

Final Report 2nd R.S.G.

Developing a conservation program for *Atelognathus patagonicus* (Anura, Leptodactylidae) an endangered endemic frog of northwestern Patagonia, Argentina: population status, demographic structure and public education. Country: Argentina

Maria Elena Cuello

Abstract

Atelognathus patagonicus was not found in six new lagoons in Neuquén province in the summer of 2006. Introduced fish (Salmonids) and *A. patagonicus* have been found in Laguna Colorada (Neuquén). The species have not been found in Laguna Agnata and Laguna Del Toro (Neuquén). Fish have not been found in these lagoons.

A high proportion of total surface of lagoons of Laguna Blanca System were occupied by *A. patagonicus* (78.6 %). A low proportion (27.8 %) of total surface of lagoons which were sampled from 2004-2006 were occupied by *A. patagonicus*. The rest (72.2 %) was occupied by introduced fish but *A. patagonicus* was not found.

The tadpoles of *A. patagonicus* chose shelter (coils of *Myriophyllum quitense*) during outdoor experiments. Tadpoles of *A. patagonicus* did not show changes in treatment with water from tank with perch. They did not increase activity neither uses of shelter.

Laguna Batea (N.P.L.B.), which is a temporary pond, remained with water in dry season in 2005 (about 0.70 cm depth). The overwintering tadpoles were not found. This population showed only one cohort that finished its metamorphosis in one breeding season.

Laguna Verde is a permanent lagoon. There were not significant differences between three breeding season (2004-2005-2006). Although samples were intensive in January 2006 in Laguna Verde the ovoposition sites were not found. Early larvae (a few amount) were found vegetated area near shore and in deep vegetated area. Frogs and tadpoles choose complex habitat to breed and develop as coarse fragment (gravel, boulder and bedrock) submerged in shallow water and vegetated areas (largely *M. quitense*). The captures were more abundant in vegetated area than in an-vegetated area in deep area of lagoon (about 4.5 m).

A new design for brochure has been made. New information has been incorporated in it. It will be an important tool in school, for tourist trade and for landholders in the region.

Landholders in the region have made evident especially interest in fish sowing. There is no prohibition of fish sowing nowadays. The actions at the provincial and national levels have to try a different approach to the problem. A legal norm must be developed to protect these wetlands.

1. Goals and objectives of the project

The two major goals of this project are to (a) establish what the global risks of vulnerability are to this frog and (b) start a program of public education about the frogs, fishes, and health of the Laguna Blanca aquatic ecosystem.

For goal (a) I will determine the demographic characteristics of frog populations in lakes with different durations of permanent water ; (ii) I will determine the presence (or absence) and relative abundances of *A. patagonicus* and of introduced predatory fishes in the lakes of the Laguna Blanca hydrological system and (iii) perform experiments to determine if/how the fish alter the behavior of the larval frogs and the possibility that the aquatic vegetation is an important habitat (and refuge) for larval and adult frogs. For goal (b) I will increase public awareness about the problems and values of conserving these unusual animals at a workshop with the local stakeholders and distributing a colorful brochure about the biology of the frogs and this special ecosystem.

2. Methodology

Outdoor experiments

The design consisted of a factorial experiment to measure the behavior of tadpoles of *A. patagonicus* in water coming from tanks with and without perch (control). The variables measured were the activity (swimming versus stationary activity) and the use of shelters (branches of *Myriophyllum quitense*).

The tadpoles of *A. patagonicus* were captured at Laguna Verde (Laguna Blanca National Park) under National Park Administration permission .

The experiment was run under control conditions and it consisted of four treatments. All treatments were replicated five times. Each replicate included one tadpole totaling 20 tadpoles Stages 35-38) (Gosner, 1960).

The treatments were:

1. Water from tank without perch (control)
2. Water and clumps *M. quitense* (control)
3. Water from tank with perch
4. Water from tank with perch and clumps *M. quitense*

The twenty boxes (30 x 50 cm) were arranged at random. Tadpoles remained in the boxes during fifteen minutes before the experiment began. Temperature and dissolved oxygen were measured.

