Original Research

Associations between Optical Properties and Mixotrophic Ciliates Abundances Using Remote Sensing Techniques in Two North Patagonian Lakes (Villarrica and Caburgua, 38°S, Araucania, Chile)

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Abstract

The north Patagonian lakes in their original stage were characterized by their marked oligotrophy, with high abundance of mixotrophic ciliates in lakes with native forest in their surrounding basins. Nevertheless, in the last decades, it was a replace of native forest of different kind of human activities, such as towns and agricultural zones, that generate a transition from oligotrophy to mesotrophy, being replaced the mixotrophic ciliates by different kind of phytoplankton. The aim of the present study was to propose a descriptive model using remote sensing techniques for determining the best model for predict the mixotrophic ciliates abundance in two North Patagonian Chilean lakes.

In studies sites, only Caburgua lake has mixotrophic ciliates, that belong to the species *Ophyridium naumanni, Stentor amethystinus* and *S. araucanus*, whereas Villarrica lake has not mixotrophic ciliates. The multiple regression analysis revealed that for *O.naumanni* and *S. amethystinus* have significant direct associations between temperature, B01, B07 reflectances, *S. araucanus* abundances, and inverse associations with B02, B04 and B07, and the abundance of both species was significantly inverse. Finally, for *S. araucanus* was positive associations with B04 and *S. amethystinus*. The exposed results would be similar to the first descriptions of mixotrophic ciliates abundances for Argentinean and

Chilean Patagonian lakes. On this basis, we propose the use of remote sensing techniques would be an important key tool for study the presence of these organism.

Keywords: patagonian lakes, spectral properties, mixotrophic ciliates, chlorophyll, remote sensing

Introduction

The Argentinean North Patagonian lakes originally were described as oligotrophic, and with native forest in their surrounding basins [1,2], these lakes in their original stage would have abundant mixotrophic ciliates [3] specifically of species *Ophyridium naumanni*, Stentor amethystinus, and S. araucanus that currently are abundant in lakes with low human alterations [4-7]. In opposite scenario, many Chilean north Patagonian lakes have marked human intervention in their surrounding basins, due towns and agricultural zones [8, 9], that generate transition from oligotrophy to mesotrophy in a period of two decades [10] with consequent changes in the zooplankton assemblages in function to trophic status even in different bays in the same lake such as was described for Llanguihue lake (41°S) [8, 9].

On the view point of mixotrophic ciliates, these would be abundant under low chlorophyll concentration, that would be associated to low crustacean zooplankton species richness and abundances, and in this scenario mixotrophic ciliates would be indicators of ultraoligotrophic or oligotrophic status in Patagonian lakes [3-5]. But under chlorophyll increase due transition from oligotrophy to mesotrophy mixotrophic ciliates are replaced due predation by crustacean zooplankton species such as Mesocyclops araucanus (= Mesocyclops longisetus) [3, 4, 11, 12]. Also, under original oligotrophic status, the mixotrophic ciliates would graze on bacteria, and simultaneously it has endosymbiontic activity due endosymbiotic algae [12, 13] and it would be prey of native juvenile fishes [14]. If it is considered that mixotrophic ciliates are key species as bioindicators in Patagonian lakes with ultraoligotrophic or oligotrophic status, probably the trophic status variations can be detectable using spectral properties by remote sensing techniques [15].

This scenario of trophic gradient of different lakes could be studied using their spectral properties. The scarce studies for Patagonian lakes, involves marked associations between LANDSAT ETM+ sensor for obtain optical image properties and zooplankton assemblages in lakes with marked glacier influence that would be associated with natural changes in water colour, such as was observed for General Carrera (46°S) and Tagua-Tagua (42°S) lakes [16-18]. In this context, it would be possible found differences between optical properties in lakes with presence and absence of mixotrophic ciliates considering their trophic differences associated [15]. The aim of the present study was determining the potential associations between optical properties obtained by remote sensing techniques and mixotrophic ciliates abundances in two Chilean North Patagonian lakes with presence of mixotrophic ciliates (Caburgua), and absence of mixotrophic ciliates (Villarrica) [15]. The hypothesis of the present study would be that associations between the different kind of mixotrophic ciliates abundances with reflectance values of different bands of LANDSAT/OLI in both mentioned lakes.

