fine sediment, sometimes combined with coarse sediment (Cei and Roig 1966; Roquero 1968; Villamil and Testoni 2012). These different substrates create a variety of aquatic habitats in the peripheral zone of the pond. In particular, the rocky bottom provides shelter and enables the development of periphyton and a variety of microfauna (Cuello et al. 2009c). In the central zone of the pond, the noticeable development of submerged rooted Andean Watermilfoil (*Myriophyllum quitense*) increases the structural complexity of these ponds (Cei and Roig 1966; Daciuk 1968).

Knowledge of the microdistribution of *A. patagonicus*, particularly in water, is limited. Available information is particularly incomplete regarding potential differing use of pond microhabitats, and associated micro-distribution patterns with ontogeny.

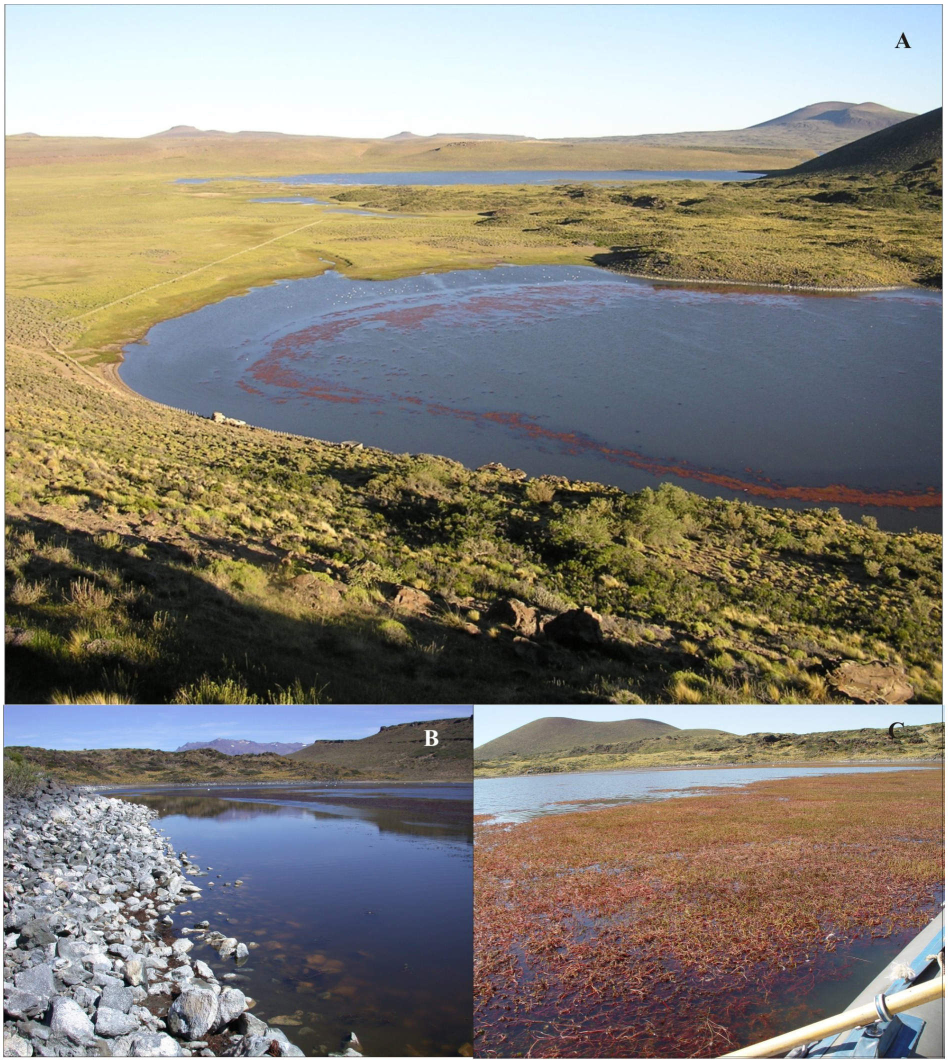

Figure 2. Laguna Verde Pond, Laguna Blanca National Park, Neuquén Province, Argentina. A) General view. Notice the reddish ring of the macrophyte *Myriophyllum quitense*. B) Rocky microhabitat in the peripheral zone. C) Segment of the *M. quitense* ring in the central zone, where the macrophyte grows and forms a complex three-dimensional structure. (A, photographed by Gonzalo Ignazi; B and C, photographed by María Elena Cuello).

In ponds with environments where there are different types and levels of structural complexity, the different *A. patagonicus* developmental stages could be expected to use a range of microhabitats in distinctive ways. Thus, the overall aim of this study is to investigate the spatial distribution of various developmental stages of *A. patagonicus* in the aquatic environment, and assess the relative importance of each microhabitat for providing conditions appropriate for the persistence of individuals and the population. Such information would guide the implementation of measures to preserve the environmental conditions essential to the conservation of the species. With this aim, we analyzed the use of the aquatic environment in a pond typical of the system and inhabited by *A. patagonicus*, and characterized by high environmental complexity.

Materials and Methods

Study site.—We conducted the study in Laguna Verde pond in Laguna Blanca National Park, Neuquén Province, Argentina (39°01'S, 70°23'W, 1,250 m above sea level; Fig. 2). This pond is located in a flat basin. The water level varies noticeably, both seasonally and from year to year, with complete drying in some exceptional years. As a result of fluctuation in level, during the decade 2000−2010, pond surface area ranged from 15 to 21 ha and the perimeter ranged from 1.5 to 2.5 km. We recorded maximum depth (4.5 m) in February 2004 and January 2006. The pond freezes over in winter, while at midday in summer the water temperature at the surface may be over 20° C. Westerly winds, which favor erosion, are frequent and particularly strong and dry in spring and summer. Gusts of wind stronger than 100 km/h increase the waves. Plentiful macrophytic vegetation tends to reduce water movement and promote the capture and stabilization of nutrients, forming sediments (Cronin et al. 2006). The water is turbid due to suspended matter and microscopic algae (Cuello et al. 2009c).