Habitat sampling and amphibian surveys

XX wetlands (XX permanent and XX temporary) located in XX ha area (coordinates) were surveyed from January through April 2006.

A wetland is considered temporary when it dries during most summers. The assessment of wetlands was based on inspection of aerial photographs and observations carried out, in previous years. Water bodies were identified from topographic maps by Geographic Military Institute, Argentina (IGM chart 3969-19-scale 1:100.000) and aerial photographs (scale 1:50.000). The perimeter and area of every lagoon were calculated with a geographic information system (Arc View Gis 3.1 and LANDSAT TM PATH 232 ROW

88) Satellite Image.

Photointerpretation

Habitat and biotic communities were quantified at each site. The percentage of within-wetland habitat that was emergent-*Myriophyllum*, submerged vegetation, and open water (lacking macrophyte vegetation) was visually estimated.

The percentage of the area covered by *Miriophyllum* at every lagoon was calculated using Arc View Gis 3.1 and LANDSAT TM PATH 232 ROW 88 Satellite Image. The coverage categories considered were low (< 20 %), moderate (20-60 %), and high (60 –100 %)

Wetland amphibians and fish were sampled using standardized dip-netting combined with visual encounter. The bundance of aquatic birds was estimated by the use of binoculars and the bird list of Laguna Blanca National Park

Phenology and microhabitat use

A Datta Logger was placed at both lagoons from April 2004 to April 2006. Data on temperature, rain and wind were provided by the Meteorological Office Airport Zapala (Neuquen)

Frog and tadpoles were captured using funnel aquatic traps (11 cm diameter, 30 cm long) (Smith and Retting, Herp Review 27 (4), 1996). Moreover, two or three transects 50-m² were set up along the shore. Each transect was 50 m long and 1 m in wide. After capturing, body length and stage were determined (Gosner 1960. Herpetologica 16:183-190). Individuals whose body length was > 27 mm were considered adults (Cei & Roig 1967). Six classes were considered for tadpoles (Table 1). All captured animals were returned unharmed to their original places after data collection. Traps were placed parallel to the shoreline from 0.20 cm to 0.80 cm depth with 5 m interval between each one in each microhabitat. Because of the hard weather conditions, the number of traps employed per month and per microhabitat differed. Traps were left in place overnight.

The shoreline was sampled selecting the most representative microhabitats: a) coarse fragments (gravel, boulder and bedrock) b) mix (gravel and sand) and c) fine sediment (dominated by sand) from January 2004 through April 2006 in Laguna Verde and a) mix (gravel and sand) and b) fine sediment (dominated by clay) from January 2004 through April 2006 in Laguna Batea.

Habitat use in areas with abundant macrophytes.

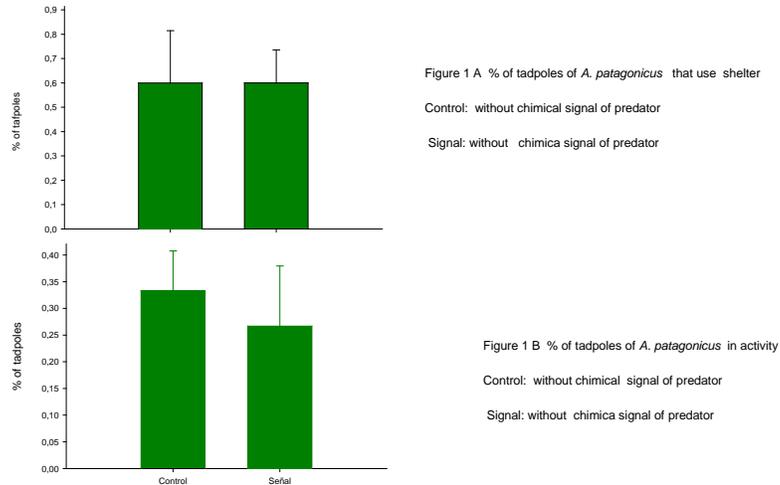
Frogs were captured with aquatic funnel traps, similar to the one used for phenology, placed at three different depths: superficial (at 20 cm), middle (at 70 cm) and maximum (approximately 350-450 cm), in areas with and without vegetation. Traps were left 12 hours at three different depths during the night. The traps in deep areas were placed from a boat or by swimming when the weather was very windy.

