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*Short Communication*

**Regulatory factors in crustacean zooplankton assemblages in mountain lakes of northern Chilean Patagonia (38-41°S): a comparison with Bulgarian counterparts (42°N)**

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**ABSTRACT.** Chilean Patagonia has protected mountainous areas with evergreen native forests; in which the lakes and rivers, of volcanic or glacial origin, are oligotrophic. In Bulgaria, there are mountainous zones with native forests and associated lakes of volcanic origin. The aim of the present study is to carry out a preliminary comparison of zooplanktonic crustaceans in lake ecosystems associated with native forests of Chilean Patagonia and of Bulgarian mountains. The study revealed that the lakes studied in Chilean Patagonia are associated mainly with *Nothofagus* forests; they are oligotrophic, with a low number of zooplanktonic crustacean species. Similar results were observed for Bulgarian mountain lakes associated with *Fagus* forests. A null model analysis of species co-occurrence was applied to the two groups of lakes, and the result revealed the absence of regulatory factors in species associations. These studies agree with similar descriptions of lakes in Andean Patagonia and New Zealand. They highlight the important role of native *Nothofagus* forests in Argentina and Chile, and of *Fagus* forests with associated soil properties in Bulgaria, in the oligotrophy of the lakes studied.

**Keywords:** *Nothofagus*, *Fagus*, native forests, lakes, oligotrophy, zooplankton, Bulgaria, Chile.

**Factores reguladores en ensambles de crustáceos zooplanctónicos en lagos de montaña del norte de la Patagonia chilena (38-41°S): una comparación con sus contrapartes de Bulgaria (42°N)**

**RESUMEN.** La Patagonia de Chile tiene una serie de áreas protegidas con bosques nativos perennes asociados a lagos y ríos oligotróficos y de origen glacial. Por otro lado en Bulgaria hay una serie de zonas montañosas con lagos asociados de origen volcánico o glacial. El objetivo del presente trabajo es realizar una primera descripción de especies de crustáceos zooplanctónicos en ecosistemas lacustres asociados a bosques nativos en la Patagonia de Chile y en las montañas de Bulgaria. Los estudios indican que los lagos de la Patagonia de Chile están asociados principalmente con bosques de *Nothofagus*, mientras que similares resultados fueron observados en lagos de Bulgaria con bosques de *Fagus*. La regresión lineal entre concentración de clorofila y número de especies para lagos chilenos, fue significativa mientras que en lagos de Bulgaria el análisis de regresión no indicó diferencias significativas. Se aplicó un análisis de co-ocurrencia de especies para ambos grupos de lagos y los resultados indicaron la ausencia de factores reguladores en las asociaciones de especies. Estos estudios concuerdan con descripciones similares para lagos de la Patagonia andina y Nueva Zelanda, y remarcen el rol de los bosques nativos de *Nothofagus* en Argentina y Chile, así

como la presencia de bosques de *Fagus* y las propiedades del suelo en Bulgaria, como regulador importante de la oligotrofia asociada a los lagos analizados.

**Palabras clave:** *Nothofagus*, *Fagus*, bosques nativos, lagos, oligotrofia, zooplancton, Bulgaria, Chile.

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The mountain lakes of Chilean North Patagonia (38-42°S) are oligotrophic, of glacial origin, with low numbers of zooplanktonic crustacean species. They are associated with native *Nothofagus* Blume forest, particularly *N. antarctica* (G. Forst.) Oerst., *N. pumilio* (Poepp. et Endl.) Krasser, and *N. dombeyi* (Mirb.) Oerst. At altitudes greater than 1000 m.a.s.l., these species coexist with *Araucaria araucana* (Molina) K. Koch, between 38-39°S (Hauenstein *et al.*, 2011; De los Ríos-Escalante *et al.*, 2011). South of 39°S, *Nothofagus* species predominate, and south of 41°S, these *Nothofagus* forests coexist with *Fitzroya cupressoides* forest (Steinhart *et al.*, 1999, 2002). Access to the lakes is difficult since they are in mountainous areas, accessible only by long mountain paths (De los Ríos *et al.*, 2007; De los Ríos & Roa, 2010). Bulgarian mountain lakes are also oligotrophic, and are associated with native *Fagus* L. forest. Their origin is volcanic below 2200 m.a.s.l., and glacial at higher altitudes, with a relatively high number of crustacean zooplankton species and low human intervention (Kalchev *et al.*, 2004; Hristozova *et al.*, 2004). The aim of the present study is to compare data obtained from the literature of chlorophyll-*a* concentration and crustacean zooplankton populations in North Patagonian mountain lakes and Bulgarian mountain lakes, considering the geographical and ecological differences between the two regions.