In the pond we identified a peripheral zone and a central zone, each with its own particular microhabitats (Fig. 2; Appendix). The peripheral zone goes from the shoreline to the outer edge of the rooted *Myriophyllum quitense* aquatic vegetation. The central zone goes from the latter point to the interior of the pond. The peripheral zone is composed of 32% large overlapping boulders (≥ 45 cm; Fig. 2B), 42% fine sediment with herbaceous plants, 17% boulders and cobbles scattered over fine sediment, and 9% *M. quitense* and *Tetrachondra patagonica*. *Myriophyllum quitense* (Haloragaceae) is a submerged rooted macrophyte that grows in small clumps. *Tetrachondra patagonica* (Tetrachondraceae) is a pygmy species typical of Mallines (flood meadows) surrounding the ponds with decumbent stems, forming dense turf (Villamil and Testoni 2012).

In the central zone, *M. quitense* flourishes, forming a ring parallel to the shore, which can be seen very clearly in summer (Fig. 2C). Within the ring it forms dense clumps, branching out in a complex three-dimensional structure. Branches are often broken off by waves and washed to shallow waters and eventually to the shore, where they create a drift line of rotting vegetation. In late summer and early autumn the plants become senescent, with leaves and stems dying, contributing to additional detritus and water turbidity. The following spring they sprout again and recover their foliage.

The most abundant aquatic vertebrates are the frog (*A. patagonicus*), which lives in the pond permanently, and many migratory aquatic birds, among which are grebes (*Podiceps occipitalis* and *Rollandia roland*) that prey on *A. patagonicus* (Cuello et al. 2005). Like all ponds inhabited by *A. patagonicus*, Laguna Verde hosts no fish. Laguna Verde pond is protected from grazing and trampling by livestock present in the area by a woven wire perimeter fence built by the Laguna Blanca National Park in 2004 (Trova and Galván 2012).

Field methods. —We characterized the microhabitats in the peripheral and central zones in the pond by three physical or abiotic variables (depth, slope, and substrate type) and three biotic variables (*M. quitense* plants, drifting *M. quitense* branches, and filamentous algae). We calculated the bottom slope in degrees and describe substrate types according to grain size following Wentworth's grain size classification (Wentworth 1922). We classified 10 aquatic microhabitats in this pond: four in the peripheral zone and six in the central zone (Appendix). In the central zone, which is deeper, we distinguished three microhabitats at different depths within the ring of aquatic vegetation and another three microhabitats at different depths of the water column in non-vegetated open areas.

We sampled tadpoles and frogs in January 2006, using plastic funnel traps (11 cm diameter, 30 cm long) with a single funnel. Animals enter traps via an inward-directed plastic cone 8 cm long. Mouth and apical openings were 11 and 2.5 cm in diameter, respectively. This method has proven to be effective for sampling aquatic amphibians (Shaffer et al. 1994; Smith and Rettig 1996; Holomuzki 1986) and was used in previous *A. patagonicus* sampling for capturing different aquatic stages (Cuello et al. 2008, 2009a,c, 2014). All sampling sessions lasted from late afternoon to early morning.

Sampling.—We placed 30 aquatic traps in each microhabitat type in the peripheral zone, arranged in 10 sets of three traps. We placed each set perpendicular to the shoreline. The sets rested horizontally on the bottom, not deeper than 0.8 m, and were spaced at 5 m intervals from each other (Shaffer et al. 1994).

We sampled in the central zone in vegetated areas and open areas devoid of vegetation. We arranged funnel traps in vertical sets of three traps to provide data from three levels: top (0.2 m), mid-depth (0.8 m), and bottom (2.5−4.5 m deep). We used a row boat to place the sets of traps at 5 m intervals: eight in vegetated areas and seven in areas without aquatic vegetation. The traps in each set were joined by a rope. In each set, the surface trap was attached to two buoys and the deep trap was anchored to the bottom with two weights.

We counted the individuals collected and recorded their developmental stage (Gosner 1960) with the aid of a magnifying glass (8 ×), after which we released them at the sites where we had captured them. We grouped the tadpoles and metamorphs into six classes, following Cuello et al. (2014): tadpoles: Class I (stages 26−30), Class II (stages 31−34), Class III (stages 35−37), Class IV (stages 38−41), and Class V (stages 42−45); and metamorphs: Class VI (stage 46). Post-metamorphs, including juveniles and adults, formed Class VII, in which we included both aquatic and littoral morphotypes.

Statistical analysis.—We calculated relative abundance (expressed as individuals/trap) for each developmental class in each microhabitat. To test whether there were differences in relative abundance of individuals among the different microhabitats in each zone, we used the non-parametric Kruskal-Wallis test (because the data did not meet the assumptions of normality) and Tukey's test for *a posteriori* multiple comparison (Zar 1984). In the central zone, we compared relative abundance of individuals between

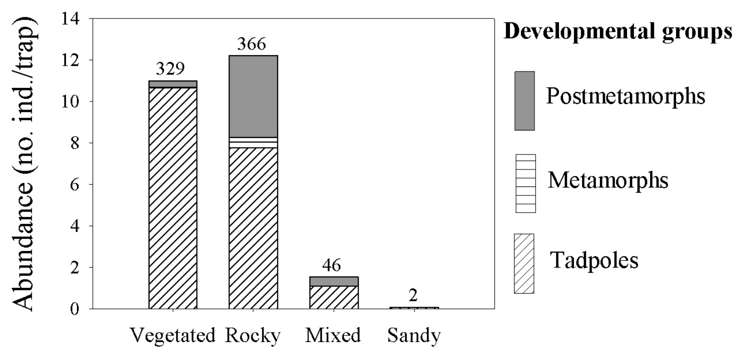

FIGURE 3. Abundance (number of individuals per trap) of *Atelognathus patagonicus* in microhabitats in the peripheral zone of the Laguna Verde pond, Laguna Blanca National Park, Neuquén Province, Argentina, by developmental group (tadpoles, metamorphs, and post-metamorphs) in January 2006. Total number of individuals in each microhabitat is indicated at top of each bar. The designation Mixed is a habitat that is a combination of rocks and sand.

vegetated and non-vegetated areas, as well as among depths.

