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NATIVE FOREST LOSS IMPACT'S ON ANURAN DIVERSITY: WITH FOCUS ON *RHINODERMA RUFUM* (PHILIPPI 1902) (RHINODERMATIDAE) IN COASTAL RANGE, SOUTH-CENTRAL CHILE

Impacto de la pérdida del bosque nativo sobre la diversidad de anuros: focalizado sobre *Rhinoderma rufum* (Philippi 1902) (Rhinodermatidae) en la Cordillera de la Costa, en el centro-sur de Chile

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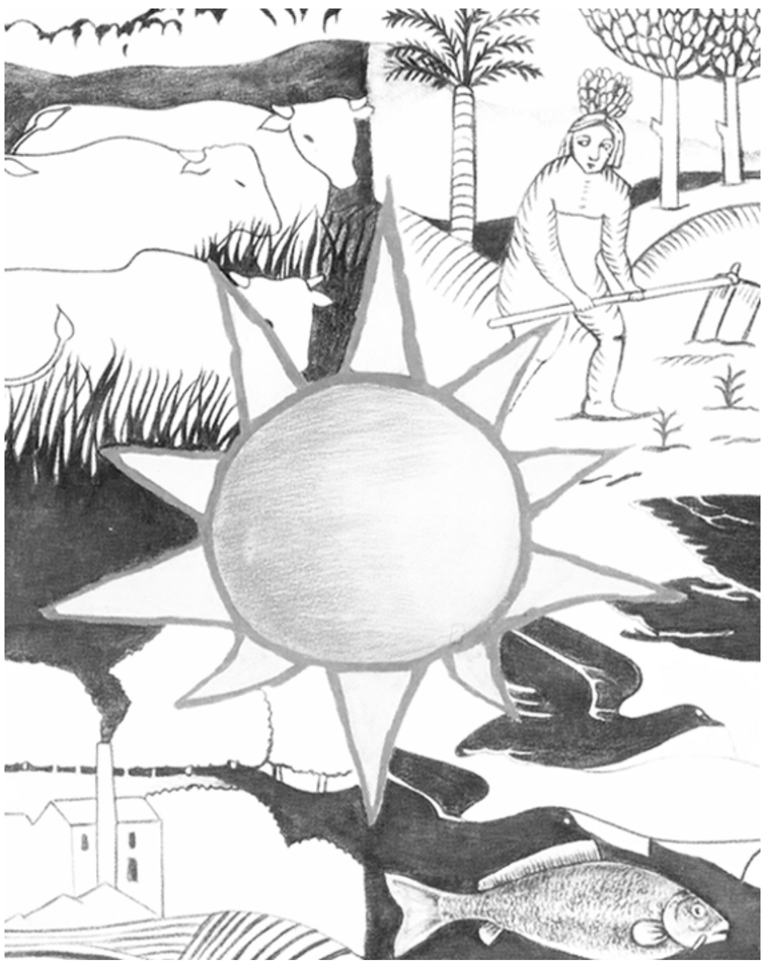

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ABSTRACT

Through intensive fieldwork along Cordillera de la Costa in Central Chile, basic biological data related to habitat loss and its possible impact on the diversity of amphibians (frogs and toads) were collected, with special emphasis to the situation of the critically endangered species *Rhinoderma rufum*. Social surveys, physical, chemical and biological data appraisals led to proposal a hypothesis regarding the conservation status of ten frog's species. The most consistent explanation for species low numbers and near extinction of *Rhinoderma rufum* appears to be lack of suitable habitat because replacement of native forest with exotic species; added to the loss of water supply, and their synergism with frog life strategies. Despite these results, and based on anecdotal observations, some hypotheses are pointed out explaining the chance of finding specimens of *R. rufum* in remnant Coastal Range forest.

Key words: endemic frogs, habitat degradation, declining population, *Rhinoderma rufum*, South Central Chile.

RESUMEN

Mediante trabajo de campo intensivo a lo largo de la Cordillera de la Costa en Chile central, se recogieron datos biológicos básicos relacionados con la pérdida de hábitat y su posible impacto en la diversidad de los anfibios (ranas y sapos), con especial atención a la situación de la especie en peligro crítico *Rhinoderma rufum*. Las encuestas sociales, y la evaluación de datos físicos, químicos y biológicos condujeron a proponer una hipótesis sobre el estado de conservación de 10 especies de rana. La explicación más coherente para los números bajos y casi extinción de *R. rufum* parece ser la falta de hábitat adecuado debido a la sustitución del bosque nativo por especies exóticas; añadido a la pérdida de agua, y su sinergia con las estrategias de vida de las ranas. A pesar de estos resultados, y sobre la base de observaciones anecdóticas, se proponen algunas hipótesis para explicar la posibilidad de reencontrar ejemplares de *R. rufum* en remanentes de bosque nativo de la Cordillera de la Costa.

Palabras clave: ranas endémicas, degradación del hábitat, disminución poblacional, *Rhinoderma rufum*, Chile Central.

INTRODUCTION

Among Coastal Range's woody ecosystems of Central Chile, five different forest formations have been differentiated from north to south: desert steppe, sclerophyllous forests, maulino forest, sclerophyllous bushes, and *Nothofagus* forest (Formas 1979; Donoso 1982). Although each one possesses their own dynamic, floristic and faunistic components, as a whole they are included within the Chilean winter rainfall forest biodiversity hotspot (Myers et al. 2000). Cei (1962) and Formas (1979) documented ten anuran species to partially overlap [*Alsodes nodosus* (Duméril & Bibron 1841), *A. vanzolinii* Donoso Barros 1976, *Batrachyla taeniata* Girard 1854, *Eupsophus septentrionalis* Ibarra-Vidal, Ortiz & Torres-Pérez 2004, *E. queulensis* Veloso, Celis-Diez, Guerrero, Méndez, Iturra & Simonetti 2005, *Rhinella arunco* (Guichenot, 1848), *Rhinoderma darwinii* Duméril & Bibron 1841)] or fully overlapping their distribution range within this area [*Calyptocephalella gayi* (Duméril & Bibron 1841), *Rhinoderma rufum* (Philippi 1902) and *Pleurodema thaul* (Lesson 1826)].