Data about the trophic status and zooplanktonic crustacean and littoral species from Chilean mountain lakes were obtained from the literature (n = 8; Steinhart *et al.*, 2002; De los Ríos-Escalante *et al.*, 2011) and from field-work in Alerce Andino National Park. The data from Bulgaria (n = 9) were taken from the literature (Kalchev *et al.*, 2004; Hristozova *et al.*, 2004).

Firstly a regression analysis between chlorophyll-*a* concentration and number the species of was applied using the xlstat 5.0 software (www.adinsoft.com) in order to determine the potential relationship between the two variables. Next, a species absence/presence matrix was constructed, with the species in rows and sites in columns. When this matrix was complete, the Bray-Curtis Index with single link for similarity was obtained to determine potential similarities between sites, on the basis of species associations (Gotelli & Graves, 1986); this analysis used the Biodiversity Pro.

Version 2.0 software (McAleece *et al.*, 1997). The next step was to calculate a Checkerboard score ("C-score"), a quantitative index of occurrence that measures the extent to which species co-occur less frequently than expected by chance (Gotelli, 2000). A community is structured by competition when the C-score is significantly larger than expected by chance (Gotelli, 2000). Finally, co-occurrence patterns were compared with null expectations by simulation. Gotelli & Entsminger (2007) and Gotelli (2000) suggest the following robust statistical null models: (1) Fixed-Fixed: in this model the row and column sums of the matrix are preserved. Thus, each random community contains the same number of species as the original community (fixed column), and each species occurs with the same frequency as in the original community (fixed row). (2) Fixed-Equiprobable: in this algorithm only the row sums are fixed, while the columns are treated as equiprobable. This null model considers all the samples (column) as equally available for all species. (3) Fixed-Proportional: in this algorithm the species occurrence totals are maintained as in the original community, and the probability that a species will occur at a site (column) is proportional to the column total for that sample. The null model analyses were performed using the software Ecosim version 7.0 (Gotelli & Entsminger, 2007).

The results revealed a significant correlation between chlorophyll-*a* concentration and species number for Chilean lakes (Fig. 1); however this significant relationship did not exist for their Bulgarian counterparts (Fig. 1). The cluster analysis for Chilean lakes revealed the existence of two sites with approximately 80% similarity, Icalma and Galletué lakes, whereas the similarities between the remaining sites was approximately 40-60% (Fig. 2). Of the Bulgarian lakes, Alekovo with Gorno Marichino, and Salkata, Babreka and Okoto, formed two different groups with approximately 75% similarity, whereas for the remaining sites the similarities varied between 50-75% (Fig. 2). In the Chilean lakes there are very few species, and many of these are repeated in practically all of the sites studied (Table 1). The Bulgarian lakes contain many species in comparison with their Chilean counterparts, nevertheless there are also many repeated species

**Table 1.** Location, geographical parameters and species reported for water bodies analysed in the study.**Tabla 1.** Localización, parámetros geográficos y especies reportadas para los cuerpos de agua analizados en el presente estudio.