Within each zone (peripheral or central) we compared relative abundance of individuals from each developmental class among microhabitats using Kruskal-Wallis. For *a posteriori* multiple comparisons, we used Tukey's test in the peripheral zone and Dunn's Method in the central zone. In the central zone, we used Dunn's Method to compare between vegetated and non-vegetated areas, as well as among depths (Zar 1984).

RESULTS

Distribution of individuals per microhabitat in the peripheral zone.—We captured 743 individuals in the peripheral zone, mainly in the rocky microhabitat and the vegetated microhabitat (Fig. 3). We found tadpoles, metamorphs and post-metamorphs in the rocky microhabitat, and mainly tadpoles in the vegetated microhabitat. All post-metamorphic individuals captured in the peripheral zone were of the aquatic morphotype. Overall, the relative abundance of individuals differed significantly among microhabitats (H = 57.36, df = 3, P < 0.001). The number of tadpoles in rocky and vegetated microhabitats differed significantly from the number in mixed and sandy microhabitats (Table 1).

TABLE 1. *A posteriori* multiple comparison method (Tukey test; q) of relative abundance (individuals/trap) of the microhabitats of *Atelognathus patagonicus* in the peripheral zone of the Laguna Verde pond, Laguna Blanca National Park, Neuquén Province, Argentina, in January 2006. The designation Mixed is a habitat that is a combination of rocks and sand. Asterisks indicate a significant difference at $P \leq 0.05$.

Comparison	*q*
Rocky vs. Mixed	4.28*
Rocky vs. Sandy	7.59*
Rocky vs. Vegetated	1.65
Vegetated vs. Mixed	5.93*
Vegetated vs. Sandy	9.24*
Mixed vs. Sandy	3.31*

We found a high proportion of Class I tadpoles in the vegetated microhabitat (Fig. 4). Class I was very poorly represented in the whole capture sample. Abundance of Class II tadpoles was highest in the vegetated microhabitat (Fig. 4). Overall, the relative abundance of Class II tadpoles differed significantly among microhabitats (Table 2). The number of individuals in vegetated and other microhabitats differed significantly (Table 2). Abundance of Class III tadpoles was highest in the vegetated microhabitat followed by rocky (Fig. 4). Relative abundance of individuals differed significantly among the four microhabitats (Table 2). For Class III tadpoles, there were significant differences in the number of individuals between vegetated and the other three microhabitats, and between rocky and sandy microhabitats (Table 2).

We captured Class IV tadpoles in three microhabitats in the peripheral zone. Their abundance was highest in the rocky and vegetated microhabitats (Fig. 4). There were significant differences in the number of Class IV tadpoles recorded among microhabitats and the number in vegetated and rocky microhabitats differed significantly from the other two microhabitats (Table 2). We captured Class V tadpoles in three microhabitats. They were most abundant in rocky and mixed microhabitats (Fig. 4). The number of Class V tadpoles in different microhabitats differed significantly and the relative abundances differed significantly between rocky and other microhabitats (Table 2).

We captured a high proportion of the metamorphs (Class VI) in the rocky microhabitat (Fig. 4). Class VI was very poorly represented in the captures. We recorded post-metamorphic individuals (Class VII) mainly in the rocky microhabitat. Captures in mixed and in vegetated microhabitats were low (Fig. 4). The

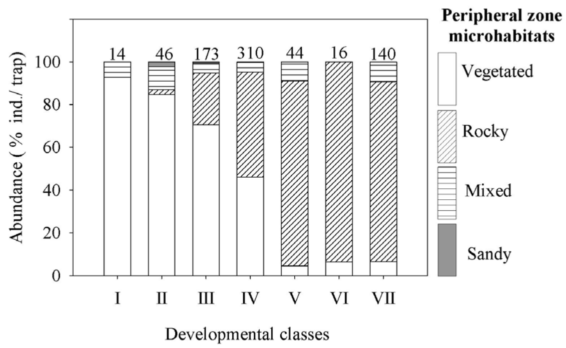

Figure 4. Abundance (% of individuals per trap) of each *Atelognathus patagonicus* developmental class in microhabitats in the peripheral zone of the Laguna Verde pond, Laguna Blanca National Park, Neuquén Province, Argentina, in January 2006. Total number of individuals of each developmental class is indicated at the top of each bar. The designation Mixed is a habitat that is a combination of rocks and sand.

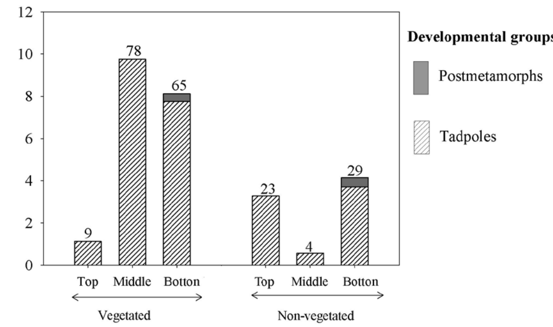

Figure 5. Abundance (number of individuals per trap) of *Atelognathus patagonicus* in microhabitats in the central zone of the Laguna Verde pond, Laguna Blanca National Park, Neuquén Province, Argentina, for tadpoles and post-metamorphs in January 2006. Metamorphs were absent and the total number of individuals captured in each microhabitat is indicated at the top of each bar.

number of post-metamorphic individuals in the four peripheral microhabitats differed significantly with the number in rocky microhabitats differing significantly from sandy and vegetated microhabitats (Table 2).