Moreover, the traps were placed in areas with macrophyta near the shore from January to April 2006.

3. Results

3.1 Outdoor experiments

Both the activity and the use of shelters were higher in tadpoles in water coming from tanks with perch (Figure 1 A and B). The differences were not significant in either of the treatments ($t_{4}= 0.590$, $P=0.587$; $t_{4}= -0.497$, $P=0.646$). The use of shelters (coils of *M. quitense*) was high in both treatments.



3.2 Habitat samplings and amphibian surveys. Photointerpretation.

Eight new lagoons were sampled. The species has been found in six of them but it has not been found in two of them. These are Laguna Del Toro (Neuquén) and Agnata (Neuquén).

The introduced fish, trout, was found sharing the habitat with *A. patagonicus* in Laguna Colorada (Neuquén, Argentina).

| Lagoons | Jurisdiction National Park | Wetland ha | <i>A. patagonicus</i> | Fish introduced | Macrophyte abundance |
|--------------|----------------------------|------------|-----------------------|-----------------|----------------------|
| Un-named | not included | 8.7 | present | absent | high |
| Un-named | not included | 4.5 | present | absent | high |
| El Toro | not included | 14 | absent | absent | moderate |
| Los Alamitos | not included | 16.7 | present | absent | high |
| Un-named | not included | 4.5 | present | absent | high |
| Los Juncos | not included | 20.8 | present | absent | high |
| Agnata | not included | 8.9 | absent | absent | low |
| Colorada | not included | 39.8 | present | present | high |

Table 1 New record of lagoons in the Laguna Blanca System (Neuquén). January - March 2006

Laguna Del Toro (Neuquén) was used to provide water to Zapala, a neighbor city, during almost 15 years. At present, it is being used to irrigate vegetable gardens.

The abundance of aquatic birds was high in six lagoons but low in two. The areas with macrophyte were high and moderate in six lagoons but low in two (Table 1).

3.3 *Phenology in two lagoons with different hydroperiod.*

Laguna Batea (LBNP), a temporary wetland, has 1.2 ha in October 2005 and 0.5 ha in April 2006 surface. It dried from February to May 2004. The traps could not be placed in that period. The lagoon was not sampled from June to August because of snow and ice on roads. It had a 2-m depth in winter and spring 2004-2005 and a 0.80-m depth in summer and autumn 2005-2006. The rainfall was more abundant in winter 2004 than in 2005. (Figure 2)

In Laguna Batea the captures were abundant from November to April in both years and low from May to October in 2005 (Figure 2). The differences (average individual/traps h) between 2004-2005 were significant (Mann-Whitney Test $Z_1 = 4.257$; $P < 0.005$). The activity of the species was restricted from October to April in both years. The first capture of early larvae happened in November in both years. Early larvae were captured from November 2004 to January 2005 and in November 2005. The findings showed that several egg-laying could have happened in the first period (Nov 2004-Jan 2005) and only one in the second (Nov 2005).

Traps were placed under the ice (5 cm wide) during two winters but no larvae were captured.

Laguna Verde, a permanent wetland was 4.5 m deep in April 2005. The lagoon has a 20 ha in October 2005 and 1.5 ha in April 2006 surface.

The captures were abundant from November to April and low from May to October in both years. The highest captures were in February 2004-2005 and January 2006 (Figure 2). The differences (average individual/traps h) between 2004-2005 were not significant (Mann-Whitney Test $Z_1 = -1.343$; $P = 0.179$).

Adults, juveniles and larvae were captures even under the ice in winter, in that lagoon over the year.

