



## Influence of biotic and abiotic factors on the metazoan parasite communities of a native prey fish: study in 28 Andean Patagonian lakes

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**ABSTRACT.** *Galaxias maculatus* (small puyen) is a prey fish which plays a main role in lake food webs of Patagonia. Previous studies, at local scale, have shown that the richness and composition of parasitic metazoan component communities associated to *G. maculatus* in a group of small shallow lakes in the surroundings of the Nahuel Huapi Lake, are affected by the composition of the native fish assemblage in each lake. The aims of this work were: a) to characterize the helminth community of *G. maculatus* at regional scale, including data of 28 Andean lakes of the Neuquén and Río Negro Provinces, and b) to identify biotic and abiotic factors of these lakes influencing the occurrence and prevalence of the different helminth species parasitizing this highly abundant and widely distributed prey fish in Patagonia. The analysis indicates that the parasite community of different populations of *G. maculatus* varies according to the basin, lake area, altitude and the fish assemblage of the lake.

[Keywords: *Galaxias maculatus*, parasites, helminth communities, Patagonia, *Percichthys trucha*]

**RESUMEN.** **Influencia de factores bióticos y abióticos sobre las comunidades de parásitos metazoos de un pez presa nativo: estudio en 28 lagos andino patagónicos.** *Galaxias maculatus* (puyen chico) es una especie de pez presa que tiene un rol importante en las redes tróficas de los lagos de la Patagonia. Estudios previos, a escala local, muestran que la riqueza y la composición de las comunidades componentes de parásitos metazoos de *G. maculatus* en un grupo de pequeños lagos someros en los alrededores del lago Nahuel Huapi, están afectadas por la composición del ensamble de peces nativos en cada lago. Los objetivos del presente estudio fueron: a) caracterizar la comunidad de helmintos de *G. maculatus* incluyendo datos de 28 lagos andinos de las provincias de Río Negro y Neuquén, y b) identificar los factores bióticos y abióticos de estos lagos que afectan la presencia y la prevalencia de las diferentes especies de helmintos que parasitan a este pez presa abundante y ampliamente distribuido en la Patagonia. El análisis indica que las comunidades de helmintos de las diferentes poblaciones de *G. maculatus* varían de acuerdo a la vertiente, al área lacustre, a la altitud y al ensamble de peces del lago.

[Palabras clave: *Galaxias maculatus*, parásitos, comunidades de helmintos, Patagonia, *Percichthys trucha*]

### INTRODUCTION

Some ecological studies on fish parasites, mainly in marine fishes, have attempted to relate host features such as body size, habitat, distributional range, and feeding and schooling habits with parasite diversity (Rodhe 2010; Timi et al. 2010). These studies have pointed out that the diversity of parasites is greater in hosts with large body size, high population density or extended geographical range; all features increasing the likelihood of encounter between an infective parasite stage and a potential host (Rodhe 2010). In freshwater systems, the helminth communities of fish show considerable spatial variation among lakes of the same region. For this reason, the main aim of studies on the ecology

of helminths parasitizing freshwater fish have involved the recognition of patterns and identification of processes affecting richness (Carney & Dick 1999; Barger & Esch 2001; Barger 2006), distribution (Kennedy 1990), diversity (Kennedy et al. 1986), and abundance of parasite communities (Kennedy et al. 1986). Other studies have related habitat features, such as trophic status of the lake, type of bottom, and presence of macrophytes, with parasite species abundance (Kennedy 1975; Marcogliese & Cone 1991). In addition, local processes in parasite communities are affected by biogeographical patterns and historical processes (Kennedy & Guégan 1994; Barker et al. 1996). Other studies have stressed out that lake differences in size, isolation, and fauna can reduce the similarity among component

Editora asociada: María Dieguez

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Recibido: 8 de abril de 2015, Fin de arbitraje: 8 de agosto de 2015, Última versión revisada: 4 de septiembre de 2015, Aceptado: 25 de septiembre de 2015.

communities of parasites. In contrast, extended geographical range of the host, physical proximity, movement of piscivorous birds, and similar fish assemblage can promote similarities (Esch et al. 1988; Hartvigsen & Kennedy 1993; Anderson & Sukhdeo 2009; Fernandez et al. 2010; Timi et al. 2010).