Distribution of individuals per microhabitat in the central zone.—We captured 208 individuals, mostly tadpoles, in the central zone, which comprised vegetated and non-vegetated microhabitats at three depths. Relative abundance was highest in the vegetated middle and vegetated bottom microhabitats (Fig. 5). All post-metamorphic individuals captured in the central zone were of the aquatic morphotype. Overall, the relative abundance of individuals differed significantly among microhabitats (H = 127.95, df = 2, P < 0.001). The abundance of individuals differed between vegetated and non-vegetated microhabitats (q =10.11, P < 0.05), among depths (q = 15.15, P < 0.05) and between vegetated and non-vegetated microhabitats at different depths (q = 5.05, P < 0.05).

We recorded Class I tadpoles in two microhabitats: vegetated bottom and vegetated middle, with very low abundance (Fig. 6). Class II was poorly represented in the captures, being recorded in four microhabitats (Fig. 6). Abundance was highest for vegetated bottom and vegetated middle. We captured Class III tadpoles in all six microhabitats in the central zone and mean numbers ranged from 1.3 to 3.3 individuals/trap (Fig. 6). Differences in the number of tadpoles among microhabitats were not significant (H = 7.57, df = 5, P = 0.275).

We also captured Class IV tadpoles in all the microhabitats in the central zone (Fig. 6). Abundance was highest for vegetated middle and lowest in vegetated top and non-vegetated middle microhabitats (Fig. 6). Overall, the abundance of Class IV tadpoles differed significantly among the six microhabitats (H = 16.49, df = 5, P = 0.006) and there were significant differences between vegetated middle and non-vegetated middle (q = 2.99, P < 0.05). We did not capture any individuals

Table 2. Results of Kruskal-Wallis tests, H (values across the top) and *a posteriori* multiple comparison method Tukey tests, q (in body of table), of relative abundance (individuals/trap) of *Atelognathus patagonicus* in the peripheral zone microhabitats for each developmental class in the Laguna Verde pond, Laguna Blanca National Park, Neuquén Province, Argentina, in January 2006. Developmental classes are defined in text. Classes I and VI are not included in the table due to small sample size. The designation Mixed is a habitat that is a combination of rocks and sand. Asterisks indicate a significant difference at $P \leq 0.05$ (*) or $P \leq 0.001$ (**).

	Developmental Classes				
	II	III	IV	V	VII
Comparisons	47.55**	52.97**	46.33**	37.50**	29.08**
Rocky vs. Mixed	0.65	3.35	4.84*	4.19*	3.55
Rocky vs. Sandy	0.28	4.41*	6.64*	5.11*	5.90*
Rocky vs. Vegetated	5.43*	3.72*	0.35	4.53*	3.77*
Vegetated vs. Mixed	4.78*	7.07*	5.19*	0.333	0.22
Vegetated vs. Sandy	5.72*	8.13*	6.99*	0.583	2.13
Mixed vs. Sandy	0.93	1.06	1.8	0.92	2.35

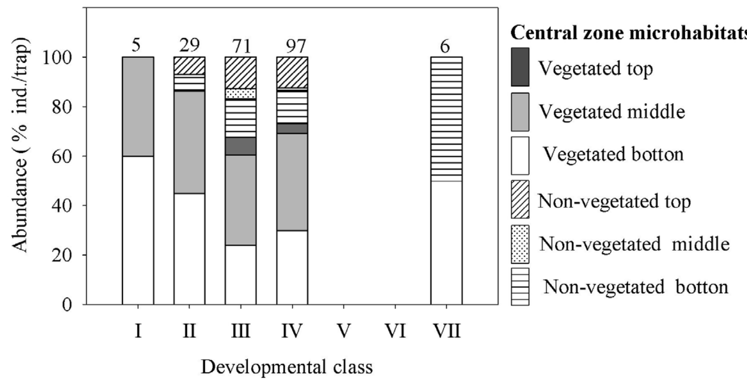

Figure 6. Abundance (% of individuals per trap) of each *Atelognathus patagonicus* developmental class in microhabitats in the central zone of the Laguna Verde pond, Laguna Blanca National Park, Neuquén Province, Argentina, in January 2006. Classes V and VI absent. The total number of individuals in each developmental class is indicated at the top of each bar.

belonging to Classes V and VI in the central zone (Fig. 6). We captured Class VII (post-metamorphic) individuals in equal numbers in vegetated bottom and non-vegetated bottom microhabitats (Fig. 6). Class VII was very poorly represented in the captures (Fig. 6).

Discussion

This is the first investigation of the distribution of *Atelognathus patagonicus* frogs and tadpoles among different aquatic microhabitats in a typical pond. Some of the microhabitats were highly complex, particularly regarding substrate heterogeneity and macrophyte development. Frog and tadpole spatial distribution is thus associated with a wide range of microhabitats. The use of funnel traps enabled a wide range of aquatic habitats in the pond to be sampled, even those where particular environmental complexity makes the use of other sampling techniques difficult. This methodology enabled efficient sampling of all *A. patagonicus* developmental stages except metamorphs, which must be captured by land sampling (Cuello et al. 2014).

The different types of substrate in the peripheral zone are strongly associated with abundance and distribution of individuals according to ontogenetic stage. The rocky microhabitat is composed of numerous overlapping boulders of different sizes that provide a large number of interstices where frogs and more advanced tadpoles are usually located, as previously noted by Fox et al. (2005). In the mixed microhabitat, rocks are fewer and smaller, which may be why *A. patagonicus* used it less. The sandy microhabitat consists of smaller, evenly distributed particles and none of the ontogenetic stages of *A. patagonicus* used it.