Early larvae were found from December to March, advance larvae were captured every month of the year and metamorphs were captured particularly in summer.

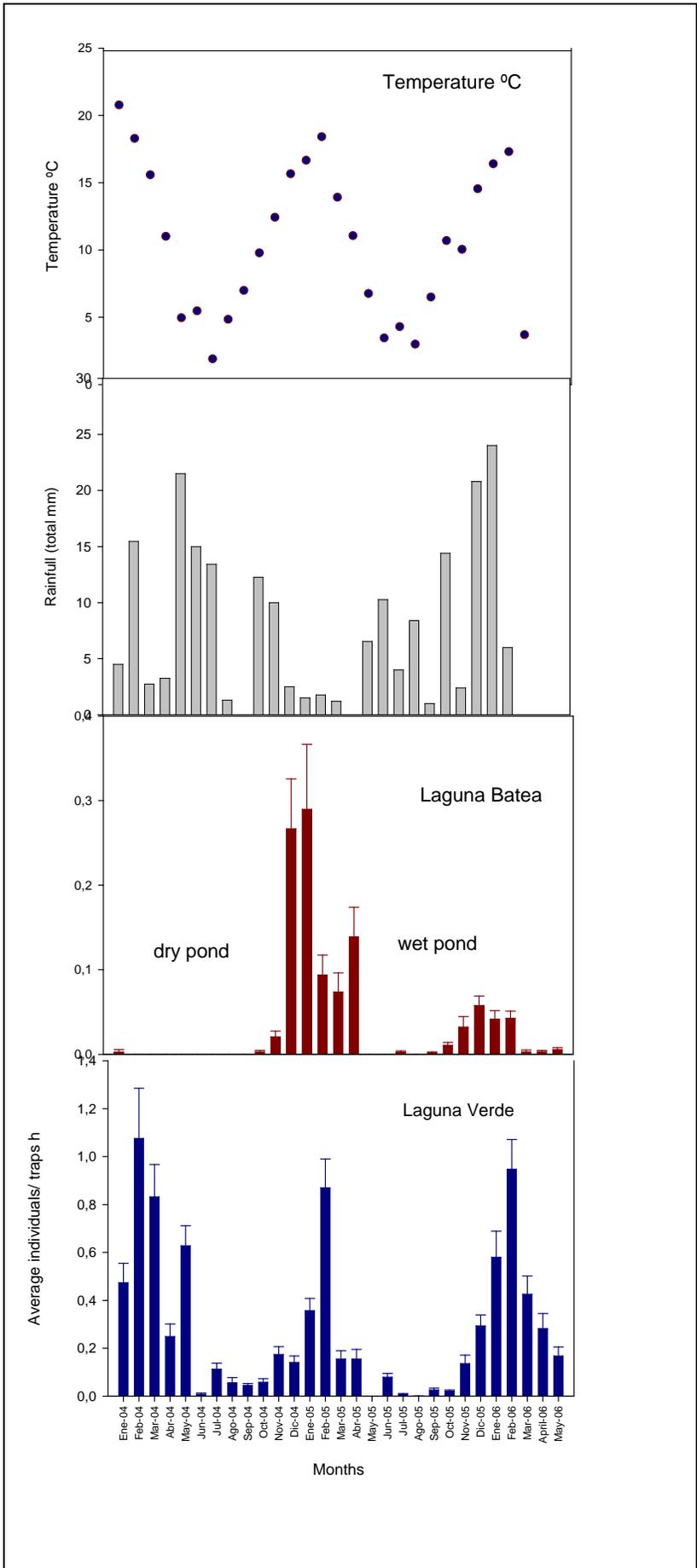


Figure 2 Average individuals/trap h of *A. patagonicus* captured in Laguna Verde and in Laguna Batea (Laguna Blanca National Park), from January 2004 through May 2006. Temperature and precipitation data were supplied by the Zapala Meteorological Station, Neuquén (30 Km. from the sample sites)

No egg-laying was found but the captures of early larvae led to the assumption that it would have been from November to March.

