Phytoplankton absorption spectra along the water column in deep North Patagonian Andean lakes (Argentina)

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Abstract

During a summer period we studied the vertical variation of in vivo and chlorophyll *a* specific phytoplankton absorption spectra in relation to the underwater light climate of ten deep North Patagonian Andean lakes of Argentina. The lakes were thermally stratified, and the underwater light climate was characterized by extended euphotic zones which included highly illuminated epilimnetic layers (both UVR and PAR) and metalimnia exposed to dim blue-green light. Most of the lakes presented the development of Deep Chlorophyll Maxima (DCM) at the metalimnetic layers, near 1% of surface PAR irradiance. Analyzing the fourth-derivative plots of in vivo phytoplankton absorption spectra [$d^{IV}a_{ph}(\lambda)$], we were able to identify several maxima absorption values attributed to different pigments. Considering lakes with DCM, a significant positive linear relationship was found between $d^{IV}a_{ph}$ (495–500 nm) normalized by chlorophyll *a* and downward irradiance. Indeed, a negative significant relationship was found between $d^{IV}a_{ph}$ (495–500 nm) normalized by chlorophyll *a* and diffuse PAR attenuation coefficients. These results point out an increase in the relative concentration of different carotenoids at surface layers indicating the role of photoprotection of these pigments. On the other hand, significant negative linear relationships were found between fourth-derivative spectra normalized by chlorophyll *a* at 650, 590–595, 560–565 and 520–525 nm and downward irradiance. These results indicated an increase in the relative concentration of photosynthetic accessory pigments at deep layers of the euphotic zone. Furthermore, we found a decrease in depth of specific absorption spectra at 440, 670 nm and in the ratio a_{ph}^* (440 nm) to a_{ph}^* (670 nm). This pattern was associated with the package effect concept. The increase in relative photosynthetic accessory pigment concentrations and the decrease in values of specific absorption spectra at the bottom of the euphotic zone were attributed to changes in phytoplankton communities between surface and deep layers. These outcomes pointed out that the underwater light climate and temperature water structure are, like in marine systems, very important factors governing the distribution of phytoplanktonic organisms.

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In addition, the possession of specific photosynthetic accessory pigments suggests that dominant species in the DCM are well adapted to these dim blue-green light scenarios.

Introduction

Phytoplankton absorption spectra have been extensively used in regional comparison (Harimoto, Ishizaka, & Itsuda, 1999). Different works have shown that changes in pigment composition and pigment package effects are the primary source of variability in specific absorption of phytoplankton (Bricaud & Stramski, 1990; Fujiki & Taguchi, 2002; Mitchell & Kiefer, 1988). These sources of variation were associated with changes in the species structure of primary producers' community and/or due to changes in the internal concentration of pigment in responses to photo-acclimation process (Bricaud & Stramski, 1990; Morel & Bricaud, 1981). The analysis of in vivo and specific absorption spectra is difficult considering that they are complex spectra due to high overlapping bands of different pigments and associated proteins. Derivative analysis is an objective tool for isolating absorption peaks in phytoplankton absorption spectra (Aguirre-Gómez, Weeks, & Boxall, 2001; Bidigare, Morrow, & Kiefer, 1989; Millie et al., 1997; Staehr & Cullen, 2003).

The package effect predicts that the absorption spectrum is flattened due to increasing cell or colony size or pigment concentration (Morel & Bricaud, 1981), so changes in these features along the water column will drive the specific absorption of phytoplankton to different patterns. On the other hand, phytoplankton pigments include groups of compounds with different chemical and physical properties (Kirk, 1994). Basically, these pigments can be divided into three groups: chlorophylls (*a*, *b* and *c*), carotenoids (carotenes and their oxygenated derivatives known as xanthophylls), and biliproteins (allophycocyanins, phycocyanins and phycoerythrins). Pigments can also be separated according to their function in phytoplankton cells (Bidigare et al., 1990; Majchrowski & Ostrowska, 2000; Stón & Kosakowska, 2000). The photosynthetic accessory pigments absorb radiation energy in different wavelengths than chlorophyll *a*, transferring part of this energy onto chlorophyll *a* for photosynthesis (Majchrowski & Ostrowska, 2000). A second group of accessory pigments are the photoprotecting (non-photosynthetic) ones (Karentz et al., 1994; Majchrowski & Ostrowska, 2000). These pigments are mainly carotenoids and are important in absorbing low-wavelength PAR (400–500 nm) as well as near UV irradiation (360–400 nm) acting as direct sun-screens (Laurion, Lami, & Sommaruga, 2002) and providing protection against photooxidative stress (Karentz et al., 1994).

In natural environments, absolute levels of radiation and underwater light spectra, vertical temperature and density gradients and nutrient availability, determine phytoplankton distribution along the water column (Majchrowski & Ostrowska, 2000; Sharples et al., 2001). Phytoplankton pigments respond to changes in the light field presenting quantitative and qualitative diversity that depend on species composition, the photo-adaptive state of the phytoplankton and physiological changes of phytoplankton cells which undergo photo-acclimation (Kirk, 1994; Stón & Kosakowska, 2000).

In oligotrophic oceans, photoprotective pigments have shown a decrease with increasing depth (Bidigare et al., 1989; Hoepffner & Sathyendranath, 1992; Majchrowski & Ostrowska, 2000), because in deep waters the absolute levels of short-wave radiation are lower (Majchrowski & Ostrowska, 2000). In contrast, both chlorophyll *a* and photosynthetic accessory pigment concentrations have shown an increase with increasing depth in response to lower light levels in deep waters (Bricaud & Stramski, 1990; Kirk, 1994; Majchrowski & Ostrowska, 2000).

A raise in Chl *a* concentration with depth was observed in different oligotrophic and very transparent lakes around the world (Coon, López, Richerson, Powell, & Goldman, 1987; Fennel & Boss, 2003; Gervais, Padisak, & Koschel, 1997). In some large and deep North Patagonian Andean lakes of Argentina a deep chlorophyll maximum (DCM) was described at metalimnetic layers near the 1% surface irradiance (Modenutti, Balseiro, & Queimaliños, 2000; Pérez, Queimaliños, & Modenutti, 2002; Queimaliños, Modenutti, & Balseiro, 1999). In these lakes, where epilimnetic layers are characterized by very high irradiances and the metalimnetic strata are even illuminated (Modenutti, Pérez, Balseiro, & Queimaliños, 2000; Morris et al., 1995; Pérez et al., 2002; Queimaliños et al., 1999), important differences in the accessory pigment structure between surface and deep layers could be expected. During summer, when thermal stratification occurred, we studied a set of 10 lakes belonging to both Atlantic and Pacific watersheds. We analyzed the vertical variations of the in vivo absorption spectra in all lakes, and applying the fourth-derivative method on these spectra we were able to identify different pigment structures. In addition, we calculated specific absorption

spectra in order to estimate the magnitude of package effect. The aim of the present work was to analyze phytoplankton specific absorption spectra in relation to changes in pigment composition and package effects under contrasting optical scenarios of deep Andean Lakes.