At these Coastal Range latitudes (33º - 37º LS) water is naturally scarce (Oyarzún et al. 2003, Falvey & Garreaud 2007). Consequently, native frogs display at least four different reproductive larval adaptive strategies (Formas 1981, Cuevas & Cifuentes 2009) along this area; which are strongly determined by local rainfall and temperatures, all linked to predominant seasonal weather. In this context, ancient wooded formations historically have played a key role in safeguarding this reduced assemblage of endemic anuran species maintaining habitat, environmental heterogeneity and suitable micro-climatic conditions (Gutiérrez & Squeo 2004).

Currently, amphibian declining has become a worldwide scientific and public matter of awareness (Pechmann et al. 1991, Collins & Halliday 2005, Lips et al. 2006). Among a long list of threats (see www.iucn.org), habitat degradation and synergisms between two or more alterations, emerge as the most distressing factors to frog's survival (IUCN 2010a). In many extreme cases this implies a loss of the ecosystem's functionality and resilience (Grant et al. 1994, Chapin et al. 2003, Gibbs 2008). In this storyline, Smith-Ramírez (2004) has brought attention to the loss of biodiversity and endemism in the Chilean Coastal Range (40º to 42º S) due to native forest degradation. Not surprisingly, at least six of the frog species above mentioned has been categorized as Threatened (IUCN 2010b); furthermore, specimens of *R. rufum* has not been collected since 1981 being catalogued as Critically Endangered (A2ace) representing a major concern in this study. However, ecological impacts on amphibians assembly as a result of progressive deterioration of native forest along the Cordillera de la Costa (67 % from 1975 to 2000) (Gonzalez-Espinoza et al. 2007), are still largely unknown.

Bio-ecological base line data attained in 10 fieldwork campaigns (see Table 1) conducted between August 2003 and December 2009 are analyzed herein. Thus, assuming that in degraded places, water availability is scarcest than in well preserved ones (Little et al. 2009), in this work it is hypothesized that a negative synergism occurs between free larval adaptive strategies and degraded habitat conditions.

MATERIAL AND METHODS

Study area

This research was conducted along Coastal Range forest of Central Chile (32º to 37º S) (Fig. 1). This area is included into the semi arid and sub humid Mediterranean regions

Forest	Localities	Coordinates	Rch	An	Av	Asp1	Asp2	Bl	Bt	Cg	Eq	En	Es	Er	Tb	Ta	Rr	Rd	Pt
D. Steppe	Zapallar* (1)	32º34’ S; 71º28’ W	-	5	-	-	-	-	0	0	-	-	-	-	-	-	0	-	8
	Laguna Verde (4)	33°06’ S; 71°39’ W	-	15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7
	La Campana (3)	33º02’ S; 71º15’ W	-	13	-	-	-	-	0	0	-	-	-	-	-	-	-	-	5
	Aguas Claras (2)	32º33’ S; 71º26’ W	-	11	-	-	-	-	4	1	-	-	-	-	-	-	-	-	12
Scl. Forest	Q. Ranguilí* (9)	34º50’ S; 71º15’ W	2	0	-	-	-	-	0	1	-	-	-	-	-	-	0	-	16
	Barranca Alta* (7)	34º41’ S; 71º43’ W	0	2	-	-	-	-	-	0	-	-	-	-	-	-	0	-	7
	Nilahue Alto* (8)	34º41’ S; 71º35’ W	0	3	-	-	-	-	-	0	-	-	-	-	-	-	0	-	0
	Tanumé (6)	34º12’ S; 71º57’ W	0	2	-	-	-	-	-	0	-	-	-	-	-	-	0	-	0
	Vichuquén* (10)	34º53’ S; 71º58’ W	0	2	-	-	-	-	-	4	-	-	-	-	-	-	0	-	5
Scl. Bushes	Villa Alhué (7)	34°00’ S; 71°14’ W	1	0	-	-	-	-	-	0	-	-	-	-	-	-	0	-	0
M. Forest	Los Ruiles (12)	35°59’ S; 72°14’ W	-	0	-	4	-	-	-	0	8	-	-	-	-	-	-	-	6
	Los Queules (13)	35º59’ S; 72º41’ W	5	0	-	10	-	18	-	8	7	-	24	-	13	-	-	-	12
	Trehualemu (11)	35°56’ S; 72°43’ W	-	0	-	7	-	0	-	0	0	-	-	-	-	-	-	-	17
Noth. forest	Chiguayante* (16)	36º51’ S; 72º59’ W	0	-	-	-	-	0	0	0	0	-	-	15	-	-	0	0	9
	Ramadillas* (17)	37º18’ S; 73º17’ W	0	-	5	-	-	12	6	0	0	12	-	-	0	-	0	0	9
	Nonguén (14)	36º46’ S; 73º03’ W	0	-	-	-	-	6	5	0	0	-	-	5	-	-	0	0	7
	SP Tregua (15)	39°36’ S; 72°03’ W	0	-	-	-	12	10	9	0	0	-	-	25	-	4	-	5	15

TABLE 1. SUMMARY OF SURVEY LOCALITIES DATA (COORDINATES AND REGIONS) AND NUMBER OF SPECIMENS DETECTED DURING THE FIELDWORK. * Places where *R. rufum* was previously collected. Note: Number among parenthesis indicate the locality name assigned in yellow in the map (A) in figure 1. % Species never detected, 0 species reported but not detected in this study. An = *Alsodes nodosus*, Av = *A. vanzolinii*, Asp1= *Alsodes* sp1., Asp2= *Alsodes* sp2, Bl = *Batrachyla leptopus*, Bt = *B. taeniata*, Cc = *Calyptocephalella gayi*, Es= *Eupsophus septentrionalis*, Eq= *Eupsophus queulensis*, En= *Eupsophus nahuelbutensis*, Er = *Eupsophus roseus*, Pt = *Pleurodema thaul*, Rr = *Rhinoderma rufum*.

Resumen de datos de las localidades prospectadas (coordenadas y regions) y números de especímenes detectados en el trabajo de campo. * Lugares donde *R. rufum* fue colectado previamente.