Material and Methods

Study sites: lake Caburgua is located in Andes mountains in Araucania region, its surrounding basin has perennial native forest with mountains, and very few human altered zones, this lake is oligotrophic with marked mixotrophic *Stentor* protozoa abundances [4, 5] (Table 1, Fig. 1). Lake Villarrica is located at west of Caburgua lake [4], its surrounding basin is characterized by the presence of Villarrica and Pucon towns, small recreational residences at south, native forest at north [15], and the presence of Villarrica volcano (Fig. 1).

Sampling procedures: both sites were visited between November 2018 and January 2019 that is the period with maximum zooplankton abundances [3, 5]. For Caburgua lake, four sites were sampled in a transect of 4 km at north of the lake (Fig. 1), whereas for Villarrica lake it considered two bays with towns in the south shore (Villarrica and Pucon), one site at center of the lake, and the fourth site in the northern shore of the lake where there is native forest (Fig. 1). For each site was measured in situ temperature using sensor YSI Pro Plus, at surface, concentration of total bacteria (AMR = aerobic mesophilic recount) were quantified in laboratory [19]. Chorophyll a, from samples were analysed using acetone extraction [20], whereas mixotrophic ciliates were quantified and identified based on literature [5, 14].

The spectral properties were obtained from satellite LANDSAT/OLI, it got from Land Processes Distributed Active Archive Center (LP DAAC) from U.S. Geological Survey (http://LPDAAC.usgs.gov). The bands of visible light used were B01 coastal spray (0.430-0.450 μ m), B02 blue (0.450-0.510 μ m), B03 green (0.530-0.590 μ m), B04 red (0.640-0.670 μ m), for near infrared were B05 (0.850-0.880 μ m), and for short wave infrared (SWIR) were B06 (1.570-1.650 μ m) and B07 (2.100-2.290 μ m) were calibrated radiometrically to spectral irradiance and then to reflectance, with atmospheric correction being applied [15-17, 20-22].

Exploratory multivariate data analysis: all data analysis was applied using software R [23]. As first step, data analysis a first step was applied a matrix correlation

udy.			La Poza Pucon bay	39°16'33''S 71°59'47''W	0.104	0.082	0.056	0.027	0.012	0.004	0.003	19.500	7.0	2.000	0.000	0.000	0.000			39°16'33'S 71°59'47''W	Cb4	0.097	0.075	0.053	0.030	0.012
ided in the present st		ember 2018	Villarrica Port	39°16°05°'S 72°23°20°'W	0.104	0.081	0.055	0.028	0.013	0.004	0.003	18.100	25.0	8.300	0.000	0.000	0.000		ember 2018	39°16°05°'S 72°23°29°'W	Cb 3	0.096	0.074	0.048	0.025	0.012
indL ⁻¹) for sites inclu		10 th Dec	Center	39° 14'39'' S 72° 08'06'' W	0.104	0.081	0.054	0.026	0.013	0.004	0.002	17.800	7.0	1.400	0.000	0.000	0.000		15 th Dec	39°10'14''S 71°47'47'W	Cb 2	0.097	0.074	0.051	0.028	0.012
ciliates abundances (North littoral	39°12'40''S 72°08'26''W	0.108	0.084	0.058	0.029	0.014	0.006	0.004	17.900	3.0	6.600	0.000	0.000	0.000			39°09'43''S 71°47'41''W	Cb 1	0.099	0.078	0.049	0.030	0.020
(µgL ⁻¹) and mixotrophic (Villarrica lake		La Poza Pucon bay	39°16'33''S 71°59'47''W	0.104	0.082	0.056	0.027	0.012	0.004	0.003	13.000	27.0	2.400	0.000	0.000	0.000	Caburgua lake		39°16'33'S 71°59'47''W	Cb4	0.097	0.075	0.053	0.030	0.012
phyll concentration		vember 2018	Villarrica Port	39°16'05''S 72°23'20''W	0.104	0.081	0.055	0.028	0.013	0.004	0.003	14.000	2.0	2.320	0.000	0.000	0.000		vember 2018	39°16'05''S 72°23'29''W	Cb 3	0.096	0.074	0.048	0.025	0.012
unces (CFU), chloroj		10 th No	Center	39° 14'39'' S 72° 08'06'' W	0.104	0.081	0.054	0.026	0.013	0.004	0.002	14.200	4.0	1.670	0.000	0.000	0.000		15 th No	39°10°14°'S 71°47°47°W	Cb 2	0.097	0.074	0.051	0.028	0.012
otal bacteria abunda			North littoral	39°12'40''S 72°08'26''W	0.108	0.084	0.058	0.029	0.014	0.006	0.004	12.900	4.0	1.975	0.000	0.000	0.000			39°09'43''S 71°47'41''W	Cb 1	0.099	0.078	0.049	0.030	0.020
Table 1. Optical properties,			Site	Geographical location	B01	B02	B03	B04	B05	B06	B07	Temperature	Total bacteria	Chlorophyll a	Ophyrydium naumanni	Stentor amethystinus	S. araucanus			Geographical location	Site	B01	B02	B03	B04	B05