Study area

The studied lakes are located between $40°27'$ S and $42°49'$ S in the North Andean Patagonian region (Argentina) (Fig. 1) corresponding to the Glacial lakes district of the Southern Andes (Iriondo, 1989). The climate is temperate cool (mean annual temperature: $8.7\,°C$) with predominance of westerly winds, and annual precipitation of 1500 mm (Paruelo, Beltrán, Jobbágy, Sala, & Golliuscio, 1998). The vegetation corresponds to the Andean-Patagonic temperate forest represented in the lakes' shores mainly by *Nothofagus dombeyi* (Mirb.) Blume and *Austrocedrus chilensis* (D.Don) Florin et Boutleje. The area is included within three National Parks: Nahuel Huapi, Puelo and Los Alerces (Fig. 1), and is characterised by a profuse hydrographic system including large deep lakes (area $> 5\,\mathrm{km}^2$; $Z_{max} > 100\,\mathrm{m}$). The main rivers fed from these Andean waters cross the plateau steppe and outflow to the Atlantic Ocean, but there are also other rivers that cross the Andes flowing towards the Pacific Ocean (Modenutti et al., 1998). The ten studied lakes are large and deep: Lakes Falkner, Villarino, Correntoso, Nahuel Huapi and Gutiérrez of Atlantic watershed, and Lakes Mascardi Catedral, Mascardi Tronador, Puelo, Rivadavia and Futalaufquen of Pacific watershed (Fig. 1). The two arms of Mascardi lake (Catedral and Tronador) were considered as two lakes since this system has a different hydrology from the other study basins due to the three main glaciers (Manso, Overo and Blanco glaciers) present on headwaters that only drains into Mascardi Tronador (Modenutti, Pérez et al., 2000).

All the studied lakes exhibit a warm monomictic thermal regime, with thermal stratification during late spring and summer (Pérez et al., 2002; Quirós & Drago, 1985). PAR and UVR transparency is high in North Patagonian Andean lakes with low dissolved organic carbon concentration (Morris et al., 1995). The trophic status was described as ultra-oligotrophic or oligotrophic (Markert et al., 1997; Modenutti, Balseiro et al., 1998; Modenutti et al., 2000; Pedrozo, Chillrud, Temporetti, & Diaz, 1993).

Materials and methods

Sampling

Sampling was carried out in summer (December–January 2001–2002) in a central sampling point of each lake. Vertical profiles (0–50 m) of UV bands (305, 320, 340 and 380 nm) and PAR downward irradiance (400–700 nm) and temperature were measured with a
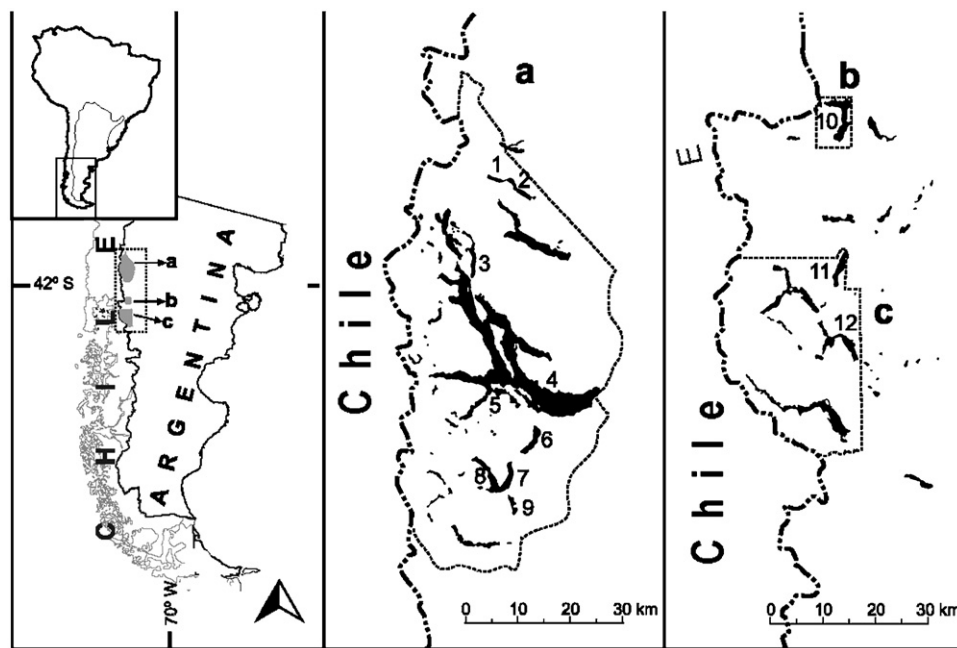

Fig. 1. Map of the Patagonian Andean region of Argentina showing the three National Parks (a: Nahuel Huapi, b: Puelo and c: Los Alerces) Lakes of Atlantic watershed, 1: Falkner; 2: Villarino; 3: Correntoso; 4: Nahuel Huapi; 5: Gutiérrez. Lakes of Pacific watershed, 6: Mascardi Catedral; 7: Mascardi Tronador; 8: Puelo; 9: Rivadavia, and 10: Futalaufquen.

PUV 500B submersible radiometer (Biospherical Instruments). Underwater light quality was measured with the radiometer equipped with coloured glass filters. Blue and green filters were pass band filters with a maximum transmittance at 484 and 520 nm, respectively; while the red one was a high pass filter with a maximum transmittance over 600 nm. Separate profiles were conducted with each filter. In situ chlorophyll *a* profiles were determined with the PUV 500B (PUV 683) calibrated against measurements in the laboratory on the basis of ethanol extractions. Water samples of 12 l were obtained with a Schindler-Patalas trap from 0 to 50 m at each 10 m interval. The water obtained was transferred to 10 l polypropylene containers. Containers were kept in darkness and thermally isolated and were carried immediately to the laboratory. All sampling was carried out in triplicates, at mid-day, 1 h before astronomic noon.