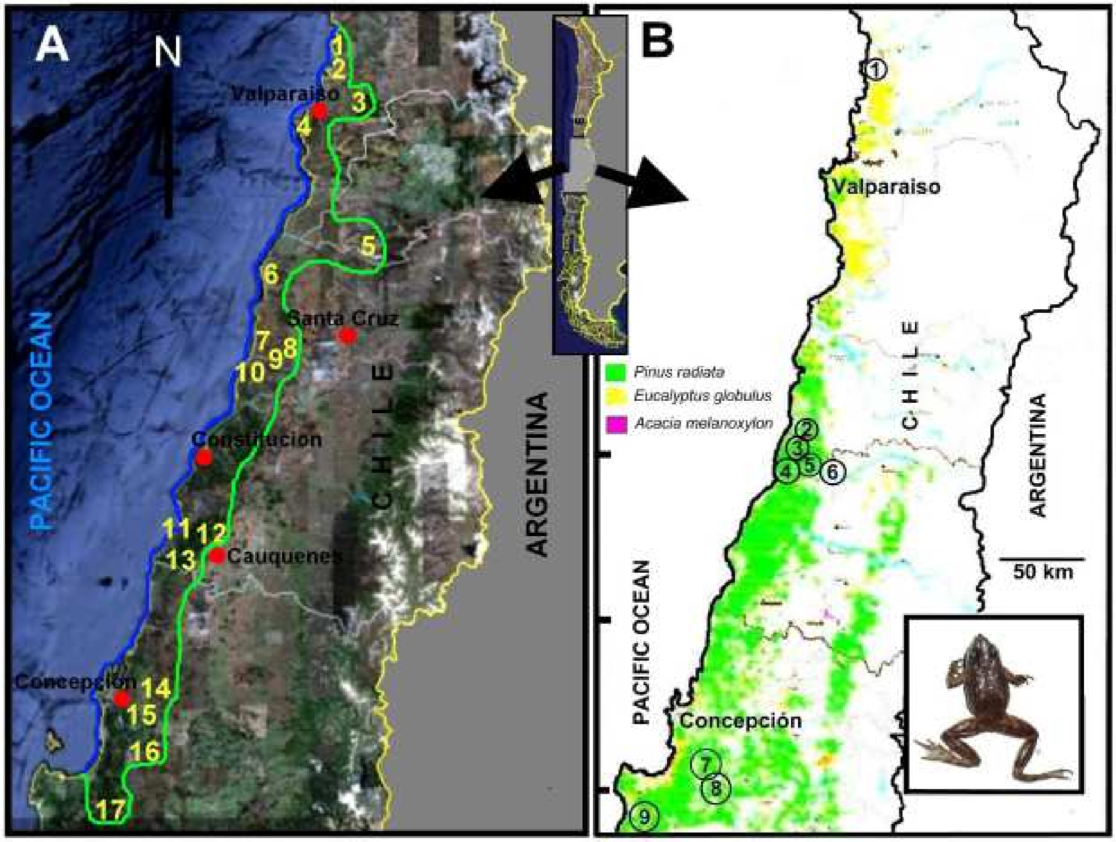

FIGURE 1: MAPS OF CENTRAL CHILE SHOWING IN: A THE SAMPLED LOCATIONS ALONG COASTAL RANGE, AND IN B THE AREA COVERED WITH EXOTIC PLANTATIONS OF *Pinus* And *Eucalyptus* Coinciding With *R. rufum* RANGE. INFERIOR RIGHT FRAME SHOWS A *R. rufum* photo.

Mapa de Chile central mostrando en: A los lugares muestreados a lo largo de la Cordillera de la Costa, y en B el área cubierta con plantaciones exóticas de *Pinus* and *Eucalyptus* coincidiendo con la distribución de *R. rufum*. Recuadro inferior derecho muestra una foto de *R. rufum*.

sensu Di Castri (1968). It presents average annual temperatures among 15.6 and 22.1ºC, and an annual mean rainfall ranging from 424.2 mm to 942.8 mm (Di Castri & Hayek 1976). Hottest months extends from November to February (25º C average temperature), and the coldest from March to August (7º C). Woody formations considered in this study are located between the drainage of the Biobio and the Aconcagua Rivers. Human settlements (cities and small villages), agriculture, and forestry activities are predominantly land uses (70%). Agriculture is devoted to wine-related activities (10%) fruit culture (5%) and other food production activities. The forestry industry is devoted to *Pinus radiata* D. Don and *Eucalyptus globulus* Labill and to a minor scale to *Populus alba* L. plantations (Fig. 1B). The wild grasses *Equisetum bogotense* Kunth, *Lamium album* L. and *Sylibum marianum* (L) Gaertn are very common at the border of stream or humid areas.

Fieldtrips, sampling and collection efforts

Among September of the year 2003 and December of 2009, 17 localities were surveyed (Fig. 1A). Sexagesimal co-ordinates (Datum WGS 84) were obtained with a GPS (Global Positioning System) (Table 1). Ten of the sites were selected based on historical species distribution (1918–1981) (Table 2) and voucher specimens information (Table 3) for *R. rufum* (Instituto de Zoología Universidad Austral: IZUA and Zoologishes Forschunginstitut Museum Koenig: ZFMK), as one of the purposes of this research was to re-find specimens of this species. Seven additional localities were selected using additional bibliographic sources accounting for the total anuran fauna described for this area up to date (Table 2).

Habitat quality assessing

Vegetation and the richness for each site it was determined using a qualitative scale ranging from one to five (Coneza Fernández - Vitora 1997), with One equating to monospecific vegetation and Five indicating a high diversity of plant species. In order to analyse the results, survey localities were classified as degraded and conserved places. A degraded place was that which lost an important fraction of their original vegetation components (1 to 2.5), and therefore, also their capacity to generate a propitious micro climate for frog's life. In contrast, a conserved place was that which nonetheless to be altered, maintain native vegetation elements (2.51 to 5), allowing water production and moisture.

Forest ecosystem	Taxa	References
Desert Steppe	*Rhinella* (1 sp), *Calyptocephalella* (1 sp), *Pleurodema* (1 sp), *Batrachyla* (1 sp).	Philippi 1902, Cei 1962, Formas & Brieva 1999
Sclerophylous Forest	*Alsodes* (1 sp), *Rhinoderma* (1 sp), *Rhinella* (1 sp), *Calyptocephalella* (1 sp). 1979, Díaz & Veloso 1979.	Barros 1918, Cei 1962, Formas et al. 1975, Formas
Maulino Forest	*Alsodes* (1 sp), *Eupsophus* (2 sp), *Pleurodema* (1 sp), *Calyptocephalella* (1 sp).	Cei 1962, Formas 1979, Ibarra-Vidal et al. 2004, Veloso et al. 2005.
Sclerophylous shrub	*Alsodes* (1 sp), *Pleurodema* (1 sp), *Calyptocephalella* (1 sp), *Rhinella* (1 sp).	Cei 1962, Philippi 1902, Navarro & Veloso 1988.
Nothofagus forest	*Alsodes* (1 sp), *Batrachyla* (3 sp), *Rhinoderma* (2 sp), *Eupsophus* (2 spp), *Calyptocephalella* (1sp), *Pleurodema* (1 sp), *Rhinella* (1 sp), *Telmatobufo* (1 sp).	Formas 1979, Cuevas & Formas 2005, Cuevas & Ugarte 2008, Cuevas & Cifuentes 2009.