North Patagonian Chilean lakes						
Site (local name)	Geographical location	Zmax (m)	Altitude (m)	Surface (km <sup>2</sup> )	Chl- <i>a</i> (µg L <sup>-1</sup> )	Species reported
Tinquilco <sup>1</sup>	39°10'S, 71°43'W	40	840	10	2.0	<i>Boeckella gracilis</i> <i>Mesocyclops longisetus</i> <i>Daphnia pulex</i> <i>Ceriodaphnia dubia</i> <i>Neobosmina chilensis</i>
Galletué <sup>1</sup>	38°40'S, 71°15'W	43	1144	13	2.8	<i>B. gracilipes</i> <i>M. longisetus</i> <i>D. pulex</i> <i>C. dubia</i>
Icalma <sup>1</sup>	38°47'S, 71°16'W	135	1154	10	0.8	<i>B. gracilipes</i> <i>M. longisetus</i> <i>D. pulex</i>
Verde <sup>1</sup>	38°40'S, 71°37'W	3	1100	1	0.1	<i>B. gracilis</i> <i>D. pulex</i>
Captrén <sup>1</sup>	38°38'S, 71°42'W	6	1100	1	0.1	<i>T. p T. prasinus</i> <i>D. pulex</i> <i>C. dubia</i>
Chaiquenes <sup>2</sup>	41°34'S, 72°32'W	N.D	N.D	N.D		<i>B. gracilis</i> <i>Alona</i> sp.
Triangulo <sup>2</sup>	41°36'S, 72°28'W	N.D	N.D	N.D		<i>Scapholeberis exspiniifera</i>
Sargazo <sup>2</sup>	41°30'S, 72°36'W	N.D	N.D	N.D		<i>B. gracilis</i> <i>T. prasinus</i> <i>Alona</i> sp. <i>S. exspiniifera</i>
Bulgarian mountain lakes						
Okoto <sup>3,4</sup>	42°11'N, 23°18'E	37.5	2440	0.68	0.48	<i>Daphnia rosea</i> <i>Alona affinis</i> <i>Alona rectangula</i> <i>Alonopsis elongata</i> <i>Alonella excisa</i> <i>Chydorus sphaericus</i> <i>Ch. piger</i> <i>Eurycercus lamellatus</i> <i>Arctodiaptomus alpinus</i> <i>Eucyclops serrulatus</i> <i>Megacyclops gigas</i>
Babreka <sup>3,4</sup>	42°12'N, 23°18'E	28.0	2282	0.85	1.52	<i>D. obtusa</i> <i>D. rosea</i> <i>Alonopsis elongata</i> <i>Alona affinis</i> <i>Alonella excisa</i> <i>Ch. piger</i> <i>Ch. sphaericus</i>