Optical properties

Diffuse vertical attenuation coefficients of PAR downward irradiance, UV, blue, green and red radiations (Kd_{PAR}, Kd_{305}, Kd_{BLUE}, Kd_{GREEN} and Kd_{RED}) for each lake were calculated by regressing log-transformed light with depth. Absorption measurements of the suspended particulate matter components were carried out on each water sample (each depth of every lake), within 2 h after sampling. Optical densities were measured with a Metrolab 1700 spectrophotometer in the spectral range of 400–750 nm at 1 nm intervals. Light absorption spectra by lake particles [$a_p(\lambda)$] were determined after concentration of the particles on GF/F glass-fiber filters following the filter pad technique (Trüper & Yentsch, 1967). Two or three liters of water of each lake were filtered, and optical densities were measured directly on the wet filters against a blank clean filter, wetted with distilled water used as reference. Instead of analyzing the amplification factor [$\beta(\lambda)$] as a function of optical densities of particles retained in the filters [$OD_{filt}(\lambda)$], we examined optical densities of suspended particles [$OD_{susp}(\lambda)$] as a function of $OD_{filt}(\lambda)$ according to the relationship obtained by Cleveland and Weidemann (1993). During the scanning, the wetness of the filters remained unchanged. The absorption at 750 nm, where absorption can be considered negligible, was assumed to be due to residual scattering in the filtrate, and was subtracted from the absorbance values at all other wavelengths in order to calculate [$a_p(\lambda)$] (Bricaud & Stramski, 1990). After measuring the absorption of total particulate matter, the spectral absorption of non-algal material [$a_d(\lambda)$] was measured separately following Kishino, Booth, and Okami (1984). In this case, the GF/F filter was placed in absolute methanol at 4 °C for 45–60 min in order to extract pigments. The bleached filter was dried and then soaked again in filtered lake water for 1 h. The same treatment was applied in parallel to a reference filter used as blank. The absorption coefficients of viable phytoplankton [$a_{ph}(\lambda)$] were obtained of subtracting the absorption by the bleached matter [$a_d(\lambda)$], from the absorption of total particulate matter [$a_p(\lambda)$].

A *t*-test was applied to test differences in DCM depth and vertical attenuation coefficients (Kd_{305}, Kd_{PAR}, Kd_{BLUE}, Kd_{GREEN} and Kd_{RED}) between deep lakes of Atlantic and Pacific watersheds. Prior to analysis a Kolmogorov-Smirnov test (with Lilliefors' correction) and a Levene Median test were applied to test data for normality and homoscedasticity respectively.

Analyses of in vivo and specific absorption coefficients

Phytoplankton in vivo absorption coefficients were converted into chlorophyll specific absorption coefficients [$a_{ph}^*(\lambda)$] dividing them by extracted chlorophyll *a* concentration (Bricaud & Stramski, 1990). Chlorophyll *a* (Chl *a*) concentration was immediately determined for samples carried from the field. A volume of 200 ml of water of each level was filtered onto GF/F filters and extracted with hot ethanol (Nusch, 1980). Chl *a* concentrations were determined with a fluorometer Turner AU-10.

The analysis of in vivo and chlorophyll *a* specific absorption spectra allows the evaluation of the true absorption of phytoplankton community. The absorption spectra of phytoplankton are complex due to the mixtures of pigments and associated proteins. To solve this problem the derivative analysis of spectra curves proved to be a powerful method (Aguirre-Gómez et al., 2001; Bidigare et al., 1989; Kirkpatrick, Millie, Moline, & Schofield, 2000; Millie, Schofield, Kirkpatrick, Johnsen, & Evens, 2002). Derivative analysis provides information regarding the convexity and concavity of a given absorption curve, and it is used for separating secondary absorption peaks and shoulders produced by algal pigments in regions of overlapping absorption (Bidigare et al., 1989). Smoothed data and the fourth derivative spectra were obtained with the modified Savitsky-Golay algorithm provided by PeakFit®, Systat software inc. The possible generation of spurious (artificial bands) was kept in mind when analyzing high derivative spectra of obtained data. Suitable information was obtained by taking different derivative curves including the odd-numbered derivatives (Butler & Hopkins, 1970a).

The fourth-derivative spectra (d^{IV}) (Butler & Hopkins, 1970a, b) were computed for each in vivo absorption spectrum to solve the number and position of the different absorption bands. The amplitude of any

maximum obtained from the fourth-derivative spectrum of in vivo phytoplankton absorption ($d^{IV}a_{ph}$) is proportional to the concentration of the chromoprotein that absorbs at that wavelength (Bidigare et al., 1989). In order to compare the variations of pigments or group of pigments in relation with chlorophyll a between surface and deep layers of the water column, derivative spectrum of in vivo absorption spectrum was normalized by the corresponding chlorophyll a concentration ($d^{IV}a_{ph}^{**}$). In the present work the in vivo absorption spectra of phytoplankton was not corrected by package effect; nevertheless, this effect was considered and discussed in quantitative analyses.

In order to identify the major contributing pigments of the in vivo absorption spectra of phytoplankton in natural samples, we compared measured wavelength positions of the fourth-derivative spectra with published values. In addition, the derivative method was applied to in vivo absorption spectra of four algal cultures with different known pigment assemblages. Two picocyanobacteria cultures, Synechococcus sp. rich in phycocyanin (PC) and Synechococcus sp. rich in phycoerythrin (PE); one Chlorophyceae, Chlorella minima and one diatom Thalassiosira fluviatilis were used for this analysis. Synechococcus and Chlorella cultures were provided by L'Istituto per lo Studio degli Ecosistemi (Pallanza, Italy), and the diatom culture was provided by the Photobiology Station of Playa Unión (Chubut, Argentina). The selection of these species was due to previous studies on phytoplankton composition in Andean lakes (Pérez et al., 2002).

Reference wavelengths used in this study were: Chlorophyll a (415–425, 440–455, 620, 675 nm) (Aguirre-Gómez et al., 2001); Chlorophyll b (465–470, 585–595 and 650 nm) (Millie et al., 1997) and (483, 650 nm) (Bidigare et al., 1989); total chlorophyll c (470, 630 nm) (Bidigare et al., 1989) and (465–470, 589, 639 nm) (Millie, Kirkpatrick, & Vinyard, 1995; Millie et al., 1997); phycoerythrin (543–550, 566–568 nm) (Payri, Maritorena, Bizeau, & Rodière, 2001; Smith & Alberte, 1994); phycoerythrocyanin (~550 nm, ~575 nm) (Millie et al., 2002); phycocyanin (625–630 nm) (Millie et al., 2002; Payri et al., 2001); fucoxanthin (521–531 nm) (Bidigare et al., 1989); different carotenoids (490–495 nm) (Millie et al., 1997; Owens, Gallagher, & Alberte, 1987).

Linear regression models were used to analyze in vivo absorption spectra of phytoplankton in relation with apparent optical properties (upward PAR irradiance and PAR vertical attenuation coefficients).

Floristic analysis

A volume of 250 ml of lake water of each depth (from 0 to 50 m at each 10 m interval) was fixed with acid Lugol solution for phytoplankton identification. In the laboratory, floristic assessment was carried out with an inverted microscope using 50 ml Utermöhl chambers.

Results

During the studied summer season, a direct thermal stratification was observed in all the studied lakes, with varying epilimnetic layers which ranged between 15 and 40 m depth (Fig. 2). Solar radiation was observed to be high (underwater surface downward irradiance 1600–1800 μmol photons m^{-2} s^{-1}) since summer is a dry season in North Patagonia with few cloudy days.