TABLE 2. SUMMARY OF THE FOREST ECOSYSTEMS, FROG'S GENERA AND SOURCES OF PREVIOUS DATA IN THE STUDY AREA.

Resumen de los ecosistemas boscosos, géneros de ranas y fuentes de datos previos en el área de estudio.

Locality	Region of Chile	Nº catalogue	N	Source
Zapallar	De Valparaíso	IZUA 1800	1	Formas et al.1975
Santiago (Zapallar)	De Valparaíso	ZFMK 8344-8349	6	Busse & Werning 2004
Barranca Alta	De O'Higgins	IZUA 1294-1297	4	Torres & Castillo 1973
Vichuquén	De Maule	ZUA 1323-1332	10	Philippi 1902, Formas et al. 1975
Cerro Caracol	Del Biobío	IIZUA 535	8	Formas et al. 1975
Chiguayante	Del Biobío	IZUA 1235-1249	14	Formas et al. 1975

TABLE 3. SUMMARY OF LOCALITIES, VOUCHER SPECIMENS OF *RHINODERMA RUFUM* IN CENTRAL CHILE, AND LITERATURE SOURCE OF THESE DATA. IZUA= Instituto de Zoología Universidad Austral de Chile, ZFMK = Zoologische Forschungsinstitut und Museum Köenig.

Resumen de las localidades, números de catálogos de los especímenes de Rhinoderma rufum en Chile Central y fuente literaria de estos datos.

Physical parameters such as stream water temperature (Tº), air temperature and relative humidity (HR) were obtained with an alcohol thermometer (graduated -15 to 50ºC) which was submerged during 10 minutes to obtain a reliable measure, and a digital hygrometer. Related chemical parameters such as water pH and dissolved (oxygen) O_2 were registered with a portable pH-meter only in those places where small streams or springs were present. UV-B measurements were obtained from www.meteochile.cl (Meteorological Direction of Chile).

Sampling and Data analysis

In each locality, samplings in random quadrants (20 x 20 m) were carried on. Time-limited searches for adults, juveniles and tadpoles in the water (hand nets) were performed for 60 minutes in three related areas: a) forest, b) prairie, and c) ecotone. In each place, three replicates per each type of sample were done, an effort that demanded at least two days per site. In highly heterogeneous areas collections were not time-constrained, rather, each researcher covered a transect trying to complete a similar collection time. To evoke mating calls of *R. rufum*'s males, during breeding season a male calling playback was executed six times for 10 minutes (60 minutes) with 5 minute intervals.

Based on fieldwork data the following diversity indexes were determined. 1 *Efficiency of detection*: Ed = N/Np x 100; where: N = number of captured species, and Np = number of potentially present species (Corn & Bury 1990). 2 *Specific diversity*: DMg = (S-1)/lnN, where (S-1) = total number of species, and N = total number of collected individuals. 3 *Density of species* (Ds): Spp/A the number of species or individuals by unit of area (Heyer et al. 1994).

In order to evaluate the impact of the habitat quality on the amphibian diversity along the study area a multivariate non metric multidimensional scaling (NMMDS) analysis was developed with the program PAST V 2.00 (Paleontological statistics) to assess: a) frog diversity (species) v/s forest ecosystem; b) frogs composition v/s degraded and conserved places; and c) frogs larval strategy v/s degraded and conserved places. Larval strategy follows Cuevas & Cifuentes (2009) where LMS (Lenthic mountain stream) *Alsodes*; LeMS (Lothic mountain stream with mouth in like sucker form) *Telmatobufo*; LFTL (Lenthic free tadpole in permanent ponds with a large period to reach the metamorphosis) *Calyptocephalella*, *Batrachyla*; LFTSh (Lenthic free tadpole in temporal ponds with warm water with very short larval period) *Pleurodema*, *Rhinella*; DD (Direct development, Neomely) *Rhinoderma darwinii;* ID (Indirect development), R. rufum; TD (Terrestrial development, nidiculous) *Eupsophus*.

The non-metric MDS was complemented with an analysis SIMPER («Similarity Percentages-species contribution») to each previous analysis, in order to evaluate the percentage of similarity of the fauna between different sampled localities based on the similarity matrix calculated with the coefficient of Bray-Curtis, previous transformation of the data with double square root (Zar 1999)

RESULTS

Collection efforts and species richness

A 520 total man hours were logged for all fieldtrips, with the greatest efforts being displayed in Barranca Alta and Nilahue Alto (48 hours/man in two days), Los Queules (60 hours/man in five days), and Chiguayante (60 hours/man in three days). A total of 14 anuran species belonging to five families (Batrachylidae, Alsodidae, Calyptocephalellidae, Bufonidae, and Leptodactylidae) (sensu Frost 2014), were detected along the study area. Details of the localities, genera, species, and amount of specimens detected in each forest ecosystem are presented in Table 1.

The most common species observed was *P. thaul*, recorded in 72.2% of the total localities, following by *A. nodosus* (36.36%), and *C. gayi* (18.18%). Eggs and tadpoles in the border of streams were recorded for *E. nahuelbutensis*, and *P. thaul*. In a swimming pool in Ranguilí, eggs of *Rhinella arunco* were detected. First time records were obtained for *A. nodosus* in Barranca Alta, Vichuquen, and Nilahue Alto; for *Batrachyla leptopus* and *Telmatobufo ignotus* Cuevas, 2010 in Los Queules; and an unnamed species of *Alsodes* (called here *A. aff vanzolinii* because chromosome attributes) in Los Ruiles, Los Queules, and Tanumé. The only species never detected during the fieldwork was *R. rufum.* However, according to data *registered* (Table 1) during fieldwork, *R. rufum* might be re found in Barranca Alta, Nilahue Alto, and Chiguayante. Seven populations could not be found again and are likely extinct.

Playback stimulation

When mating call was played outdoors to evoke the presence of *R. rufum*, positive results were not obtained. Frog call responses corresponded mainly to males of *Eupsophus roseus* (Nonguén), *E. queulensis* (Los Queules), *Batrachyla leptopus* (Ramadillas), and *Pleurodema thaul* (Ranguilí).