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0.004	0.003	18.70	18.0	1.0	7.0	3.0	6.0
0.003	0.002	18.40	4.0	1.5	11.0	4.0	3.0
0.003	0.002	19.20	8.0	0.2	3.0	2.0	2.0
0.011	0.006	19.70	19.0	0.2	2.0	2.0	2.0
0.004	0.003	12.80	19.0	0.0	6.0	3.0	4.0
0.003	0.002	12.90	4.0	0.1	3.0	3.0	2.0
0.003	0.002	13.20	2.0	0.2	2.0	3.0	3.0
0.011	0.006	13.40	2.0	0.6	2.0	0.0	1.0
B06	B07	Temperature	Total bacteria	Chlorophyll a	Ophyrydium naumanni	Stentor amethystinus	S. araucanus

analysis using Hmisc R package [24], for determine the associations between studied variables. As second step a principal component analysis (PCA), this statistical analysis was applied, factoextra [25] and ggplot2 [26] R packages. As second step, a multiple regression analysis [27] was applied considering as dependent variables, the abundance of the mixotrophic ciliates abundances, and independent variables the optical properties, temperature, chlorophyll *a* concentration, and bacterial abundances, all data analysis was applied using software R [23].

## Results

The obtained results revealed that chlorophyll concentration and reflectance values of LANDSAT OLI, are more high at Villarrica lake in comparison to Caburgua lake, therefore the mixotrophic ciliates were absent in Villarrica lake (Table 1). The temperature and total bacteria values were not relatively different for studied sites (Table 1).

The correlation matrix revealed the existence of significant direct associations between B01 with B02, B01 with B03, B01 with chlorophyll a, B02 with B03, B02 with chlorophyll a, B03 with chlorophyll a, B04 with B05, B04 with B06, B04 with B07, B05 with B06, B05 with B07, B06 with B07, total mixotrophic ciliates with O. naumanni, total mixotrophic ciliates with S. amethystinus, total mixotrophic with S. araucanus, O. naumanni with S. amethystinus, O. naumanni with S. araucanus and S. amethystinus with S. araucanus (Table 2). Finally, inverse significant associations were found between B01 with total mixotrophics ciliates, B01 with O. naumanni, B01 with S. amethystinus, B01 with araucanus, B02 with total mixotrophics ciliates, B02 with O. naumanni, B02 with S. amethystinus, B02 with S. araucanus, B03 with total mixotrophics, B03 with O. naumanni, B03 with S. amethystinus, B03 with S. araucanus, and Chlorophyll a with S. araucanus (Table 2).