Considering lake light climate, the lakes of Atlantic watershed were more transparent (t-test: Kd_{305} $p = 0.018$, Kd_{PAR} $p = 0.021$, Kd_{Blue} $p = 0.013$, Kd_{Green} $p = 0.011$ and Kd_{Red} $p = 0.015$) and showed more homogeneous patterns than those of Pacific one (Fig. 2). In particular, the former presented Kd_{PAR} values between 0.09 and 0.14 m^{-1} (Table 1) and their euphotic zones ranged from 32.6 to 46.8 m (Fig. 2, upper panel). In contrast, the lakes of Pacific watershed presented statistically higher Kd_{PAR} values (0.13–0.22 m^{-1}) (Table 1), with euphotic zones between 20.3 and 36.0 m (Fig. 2, lower panel). In most of the studied lakes the euphotic zone was deeper than the epilimnion, with the exception of Lake Puelo, which presented both strata of the same width (Fig. 2, Lake Puelo). Optical quality in the studied lakes was characterized by a rapid attenuation of red light, while blue and green light penetrated deepest (Table 1). The two lakes receiving the input of glacial clays (Lakes Mascardi and Puelo) exhibited the higher attenuation of the blue light (Table 1). On the other hand, attenuation coefficients of UV-B waveband (Kd_{305}) ranged from 0.44 to 1.07 m^{-1} (Table 1). Thus, the upper part of the epilimnion was exposed to this potential hazardous radiation since the 1% of the 305 nm waveband was observed between 4.3 and 11 m.

Most of the lakes presented a DCM at the metalimnion in coincidence with the 1% of surface PAR irradiance (Fig. 2, upper and lower panels). In Lakes Correntoso and Nahuel Huapi, both of Atlantic watershed, the distribution of chlorophyll a was observed to be deeper than the other study lakes (DCM~45 m) (Fig. 2, upper panel, t-test $p < 0.001$, Lake Puelo not considered in this analysis, see below). In particular, in Lake Correntoso this level was included in the hypolimnion (Fig. 2, Lake Correntoso). On the contrary, in the two lakes of Pacific watershed receiving glacial clays a different pattern was recorded. In Lake Puelo, the development of the DCM was not observed and in Lake Mascardi Tronador the shallowest DCM was registered (Fig. 2, lower panel).

Lakes of both watersheds presented chlorophyll a specific absorption spectra a_{ph}^{*} (λ) with two maxima at
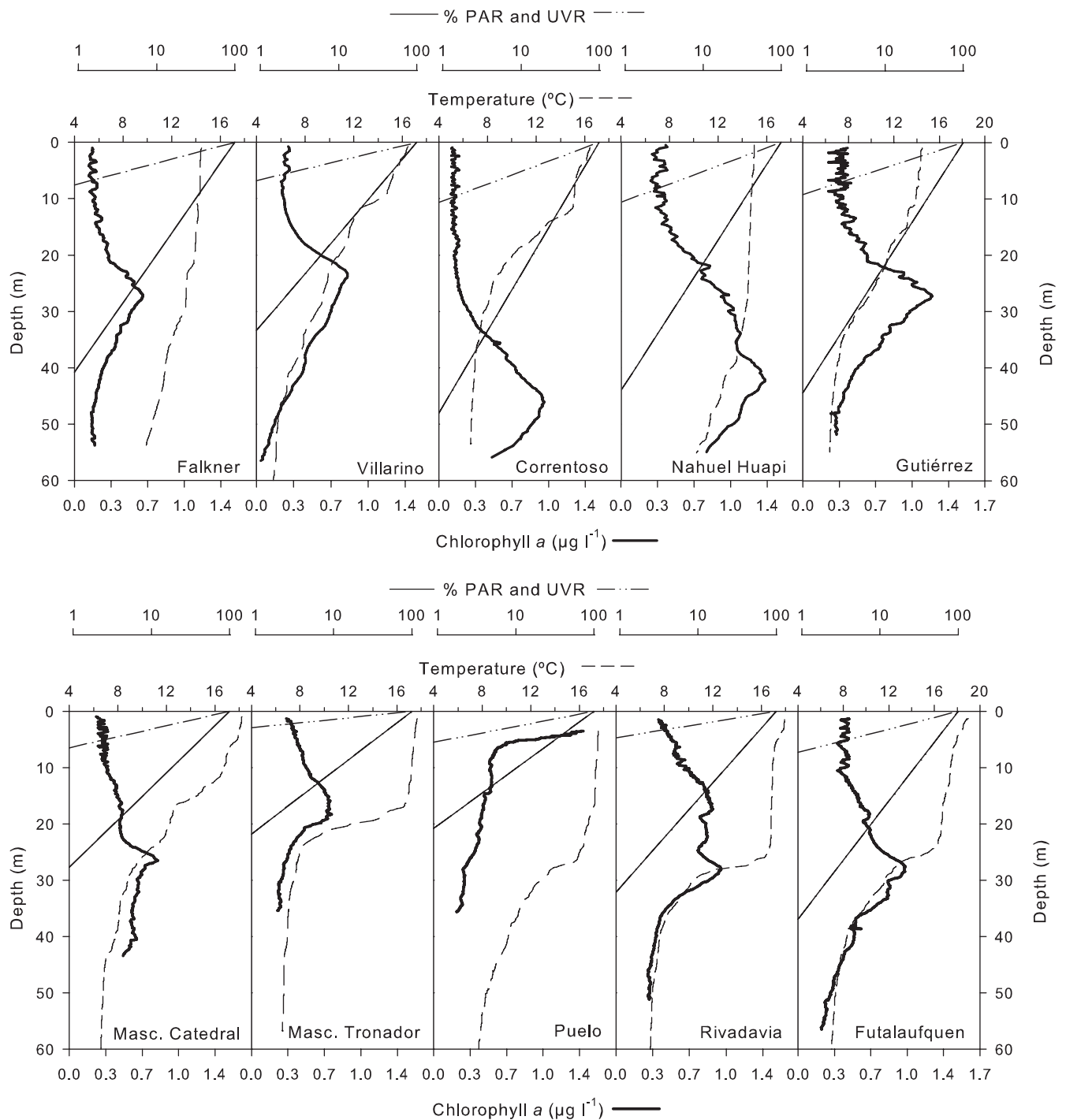
Fig. 2. Vertical profiles of temperature, chlorophyll *a* concentration and Photosynthetically Active Radiation (PAR) (400–700 nm) and Ultraviolet radiation (UVR) at 305 nm in the ten studied lakes. Upper panel: lakes of Atlantic watershed; lower panel: lakes of Pacific watershed.