Habitat characterization

According to Conesa Fernandez - Vitora (1997) indexes, Barranca Alta, Nilahue Alto and Ramadillas reached minimal values (1 to 2), and only La Campana, Los Queules, Los Ruiles and San Pablo de Tregua (control site) all of which are protected areas, reach top values (5). Consequently classified by a woody ecosystem the following localities were considered as degraded: *Dessert Steppe*: Zapallar and Laguna Verde; *Sclerophyl forest*: Barranca Alta, Quebradas de Ranguilí, and Nilahue Alto; *Maulino Forest*:

Trehualemu; *Sclerophyl Bushes*: Villa Alhué; *Nothofagus* Forest: Ramadillas, Chiguayante. In almost all these localities the native forest has been replaced by exotic commercial plant species (*Pinus radiata* and *Eucalyptus globulus*) and also exotic bushes such as *Rubus* sp., *Rosa* aff. *rubiginosa*, *Spartium junceum* and *Ulex europaeus*. In contrast, those localities that based on their flora presented a more healthy condition were considered as conserved, and included: *Dessert Steppe*: Aguas Claras, La Campana; *Sclerophyl Forest*: Tanumé, Vichuquén; *Maulino Forest:* Los Ruiles, Los Queules; *Sclerophyl Bushes*: Quirihue; Nothofagus *forest*: Nonguén, SP Tregua. These localities were characterised by remnants of native forest such *Acacia caven* (Molina) Molina, *Prosopis chilensis* (Molina) Stuntz, *Quillaja saponaria* Molina, *Cryptocarpa alba* (Molina) Looser, *Peumus boldus* Molina, *Aristotelia chilensis* (Molina) Stuntz, and remnants of *Aextoxicon punctatum* Ruiz et pav. forest. They all contained small watershed surrounded by wild native grasses and bushes.

Measures of physical parameters (water and air Tº) (Table 4) were relatively high. Extreme values of water Tº were 11.5ºC (La Campana) and 18ºC (Barranca Alta). Extremes values of air Tº were 20.5 ºC (Ranguilí) and 15.0 ºC (Zapallar). Maximum extremes values were 37 ºC (Aguas Claras) and 24.6 ºC (Chiguayante). In Barranca Alta, water and air temperatures were the same (18 ºC). Measures of chemical parameters indicate a more alkaline (8.90) pH in Vichuquén and minor (7.5) in Barranca Alta. Values of relative humidity fluctuate between 38% in Ranguilí (degraded) and 85% in Nonguén (conserved). UV-B indexes are showed in Fig. 2.

Localities	Tº water	Tº air	Tº air max	pH	HR	0_2
Zapallar	12.2 ºC	15.0 ºC	35.5 ºC	8.00 (-67 mv)	67%	10.00 mg L^{-1}
Aguas Claras	12.4 ºC	15.6 ºC	37.0 ºC	8.36 (-68 mv)	78%	10.00 mg L^{-1}
La Campana	11.5 ºC	16.8 ºC	32.7 ºC	7.80 (53 mv)	75%	10.98 mg L^{-1}
Barranca Alta	18.0 ºC	18.0 ºC	33.0 ºC	7.50 (-55 mv)	69%	10.70 mg L^{-1}
Nilahue Alto	15.0 ºC	20.0 ºC	29.2 ºC	8.30 (-65 mv)	39%	9.98 mg L^{-1}
Ranguilí	16.0 ºC	20.5 ºC	30.0 ºC	8.15 (-67 mv)	38%	9.70 mg L^{-1}
Hualañé	12.3 ºC	18.5 ºC	27.8 ºC	8.90 (-69 mv)	78%	10.00 mg L^{-1}
Vichuquén	*	18.6 ºC	30.8 ºC	*	77%	*
Cerro Caracol	*	17.9 ºC	27.9 ºC	*	85%	*
Chiguayante	12.5 ºC	16.5 ºC	24.6 ºC	8.50 (-65 mv)	75%	9.50 mg L^{-1}
Ramadillas	15.0 ºC	16.5 ºC	25.0 ºC	8.45 (-64 mv)	75%	9.45 mg L^{-1}

TABLE 4. SUMMARY OF PHYSICAL AND CHEMICAL PARAMETERS TAKEN DURING THE FIELDWORK. (*) PLACES WITHOUT WATER DURING THE FIELDTRIP TIME. Tº WATER AND AIR (FIRST TWO COLUMNS) AT SAME HOUR.

Resumen de los parámetros físicos y químicos tomados durante el trabajo de campo. (*) Lugares sin agua durante las actividades de terreno. Tº agua y aire (primeras dos columnas) a la misma hora.

Data analysis results

Diversity indexes: In the Table 5 are presented a summary of some diversity indexes estimations for each locality.

NMMDS Batrachofauna v/s forest ecosystems: Figure 2A is an acceptable graphic representation of the relations between forests ecosystems based on batracofauna composition [stress 0.17 (Fig. $2a_1$) Kruskal 1964]. Localities linked to the *Nothofagus* forest (green circles) have more similarities among themselves than when they are compared to the rest of the localities, which were displayed separated of the preceding group and among themselves. Those localities from Dessert Steppe (red circles) (AC, LC, ZAP, and LV) are grouped besides to those associated to Sclerophyl forest (violet circles) (BARR, VICH, RAN), Sclerophyl shrub forest (yellow circle) (ALH) and one from Maulino forest (blue circles) (TREH). All the remaining places were separated from the previous groups and among themselves, i.e., LQ and LR (Maulino Forest) and TAN and NALT (Sclerophyl forest). The cluster shown in Fig. $2a_2$ supports these results, with localities associated to *Nothofagus* forest grouped alone. The SIMPER analysis indicated a percentage of dissimilarity of 76.27 % among all the localities; the species contribution to similarities are supported mainly by *P. thaul* with 16.2 %, *A. nodosus* 15.16 %, *E. roseus* 9.17 %, *Alsodes* sp (1) 5.64 % with a cumulative percentage of 70.1 %.

Localities	Ed N/Npx100	DMg (S-1)/lnN	*Ds* Spp/A
La Campana	25%	0.691	16
Zapallar	60%	0.779	21
Aguas Claras	60%	1.200	10.5
Barranca Alta	50%	0.910	3.33
Ranguilí	50%	1.010	4.33
Los Quillayes	25%	0	0.33
Nilahue Alto	25%	0.910	3
Hualañé	0	0	0
Vichuquén	0	0	0
Cerro Caracol	75%	1.275	13
Chiguayante	80%	0.629	15
Ramadillas	71,4%	1.231	11

TABLE 5. SUMMARY OF SOME DIVERSITY INDEXES DETERMINED ON THE BASE OF DATA REGISTERED DURING THE FIELDWORK.