The native Patagonian freshwater fish fauna comprises 26 species including galaxiids, percichthyids, silurids, characids, atherinopsids, and mugilids (Pascual et al. 2007). *Galaxias maculatus*, the most world widely distributed galaxiid (McDowall 2006), is also the most abundant native fish in Andean Patagonian lakes, and a frequent prey of co-occurring piscivorous fish. On the other hand, *Percichthys trucha*, the largest native piscivorous in these lakes, feeds on *G. maculatus* (Macchi et al. 1999; Ruzzante et al. 2011). Other piscivorous species in the fish assemblages are the native *Galaxias platei* and introduced salmonids. *Galaxias maculatus* is an important link in the transmission of helminths, due to its intermediate trophic position in aquatic Patagonian food webs, and being involved in different food chains of fish and birds (Rasmussen et al. 1993; Macchi et al. 1999; Alarcón et al. 2012; Frixione et al. 2012).

The aims of this work were: a) to characterize the helminth community of *G. maculatus* at regional scale, including 28 Andean lakes of the Neuquén and Río Negro provinces, and b) to identify biotic and abiotic factors of the lakes influencing the occurrence and prevalence of the different helminth species parasitizing this highly abundant and widely distributed prey fish in Patagonia.

## MATERIALS AND METHODS

The studied area is included within two National Parks: Nahuel Huapi, and Lanín, and is characterized by a profuse hydrographic system including mainly ultraoligotrophic or oligotrophic glacial deep lakes. They are generally surrounded by subantarctic forest dominated by *Nothofagus dombeyi*, and the shoreline of the lakes can be colonized by the reed, *Schoenoplectus californicus* (= *Scirpus californicus*). Generally, these lakes have extended euphotic zone, oxygenated bottom, and the littoral offers many habitats promoting an increase in species richness. These freshwater systems have pelagic food webs with scarce native top predators although introduced salmonids in

some lakes exert top predation (Modenutti et al. 2010).

*Parasitological terminology* (Esch et al. 1990; Esch & Fernández 1993, Rauque et al. 2002; 2003)

- Allogenic species: parasite species that uses fish or other aquatic vertebrates as intermediate hosts and matures in birds or mammals.
- Autogenic species: parasite species with the entire life cycle completed within an aquatic ecosystem.
- Prevalence: percentage of hosts infected in a given sample.
- Infrapopulation: all parasites of a single species on/within one host.
- Infracommunity: infrapopulations on/within one host.
- Component community: infracommunities on/within a host population.
- Postcyclic transmission: when the adult parasite survives and reproduces in the predator of its definitive host.

### Data collection and statistical analysis

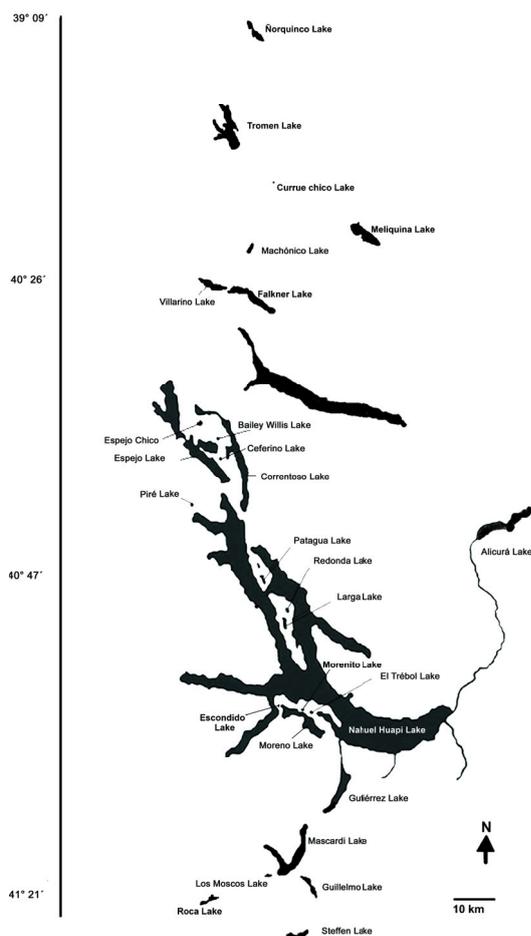
Studied lakes are located between 39°09' S and 41°31' S (Table 1, Figure 1), and flow either to the Pacific (Manso river Basin) or to the Atlantic (Limay river Basin). Between 20 and 30 fish were collected with seine nets and baited traps in the littoral zone of each lake during the summer of 2008-2009. Fishes were transported alive to the laboratory where they were maintained in aerated aquaria for no more than two days until they were killed by severing the spinal cord, and examined. Prior to dissection, the individual length was recorded with a digital caliper (to the nearest 1 mm), from the mouth to the end of the caudal fin. The fish were dissected to search for helminths. Examination included fins, skin, eyes, brain, gills, heart, abdominal cavity, gastrointestinal tract, liver, gall bladder, gonads, and kidney. Helminth parasites found during the examination were collected, identified, and counted.