The PCA results revealed that the main contributor variables for axis 1, were B01. B02, total mixotrophic ciliates and S. amethystinus, whereas for second axis, the main contributor variables were B05, B06, and B07 (Table 3, Fig. 2). The PCA results revealed the existence of two main group, the first group joined Villarrica lake sites, within these sites, the north littoral sites have high of B01 and B02 reflectances and low chlorophyll a concentration in comparison with other sites of this lake whereas the remaining sites of Villarrica lake have high B03 reflectance and marked high chlorophyll concentration (Fig. 2). A different situation was reported for Caburgua lake, where the first site corresponding to nearby to littoral in both sampled dates was different, because both sites have high B04, B05, B06 and B07 reflectance values, and low mixotrophic abundances considering total mixotrophics ciliates and each recorded species (Fig. 2). Whereas



Fig. 1. Map with sites included in the present study [28].

the remaining sites of Caburgua lake that have low reflectance values, and high mixotrophic abundances considering total mixotrophics and each recorded species (Fig. 2).

The results of multiple regression, revealed first that the most robust model for O. naumani has direct significant associations with B01 and B07 reflectances, temperature, total mixotrophic ciliates abundances and S. araucanus abundances, whereas it has inverse significant association between B02, B04 and B07 reflectances, and with S. amethystinus abundances (Table 4). As second step, the results of multiple regression, revealed first that the most robust model for S. amethystinus has direct significant associations with B01 and B07 reflectances, temperature, total mixotrophic ciliates abundances and S. araucanus abundances, whereas it has inverse significant association between B02, B04 and B07 reflectances, and with O. naumanni abundances (Table 2). Finally, for S. araucanus the robust model had significant association between B04 reflectance and S. amethystinus abundances (Table 4).

#### Discussion

The obtained results would be similar to first descriptions of North Patagonian lakes of Argentina and Chile about the presence of *O. naumanni, S. amethystinus* and *S. araucanus* [4, 5]. The dominance of the all species in the second sampled period would be similar to the literature descriptions that mentioned

the marked dominance of this species in southern summer (December, January, and February) [5], that would be explained probably to temperature increase. Although literature mentioned that mixotrophic ciliates graze on bacteria [3, 13], the literature published about bacterial abundances in North Patagonian lakes are very preliminary, and denotes differences in function to human intervention for Villarrica lake [15, 28]. The marked differences in spectral properties in both lakes (Table 1), is agree with literature descriptions for a central Chilean lake with marked gradient of trophic status in different sites [28]. In this context, the marked associations of different ETM+ remote sensor bands between S. araucanus with the other two species would be due the pigmentation of the first specie that is blue-green, whereas O. naumanni and S. amethystinus have green-brown pigmentation [14, 29]. Also, S. *araucanus* is dominant in surface layers (epilimnion), due its marked photoprotective strategies, whereas O. naumanni and S. amethystinus prefers metalimnion due their vulnerability to natural solar radiation [5].

Similar results about the same mixotrophic ciliates species abundances and trophic interactions, has been described for Argentinean north Patagonian lakes [6, 7, 30-32]. The Argentinean lakes has more studies about interactions between bacteria and pyco-phytoplankton, for these lakes have descriptions of pyco-cianobacteria that are abundant in metalimnion zones [33] and other kind of bacteria [34, 35]. The results obtained for Argentinean lakes about mixotrophic ciliates, and ultraoligotrophic lakes would give support to the present