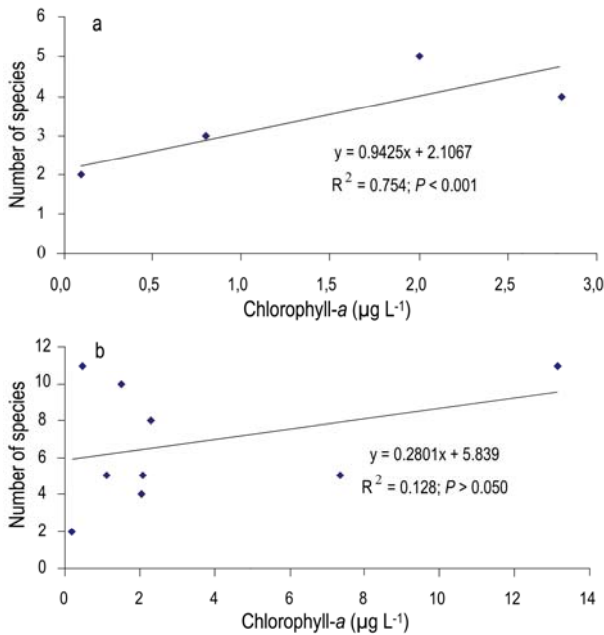
Bulgarian mountain lakes						
Site (local name)	Geographical location	Zmax (m)	Altitude (m)	Surface (km <sup>2</sup> )	Chl- <i>a</i> (µg L <sup>-1</sup> )	Species reported
						<i>Arctodiaptomus alpinus</i> <i>Megacyclops gigas</i> <i>Acanthocyclops vernalis</i>
Salzata <sup>3,4</sup>	42°11'N, 23°18'E	4.5	2535	0.07	2.30	<i>Chirocephalus diaphanus</i> <i>D. rosea</i> <i>Alonopsis elongata</i> <i>Alona affinis</i> <i>Ch. sphaericus</i> <i>A. alpinus</i> <i>Megacyclops gigas</i> <i>Acanthocyclops vernalis</i>
Bliznaka <sup>3,4</sup>	42°12'N, 23°18'E	27.5	1143	0.91	13.14	<i>D. rosea</i> <i>Alonopsis elongata</i> <i>Alona affinis</i> <i>Alona quadrangularis</i> <i>Alonella excisa</i> <i>Chydorus sphaericus</i> <i>A. alpinus</i> <i>A. nietammeri</i> <i>Eucyclops serrulatus</i> <i>Cyclops abyssorum tritricus</i> <i>A. vernalis</i>
Alekovo <sup>3,4</sup>	42°11'N, 23°34'E	14.5	1545	0.24	1.12	<i>D. rosea</i> <i>Alonopsis elongata</i> <i>Alona affinis</i> <i>Chydorus sphaericus</i> <i>Acanthocyclops vernalis</i>
Ledeno <sup>3,4</sup>	42°07'N, 23°34'E	16.4	2709	0.18	0.53	<i>D. rosea</i> <i>Ch. sphaericus</i>
Karakashevo <sup>3,4</sup>	42°10'N, 23°35'E	6.6	2391	0.26	7.34	<i>Eurycercus lamellatus</i> <i>Alonella excisa</i> <i>Alona affinis</i> <i>Ch. sphaericus</i> <i>Eucyclops serrulatus</i>
Dolno Marichino <sup>3,4</sup>	42°09'N, 23°35'E	5.5	2368	0.11	2.03	<i>D. rosea</i> <i>A. affinis</i> <i>Ch. piger</i> <i>Ch. sphaericus</i>
Gorno Marichino <sup>3,4</sup>	42°09'N, 23°35'E	10.8	2378	2.15	2.06	<i>Daphnia longispina</i> <i>D. rosea</i> <i>Alona affinis</i> <i>Ch. sphaericus</i> <i>Acanthocyclops vernalis</i>

<sup>1</sup>De los Ríos-Escalante *et al.* (2011), <sup>2</sup>De los Ríos-Escalante, unpublished data, <sup>3</sup>Kalchev *et al.* (2004), <sup>4</sup>Hristozova *et al.* (2004).

**Table 2.** Results of null model analysis for co-occurrence of species for studied sites ( $P$  values lower than 0.05 denotes random absence).

**Tabla 2.** Resultados del modelo nulo de análisis de co-ocurrencia de especies para los sitios estudiados (valores de  $P$  inferiores a 0,05 indican ausencia de azar).

North Patagonian Chilean mountain lakes					
	Observed index	Mean index	Standard Effect Size	Variance	$P$
Fixed-Fixed	2.861	2.845	0.151	0.010	0.447
Fixed-Proportional	2.861	2.365	1.108	0.200	0.137
Fixed-Equiprobable	2.861	2.694	0.459	0.131	0.372
Bulgarian mountain lakes					
	Observed index	Mean index	Standard Effect Size	Variance	$P$
Fixed-Fixed	1.397	1.349	0.829	0.003	0.200
Fixed-Proportional	1.397	1.654	-1.081	0.056	0.862
Fixed-Equiprobable	1.397	2.012	-3.729	0.027	0.998