To characterize the parasite community of *G. maculatus* in each lake, both autogenic and allogenic species were considered. The prevalence and component and infracommunity richness were calculated (Bush et al. 1997). The prevalence of parasites was evaluated in relation to abiotic and biotic factors of the lakes. The following geographic and morphometric parameters of the lakes were considered: altitude, basin (dichotomous variable

**Table 1.** Characteristics of the lakes and of *Galaxias maculatus* analyzed (N: number of puyen captured and individual total length; Pt: *Percichthys trucha*; Gp: *Galaxias platei*; Oh: *Odontestes hatcheri*; Ov: *Oliveichthys viedmensis*; Hm: *Hatcheria macraei*; Om: *Oncorhynchus mykiss*; Sf: *Salvelinus fontinalis*; Ss: *Salmo salar*; St: *Salmo trutta*).

**Tabla 1.** Características de los lagos y de los especímenes de *Galaxias maculatus* analizados. (N: número de puyenes capturados y largo total; Pt: *Percichthys trucha*; Gp: *Galaxias platei*; Oh: *Odontestes hatcheri*; Ov: *Oliveichthys viedmensis*; Hm: *Hatcheria macraei*; Om: *Oncorhynchus mykiss*; Sf: *Salvelinus fontinalis*; Ss: *Salmo salar*; St: *Salmo trutta*).

Lake	Latitude	Longitude	Size (ha)	Depth (m)	Basin	Macrophyta	Native predator	Other native fishes	Exotic predators	N	Total length of <i>G. maculatus</i> Range (mm)
Ñorquinco	-39.15	-71.28	7.09	87.2	Limay	no	Pt		Om Sf St	26	36-63
Tromen	-39.51	-71.43	28.99	>100	Limay	no	Pt	Gp Oh Dv	Om Sf St	24	17-55
Currué Chico	-39.90	-71.33	0.51	<20	Limay	yes	Pt	Gp	Om Ss	21	60-76
Machónico	-40.33	-71.48	1.45	<20	Limay	yes	Pt		Om St	30	39-62
Meliquina	-40.38	-71.28	13.93	90	Limay	no		Gp	Om Sf St Ss	20	42-52
Villarino	-40.43	-71.41	6.21	98	Limay	yes	Pt		Om Sf	20	49-66
Falkner	-40.45	-71.53	10.4	120	Limay	yes	Pt	Gp	Om Sf St	20	45-58
Espejo Chico	-40.60	-71.70	0.45	100	Limay	yes			Om Sf	30	41-58
Bailey Willis	-40.63	-71.66	0.5	10	Limay	yes			Om	30	34-59
Ceferino	-40.65	-71.70	0.031	<20	Limay	yes			Om	30	26-85
Correntoso	-40.66	-71.66	20.05	87.2	Limay	no	Pt		Om Sf St	30	39-64
Alicura	-40.66	-71.00	67.5	110	Limay	no	Pt	Oh Dv Hm	Om Sf St Ss	30	53-71
Espejo	-40.64	-71.72	38.83	245	Limay	yes	Pt	Gp Dv	Om Sf St	30	34-59
Pré	-40.71	-71.65	0.244	15	Limay	yes	Pt		Om Sf St	30	48-70
Patagua	-40.78	-71.61	1.077	<20	Limay	yes		Oh		30	25-62
Redonda	-40.90	-71.56	0.009	<20	Limay	yes			Om	30	34-98
Larga	-40.90	-71.55	0.504	<20	Limay	yes		Oh		30	37-66
Morenito	-41.03	-71.53	0.29	12	Limay	yes	Pt	Gp Oh	Om Sf	30	48-79
Escondido	-41.04	-71.31	0.08	8.3	Limay	yes	Pt			30	40-63
Nahuel Huapi	-41.06	-71.40	557	464	Limay	no	Pt	Gp Oh Dv	Om Sf St Ss	30	39-58
El Trébol	-41.06	-71.50	0.3	12	Limay	yes			Om	30	35-71
Moreno	-41.06	-71.55	11.34	112	Limay	yes	Pt	Gp Oh Dv	Om Sf St	30	34-52
Gutiérrez	-41.17	-71.41	16.4	111	Limay	no		Gp Dv	Om Sf St	30	46-72
Roca	-41.23	-71.55	3.5	38	Manso	yes			Om Sf St	20	49-70
Mascardi	-41.26	-71.64	39.26	218	Manso	yes		Gp	Om Sf St	30	44-72
Los Moscos	-41.35	-71.62	2.3	50	Manso	yes		Gp	Om Sf St	27	50-74
Guillelmo	-41.37	-71.49	6.5	107.1	Manso	yes			Om Sf	30	39-59
Steffen	-41.51	-71.55	5.39	77	Manso	yes	Pt	Oh Dv Hm	Om Sf St Ss	24	54-80