430–440 and 665–675 nm (Fig. 3). All spectra showed an important variation with depth (Fig. 3). A significant decrease in values of a_{ph}* (440 nm) was observed between surface layers (10 m) and the depth were DCM developed (*t*-test, $p < 0.001$). This pattern was not observed in lakes Rivadavia and Puelo (Fig. 3). Values of a_{ph}* (440 nm) ranged from 0.183 m² (mg Chl

$a)^{-1}$ in lake Correntoso at 0–0.041 m² (mg Chl $a)^{-1}$ in lake Nahuel Huapi at 40 m depth (Fig. 3). Although a_{ph}* (670 nm) also diminished with increasing depth (Fig. 3), this decrease was not significant (*t*-test, $p > 0.05$). In addition, the ratio a_{ph}* (440 nm) to a_{ph}* (670 nm) also showed a decrease with increasing depth, being this variation more accentuated at metalimnetic

Table 1. Morphometric characteristics, light vertical attenuation coefficients of Photosynthetically Active Radiation (Kd_{PAR}), Ultraviolet Radiation at 305 nm (Kd_{305}) and of the three studied colours (Kd_{BLUE}, Kd_{GREEN}, Kd_{RED}) of the ten lakes grouped by watershed (Atlantic upper panel and Pacific lower panel)

Lakes	Area (km^2)	Z_{max} (m)	Kd_{PAR}* (m^{-1})	Kd_{305}* (m^{-1})	Kd_{BLUE}* (m^{-1})	Kd_{GREEN}* (m^{-1})	Kd_{RED}* (m^{-1})
Falkner	11.34	>100	0.14	0.67	0.15	0.13	0.17
Villarino	5.25	>100	0.14	0.58	0.13	0.11	0.15
Correntoso	19.50	>100	0.09	0.44	0.10	0.09	0.12
Nahuel Huapi	557.00	464	0.10	0.44	0.10	0.09	0.12
Gutiérrez	16.40	111	0.10	0.48	0.10	0.09	0.12
Mascardi Catedral	39.20	118	0.17	0.89	0.21	0.17	0.22
Mascardi Tronador	39.20	218	0.22	1.07	0.31	0.25	0.31
Puelo	44.00	180	0.21	0.91	0.24	0.21	0.24
Rivadavia	21.70	147	0.14	0.65	0.16	0.14	0.17
Futalaufquen	44.60	168	0.13	0.58	0.15	0.13	0.17

Area and Z_{max} from Quirós & Drago (1985)

*Indicates significant differences ($p < 0.05$) between lakes of different watersheds.

and hypolimnetic layers of lakes of Atlantic watershed (Fig. 4a). The lakes of Pacific watershed are more heterogeneous, and the diminishing pattern of the ratio a_{ph}* (440 nm)/a_{ph}* (670 nm) was not observed in lakes Puelo and Rivadavia. Indeed, in the former this ratio increased with increasing depth (Fig. 4b).

Derivative analysis was applied to in vivo absorption spectra of four algal cultures. We were able to identify 11 maximum absorption values (Table 2). Peaks 1, 2 and 11 were observed in the four algal cultures, and corresponded to the in vivo absorption of chlorophyll a (Table 2). Peak 3, located between 464 and 468 nm, was observed in *Chlorella minima*, *T. fluviatilis* and *Synechococcus* rich in PE. In *Chlorella minima* this maximum was attributed to the presence of chlorophyll b and carotenoids; in *T. fluviatilis* to total chlorophyll c and carotenoids, while in *Synechococcus* rich in PE corresponded to the presence of different carotenoids (Table 2). Peak 4 corresponded to the presence of β-carotene, diadinoxanthin or fucoxanthin, depending on algal species. Peaks 5 and 6, with their maxima at 527 and 530, were attributed to the presence of fucoxanthin or fucoxanthin-derivates in *T. fluviatilis* and to phycoerythrin in *Synechococcus* rich in PE. In addition, peak 7 was observed for *Synechococcus* rich in PE and rich in PC (Table 2) and corresponded to the presence of phycoerythrin, being higher in the former strain. In *Chlorella minima*, peak 8 was attributed to the presence of chlorophyll b, and in *T. fluviatilis* this maximum was attributed to total chlorophyll c and a secondary absorption band of chlorophyll a. Peak 9 was solved by the fourth derivative analysis in different spectral components (Table 2). In *Synechococcus* rich in PE and rich in PC corresponded to the presence of PE, PC and a secondary band of chlorophyll a. In *Chlorella minima* and *T. fluviatilis* this peak was attributed to the

secondary band of chlorophyll a and to the presence of chlorophylls $c_1 + c_2$, respectively. Finally, peak 10 with maximum at 645 nm was only observed in *Chlorella minima* and corresponded to the presence of chlorophyll b (Table 2).

Analyzing the fourth-derivative plots of in vivo phytoplankton absorption spectra of lake natural samples, we were able to identify twelve maxima absorption values. Maxima of $d^{IV}a_{ph}$ located at 495–500 nm were attributed to different carotenoids, at 520–525 nm to fucoxanthin, fucoxanthin derivates and peridinin, and both at 534–540 and 560–565 nm to phycoerythrobilin chromophore of phycoerythrin and phycoerythrocyanin. Finally, the bands between 590 and 595 nm were assigned to total chlorophyll c, chlorophyll b and a secondary band of chlorophyll a, between 630 and 640 nm to chlorophyll $c_1 + c_2$, and at 650 nm to chlorophyll b.