Females with pigmented oocytes observed through the thin, transparent ventral skin and males with nuptial pads were captured from November to April.

3.4 Use of habitat in three different microhabitats.

The captures were more abundant in the mix microhabitat in Laguna Batea and coarse and mix in Laguna Verde (Figure 3). The differences (average individuals/traps h) between two microhabitats in Laguna Batea and among three microhabitats in Laguna Verde were significant ($Z_{1,932} = -3.24$, $p < 0.001$; $K_{2,975} = 87.46$, $p < 0.0001$)

Larvae, juveniles and adults were more abundant in complex microhabitat in Laguna Verde. Larvae were abundant in mix microhabitats in this lagoon.

Larvae, juveniles and adults were abundant in mix microhabitat in Laguna Batea. Larvae were abundant in low complex microhabitat (Figure 3).

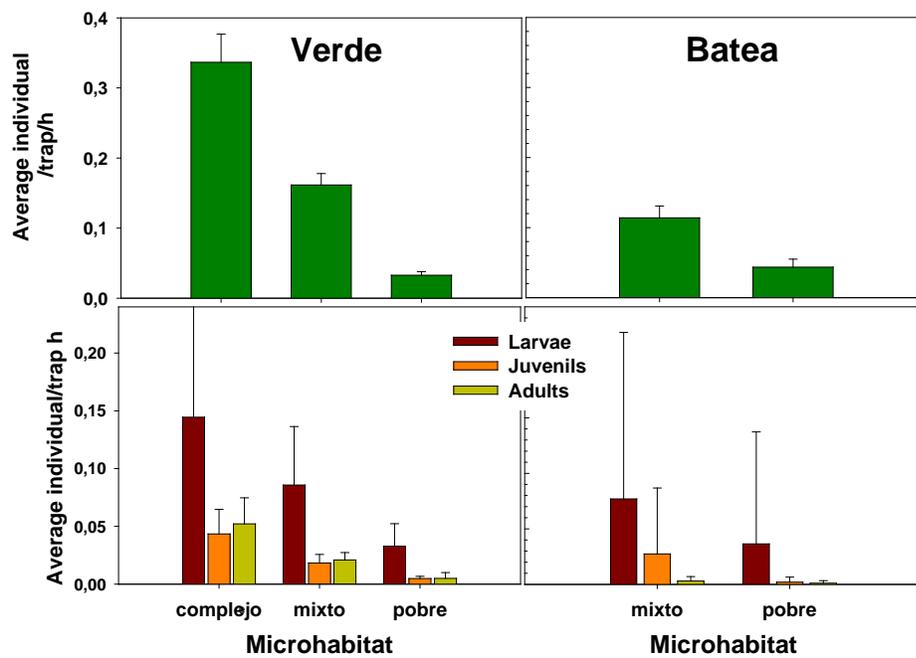


Figure 3 Average individual/trap h in three microhabitat : complex, mix and low complex (sand or clay)

3.5.1 Macrophyte near shore

In January 2006 the captures were as high in areas with macrophyte near the shore as in both coarse fragment and mix microhabitat. They were low in the sand microhabitat. The differences (average

individuals/traps h) among four microhabitats were significant ($K_{3, 460} = 113.07$, $p < 0.0001$). The sand microhabitat was significantly different from the other three.

3.5.2 Macrophyte in deep area

Atelognathus patagonicus was found in the three water depths and in vegetated and non-vegetated areas as well. Significant differences were found in the number of individuals (Figure 4). between vegetated and non-vegetated area (Mann Whitney $Z = -2.439$ $P = 0.015$). The “aquatic form” female (body length = 4.5 cm) was found at 4.5 m of depth in an area with high abundance of *Miriophyllum quitense*.