orrelation	n matrix for vari	iables considered i	in the present stud	ly ("P" value:	s lower than	0.05 denotes	significant as:	sociations).					
	S. araucanus	S.amethystinus	Onaumanni	Total Mixotr.	$Chl_a$	Total bacteria	(°C) Temp	B07	B06	B05	B04	B03	B02
	-0.802 P<0.001	-0.873 P<0.001	-0.750 P<0.001	-0.849 P<0.001	0.635 P = 0.008	-0.043 P = 0.872	0.026 P = 0.921	0.163 P = 0.544	0.099 P = 0.714	0.029 P = 0.913	0.020 P = 0.941	0.876 P<0.001	0.982 P<0.001
	-0.812 P<0.001	-0.903 P<0.001	-0.750 P<0.001	-0.860 P<0.001	0.602 P = 0.013	0024 P = 0.928	-0.012 P = 0.963	0.278 P = 0.296	0.215 P = 0.423	0.140 P = 0.604	0.052 P = 0.845	0.829 P<0.001	
	-0.513 P = 0.042	P-0.691 <0.001	-0.594 P = 0.015	-0.661 P = 0.005	0.581 P = 0.018	0.132 P = 0.625	-0.042 P = 0.876	P = -0.030 0.910	-0.171 P = 0.524	-0.287 P = 0.280	0.159 P = 0.554		
	0.290 P = 0.275	-0.062 P = 0.818	-0.041 P = 0.878	0.009 P = 0.971	0.009 P = 0.971	0.272 P = 0.307	0.021 P = 0.938	0.706 P = 0.002	0.594 P = 0.015	0.507 P = 0.044			
	-0.145 P = 0.590	-0.232 P = 0.387	-0.173 P = 0.521	-0.180 P = 0.504	-0.089 P = 0.742	-0.036 P = 0.894	0.062 P = 0.819	0.908 P<0.001	0.981 P<0.001				
	-0.130 P = 0.629	-0.264 P = 0.321	-0.182 P = 0.498	-0.196 P = 0.465	-0.063 P = 0.814	0.022 P = 0.935	0.053 P = 0.842	0.961 P<0.001					
	-0.108 P = 0.690	-0.283 P = 0.287	-0.182 P = 0.499	-0.202 P = 0.451	0.047 P = 0.861	0.125 P = 0.642	0.043 P = 0.872						
	0.098 P = 0.717	0.070 P = 0.795	0.173 P = 0.521	0.110 P = 0.684	0.243 P = 0.363	0.171 P = 0.525							
ia	0.181 P = 0.503	0.026 P = 0.923	0041 P = 0.880	0.052 P = 0.846	0.231 P = 0.388								
	-0.478 P = 0.055	-0.512 P = 0.042	-0.374 P = 0.152	-0.473 P = 0.063									
н	0.889 P < 0.001	0.953 P < 0.001	0.965 P< 0.001										
ui	0.809 P< 0.001	$\begin{array}{c} 0.855\\ P<0.001 \end{array}$											
ns	0.867 P< 0.001												

	Axis 1	Axis 2
B01	-0.375	0.087
B02	-0.380	0.027
B03	-0.305	0.176
B04	-0.037	-0.392
B05	-0.083	-0.506
B06	-0.103	-0.516
B07	-0.121	-0.507
Temp (°C)	0.021	-0.041
Total_bacteria	<-0.001	-0.061
Chlorophyll_a	-0.242	0.111
Total Mixotrophic	0.377	-0.032
O. naumanni	0.347	-0.021
S. amethystinus	0.381	0.006
S. araucanus	0.346	-0.09

Table 3. Contribution of considered variables for PCA.

results observed for Caburgua lake, nevertheless, the available methodology to determine bacterial abundances is more developed for Argentinean lakes than for Chilean lakes, and in the present status the field study of the role of bacterial in Chilean lakes would be uncertain [3]. The trophic interactions that involve mixotrophic ciliates, reported that this group together crustacean zooplankton would graze on bacteria, such as been observed for Argentinean lakes [36], and in this scenario, it would have direct association between mixotrophic ciliates with crustacean zooplankton such as been observed for Argentinean Patagonian lakes [34, 36]. Similar results have been observed for marine environments [37, 38-40]. Also, important topic about mixotrophic ciliates, is related with phytoplankton decreasing, this result was observed in the present study for Caburgua Lake, and also was described for Baikal lake [41] and Alpine lakes [42]. In this context, it has been reported for Argentinean Patagonia and for Alpine lakes, that it is possible found abundant mixotrophic ciliates under presence of glacier sediments that would affect water transparency affecting the phytoplankton activity, and enhancing mixotrophic ciliates activity



Fig. 2. Results of PCA for sites and variables included in the present study (Legend for sites: 1 = Villarrica North littoral, November; 2 = Villarrica centre, November; 3 = Villarrica port November; 4 = Villarrica La Poza Pucon bay November; 5 = Villarrica North littoral, December; 6 = Villarrica centre, December; 7 = Villarrica port, December; 8 = Villarrica La Poza Pucon bay, December; 9 = Caburgua 1, November; 10 = Caburgua 2 = November; 11 = Caburgua 3, November; 12 = Caburgua 4, November; 13 = Caburgua 1, December; 14 = Caburgua 2, December; 15 = Caburgua 3, December; 16 = Caburgua 4, December).