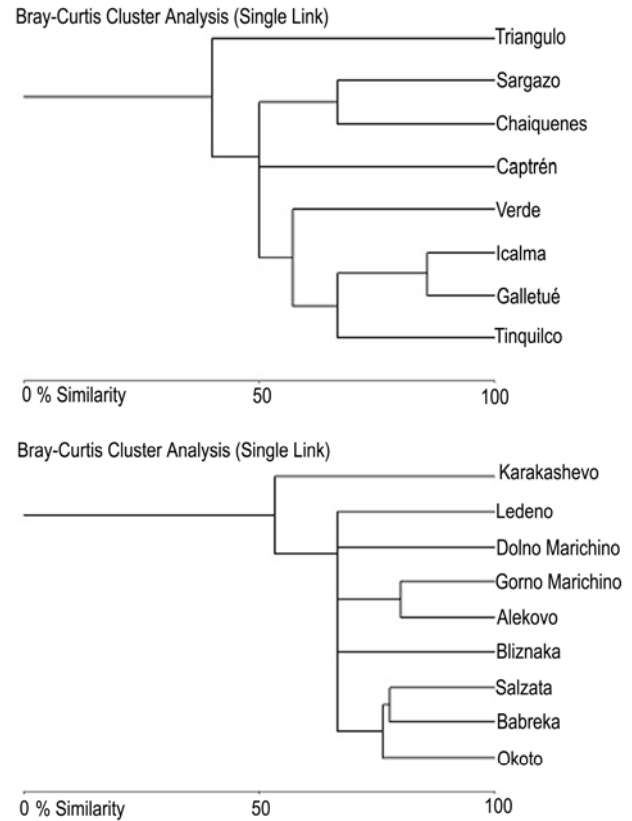


**Figure 1.** Regression analysis between chlorophyll-*a* concentration and number of species for lakes of a) northern Patagonia Chilean, and b) Bulgarian.

**Figura 1.** Análisis de regresión entre concentración de clorofil-*a* y número de especies para lagos de a) norte de la Patagonia chilena, y b) Bulgaria.

(Table 1). This would be the reason why the results of null models revealed the absence of regulatory factors in all simulations for Chilean and Bulgarian lakes (Table 2).

The literature on Chilean North Patagonian lakes and ponds revealed the marked role of oligotrophy in species richness and calanoid dominance (De los Ríos-



**Figure 2.** Dendrogram with the results of Bray-Curtis Similarity index for northern Patagonian Chilean mountain lakes (up) and Bulgarian mountain lakes (low).

**Figura 2.** Dendrograma con los resultados del análisis del índice de similitud de Bray-Curtis para lagos del norte de la Patagonia chilena (arriba) y lagos de Bulgaria (abajo).

Escalante, 2010; De los Ríos-Escalante *et al.*, 2011). Nevertheless, the null model showed absence of regulatory factors (De los Ríos & Roa, 2010). The literature on Chilean lakes revealed that the main causes of changes in the trophic status are changes in the surrounding basin, when native forest is replaced by agricultural use, towns and industries (Soto, 2002). Under these conditions, changes in the chemical properties of the soil will lead to a decrease in nutrient composition and an increase in zooplankton biomass, with the consequent generation of clear water phase (Hristozova *et al.*, 2004; Kalchev *et al.*, 2004). Similar results to those found in Chilean lakes were described for Argentinean Patagonian lakes which are oligotrophic and contain a low species number (Modenutti *et al.*, 1998); and for New Zealand lakes, where an increase in the number species of may be found directly associated with the increase in chlorophyll-*a* concentration (Jeppensen *et al.*, 2000).

Another difference between Chilean and Bulgarian mountain lakes is the fact that many Bulgarian lakes containing introduced salmonids (*Salvelinus fontinalis* and *Salmo trutta*), with consequent effects on their zooplankton composition (Kalchev *et al.*, 2004). Such effects would not be found in Chilean lakes containing no salmonids (De los Ríos-Escalante, 2010). The literature describes the effects caused by introduced salmonids on zooplankton composition, specifically the presence of small-sized species, and this effect is indeed observed in Argentinean Patagonian lakes (Reissig *et al.*, 2006), which would probably present similarities to Bulgarian mountain lakes (Kalchev *et al.*, 2004). However no studies exist about the effects of salmonids on zooplankton in Chilean lakes (De los Ríos-Escalante, 2010). This last topic (the presence of fishes, specifically salmonids) would be the main difference between the regulatory mechanisms affecting zooplankton communities in the mountain lakes of Bulgaria on one hand, and Chilean Patagonia on the other. According to descriptions of Patagonian lakes in Argentina and Chile, in the presence of fish, zooplankton assemblages are characterized by small-sized species and low number species of (Soto *et al.*, 1994; Modenutti *et al.*, 1998; Reissig *et al.*, 2006). The results obtained would indicate that more ecological studies are necessary, specifically about trophism in pelagic lake environments.

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