**Figure 1.** Map of the study area in Northwestern Patagonia (Argentina) indicating the lakes sampled in this study

**Figura 1.** Mapa del noroeste de la Patagonia (Argentina) indicando los lagos relevados en este estudio.

indicating whether a lake flows to the Pacific or to the Atlantic Ocean), area, and depth. Also, biotic factors such as the presence of macrophytes, the number of co-occurring piscivorous species, the total number of fish species, and nine dichotomous variables accounting for the presence (yes/no) of different fish species: *Percichthys trucha* (small mouth perch), *Odontesthes hatcheri* (Patagonian silverside), *Olivaichthys vielmensis* (velvet catfish), *Hatcheria macraei* (stream catfish), *Galaxias platei* (big puyen), *Oncorhynchus mykiss* (rainbow trout), *Salvelinus fontinalis* (brook trout), *Salmo trutta* (brown trout), and *Salmo salar* (landlocked salmon).

The statistical analysis of the resulting data was performed in two stages. First, a hierarchical cluster analysis was done to uncover clusters of lakes similar in terms of prevalence of the parasite species. Afterwards, the classification and regression tree analysis (Breiman et al. 1984; Therneau & Atkinson 2011) was used to characterize the clusters of lakes according to their geographical

traits and fish assemblages. The complete linkage based on Euclidean distance was used to measure intercluster dissimilarity (Everitt 2007).

The cluster analysis allocates each lake to a cluster; a categorical variable was defined to indicate the number of the cluster for each lake. In the tree analysis, the response variable was cluster allocation, and the predictor variables were the abiotic and biotic factors. In the first step of the process, the algorithm divides the whole set of lakes in two groups according to a question involving one of the explanatory variables (for example, "is the area of the lake  $\geq 3.5$  ha?"). Allowable questions involve one predictor  $x$ : if  $x$  is ordered, the question has the form "is  $x \geq c$ ", for a given value  $c$ ; if  $x$  is categorical the question has the form "is  $x$  in  $S$ ?" where  $S$  is any subset of categories of  $x$ . The question that defines the partition is automatically selected among all allowable questions based on a rule that maximizes a measure of the improvement caused by the new partition. In this study, we used the Gini measure of improvement (Therneau & Atkinson 2011). This process is repeated for each subgroup until all the subgroups reach a minimum size. Each step results in subgroups that are more homogeneous than the groups at the previous step; that is, there is less diversity in terms of number of clusters. The last step of the tree growing algorithm selects an appropriate tree size (pruning) (Therneau & Atkinson 2011). The resulting model can be represented as a binary tree whose leaves or terminal nodes correspond to the partition of the data. All analyses were performed with the R 3.1.1 package. The classification and regression tree analysis was performed using rpart3.1-54 (Therneau & Atkinson 2011).

## RESULTS

From the 28 sampled lakes, 23 belong to the Atlantic and 5 to the Pacific basin. Biotic and abiotic factors characterizing each lake are shown in Table 1. The size of the 772 studied specimens of *G. maculatus* varied between 26 and 98mm (Table 1).

A total of 17 helminth species were found parasitizing *G. maculatus* in the sampled lakes: 7 digeneans (5 larvae and 2 adults), 2 monogeneans (adults), 2 cestodes (1 larva and 1 adult), 2 acanthocephalans (adults), and 4 nematodes (2 larvae and 2 adults). Eleven of these species have autogenic cycles, the digeneans *Steganoderma szidati*, *Acanthostomoides apophalliformis*, and *Derogenes* sp., the monogeneans *Philureter trigoniopsis* and *Gyrodactylus* sp., the cestode *Ailinella mirabilis*, the acanthocephalans *Acanthocephalus tumescens* and *Pomphorhynchus patagonicus*, and the nematodes *Hysterothylacium*

**Table 2.** Characteristics of the helminth communities present in *Galaxias maculatus* populations in 28 lakes of northwestern Patagonia: species, stage (L: larvae; A: adult), life cycle, prevalence (%), and total richness.

**Tabla 2.** Características de la comunidad de helmintos presentes en poblaciones de *Galaxias maculatus* de 28 lagos del noroeste de la Patagonia: especies (L: larva; A: adulto), estadio, ciclo de vida, prevalencia (%) y riqueza total.