Resumen de algunos índices de biodiversidad determinados en base a los datos registrados en terreno.

NMMDS Batrachofauna composition v/s degraded and conserved places: Fig. 2B is a good representation of the relationship of the amphibian community to degraded and conserved places of the forest ecosystem (stress of 0.07, Fig. $2b_1$). The percentage of dissimilarity between degraded and conserved sites was 72.93 %. The species *P. thaul, A. nodosus*, *E. roseus*, *Alsodes* sp (1) were those with the highest similarity contribution (SIMPER analyses results) with a cumulative percentage of 65.86. The plot (Fig. 2B) suggests that faunal communities (abundance and composition of frogs) registered in degraded places are different to those detected in those conserved ones, except in *Nothofagus* forest where degraded and conserved showed similar fauna richness but distinct diversity composition. An exception was Ramadillas, which appeared apart from the remaining degraded places suggesting that the faunal composition was more similar to those conserved ones, in spite of being a much degraded locality.

NMMDS Frogs larval strategy v/s degraded and conserved places: The plot showed in Figure 2C is a good representation of the relationship between larval strategy and habitat degradation (stress 0.065; Fig. $2c_1$). The Cluster in Fig. $2c_2$ shows that the degraded Maulino forest (MauDeg) constitutes a distinct group. Maulino Conserved (MauCon) plus *Nothofagus* degraded and Conserved (NotDeg, NotCon) form another group based on anuran larval strategy. The SIMPER analyses indicated that these species differ in a 37.44 % based on their larval strategy. In

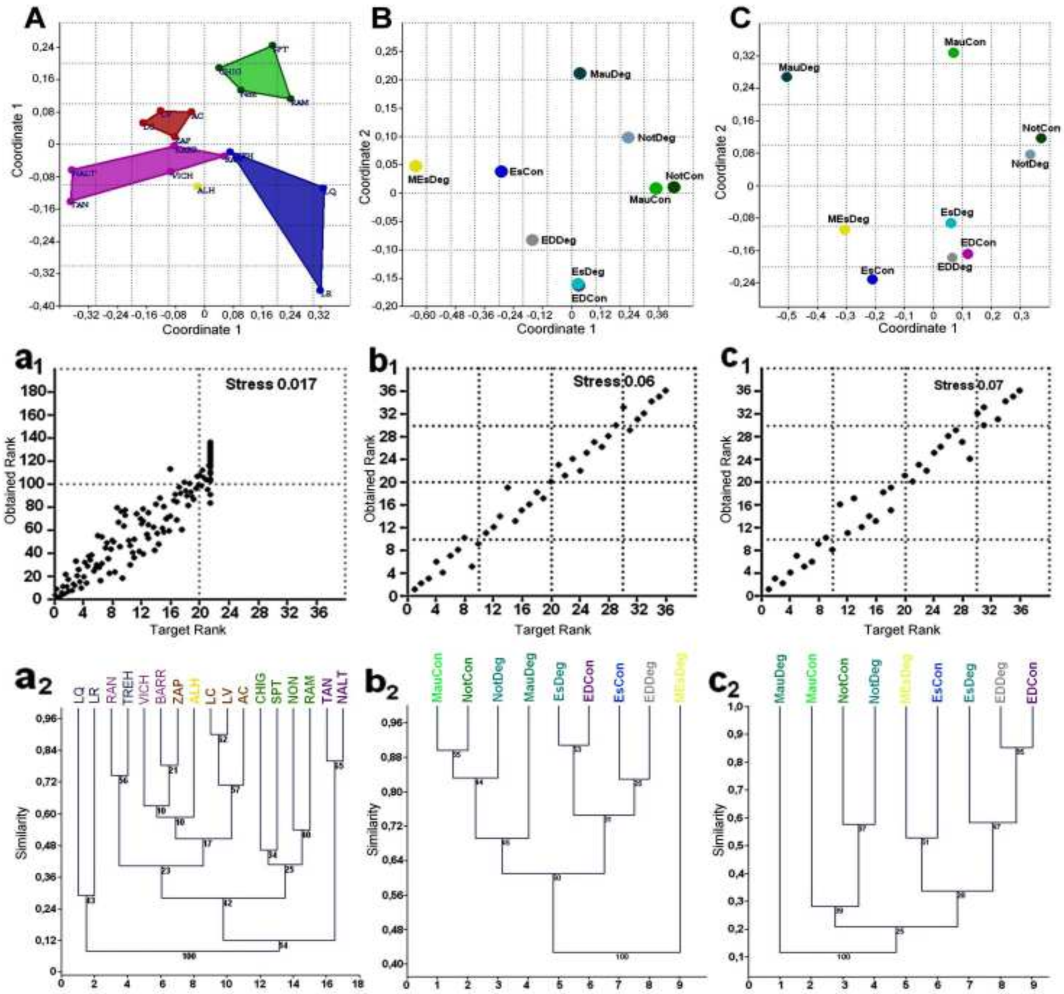

FIGURE 2. NON METRIC MULTI DIMENSIONAL SCALING (NMMDS) (BRAY CURTIS INDEX) OF ANURAN DIVERSITY IN COASTAL RANGE FOREST IN CENTRAL CHILE SHOWING IN: A, A_1 AND A_2 BATRACHOFAUNA V/S FOREST ECOSYSTEMS, B, B_1 AND B_2 BATRACHOFAUNA COMPOSITION V/S DEGRADED AND CONSERVED PLACES, AND C, C_1 AND C_2 FROGS LARVAL STRATEGY V/S DEGRADED AND CONSERVED PLACES. WHERE A_1, B_1, C_1 STRESS OF THE ANALYSIS AND A_2, B_2, C_2 CLUSTER ANALYSIS. ABBREVIATIONS ARE GIVEN IN CORRESPONDING RESULTS SECTION.