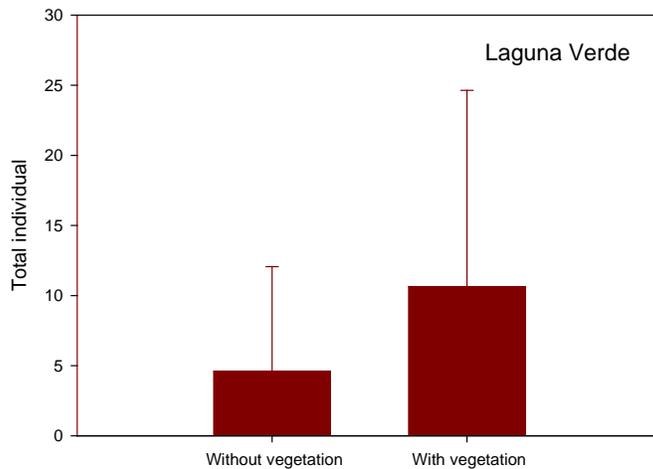


Figure 4. Total individuals captured in area with vegetation and without vegetation in Laguna Verde, January 2006.

4. Discussion

Atelognathus patagonicus tadpoles did not show changes in their behavior when they were in treatment with water from tank with perch. These results match with the fact that they have evolved without fish. Lagoons of Laguna Blanca System are characterized by the absence of fish.

Tadpoles of other species which have evolved in presence of fish show changes in their behavior (increase or lower the activity). Furthermore other species of frogs increase the use of shelter when they are in presence fish.

The absence of amphibians and fish in Laguna Agnata (as too Solitaria visited in 2004) is related to some characteristics as low depth and turbidity typical from Patagonian meadows or “mallines. Ongoing studies on the biology of *A. patagonicus* had shown that the species requires complex microhabitats typical from the basaltic lagoons present in the area. Laguna El Toro was an exception with absence of amphibians and fishes regardless of its basaltic origin. A reasonable explanation might be the anthropogenic disturbance that it suffered through reiterated drainages in a 10 years period that might influences negatively on the successful colonization of the amphibian.

The absence of amphibian was not always associated with presence of fish, however. Laguna Colorada with fish (salmonids) had presence of amphibian and high abundance of macrophyte. It might be associated to differences in food habits perch and salmonids.

A high proportion of lagoons in the studied wetland system were occupied by amphibians (78.57%). These water bodies were characterized by abundant aquatic vegetation (macrophyte coverage 60–100 %). A low proportion (27.8 %) of total surface of lagoons which were sampled from February 2004 to April 2006 were occupied by *A. patagonicus*. The rest (72.2 % of surface water) is occupied by fishes but *A. patagonicus* has not found.

These findings are very important because they are showing that reduction of area for *A. patagonicus* was about 72.2 % in the last ten years.

Laguna Batea, which is a temporary pond, remained with water during the summer of 2005 and 2006. It was dry in 2004. This wetland is directly associated with the amount of yearly rainfall.

The overwinter tadpoles were not found in Laguna Batea although the lagoon remained with a level of water around 0.70 cm. Therefore there must be other characteristics which are acting as a signal on this population. Low levels of O₂ in water, high water temperature and change in water Ph are the most common factors that regulate the time of metamorphosis in other frogs. Furthermore, this lagoon is characterized by presence of abundant predators (naiads and larvae insects). They can be an important factor in the regulation of metamorphosis.

Laguna Verde, which is a permanent pond, did not show significant different over three breeding seasons studied.

The first ovoposition must take place in Laguna Verde in November. Some tadpoles finish metamorphosis in March and April. This strategy is similar to the one that take place in temporary ponds as for instance, Laguna Batea. Other tadpoles remain as overwintering tadpoles and finish metamorphosis in the following year. It is unknown if these overwintering tadpoles come from the same or from different cohorts (early and later ovopositions). We have found at least two cohortes coexisting in spring and summer in Laguna Verde.

The species shows a remarkable phenotypic plasticity: “aquatic form” and “terrestrial form” in postmetamorphics individuals and two strategies in the development of tadpoles.