Considering all studied lakes, a significant positive linear relationship was observed between $d^{IV}a_{ph}$ (670 nm) and extracted chlorophyll a concentration ($r^2 = 0.77$, $p < 0.001$, $n = 57$) (Fig. 5). Considering the lakes with DCM, significant negative linear relationships were found between fourth-derivative spectra normalized by chlorophyll a concentration ($d^{IV}a_{ph}$**) at 650, 590–595, 560–565 and 520–525 nm and downward irradiance ($p < 0.0001$) (Fig. 6). This result indicated an increase in the Accessory Photosynthetic Pigments between surface and deep layers of the euphotic zone. Besides not apparent pattern was observed between $d^{IV}a_{ph}$** (630 nm) and downward irradiance (Fig. 6). On the other hand, at 495–500 nm a significant positive linear relationship was found between $d^{IV}a_{ph}$** and downward irradiance ($p < 0.001$) (Fig. 6). Indeed, considering all studied lakes a negative significant relationship was found between $d^{IV}a_{ph}$**
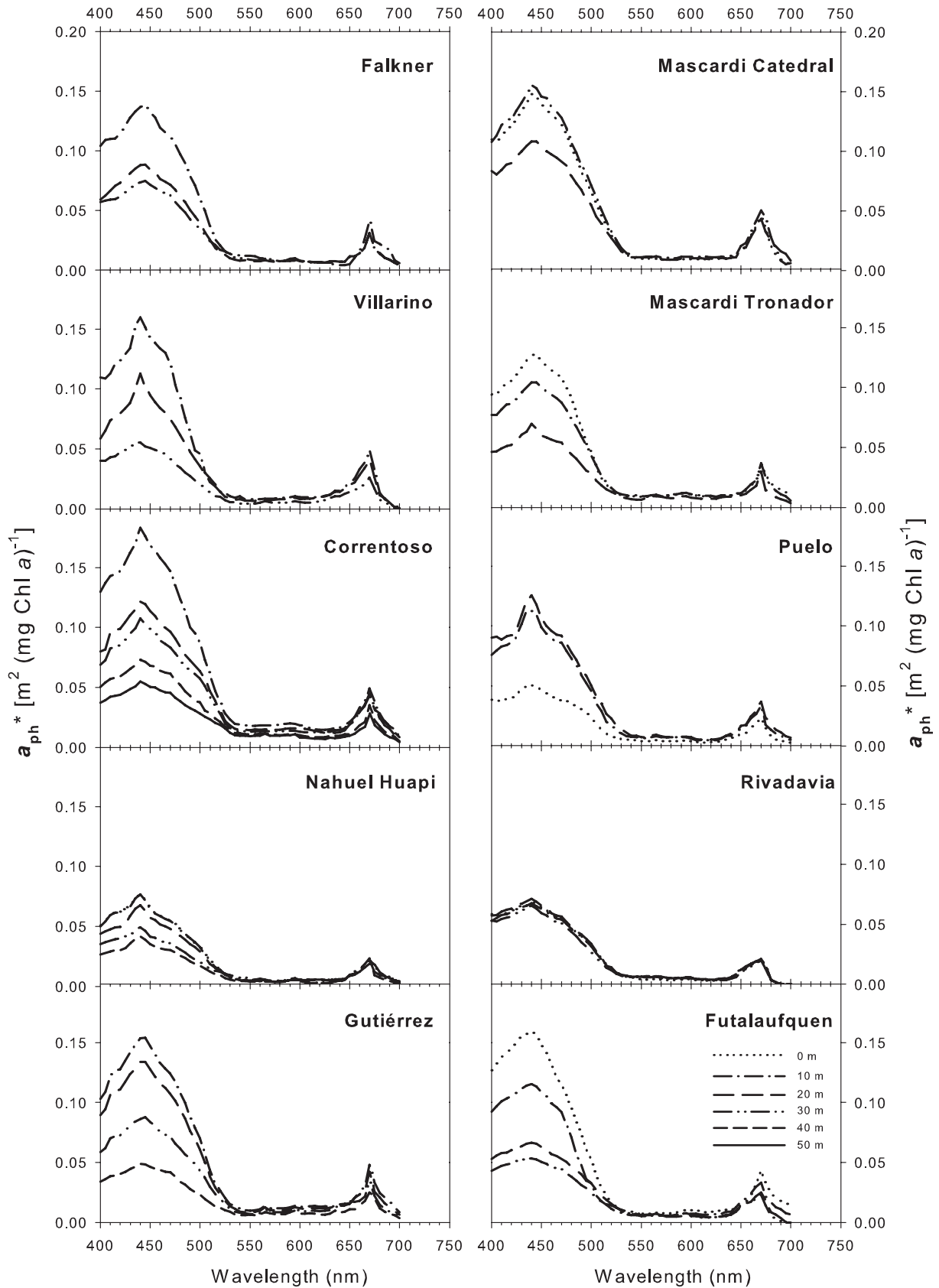
Fig. 3. Chlorophyll *a* specific phytoplankton absorption spectra (a_{ph}*) in the euphotic zone of lakes of Atlantic (left panel) and Pacific (right panel) watersheds.

(495–500 nm) and diffuse PAR attenuation coefficients (Kd_{PAR}) ($r^2 = 0.72$, $p < 0.0001$, $n = 10$) (Fig. 7). This result indicates an increase in the absorption and relative concentration of different carotenoids with the increase in transparency of lake waters, implying that these carotenoids may act as photoprotecting pigments.

Discussion

The studied North Patagonian Andean lakes exhibited high transparency ($Kd_{PAR} \leqslant 0.22 \, \text{m}^{-1}$) and extended euphotic zones that included the whole epilimnion ($Z_{1\%PAR} > Z_{therm}$). As a consequence of these features two optic scenarios can be delimited in the water column. The epilimnion characterized by irregular light regime, with high irradiances including hazardous UV-B in the upper levels, and the subsequent illuminated metalimnion which exhibited a more stable dim-light regime with a prevalence of blue-green light.

In the epilimnion, algal cells can not remain fixed in space in relation to light field thus, they are subjected to continually varying irradiances (Kirk, 1994). In the epilimnion of Andean lakes, solar radiation experienced by phytoplankton varied greatly from upper levels exposed to UV-B and high PAR surface irradiances to the thermocline depth in which UVR is absent and PAR irradiance decreases. High levels of UVR and PAR have been observed to present different deleterious effects in phytoplankton organisms (Karentz et al., 1994) and in Patagonian waterbodies diverse negative effects have been previously reported (Helbling, Villafañe, & Barbieri, 2001; Modenutti, Balseiro, Callieri, Queimaliños, & Bertoni, 2004; Villafañe, Buma,
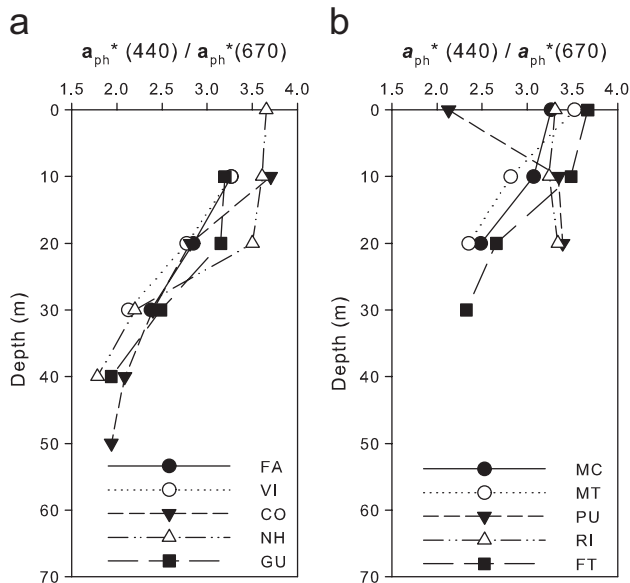

Fig. 4. Ratio $a_{ph}*(440)/a_{ph}*(670)$ in the euphotic zone of: (a) lakes of Atlantic watershed and (b) lakes of Pacific watershed. References: FA: Falkner; VI: Villarino; CO: Correntoso; NH: Nahuel Huapi; GU: Gutiérrez; MC: Mascardi Catedral; MT: Mascardi Tronador; PU: Puelo; RI: Rivadavia, and FT: Futalaufquen.