	DIGenea							MONOGenea				ACANTHO- CEPHALA	NEMATODA				Total richness
	<i>Acanthostomoides apophalliformis</i>	Heterophyidae sp.	<i>Posthodiplostomum</i> sp.	<i>Steganoderma szidati</i>	<i>Stephanoprora uruguayense</i>	<i>Tyloodelphys</i> sp.	<i>Derogetes</i> sp.	<i>Philureter trigonopsis</i>	<i>Gyrodactylus</i> sp.	<i>Diphyllbothrium</i> sp.	<i>Ailinea mirabilis</i>	<i>Acanthocephalus tumescens</i>	<i>Pomphorhynchus patagonicus</i>	<i>Camallanus corderoi</i>	<i>Contracaecum</i> sp.	<i>Hedruris suttonae</i>	
Stage (L/A)	L	L	L	A	L	L	A	A	A	L	A	A	A	A	L	A	L
Life cycle	Autogenic	Alogenic	Alogenic	Autogenic	Alogenic	Alogenic	Autogenic	Autogenic	Autogenic	Alogenic	Autogenic	Autogenic	Autogenic	Autogenic	Alogenic	Autogenic	Autogenic
Ñorquinco	77		1	2	42	88					19.2	53.8		11.5	3.8	42.31	10
Tromen	37.5			4	17	100		12			16.7					45.83	7
Currue Chico	5											14					2
Machónico	97					93					6.7	16.7		6.6	36.7		6
Meliquina	20					80		15			30			5			5
Villarino	95	5		5	55	95		10		5				5	40		9
Falkner	90				80	100		5	5	20					15		7
Espejo Chico	80		20	10	17	60		30		16.7	3.3			20	13.3		10
Bailey Willis		13	33	27		27		13		50				10			7
Ceferino			30	3		93				36.7				10			5
Correntoso	87		13	3	20	97		13	10	16.7				6.7	13.3	6.7	11
Alicurá	87					97		7	7	3.3				13.3			6
Espejo	80		20			90				26.7	3.3		3.3	10	20	10	9
Piré	23			10		100				13.3				33.3	16.7	6.7	7
Patagua			100	53	3	37									10	3.3	6
Redonda			63	10		100											3
Larga			100	10		80								13.3			4
Morenito	80		37	3	3	100		13		13			23	40	63	17	11
Escondido	80		43	3		70		7		43			43	47		3	9
Nahuel Huapi	47			17	33	80		23	20	27	20		3.3	13.3	30	10	12
El Trébol		23	7	47	20	15		13	3					3			8
Moreno	57		3		3	77		27		13.3	3.3				3.3		8
Gutiérrez	3		43			100	6.7	3	7	6.6				3.3			8
Roca			90		35	100						90		5			5
Mascardi			20	3	10	80			10	16.6		56.7		13.3	20		9
Los Moscos			26	7	26	52				7.4	70.4			22.2			7
Guillermo					3			10	27	23	3			7			6
Steffen			93			100		4		12.5		62.5		8.3	29.2		7

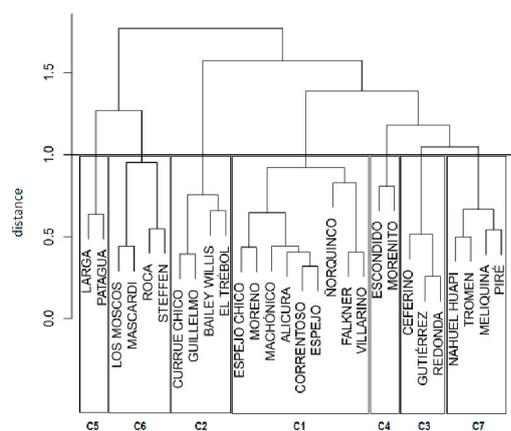
*patagonense*, *Camallanus corderoi*, and *Hedruris suttonae*. The remaining six species have allogenic cycles, the digeneans *Stephanoprora uruguayense*, *Tyloodelphys* sp., *Posthodiplostomum* sp., and Heterophyidae sp., the cestode *Diphyllbothrium* sp., and the nematode

*Contracaecum* sp. (Table 2). Parasite species richness ranged from 2 to 12 in populations of *G. maculatus* of Currue Chico Lake and Nahuel Huapi Lake, respectively (Table 2). The only parasites that reached 100% of prevalence in some lakes were the larvae of

**Table 3.** Cluster analysis summary indicating the groups of lakes, helminth assemblages occurring in all the lakes and the helminths with the maximum prevalence.

**Tabla 3.** Resumen del análisis de clúster indicando los grupos de lagos, los helmintos presentes en todos los lagos del grupo y los helmintos que presentan las prevalencias máximas.