The “terrestrial form” was found especially in dry years and in wet years it was found in summer and autumn. This “form” will let the species respond to environmental changes in the lagoons and it will probably let recolonization of temporal ponds. The “terrestrial form” was more abundant in temporal ponds as Laguna Batea and Laguna Antonio. The species showed one cohort that finished metamorphosis in one breeding season in temporary lagoon. It showed two o more cohortes that remained in the lagoon during the winter in permanent lagoons.

This study on Laguna Batea has been very important because: Laguna Batea is the only temporary pond in Laguna Blanca National Park and it was monthly sampled during 28 months, from December 2004 until may 2006.

The captures of *A. patagonicus* showed a positive correlation with water temperature. The captures of *A. patagonicus* showed a negative correlation with the amount of precipitation. The higher amount of summer captures may be related to breeding and food activity. Other species of frogs, which have been studied in laboratory and in field, showed increase in activity when the temperature went up.

There is only one cite about other overwinter tadpoles in Patagonia: *Alsodes gargola* that inhabits at 1800 m . The presence of overwinter tadpoles in *A. patagonicus* populations (1260 m) may be related with hard weather conditions as low temperature in southern winter. Furthermore, Laguna Verde showed some morphometric characteristics (surface 20 ha and depth 4.50 m) that let maintain low temperature. Low water temperature may delay development of tadpoles.

Frogs and tadpoles choose complex habitat to breed and develop as coarse fragment (gravel, boulder and bedrock) submerged in shallow water and vegetated areas (largely *M. quitense*). Moreover, these shorelines are covered by coils of *M. quitense* that waves draw onto the shore. These coils become a contribution to organic material in complex habitats. Frogs and tadpoles find shelter in the interstices of piled rocks.

Grebes population (*Podiceps rolland*) in Laguna Verde is large (about 200 specimen in January). They exert a strong predation action over frogs and tadpoles. The captures of specimens by grebes are more abundant in deep vegetated areas than in microhabitats with boulder and rocks. Boulders and rocks may be a shelter for frogs and tadpoles. Grebes can not predate in complex microhabitats. The interaction between grebes and *A. patagonicus* has not been studied yet. Nevertheless, it has been recorded and photographed several times.

The differences among the captures of classes in different microhabitats over the months may be an indicator of the movements of *A. patagonicus* population inside the lagoon. Furthermore, the differences among classes may be an indicator of a habitat selection pattern.

Although the ovoposition sites still remain unknown, some early larvae have been found in vegetated areas. Yet more sampling should be done in these areas to find ovoposition sites.

Parallel studied have shown that frogs are benthivorous. Frogs may select complex habitat because they can find the trophic resource there.

Tadpoles are detritivorous and planctonic. They may find food under the rocks and in vegetated areas.

5. Drawbacks

Objective (a) proposed was to determine the demographic characteristics of frog populations. This objective could not be developed. We marked a large amount of frogs. Recaptures were very low. These data were analyzed and we concluded that: 1. the population of Laguna Verde often migrates toward deep areas -2. the population of Laguna Verde does not fulfill requirements of the model (closed population)

We decided to make a Life Table to estimate Survival Index and other demographic characteristics so we made a weekly sampling. We could not find sites of ovopositions or a large number of early larvae. Although we could not make the Life Table the information turned out useful to understand microhabitat use pattern and phenology in a permanent pond.

Sampling in deep areas was very difficult. As we had a rowing boat we could only sample in calm days. On windy days we put traps swimming in deep areas.