Table 2. Absorption peaks (nm) for in vivo cultures of two strains of *Synechococcus* (rich in phycocyanin PC, and in phycoerythrin PE), *Chlorella minima* and *Thalassiosira fluviatilis* from the fourth derivative method, and literature references

| No.	Synechococcus PC	Synechococcus PE	Chlorella minima	Thalassiosira fluviatilis	Reference (λ)	References
1	409	410	410	410	412, 415	Millie et al., 1995; Aguirre-Gómez et al., 2001
2	434	435	438	434	435–440;	Millie et al., 2002
3	—	464	468	466	467, 465–470,	Bidigare et al., 1989; Millie et al., 1997
4	490	493	493	497	490, 492, 495, 499	Owens et al., 1987; Smith & Alberte, 1994
5	—	—	—	527	521–531	Bidigare et al., 1997
6	—	534	—	—	538, 540	Smith & Alberte, 1994; Payri et al., 2001
7	566	565	—	—	560, 566–568	Smith & Alberte, 1994; Payri et al., 2001
8	597	597	586	591	585–595	Millie et al., 1997
9	625	623	616	636	627–630, 625–630	Smith & Alberte, 1994; Millie et al., 2002
10	—	—	645	—	650, 643	Bidigare et al., 1989; Aguirre-Gómez et al., 2001
11	671	675	676	673	670–675	Millie et al., 2002
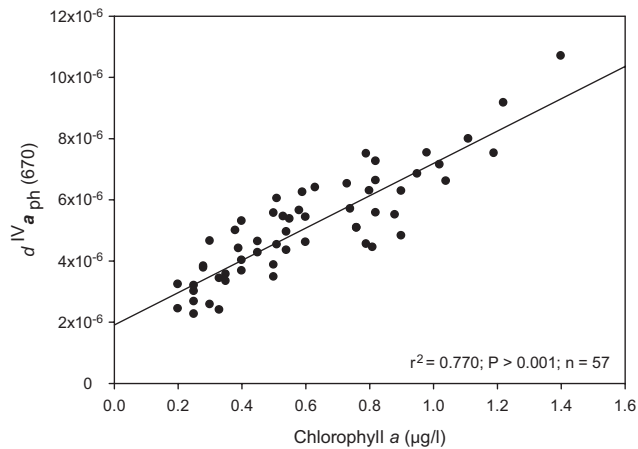
Fig. 5. Relationship between maximum fourth-derivative spectra at (670 nm) [$d^{IV}a_{ph}$ (670 nm)] and extracted chlorophyll a concentration obtained in the whole data set.

Boelen, & Helbling, 2004; Zagarese, Cravero, González, & Pedrozo, 1998). The observed maxima at 495–500 nm obtained from $d^{IV}a_{ph}$** was attributed to different carotenoids. We found two relationships between these maxima and the downward irradiance and Kd_{PAR} values, indicating that these carotenoids play a photo-protective role in these Andean lakes. In other lakes it was also shown that the increase in carotenoids in relation to chlorophyll a is an adaptive feature of surface cyanophyte populations, increasing protection against photooxidative stress from UV radiation (Paerl, 1984). These outcomes would indicate the importance of photoprotective mechanisms at the epilimnetic layers of clear North Patagonian Andean lakes. In particular, Patagonia is an area temporally influenced by the Antarctic polar vortex, and thus affected by low-ozone air masses (Orce & Helbling, 1997; Pérez et al., 1998). This situation and the optical and physical lake features would indicate a significant importance of UVR and PAR on the vertical distribution of phytoplanktonic cells in these freshwater systems (Villafañe et al., 2004).

On the other hand, the presence of dim-illuminated metalimnia would allow algal cells to remain at approximately the same depth for long periods, and in consequence, under stable light regime. The thermocline is an important physical barrier, thus the stability of vertical temperature and density gradients inhibits the transfer of heat, nutrients, algal cells and oxygen (Sharples et al., 2001). The illuminated metalimnetic layers may provide to the algae a refuge for the epilimnetic hazardous UVR and PAR intensities. Under these conditions, it is possible that algal cells have experienced chromatic acclimation or changes in phy-toplankton community structure (Harimoto et al., 1999; Majchrowski & Ostrowska, 2000; Steeman Nielsen, 1975). Development of deep chlorophyll maxima in

oceans and lakes, as in Andean lakes, has been associated with the existence of stable dim-illuminated metalimnetic layers (Kirk, 1994; Sharples et al., 2001). The $d^{IV}a_{ph}$** of in vivo phytoplankton absorption spectra showed that photosynthetic accessory pigments increased at the DCM levels, as was previously observed in marine regions (Bricaud & Stramski, 1990; Hoepffner & Sathyendranath, 1992; Majchrowski & Ostrowska, 2000; McManus & Dawson, 1994). In the studied Andean lakes, most $d^{IV}a_{ph}$ maxima were located at 520–525, 560–565 and 650 nm, and were assigned to fucoxanthin, phycoerythrin and chlorophyll b, respec-tively. The relative raise of Chl b was associated to the metalimnetic increase in the abundance of the mixo-trophic ciliate *Ophrydium naumanni* with endosymbiotic *Chlorella* sp. This green endosymbiotic alga has been previously reported as an important member of DCM in deep Andean Lakes (Modenutti & Balseiro, 2002; Modenutti, Balseiro et al., 2000; Modenutti et al., 2004; Pérez et al., 2002; Queimaliños et al., 1999). In addition, a relative increase with depth of fucoxanthin was associated with the presence of the dinoflagellate *Gymnodinium paradoxum* at DCM levels. This species was observed to be an important member of phyto-planktonic community in deep Andean lakes (Mode-nutti et al., 2004; Queimaliños, Pérez, & Modenutti, 2002). The presence of fucoxanthin was reported for some species of the genus *Gymnodinium* (Millie et al., 1995, 1997) although is generally associated with the presence of diatoms. Indeed, we were able to identify this pigment only in our diatom culture. Nevertheless, in Andean lakes diatoms are not dominant at the DCM levels (Queimaliños et al., 1999). Finally, the relative increase in phycoerythrin at deep layers is associated with higher abundances of phycoerythrin-rich *Synecho-coccus* (Callieri, Modenutti, Queimaliños, Bertoni, & Balseiro, in press; Modenutti & Balseiro, 2002).