Escalamiento multidimensional no-métrico (NMMDS) (índice de Bray-Curtis) de la diversidad de anuros en la Cordillera de la Costa en Chile central, mostrando en: A, a_1 y a_2 Fauna de batracios v/s ecosistemas forestales, B, b_1 y b_2 Composición fauna de anuros v/s lugares conservados y degradados, y C, c_1 y c_2 Estrategia larval de las ranas v/s lugares degradados y conservados. Donde a_1, b_1, c_1 es el Stress del análisis y a_2, b_2, c_2 el análisis de Cluster. Las abreviaciones están dadas en la sección resultados.

contrast, terrestrial development (TD), lothic free tadpole with large period of larval phase (LFTL), and lenthic mountain stream (LeMS), all of them with 10.56, 10.33 and 7.90 % respectively, contributed with the 76.91 % cumulative of similarity of this analysis.

DISCUSSION

Frog reproductive activities and skin gaseous interchanges are closely related to water quality and availability, and healthy ecosystems (Duellman & Trueb 1986). Thus, they are considered key bio-indicators of the environmental health (Hazell 2003, Blaustein & Johnson 2006) given their extreme vulnerability to habitat degradation and fragmentation (Crump 2002, Busse 2002, Lips et al. 2006). The main processes driving extinction are the «evil quartet», i.e. habitat loss, over-exploitation, introduced species and chains of extinction (Diamond 1984). Forest ecosystems of the Coastal Range in central south of Chile have been seriously degraded because of large-scale anthropogenic intervention (Smith-Ramírez 2004, Bustamante & Simonetti 2005) (Fig. 1B). Accordingly, severe impacts on frog habitats and viability were expected in this investigation.

Regardless, when we compare these results against historic amphibian data for the study area (see Table 2), it is clear that most of the species, with the exception of *R. rufum*, were still there. Southern forest ecosystems (e.g. Maulino forest, Los Queules) *B. leptopus* (Cuevas & Cifuentes 2011), *Telmatobufo ignotus* (Cuevas 2010) and a new species of *Alsodes* previously confounded with *A. nodosus* (Cuevas & Cifuentes 2009) were also discovered. Thus, the alpha diversity sensu Meffe & Carrol (1997) showed values just under 2 indicating lower diversity of species (5 are indicative of high diversity). This is consistent with previous antecedents (Formas 1979, Correa et al. 2011) and unlike with higher levels of diversity of Chilean frogs reported among 38º and 40º LS within the temperate *Nothofagus* forest (Formas 1979, Mendez et al. 2005). The same trend was observed in conserved places against degraded sites in each of the abovementioned forest ecosystems, except in the case of the *Nothofagus* forest (Fig. 2B), where similar species diversity among degraded and conserved places was observed.

Some comprehensive studies (Diamond 1984, Grant et al. 1994, Chapin et al. 2000) have demonstrated that forest-ecosystem instability and the decline of diversity in local habitat are both correlated to the expansion of exotic monocultures, and to the corresponding damage linked to loss of water and refuges (Cushman 2006). Thus, more disturbed sites generally have lower species richness (Bishop et al. 1999), although in the case of the present study this is not the rule, having disturbed places (Ramadillas) higher or similar diversity index than conserved ones (Aguas Claras). Along Coastal Range forest ecosystems, hydrologic sources are mainly small springs giving rise to small fragile mountain streams (Oyarzún et al. 2003), where eventually tadpoles may complete their metamorphosis (Barros 1918, Torres & Castillo 1973). In Chile most species (80.71 %) display a free-living aquatic larval strategy, and among frogs collected along the study area this strategy reaches to 64.28 %. In forest ecosystems of Coastal Range in South Central Chile most documented amphibian localities are located within private lands. Given that currently there is not a legal framework for the protection of animal or plant species on private lands, the main threat faced by these species is habitat loss by commercial activities (e.g., wine industry, commercial fruit trees) and associated infrastructure (e.g., roads, summer centers, electrical grid) (Bustamante and Simonetti 2005). Furthermore, the replacement of native forest with exotic plantations (*Pinus*, *Eucalyptus* and *Populus*; subsided by government policy's 701 Law until the year 2003) have had negative effects in the remaining remnant ecosystems such as: accumulation of sediments by erosion and raindragging into streambeds, and reduction of water flow and increase in water and air temperature. In these scenario frogs with larval strategies are most threatened, because the loss of aquatic habitat (Becker et al. 2007, Cushman 2006).

Although our results suggest a relatively healthy assemblage (just one species was not detected) this study proposes that the current threatened conservation category to some Chilean anuran species inhabiting the study area most be maintained. There are many causal explanations (Cuevas & Cifuentes 2009) and probably synergisms between habitat degradation and life strategy requirements (see Baldwin et al. 2006). At least three conflicting issues can be identified in relation to the impact of forest loss and amphibian population viability. First human activities have created favourable habitat for *P. thaul* because its short larval period requires higher than normal temperatures to reach the end of metamorphosis (Díaz-Páez & Ortiz 2001). Because of that, apparently this species has become an excellent secondary invader showing a wide distribution. Second, landscape homogenization and the loss of suitable habitat to amphibians might affect *Rhinoderma rufum* to the greatest extent (Barros 1918, Formas et al. 1975, Busse 2002, Crump 2002). Third, because many streams are currently dry, species with less strict habitat requirements have more successfully survived (*Eupsophus*), in comparison to those with free tadpole (*Alsodes*) *Sensu* Becker et al. (2007). Incidentally, specimens of *Eupsophus queulensis* were detected in an abandoned *Pinus* plantation which presented *Aristotelia chilensis* renewals allowing conservation of moisture, by adding shade, leaf drop and refuges.

According to the IUCN Red List, a species is «Extinct» when there is no doubt that; their last member has died (IUCN 2010b). One of the purposes of this study, (to rediscover specimens of *R. rufum*) was not achieved. However, some data indicate that this species is seriously threatened but not extinct along its whole range, supporting its IUCN (2010b) assigned category. Evidence gathered during the fieldwork precludes quantitative conclusions about the conservation status of *R. rufum*. Regardless, recover data suggests that this species has vanished from some places such as, Barranca Alta, Nilahue Alto, and Chiguayante. In those places, suitable habitat and personal communications from local residents indicate that *R. rufum* was still there recently (Montecinos, Zúñiga Pers. Com.). *Rhinoderma rufum* has surely undergone local extinction in Zapallar, Hualañé, Ranguilí, Los Quillayes, Cerro Caracol, Ramadillas, and in the type locality of Vichuquén, where the original habitat has been devastated (Lara et al. 2012) having lost its resilience capacity (Chapin et al. 2000).