Cluster	Lakes	Richnes range	Helminth occurring in all the lakes	Helminth highest prevalences
Cluster 1	Espejo chico, Moreno Machonico, Alicura, correntoso Espejo Norquingo Falkner, Villarino	6-11	<i>Tylodelphys</i> sp., <i>Acanthostomoides apophalliformis</i> , <i>Ailinella mirabilis</i> and 8 out of 9 lakes have <i>Hedruris suttonae</i>	<i>Tylodelphys</i> sp. (60%-100%) and <i>Acanthostomoides apophalliformis</i> (57%-97%)
Cluster 2	Currué Chico, Guillermo, Bailey Willis, El Trébol	2-8	no species was present in all lakes, <i>Philureter trigoniopsis</i> and <i>Steganoderma szidati</i> was present in three of the four lakes	Low prevalence or absent of <i>Posthodiplostomum</i> sp. and <i>Tylodelphys</i> sp.
Cluster 3	Ceferino, Gutiérrez, Redonda	3-8	<i>Tylodelphys</i> sp. and <i>Posthodiplostomum</i> sp.	<i>Tylodelphys</i> sp. (93-100%) followed by <i>Posthodiplostomum</i> sp. (30-63%)
Cluster 4	Escondido, Morenito	9-11	<i>Acanthostomoides apophalliformis</i> , <i>Tylodelphys</i> sp., <i>Posthodiplostomum</i> sp., <i>Steganoderma szidati</i> , <i>Philureter trigoniopsis</i> , <i>Ailinella mirabilis</i> , <i>Camallanus corderoi</i> , <i>Contraecaecum</i> sp. and <i>Hysterothylacium</i> sp.	<i>Tylodelphys</i> sp. (70-100%) and <i>Acanthostomoides apophalliformis</i> (80%)
Cluster 5	Larga Patagua	4-6	<i>Tylodelphys</i> sp., <i>Posthodiplostomum</i> sp., <i>Steganoderma szidati</i>	<i>Posthodiplostomum</i> sp. (100% in the two lakes) followed by <i>Tylodelphys</i> sp. (37-80%) and <i>Steganoderma szidati</i> with (10-53%)
Cluster 6	Los Moscos, Mascardi, Roca, Steffen	5-9	All lake have <i>Posthodiplostomum</i> sp., <i>Tylodelphys</i> sp., <i>Acanthocephalus tumescens</i> and <i>Contraecaecum</i> sp.	<i>Tylodelphys</i> sp ranging between 52-100%), <i>Acanthocephalus tumescens</i> (57-90%) and <i>Posthodiplostomum</i> sp. (20-93%)
Cluster 7	Nahuel Huapi, Tromen, Meliquina, Piré	5-12	All lakes have <i>Tylodelphys</i> sp. and <i>Acanthostomoides apophalliformis</i>	<i>Tylodelphys</i> sp. (80-100%) followed by <i>Acanthostomoides apophalliformis</i> (20-47%)

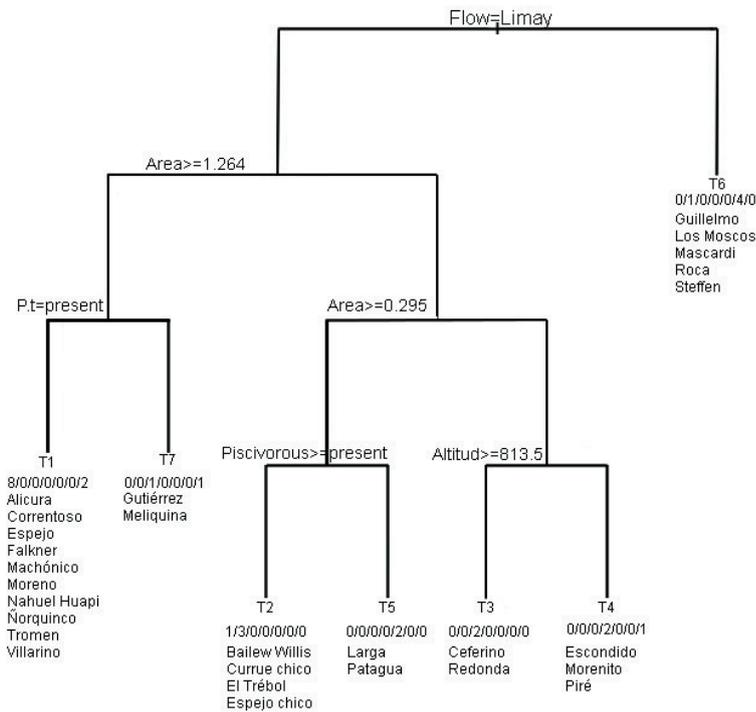


**Figure 2.** Cluster Analysis plot (based on the Euclidean distance) of helminth prevalence in populations of *Galaxias maculatus* from 28 lakes of northwestern Patagonia.