In the central zone of the pond, where the macrophyte *M. quitense* is well-developed and forms a ring of vegetation, the abundance of *A. patagonicus* tadpoles and presence of multiple ontogenetic classes was higher than in non-vegetated microhabitats of the same central zone. The physical complexity of the *M. quitense* ring of vegetation may explain this result. The ring of vegetation provides a large number of spaces for shelter, differing microhabitats, and fosters the establishment of aquatic organisms (Thomaz and Cunha 2010). Captures of *A. patagonicus* tadpoles belonging to the earliest classes (I and II) were very abundant in the vegetated microhabitat in the peripheral zone, even though *M. quitense* plants here are smaller and less structurally intricate. We therefore infer that the microhabitat structure provided by the macrophyte *M. quitense* is favorable for use by *A. patagonicus* individuals in both the central and the peripheral zones. Indeed, all the vegetated microhabitats hosted large numbers of Class I to IV tadpoles.

During our sampling efforts in Laguna Verde pond in November 2007 and December 2008, we found numerous small *A. patagonicus* tadpoles in stages 26−28 (Class I) in shallow, densely vegetated areas near the shore. The preferential distribution of Class I and II tadpoles in peripheral zones colonized by macrophytes may be related to the complexity provided by *M. quitense* and *T. patagonica*, which offers suitable structure for adherence of egg clutches (Cei 1965), at the same time ensuring shelter for early developmental stages. During visits to Laguna Verde pond subsequent to this study in October 2005, January 2007, and January 2008, one of the authors (MEC) heard *A. patagonicus* mating calls from shallow vegetated areas in the peripheral zone and vegetated areas in the central zone, indicating that these areas constitute the breeding microhabitat.

As tadpoles develop and increase in size and mobility, Classes III and IV show that in addition to occupying vegetated microhabitats, they are able to occupy others, such as the rocky microhabitat. In rocky microhabitats, rough surfaces and cracks host higher densities of algae and invertebrates that seek refuge from predators and waves (water turbulence through rough surfaces may be qualitatively different from turbulence acting on smooth surfaces). Filamentous algae may also add complexity to the rock surface and increase species richness. Downes et al. (1998) recorded a higher number of species on rocks covered in algae than on bare rocks. Similarly, in Laguna Verde pond, loose *M. quitense* branches trapped among the rocks, together with filamentous algae, increase the richness in the rocky microhabitat, where advanced *A. patagonicus* tadpoles find benthic micro-invertebrates as potential prey. Cei (1965) mentioned the omnivorous, probably suctorial habit of *A. patagonicus* tadpoles, based on the variety of small animals (amphipods, Diptera larvae, and others) and remains of green algae found in their diet and on the wide sucker-like oral disc located in ventral subterminal position. Developmental Classes III and IV did not show such marked use for a single microhabitat as did the preceding classes; indeed, Classes III and IV were evenly distributed among vegetated and rocky habitats. Further studies are needed to determine whether there are ontogenetic differences in the diet of *A. patagonicus* tadpoles that would contribute to explaining how the classes are distributed in the pond.

Owing to various mechanisms acting simultaneously, such as food availability, shelter, and foraging sites, the effects of macrophyte complexity on assemblages associated with macrophytes may be synergistic (Thomaz and Cunha 2010). In our study, the fact that we captured few individuals from the surface and middle levels of the non-vegetated water column contrasts with the high abundance in the central ring of aquatic vegetation, which provides food and shelter for individuals. *Atelognathus patagonicus* tadpoles are basically omnivorous (Cei 1965) and probably feed at the surface of and among *M. quitense* foliage. Moreover, the presence of aquatic vegetation used as shelter from predators is an important factor for tadpole survivorship. Kopp et al. (2006) showed that predator-vegetation interaction is significant for tadpole survivorship of two Hylidae species in southeastern Brazil and that the effect of predation was stronger without vegetation. Microhabitat use probably reflects a trade-off among opposing forces of selection, two of which are predation and food intake (Warkentin 1992). Accordingly, in Laguna Verde pond, we did not see *A. patagonicus* individuals by day, but captured them in funnel traps by night, suggesting that nocturnal activity protects them from daytime predators.

As in other ponds in the system, fish are naturally absent from Laguna Verde, so the main predators of *A. patagonicus* are diving birds such as the grebes *Podiceps occipitalis* and *Rollandia rolland* (Cuello et al. 2005) and Odonata larvae (*Rhionaeschna* sp.; pers. obs.). The population of *P. occipitalis* exerts the greatest predatory pressure on *A. patagonicus* post-metamorphic individuals and tadpoles, as reflected by the high capture rates by birds recorded by Cuello et al. (2005). Moreover, observations in natural environments and laboratory experiments (Casanovas and Úbeda 2006; Jara et al. 2013; Jara 2014) show that *Rhionaeschna variegata* larvae are voracious predators of Patagonian anuran tadpoles, which they catch either by ambush or by searching. The use of specific microhabitats by *A. patagonicus* that provide shelter for each development stage may reduce the risk of predation. In this regard, the diving area of the grebes does not include shallow water or rocky areas where advanced tadpoles and post-metamorphic individuals hide in interstices among and under overlapping boulders in shallow water. Moreover, in deep areas, dense macrophytic vegetation hinders the activity of diving birds. In the vegetated microhabitats, *A. patagonicus* tadpoles appear to be most at risk of predation by *Rhionaeschna* larvae, though they also find food and shelter, showing that there is a trade-off between opposite forces of selection.

Metamorphosing tadpoles (Class V) and metamorphs (Class VI) have a significantly different distribution from that of other tadpole classes. They are found almost exclusively in water along the shoreline, hiding among and under submerged rocks, and are absent from the central zone. The period during which anurans transition from tadpoles to metamorphs is critical to their survival (Arnold and Wassersug 1978; Duellman and Trueb 1986). During the final stage of metamorphosis, anurans are particularly susceptible to predation by other vertebrates (Wassersug and Sperry 1977; Arnold and Wassersug 1978; Huey 1980; Crump 1984). Highly vulnerable metamorphosing *A. patagonicus* tadpoles (Class V) probably find the shelter they need from predator pressure in the complexity of the rocky microhabitat. The time spent at this stage is short compared to their larval lifespan. Arnold and Wassersug (1978) suggested that the speed and synchronization of the transformations as well as certain behavioral features may be adaptive responses to the high risk of predation during the metamorphic transition. Metamorphs (Class VI) of *A. patagonicus* find immediate access to the air-water interface, which they need for pulmonary breathing, and access to the terrestrial environment where they spend an initial period before recruiting as aquatic or terrestrial morphotypes. In addition, sheltering under rocks minimizes the loss of water through the skin.