During the summer (December to March) when *R. rufum c*arries on its reproductive activities (Barros 1918, Torres & Castillo 1973), the air temperatures over 37º C, and the UV-B radiation reaches dangerous levels (Fig. 3A). However, the finding in Nilahue Alto (2003) of three specimens of *R. rufum* (Zúñiga, Pers. Comm.) during the building of a water pool near of a small stream, support the vanishing hypothesis (Fig. 3B). Following Lampert & Linsenmair (2002), the vanishing of some species might be explained by an alternative behaviour, involving a change of strategy when individuals face an unpredictable environment. Specimens of *R. rufum* might use the holes constructed by the freshwater shrimp's *Parastacus pugnax* to reach places with moisture and refuges to protect itself from the heat and UV-B radiation (Fig. 3B).

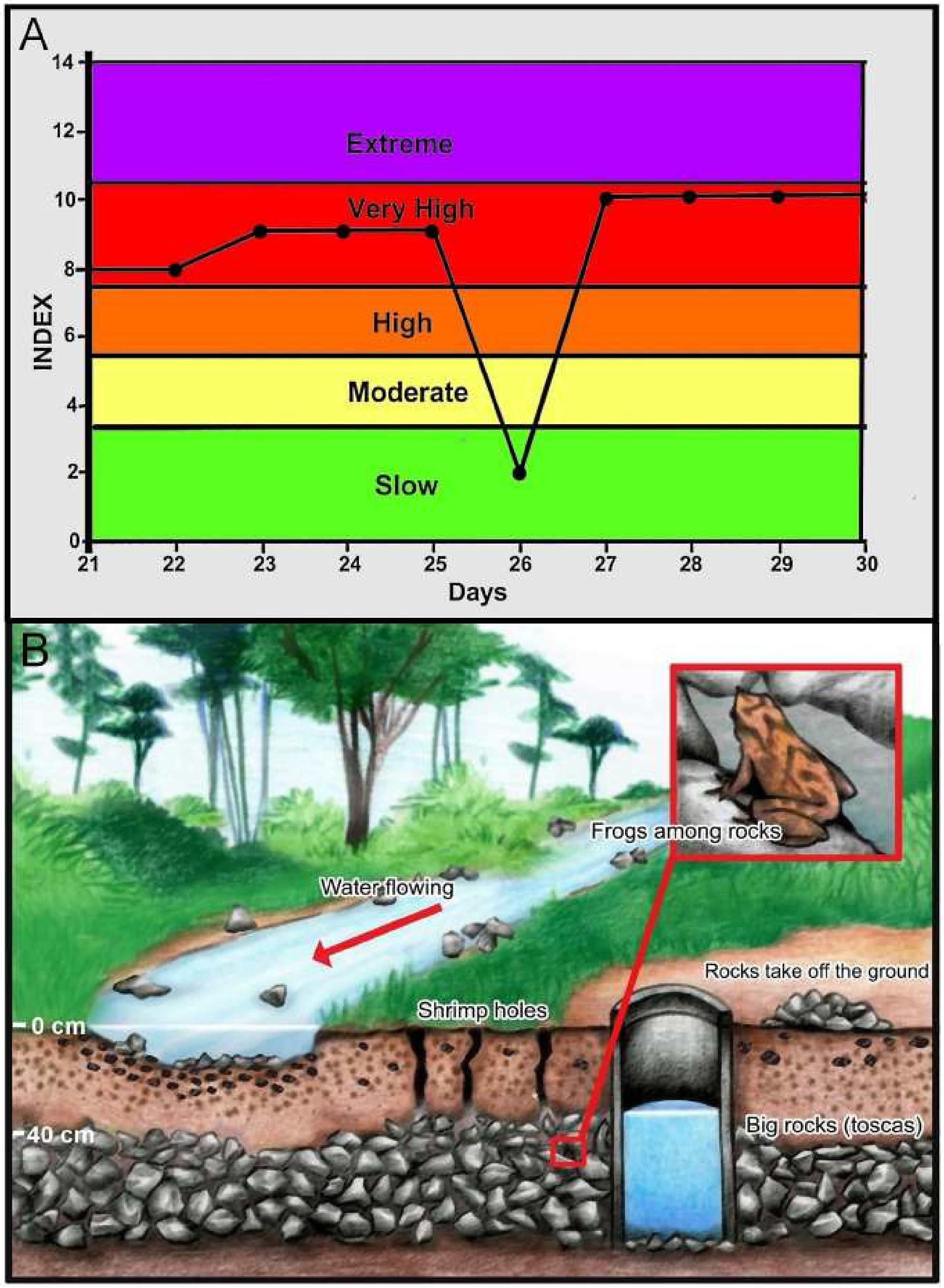

FIGURE 3: A GRAPHIC SHOWING MINIMUM AND MAXIMUM UV-B RADIATION IN CENTRAL CHILE DURING TEN DAYS OF FIELDTRIP (SUMMER 2004). B SCHEME REPRESENTING A HIDING STRATEGY HYPOTHESIS BASED ON ANECDOTAL INFORMATION.

A Gráfico mostrando valores máximos y mínimos de radiación UV-B en Chile Central durante 10 fías de trabajo en terreno (verano 2004). B Esquema representando la hipótesis «estrategia de ocultamiento de *R. rufum*» basada en información anecdótica.

CONCLUSIONS

Along the research area, has been detected six threatened anuran and at least six threatened plant species (IUCN, 2010b). Sadly, only a few small natural reserves have been created (8), most with insufficient ecosystem representation and inadequate coverage for biodiversity hotspots (Pauchard & Villarroel 2002). Most of these protected areas are wetlands (Cachagua, El Yali, Peñuelas, Federico Albert, Torca lagoon) not constituting suitable habitat for frogs with demanding habitat requirements such *Rhinoderma* (diurnal habits) or *Telmatobufo* (mountain streams environments), being these frogs in very defenceless status

In a globalization context, new economic policies by Chilean government are trying to transform our country into a world alimentary potency for the XXI century, implying that the future of our biodiversity is not hopeful. Data gathered about distributions, limiting environmental factors and the impacts of human activity is a first step towards the conservation of a species (Stuart et al. 2004). In this frame, once specimens be rediscovered, intervention of populations such as translocations most be considered.

Although, no ex situ conservation plans are in place for any anuran species in the research area, some successful attempts have been made in captivity management of *R. darwinii* in Germany between 1971 to 2002 (Busse 2002). Finally, this research is an example and of the first of the type of conservation approach conducted on Chilean anurans. Following the proposal of Diniz-Filho et al. (2005), this study calls for and promotes the idea of creating a national park or reserve to protect amphibians and their ecosystems in this part of south-central Chile.

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