**Figura 2.** Gráfico del Análisis de cluster (basado en la distancia euclídeana) de la prevalencia de helmintos en poblaciones de *Galaxias maculatus* en 28 lagos del Noroeste de la Patagonia.

*Posthodiplostomum* sp. and *Tylodelphys* sp., but the last species exhibited the highest values of prevalence in the majority of lakes (Table 2).

Figure 2 depicts the dendrogram that summarizes the hierarchical cluster analysis grouping lakes based on parasite presence and prevalence in *G. maculatus* populations, while Table 3 summarizes the characteristics of clusters. *Tylodelphys* sp. was found to be present in all the clusters, only two lakes of cluster 2 did not present this species. *Posthodiplostomum* sp. was present in all lakes of clusters 3, 4, 5, and 6. *Acanthostomoides apophalliformis* was present in all lakes of clusters 1, 4, and 7. *Acanthocephalus tumescens* and *Contraecaecum* sp. were present in all lakes of cluster 6, and *S. szidati* was present in all lakes of the cluster 5. *Tylodelphys* sp. showed the highest prevalence in all the clusters except in cluster 2. *Acanthostomoides apophalliformis* had highest prevalence in clusters 1, 4, and 7; *Posthodiplostomum* sp. in clusters 3, 5, and 6. *Steganoderma szidati* was the third species with



**Figure 3.** Tree model with the factors that affect the helminth community of *Galaxias maculatus*. Numbers between bars explain the number of lakes of the cluster that are present in branch (P.t: *Percichthys trucha*).

**Figura 3.** Modelo del árbol con los factores que afectan a la comunidad de helmintos de *Galaxias maculatus*. Los número entre barras explican la cantidad de lagos del cluster están presentes en la rama. (P.t: *Percichthys trucha*).

the highest prevalence in cluster 5. The cluster 6 was the only one in which *A. tumescens* showed the highest prevalence.

A classification tree analysis was used to identify the main abiotic and biotic characteristics associated to the lake clusters defined by parasite prevalence (Figure 3). The first split was based on basin (Atlantic vs. Pacific) and all lakes included in Terminal node 6 (T6) drain to the Pacific Ocean. Lakes flowing to the Atlantic Ocean were further separated by their areas; lakes included in T1 and T7 are larger (area  $\geq 1.3$  ha) than lakes from T2, T3, T4, and T5. The larger lakes draining to the Atlantic were separated in the next step based on the presence of *P. trucha*; lakes from T1 have populations of *P. trucha* while those from T7 do not. A new subdivision of lakes draining to the Atlantic separates those with area between 0.3 Ha and 1.3 Ha (T2 and T5) from those with area  $< 0.3$  ha (T3 and T4). Terminal nodes 2 and 5 were finally separated based on the presence of at least one piscivorous species, while T3 and T4 were divided based on their altitude. It is important to note that only the terminal nodes T3 and T5 are pure, in the sense that they comprise only lakes from one cluster (clusters 3 and 5, respectively). The terminal node T1 contains all the lakes from cluster 1 except the small lake Espejo Chico, and also the large lakes from cluster 7, Tromen and Nahuel Huapi, with *P. trucha* in their fish

assemblages. Terminal node T2 contains three of the four lakes from cluster 2, excluding the large lake Guillermo that drains to the Pacific. Terminal node T4 includes all the lakes from cluster 4 and lake Piré from cluster 7. Terminal node T6 contains all lakes from cluster 6 and one lake from cluster 7. The lakes from cluster 7 are poorly described by the tree, since the 4 lakes included are scattered in three different terminal nodes (T1, T4, T7).

## DISCUSSION

The current distribution of fish in Andean Patagonia is the result of historical processes such as the isolation of lakes from paleolakes during the Pleistocene retreat of ice (Tatur et al. 2002), the existence of refuges, and the routes of post-glacial colonization by previously displaced fishes (Cussac et al. 2004; Zattara & Premoli 2004). Since Patagonian Andean lakes vary in their physical features and fish assemblages, it could be expected that these factors be reflected in the richness and composition of the parasite communities of *G. maculatus* populations.