The observed pattern in specific absorption spectra at 440 nm, 670 nm and in the ratio a_{ph}* (440 nm) to a_{ph}* (670 nm) in Andean lakes, was associated with the package effect concept (Morel & Bricaud, 1981). This pattern has been previously described in the Sargasso Sea and in the northeastern Pacific Ocean (Bricaud & Stramski, 1990; Mitchell & Kiefer, 1988) and in laboratory experiments with cultures (Fujiki & Taguchi, 2002). This effect predicts that the specific absorption spectrum is flattened at deep layers as was observed in Andean lakes. In these lakes this difference is caused by changes in phytoplanktonic community structure, with increasing abundances of *O. naumanni* at the DCM level. The higher package effect observed at these deep layers would imply an increase in cellular absorption efficiency (Fujiki & Taguchi, 2002). Furthermore, package effect from accessory pigments could decreased the $d^{IV}a_{ph}$ maxima assigned to different pigments, being the relative increase of these pigments at the DCM levels
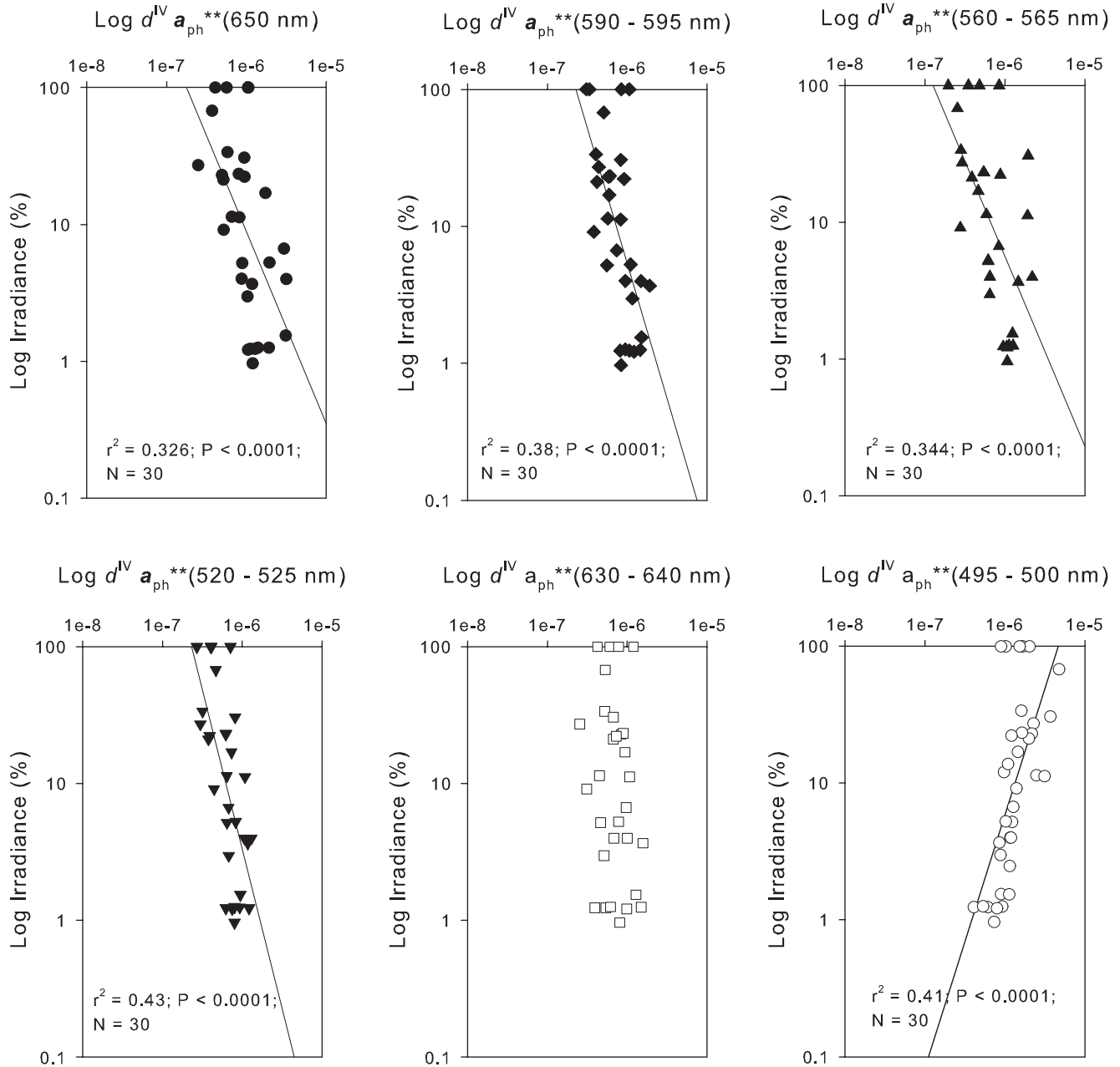
Fig. 6. Relationship between maxima fourth-derivative spectra normalized by chlorophyll a [$d^{IV}a_{ph}{**}(\lambda)$] at (650, 590–595, 560–565, 520–525, 630–640 and 495–500 nm) and downward PAR irradiance in the euphotic zone of lakes with Deep Chlorophyll Maxima.

higher than the relationships found between $d^{IV}a_{ph}{**}$ and downward irradiance.

Underwater light climate in Andean lakes is characterized by blue-green light at the metalimnetic levels. The possession of specialized photosynthetic accessory pigments that absorb in these light spectra would allow a more efficient harvesting of available light at deep levels of the water column. The presence of photosynthetic accessory pigments such as phycoerythrin, Chl b and fucoxanthin at the metalimnion of Andean lakes,

suggest that algal species presented at DCM levels are well adapted to these blue-green dim-illuminated layers.

The influence of light quality on the determination of the different algal pigments seemed to be important (Callieri, Amicucci, Bertoni, & Vöros, 1996). In general, in lakes with low light attenuation coefficients and blue light, a prevalence of phycoerythrin over phycocyanin was observed (Callieri, 1996; Kirk, 1994). In the studied Andean lakes, no phycocyanin was identified in the absorption spectra.
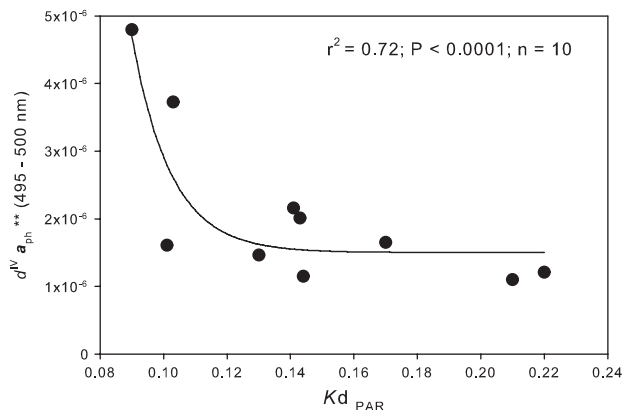
Fig. 7. Relationship between maxima fourth-derivative spectra normalized by chlorophyll a at 495–500 nm [$d^{IV}a_{ph}^{**}$(495–500 nm)] and PAR diffuse downward attenuation coefficient (Kd_{PAR}).

According to our results, in these North Patagonian Andean lakes, like in marine systems, the underwater light climate can determine the distribution pattern of the accessory pigments along the water column.

Acknowledgements

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