Juvenile and adult post-metamorphs, grouped here as Class VII, also mainly occupied the rocky microhabitat in the peripheral zone, with only a very low proportion in the vegetated bottom and non-vegetated bottom habitats of the central zone. The *A. patagonicus* aquatic morphotype, with its well-developed interdigital membranes and specialized integument with richly vascularized folds that enable cutaneous gas exchange, remains under and among submerged rocks, where it finds some protection from potential predatory birds. The aquatic morphotype characteristics enable *A. patagonicus* to be independent of the aerial environment, move about extensively in the pond, and reach deep zones.

In summary, there is a range of microhabitats in the central and peripheral zones in Laguna Verde pond, with high availability of food and space resources, enabling the establishment of *A. patagonicus* individuals of all sizes and developmental classes. The partial segregation of the different developmental stages observed in this study may reduce intraspecific competition. The ability of frogs and tadpoles to move about in the water body creates greater opportunities for selecting food and shelter. If the different developmental classes use resources differently, then a pond which has a variety of microhabitats will support a higher number of individuals than a pond with less microhabitat complexity. The high abundance of *A. patagonicus* adults, juveniles, and larvae (Fox et al. 2005), along with simultaneous presence of seasonal and overwintering tadpoles recorded during this and previous studies (Cuello et al. 2014), supports this idea.

Implications for conservation.—Within the boundaries of Laguna Verde, we identified the following two main habitat features: rocky areas with complexity provided by rock size and structure, and large areas of rooted macrophytes in both the central and peripheral zones. Vegetated and rocky areas are important to *Atelogmathus patagonicus* for different reasons and should both be prioritized for conservation. Vegetated areas are essential for breeding and supporting early larval stages, while rocky areas are important for recruitment of juveniles. The microhabitats described in this study are typical of the endorheic pond system in Laguna Blanca National Park and surroundings, where *A. patagonicus* lives. In particular, Cuello et al. (2009b) found a positive correlation in several ponds in the system between presence of *A. patagonicus* and aquatic vegetation, showing that the species requires structurally complex ponds. To date, *A. patagonicus* has not been found in any structurally simple ponds having homogenous bottoms covered in fine sediment (Cuello et al. 2009b) or in ephemeral ponds (Cuello et al. 2014).

The pond habitat analyzed herein and the habitat surrounding the pond are sensitive to deterioration caused by livestock drinking and feeding on aquatic and palustrine vegetation. The woven wire perimeter fence built by Laguna Blanca National Park Administration in 2004 around Laguna Verde pond resulted in rapid recovery of macrophytic vegetation and the marginal vegetation on the shores, providing additional shelter for *A. patagonicus* (Cuello et al. 2014). Land administrators and owners may take action to minimize future habitat loss for this frog. We highlight the need to preserve those ponds with a high degree of diversity and microhabitat complexity but which lack protection because they lie outside Laguna Blanca National Park. These ponds should be a high priority for inclusion in a regional system of protected areas.

Acknowledgments.—We would like to thank Manuela Calvo, Paula Casanovas, and Aimé Iglesias for their work as field assistants; park rangers of Laguna Blanca National Park (LBNP) and technicians from Delegación Regional Patagonia of Administración de Parques Nacionales for supporting our field work; and Daniel Testoni (LBNP) for providing information and valuable suggestions on the manuscript. Catalina Connon translated the manuscript. This project was partly supported by research grants from Universidad Nacional del Comahue (B153 and B179), Wildlife Society Conservation (Research Fellowship Program), and Rufford Small Grant for Nature Conservation. Capture of specimens was authorized by Administracion de Parques Nacionales,Argentina (Disposición DNCAP N°55/04).

Literature Cited

Alford, R.A. 1986. Habitat use and positional behavior of anuran larvae in a northern Florida temporary pond. Copeia 1986:408–423.

Alford, R.A., and M.L. Crump. 1982. Habitat partitioning among size classes of larval southern' Leopard Frogs, *Rana utricularia*. Copeia 1982:367–373.

Arnold, S.J., and R.J Wassersug. 1978. Differential predation on metamorphic anurans by garter snakes (*Thamnophis*): social behavior as a possible defense. Ecology 59:1014–1022.

Casanovas, P., and C.A. Úbeda. 2006. *Alsodes gargola* (Rana del Catedral). Predation. Herpetological Review 37:439–440.

Cei, J.M. 1965. The tadpole of *Batrachophrynus patagonicus* Gallardo. Herpetologica 20:242–245.

Cei, J.M., and V.G. Roig. 1966. Los caracteres biocenóticos de las lagunas basálticas del oeste de Neuquén. Boletín de Estudios Geográficos 13:182–201.

Cei, J.M., and V.G. Roig. 1968. Telmatobiinos de las lagunas basálticas de Neuquén (Anura, Leptodactylidae). Physis 27:265–284.

Cronin, G., W.M. Lewis, Jr., and M.A. Schiehser. 2006. Influence of freshwater macrophytes on the littoral ecosystem structure and function of a young Colorado reservoir. Aquatic Botany 85:37–43.

Crump, M.L. 1984. Ontogenetic changes in vulnerability to predation in tadpoles of *Hyla pseudopuma*. Herpetologica 40:265−271.

Cuello, M.E., M.T. Bello, M. Kun, and C.A. Úbeda. 2006. Feeding habits and their implications for the conservation of the endangered semiaquatic frog *Atelognathus patagonicus* (Anura, Neobatrachia) in a northwestern Patagonian pond. Phyllomedusa 5:67−76.