As has been pointed out previously (Viozzi et al. 2009; Fernández et al. 2010), in this study, the helminth component communities of the prey fish *G. maculatus* from small and large lakes are dominated numerically by larval digeneans, especially diplostomids

as *Tylodelphys* sp. and *Posthodiplostomum* sp. Littoral macrophytes promote the presence of pulmonate gasteropods, especially *Chilina* sp. and *Anisancylus* sp. (Flores & Semenas 2008; Ritossa et al. 2013), and also waterfowl. These factors favor the increase in the proportion of allogenic species, as *Tylodelphys* sp. and *Posthodiplostomum* sp., in relation to the total number of species in the component parasite community.

The abiotic conditions affect biotic factors, and their interaction can determine the composition of the parasite community (Anderson & Sukhdeo 2009). The major environmental factors that seem to affect the composition of helminth communities of *G. maculatus* in the Andean Patagonian region and the prevalence of the different parasite species are the abiotic factors: basin (Atlantic - Pacific), area, and altitude of the lakes, and biotic factors: presence of piscivorous fish species, especially the native *P. trucha*. One of the main structuring forces operating in Patagonian Andean lakes were glaciations that formed the deep lakes and established the current flow direction of basins, which in turn affected the distribution of fish species (Ruzzante et al. 2008; Zemlak et al. 2008). Also, connectivity of a system of lakes increases the degree of movement or flow of organisms through the landscape (Taylor et al. 2006; Crooks & Sanjayan 2006). In that sense, the Limay river basin is more complex, including more lakes and with higher connectivity compared to Manso river basin. In both basins, the big and deep lakes have a higher diversity of habitats, promoting higher fish species richness, than small and shallow lakes (unpublished own data, see also Table 1). On the other hand, at higher altitude, lake fish assemblages have generally less species due to their lower permeability to colonization. The depicted scenario along with the results of the statistical analysis suggest that an increase in fish species richness increases concomitantly *G. maculatus*'s helminth community richness, affecting also the assemblage composition and prevalence.

Predation modulates ecosystem processes playing an important role in the transmission of parasites and infection patterns in wildlife, since many parasite species use food webs as a way of transmission (Lafferty et al. 2006; Kuris et al. 2008; Byers 2009; Valtonen et al. 2010; Poulin & Leong 2011). The larvae of the allogenic species *Contracaecum* sp.,

*Tylodelphys* sp., and *Posthodiplostomum* sp. parasitize piscivorous birds using *G. maculatus* as intermediate host (Torres et al. 1992; Flores & Semenas 2002; Ritossa et al. 2013). The high vagility of these piscivorous birds promotes connectivity among aquatic environments thereby contributing to expand the distribution of allogenic helminths. Autogenic parasites, as adults of the digenean *A. apophalliformis* and the nematodes *H. patagonense* and *C. corderoi*, parasitize the intestine of the predator *P. trucha* and use *G. maculatus* also as an intermediate host (Moravec et al. 1997; Ostrowski et al. 1999; Viozzi et al. 2009), highlighting the role of the perch in the increase of species richness of helminth communities of *G. maculatus*.

In our region, the introduction of salmonids have enlarged the number of top predatory fish in aquatic trophic webs. These new predators can act as host for generalistic parasites, affecting the occurrence and prevalence of autogenic helminth species of *G. maculatus*. Fish play different roles as definitive hosts considering that some parasite species are host-generalistic and others are host-specific. For example, in the Limay river basin, *A. apophalliformis*, a host-specific digenean that uses *P. trucha* as definitive host, is well represented in the helminth communities of *G. maculatus*. In contrast, in the Manso basin where *P. trucha* is absent, the helminth communities of *G. maculatus* lack *A. apophalliformis* and show high prevalence of the generalist acanthocephalan *A. tumescens*. The presence of salmonids, which exert top predation in lake food webs of the Manso basin, increases the prevalence of *A. tumescens* since this parasite can be postcyclically transmitted from *G. maculatus* to the salmonid *Oncorhynchus mykiss* (Rauque et al. 2002, 2003).

Marcogliese & Cone (1991) pointed out that the overall parasite community within a system can be characterized by parasites of the numerically dominant host, such is the case of *G. maculatus* in Northwestern Patagonian lakes. In our region, the parasite community of different populations of *G. maculatus* varies according to the basin, lake area and altitude, and the fish assemblage. This is the first study performed on the ecology of parasite communities of a native freshwater prey fish (small puyen) at regional scale in Argentina. Overall, the study shows that abiotic factors prevail over biotic on determine helminth communities in *Galaxias maculatus*.

**ACKNOWLEDGEMENTS.** The authors would like to thank the National Park Administration for granting permission to work in protected areas. Financial support was provided by Universidad Nacional del Comahue B 165, 187, PICT 1293 (FONCYT) and PIP CONICET 112-200801-01738

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