6. Conservation Activities

1. A brochure with new information has been designed. It will be an important tool in school, for tourist business and for landholders in the region
2. There was a workshop held in Zapala. The following activities were developed there:
 - 2.1 Introductory speech about distribution, biology and conservation problem of species.
 - 2.2 Evaluation of achieved by two projects in LB.N.P.
 - 2.3 Identification of future actions and the responsible institution for each one
 - 2.31. The most important actions to be carried out will be:
 - To elaborate a legislation to prohibit fish stocking
 - To identify the lagoons that have not sampled yet
 - To establish contact with owners of land which lands basaltics lagoons can be found
 - To elaborate a billboard and a video to be distributed in schools, universities and for tourist industry
 - To continue with research on biology of *A. patagonicus*
 - To organize a meeting in 2007 for evaluate fulfillment of actions

7. Manuscripts sent and in preparation.

Cuello M.E. and M.G. Perotti Decline of the endemic frog *Atelognathus patagonicus* from Laguna Blanca, Neuquén, Argentina. Assessment of the effect of fish introduction. Froglog. IUCN/SSC DAPTF February 2006

Cuello M.E., M.G. Perotti and F. Jara Habitat (*in press* Herpetological Review)

Cuello M.E. and M.G. Perotti Overwinter. (*in press* Herpetological Review)

Cuello M.E., C. Ubeda and M.G. Perotti Vida Silvestre Revista de la Fundación Vida Silvestre Argentina. 2005 (93), pp 20-25

Fox, S., J. Yoskioka, **M. E. Cuello** C. Úbeda. 2004 Status, distribution, and ecology of a threatened semi-aquatic frog (*Atelognathus patagonicus*) of northwestern Patagonia Argentina. Copeia, 2005 (4), pp 921-929

Cuello M. E. y F. Jara 2004 *Atelognathus patagonicus*, Predation. (*in press* Herpetological Review)

Cuello, M. E., M. Kum, C. Úbeda. 2004 Dieta estival de *Atelognathus patagonicus* en la Laguna del Burro y sus alrededores (Neuquén). (*in press* Phyllomedussa)

Bello, M.T. M. E. Cuello **C. Ubeda 2004. Diet of *Pleurodema bufoninum*.**

(Herpetological Review, *in press*)

8. Reports sent to National Park Administration

Baffico Gustavo, Carmen A. Úbeda y **María Elena Cuello** Dieta larval de *Atelognathus patagonicus* (Gallardo, 1962) en Laguna Verde, Parque Nacional Laguna Blanca (Anura, Leptodactylidae) Informe de Avance. Delegación Regional Patagonia. A.P.N.

Jara Fabián y María Elena Cuello (2005) Análisis preliminar de las comunidades de Macroinvertebrados de ambientes acuáticos lenticos del Parque Nacional Laguna Blanca. Informe de Avance. Parque nacional Laguna Blanca A.P.N.

9. Congress presentations

VI Congreso Argentino de Herpetología. Paraná 2005. Evaluación del efecto de la introducción de peces en la declinación de *Atelognathus patagonicus* (Senior co-author)

VI Congreso de Herpetología. Paraná 2005. *Hedruris* sp. (Nematodo: Hedrutidae) en *Atelognathus patagonicus* (Anura Leptodactylidae) del Parque Nacional Laguna Blanca. (Senior co-author)

VI Congreso de Herpetología. Paraná 2005. Comparación de la dieta de tres poblaciones de la forma acuática de *Atelognathus patagonicus* durante el verano en tres ambientes del Sistema de lagunas del parque nacional Laguna Blanca (Anura: Leptodactylidae) (Senior co-author)

10. Project collaboration “Aprovechamiento integral de los recursos para la recuperación de Laguna Blanca. Pesquería artesanal en Laguna Blanca. Recuperación de Laguna Blanca por pesca artesanal” Parque Nacional Laguna Blanca y Centro Regional Universitario Bariloche.

11. Conservation actions proposed for 2007 and the future.

1. To design educational material in cooperation with the Park Rangers of N.P.L.B.
2. To sample lagoons which remain unsampled in Casa de Piedra Plateau
3. To develop a research project towards the evaluations of status of *A. patagonicus* population in Laguna Colorada. The structure and viability of this population is unknown. It is the only known population that cohabits with fish.
4. To a new meeting because it is needed to evaluate to go elaboration of legal norm.
5. To organize a workshop in L.B.P.N which will include a visit to the lagoons with and without fish