Cuello, M.E., F.G. Jara, and M.G. Perotti. 2009a. *Atelognathus patagonicus* (NCN). Aquatic habitat. Herpetological Review 40:198−199.

Cuello, M.E., F. Jara, and Q. Vidoz. 2005. *Atelognathus patagonicus* (NCN). Predation. Herpetological Review 36:298.

Cuello, M.E., M.G. Perotti, and G.J. Iglesias. 2009b. Dramatic decline and range contraction of the endangered Patagonian frog, *Atelognathus patagonicus* (Anura, Leptodactylidae). Oryx 43:443−446.

Cuello, M.E., C.A. Úbeda, and M.T. Bello. 2008. Relationship between morphotypes of *Atelognathus patagonicus* (Anura, Neobatrachia) and environmental conditions: evidence and possible explanation. Phyllomedusa 7:35−44.

Cuello, M.E., C.A. Úbeda, M.T. Bello, and M. Kun. 2009c. Seasonal trophic activity of the aquatic morphotype of *Atelognathus patagonicus* (Anura, Neobatrachia) and prey availability in the littoral benthos of a permanent pond in Argentinean Patagonia. Phyllomedusa 8:135−146.

Cuello, M.E., C.A. Úbeda, M.T. Bello, and M.G. Perotti. 2014. Plastic patterns in larval development of endangered endemic *Atelognathus patagonicus*: implications for conservation strategies. Endangered Species Research 23:83−92.

Daciuk, J. 1968. La fauna del Parque Nacional Laguna Blanca (estudio zoo-ecológico preliminar). Anales de Parques Nacionales (Buenos Aires) 11:225−302.

Douglas, M., and P.S. Lake. 1994. Species richness of stream stones: an investigation of the mechanisms generating the species–area relationship. Oikos 69:387−396.

Downes, B.J., P.S. Lake, E.S.G. Schreiber, and A. Glaister. 1998. Habitat structure and regulation of local species diversity in a stony, upland stream. Ecological Monographs 68:237−257.

Duellman, W.E., and L. Trueb. 1986. Biology of Amphibians. McGraw-Hill, New York, New York, USA.

Fox, S.F., J.H. Yoshioka, M.E. Cuello, and C. Úbeda. 2005. Status, distribution, and ecology of an endangered semi-aquatic frog (*Atelognathus patagonicus*) of northwestern Patagonia, Argentina. Copeia 2005:921−929.

Genova, M.I. 2011. Density and habitat preferences of Lake Titicaca Frog (*Telmatobius culeus*) at north-west of Copacabana peninsula. M.Sc. Thesis, Wageningen Universiteit, Wageningen, Güeldres, Netherlands. 73 p.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183−190.

Grenouillet, G., D. Pont, and K.L. Seip. 2002. Abundance and species richness as a function of food resources and vegetation structure: juvenile fish assemblages in rivers. Ecography 25:641−650.

Hamer, A.J., S.J. Lane, and M.J. Mahony. 2002. Management of freshwater wetlands for the endangered Green and Golden Bell Frog (*Litoria aurea*): roles of habitat determinants and space. Biological Conservation 106:413−424.

Holomuzki, J.R. 1986. Predator avoidance and diel patterns of microhabitat use by larval Tiger Salamanders. Ecology 67:737−748.

Huey, R.B. 1980. Sprint velocity of tadpoles (*Bufo boreas*) through metamorphosis. Copeia 1980:537−540.

International Union for the Conservation of Nature (IUCN). 2010. The IUCN Red List of Threatened Species. Version 2014.2. http://www.iucnredlist.org.

Jara, F.G. 2014. Trophic ontogenetic shifts of the dragonfly *Rhionaeschna variegata*: the role of larvae as predators and prey in Andean wetland communities. Annales de Limnologie-International Journal of Limnology 50:173−184.

Jara, F.G., C.A. Úbeda, and M.G. Perotti. 2013. Predatory insects in lentic freshwater habitats from northwest Patagonia: richness and phenology. Journal of Natural History 47:2749−2768.

Kopp, K., M. Wachlevski, and P.C. Eterovick. 2006. Environmental complexity reduces tadpole predation by water bugs. Canadian Journal of Zoology 84:136−140.

Menone, M.L. 2015. Encubridoras por naturaleza: las plantas acuáticas ocultan los secretos de la contaminación. Ciencia e Investigación 65:1−12.

Nájera-Hillman, E., A.C. Alfaro, S. O'Shea, B. Breen, N. Garret, and P. King. 2009. Habitat-use model for the New Zealand endemic frog *Leiopelma hochstetteri*. Endangered Species Research 9:23−31.

Parris, K.M. 2001. Distribution, habitat requirements and conservation of the Cascade Treefrog (*Litoria pearsoniana*, Anura: Hylidae). Biological Conservation 99:285−292.

Phiri, C. 2010. Ecological aspects of the macroinvertebrates associated with two submersed macrophytes in Lake Kariba. Ph.D. Thesis, University of Cape Town, Cape Town, South Africa. 302 p.

Roquero, M.J. 1968. La vegetación del Parque Nacional Laguna Blanca (Estudio fito-sociológico preliminar). Anales Parques Nacionales (Buenos Aires) 11:129–207.

Schoener, T.W. 1974. Resource partitioning in ecological communities. Science 185:27–39.

Shaffer, H.B., R.A. Alford, B.D. Woodward, S.J. Richards, R.G. Altig, and C. Gascon. 1994. Quantitative sampling of amphibian larvae. Pp. 130–141 *In* Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.A.C. Hayek, and M.S. Foster (Eds.). Smithsonian Institution Press, Washington, D.C., USA.

Smith, G.R., and J.E. Rettig. 1966. Effectiveness of aquatic funnel traps for sampling amphibian larvae. Herpetological Review 27:190–191.