Ophyridium naumanni								
	Estimate	Standard error	T value	P value				
Intercept	6.3126	3.4459	1.832	0.116				
B01	377.484	110.786	3.407	0.143*				
B02	-405.256	140.924	-2.876	0.028*				
B04	-552.330	94.589	-5.839	0.001*				
B06	-266.166	92.176	-2.888	0.027*				
B07	985.984	255.852	3.854	0.008*				
Temperature	0.044	0.016	2.617	0.039*				
Total mixotrophic	0.912	0.030	29.586	<0.001*				
S. amethystinus	-1.762	0.148	-11.862	<0.001*				
S. araucanus	0.279	0.107	2.586	0.004*				
Residual standard error: 0.180, on 6 degrees of freedom, multiple $R^2 = 0.998$ ; adjusted $R^2 = 0.996$ ; $F_{(6.9; \alpha = 0.05)} = < 0.001$								
Stentor amethystinus								
	Estimate	Standard error	T value	P value				
Intercept	0.399	0.174	2.284	0.062				
B01	0.021	0.546	4.047	0.006*				
B02	-0.024	0.685	-3.550	0.012*				
B04	-0.031	0.451	-6.962	<0.001*				
B06	-0.015	0.487	-3.140	0.020*				
B07	-0.056	0.013	4.382	0.004*				
Temperature	0.024	0.009	2.503	0.046*				
Total mixotrophic	0.501	0.037	13.554	<0.001*				
O. naumanni	-0.544	0.046	-11.862	<0.001*				
S. araucanus	-0.165	0.054	2.975	0.024*				
Residual standard error: 0.100, on 6 degrees of freedom, multiple $R^2 = 0.998$ ; adjusted $R^2 = 0.996$ ; $F_{(6.9; \alpha = 0.05)} = < 0.001$								
Stentor araucanus								
	Estimate	Standard error	T value	P value				
Intercept	-9.960	2.925	-3.404	0.004*				
B04	361.285	104.298	3.464	0.004*				
S. amethystinus	1.061	0.119	8.902	< 0.001*				
Residual standard error: 0.7041, on 13 degrees of freedom, multiple $R^2 = 0.870$ ; adjusted $R^2 = 0.851$ ; $F_{(2;13; \alpha = 0.05)} = < 0.001$								

Table 4. Results of multiple regression analysis for mixotrophic ciliates species reported in the present study.

[43]. In this scenario, if the first observations denoted for Patagonian lakes the associations between spectral properties and zooplankton composition [15-18], these results would be applicable to mixotrophic ciliates such as been observed in the present study.

The use of remote sensing techniques, for water quality studies in lakes [44], proposed marked associations in spectral properties and chlorophyll concentration [44-51]. In this context would have marked differences in watercolour due to water quality that can be detectable using remote sensing techniques [52, 53]. Also, it was proposed the use of remote sensing techniques for determine potential associations with cyanobacterial pigments in lakes [54-56]. The exposed results about associations between differences in water quality with their respective correspondence in spectral images, would be similar to the observed results in the present study. Also, in this scenario, the present results it would be possible detect differences within a same lake at large spatial scales (ten or more kilometres), that

can be detectable using remote sensing techniques [15, 57].

The exposed results revealed the presence of differences between water qualities, specifically chlorophyll concentration, between two lakes, and within each lake [15]. Nevertheless, the integration of limnological descriptions and remote sensing techniques for Chilean lakes has been described only for Vichuquén lake (34°48′42″S; 72°02′57″W), that is a coastal central Chilean lake, with marked human intervention in their surrounding basin, and it was possible propose a predictive model based in LANDSAT 8 OLI sensor images [57]. In this scenario it would be possible use remote sensing techniques for predict water quality in northern Patagonian lakes.

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#### **Conflict of Interest**

The authors declare no conflict of interest.

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