Taniguchi, H., and M. Tokeshi. 2004. Effects of habitat complexity on benthic assemblages in a variable environment. Freshwater Biology 49:1164–1178.

Tarr, T.L., and K.J. Babbitt. 2002. Effects of habitat complexity and predator identity on predation of *Rana clamitans* larvae. Amphibia-Reptilia 23:13–20.

Thomaz, S.M., and E.R. Cunha. 2010. The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. Acta Limnologica Brasiliensia 22:218–236.

Toft, C.A. 1985. Resource partitioning in amphibians and reptiles. Copeia 1985:1–21.

Trova, S., and H. Galván. 2012. Plan de Uso Ganadero, Parque Nacional Laguna Blanca-Delegación Regional Patagonia (RES. HD 206/13). Administración de Parques Nacionales, Bariloche, Río Negro, Argentina. 82 p. Available at: www.sib.gov.ar/archivos/Plan_de_uso_Ganadero_PNLB.pdf

Villamil, C.B., and D. Testoni. 2012. La Flora del Parque Nacional Laguna Blanca, Patagonia Argentina. EdiUNS, Bahía Blanca, Buenos Aires, Argentina.

Warkentin, K.M. 1992. Microhabitat use and feeding rate variation in Green Frog tadpoles (*Rana clamitans*). Copeia 1992:731–740.

Wassersug, R.J., and D.G. Sperry. 1977. The relationships of locomotion to differential predation on *Pseudacris triseriata* (Anura: Hylidae). Ecology 58:830–839

Wentworth, C.K. 1922. A scale of grade and class terms for clastic sediments. The Journal of Geology 30:377–392

Zar, J.H. 1984. Biostatistical Analysis. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, USA.

Maria Elena Cuello is a Biologist who graduated from Comahue National University, Río Negro, Argentina. Since 2003 she has been conducting research on *Atelognathus patagonicus*, an endemic endangered frog, in Laguna Blanca National Park (LBNP) and the surrounding area, thanks to grants from Rufford Small Grant Fund (1st and 2nd), Wildlife Conservation Society, Bariloche Regional University Center (CRUB), Comahue National University (Argentina), and Laguna Blanca National Park. Her recent research has focused on the response of the amphibian species endemic to ponds with different hydroperiod and especially the remarkable plasticity of *A. patagonicus* to cope with changes in the climatic conditions of the area. She has participated in activities related to raising awareness, conservation, and management for the protection of *Atelognathus patagonicus* in conjunction with LBNP, Neuquén province, and CRUB. (Photographed by Juana Aigo).

Carmen A. Úbeda began her career as a Biologist (1978) in the Department of Biology of Buenos Aires University, Argentina. She later moved to Bariloche (north-west Patagonia), where she was full-time Professor and Researcher in the Department of Zoology of Comahue National University until 2014. She earned her Ph.D. in Biological Sciences (1998) at the School of Exact and Natural Sciences of Buenos Aires University, in the field of Patagonian fauna of amphibians. Her primary research interests are the biodiversity, natural history, ecology, systematics, and conservation of the amphibians of Patagonia. At regional, national, and international levels, she has contributed to different amphibian categorization processes according to their conservation status (Argentina, IUCN). (Photographed by Ricardo Ortubay).

Maria Teresa Bello is a Biologist graduated from Córdoba National University, Argentina. As a Teacher and Researcher at Comahue National University, she directed research groups and different projects on fisheries biology and fishes in Patagonian lakes and ponds, with results reported at national and international conferences. In 2000 she joined the group studying fish populations and conservation priorities on the Valdivian Ecoregion. From 2005 to 2015, she participated in studies on the biology and conservation of the endemic anuran *Atelognathus patagonicus*. During that time she co-authored five papers and several communications on *A. patagonicus* for scientific meetings. (Photographed by Juan Carlos Mussio).

APPENDIX. Description of 10 aquatic microhabitats in Laguna Verde Pond, Laguna Blanca National Park, Neuquén Province, Argentina, in January 2006.

	PERIPHERAL ZONE MICROHABITATS				CENTRAL ZONE MICROHABITATS					
Variables	Vegetated	Rocky	Sandy	Mixed	Vegetated top	Vegetated middle	Vegetated bottom	Non-vegetated top	Non-vegetated middle	Non-vegetated bottom
Depth range (m)	0−1	0−1 Includes the water-air interface under rocks on the shore line	0−0.8	0−0.6	Upper layer of the *M. quitense* ring from the surface down to a depth of 20 cm	Middle layer of the *M. quitense* ring between the upper and lower vegetation layers	Lower layer of the *M. quitense* ring and bottom of the pond, located at 2.5−4 m	Upper layer of the water column down to a depth of 20 cm	Middle layer of the water column between the upper and lower layers	Lower layer of the water column and bottom, located at 2.5−4 m
Slope range (degrees)	20−23	12−14	7−8	8−9	–––	–––	null or almost null	–––	–––	null or almost null
Substrate type	sand	Several layers of boulders of different sizes (≥ 450 mm) with irregular surfaces, many pits and cracks	Very coarse (1−2 mm) and coarse (0.5−1 mm) sand	Boulders (250−450 mm) and cobbles (60−250 mm) scattered over a sandy bottom	––––	–––	Silt and clay, rich in fine particulate organic matter and larger plant remains	–––	–––	Silt and clay, rich in fine particulate organic matter and larger plant remains
Myriophyllum quitense plants	Isolated plants with short stems (≤ 1 m)	absent	absent	absent	Ring sector constituted of submerged and partially emergent foliage, forming a dense 3-D structure	Ring sector constituted of predominantly vertical branches	Ring sector constituted of predominantly vertical branches.	absent	absent	absent
Drifting *Myriophyllum quitense* branches	absent	plentiful, tangled	absent	absent	absent	absent	absent	absent	absent	absent
Filamentous algae	abundant	highly developed	moderately developed	plentiful	highly developed	moderately developed	absent	absent	absent	absent