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Presence, abundance and bacterivory of the mixotrophic algae *Pseudopedinella* (Dictyochophyceae) in freshwater environments

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ABSTRACT: The genus *Pseudopedinella* has been described as mixotrophic; however, ecological information about this algal stramenopile (Heterokonta) is unclear. We investigate the environmental conditions that determine the presence, abundance and bacterivory rates of this genus in freshwater systems. To this end we analyzed 54 water bodies with different limnological features distributed along a latitudinal and trophic gradient in northern Patagonia (Argentina) and the Antarctic Peninsula. In addition, 14 grazing experiments were carried out in order to estimate ingestion rates and impact on the bacterioplankton. Our results indicate that this genus is exclusively found in oligotrophic environments, and that it develops well in a wide range of temperatures. Average cell-specific grazing rate was 2.83 bacteria cell^{-1} h^{-1}, with a maximum value of 6.74 bacteria ml^{-1} h^{-1}. Interestingly, grazing increased with prey abundance and decreased with increasing nutrient availability. These patterns are common in highly bacterivorous protists that use phagotrophy as a main source of nutrient acquisition. Despite their usually low abundance (avg. 182 cells ml^{-1}), this single genus was responsible for up to 24 % (avg. 10 %) of the total grazing impact exerted by all phagotrophs in these lakes. Overall, our results support the idea that *Pseudopedinella* is a highly bacterivorous group of freshwater protists, with the ability to develop well in oligotrophic conditions and with a potentially significant impact on bacterioplankton.

KEY WORDS: *Pseudopedinella* · Bacterivory · Grazing rate · Patagonian lakes · Antarctic lakes

INTRODUCTION

Among planktonic algae the phenomenon of mixotrophic nutrition, that is the combination of phagotrophy and phototrophy, has been known for about a century (Biecheler 1936). Nevertheless, the relevance of these algae as bacterivores in pelagic trophic webs was only recognized a few decades ago (Bird & Kalff 1986, Nygaard & Tobiesen 1993). Nowadays, mixotrophy is considered a key life strategy among marine and freshwater organisms (Gasol et al. 2008, Flynn et al. 2013). Mixotrophic flagellates (MF) are major grazers of bacteria in oligotrophic systems, often being responsible for >50 % of the total bacterivory (Bird & Kalff 1986, Unrein et al. 2007, Hartmann et al. 2012).

Mixotrophy is widespread among phytoplankton (Tranvik et al. 1989, Nygaard & Tobiesen 1993, Stoecker et al. 1997) and it has been demonstrated in many algal groups, such as chrysophytes, haptophytes, dinoflagellates, cryptophytes, prasinophytes and raphidophytes (Bird & Kalff 1986, Stoecker 1999,

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Callieri et al. 2006, Unrein et al. 2007, 2014, Carvalho & Granéli 2010, Jeong et al. 2010, Maruyama & Kim 2013, McKie-Krisberg et al. 2015). Several studies have attempted to classify mixotrophs into categories based on the relative importance of their phagotrophic and phototrophic activities (Jones 1994, 2000, Stoecker 1998). While some discrepancies exist, most studies agree that each taxon differs regarding the relative magnitude of both modes of nutrition, and consequently in their potential impact on *Bacteria* and *Archaea* (from now on bacterioplankton for simplicity). However, there are relatively few investigations carried out on natural communities that comparatively analyze *in situ* bacterivory rates and grazing impact among various MF. The available data suggest that the chrysophytes are one of the most 'heterotrophic' algae, with high relative contribution of phagotrophy to their growth (Sanders et al. 1990, Olrik 1998, Rottberger et al. 2013) and high impact on the bacterial community in oligotrophic lakes (Bird & Kalff 1986, Schmidtke et al. 2006, Unrein et al. 2010). Nevertheless, some differences could be expected among genera or species belonging to the same class. In this sense, comparative analyses evaluating the physiological range of two strains of mixotrophic chrysophytes (*Dinobryon divergens* and *Poterioochromonas malhamensis*) have demonstrated marked differences in the contribution of phagotrophy to their nutritional requirements (Rottberger et al. 2013).

Heterotrophic nutrition in the stramenopile (Heterokonta) *Pseudopedinella* has been long recognized (Porter 1988, Havskum & Riemann 1996, Sekiguchi et al. 2003, Stauffer et al. 2008). However, to the best of our knowledge, its impact on the bacterioplankton has been quantified only twice (Nygaard & Tobiesen 1993, Havskum & Riemann 1996). The taxonomic status of this genus has changed during the last few decades. Within the group Stramenopiles, *Pseudopedinella* was first considered a member of the class Chrysophyceae, while since the 1980s it has been included within the class Dictyochophyceae (Hibberd 1986, Kristiansen & Sandgren 1986, Kristiansen 1990). However, several ecological studies still include this nanoplanktonic genus in the Chrysophyceae (see references in Table S1 in the Supplement at www.int-res.com/articles/suppl/a076p219_supp.pdf). Its usually low abundance in natural samples and the misplacement within the class Chrysophyceae in many studies might explain the lack of information about this genus with respect to its environmental requirements, distribution and impact on bacterioplankton.

As a general trend, the phytoplankton community of Antarctic and most Patagonian lakes is dominated by nanoplanktonic flagellated algae (Izaguirre et al. 1998, 2003, Queimaliños & Diaz 2014). In particular, *Pseudopedinella* sp. were detected in some oligotrophic Antarctic lakes using molecular fingerprinting and microscopic techniques (Unrein et al. 2005). In the present investigation, we studied 54 water bodies located along a latitudinal gradient from North Patagonia to the Antarctic Peninsula covering a wide variety of lake types. The aim of this study was to analyze in detail the cellular morphology of this genus, and identify the main environmental variables that constrain the presence, abundance, grazing rates and impact of *Pseudopedinella* on the bacterioplankton. We hypothesized that *Pseudopedinella* might exhibit high *in situ* bacterivory rates comparable with other heterotrophic organisms, and might significantly impact on bacterioplankton communities. As mentioned above, different MF might differ in the use of phagotrophy for growth and consequently in their impact on bacterioplankton. Increasing our knowledge about the ecological requirements and grazing rates of each MF taxa will help to better understand their role on microbial trophic webs. In the present manuscript, we contribute to the characterization of *Pseudopedinella* sp. as a potentially significant bacterivore.

MATERIALS AND METHODS

Study site

A total of 54 lakes were sampled along a north-south transect of 2800 km, from 41° 03′ S in North Patagonia (Argentina) to 63° 23′ S in the Antarctic Peninsula (Fig. 1). Patagonian lakes are categorized as belonging to 2 different geographical regions according to their geological origin: 'Patagonian Plateau Region' and 'Andean Patagonian Region' (Iriondo 1989, Quirós & Drago 1999). Lakes located in the first area are generally shallow and mesotrophic to eutrophic, whereas Andean Patagonian lakes are of glacial origin and consequently deeper than the Plateau lakes. Due to the rocky basin formation of the Andean lakes, they often present oligotrophic and ultraoligotrophic status. Mean annual temperature in Patagonia ranges from 12°C in the northeast to 3°C towards the south. From the Andes and eastward, total annual precipitation decreases exponentially from ca. 2000 to <200 mm yr^{-1} in the central portion of Patagonia (Paruelo et al. 1998). The studied Antarctic lakes are located in Hope Bay at the Northern end of the Antarctic Peninsula. Most of them are shallow and of glacial origin. Extensive information

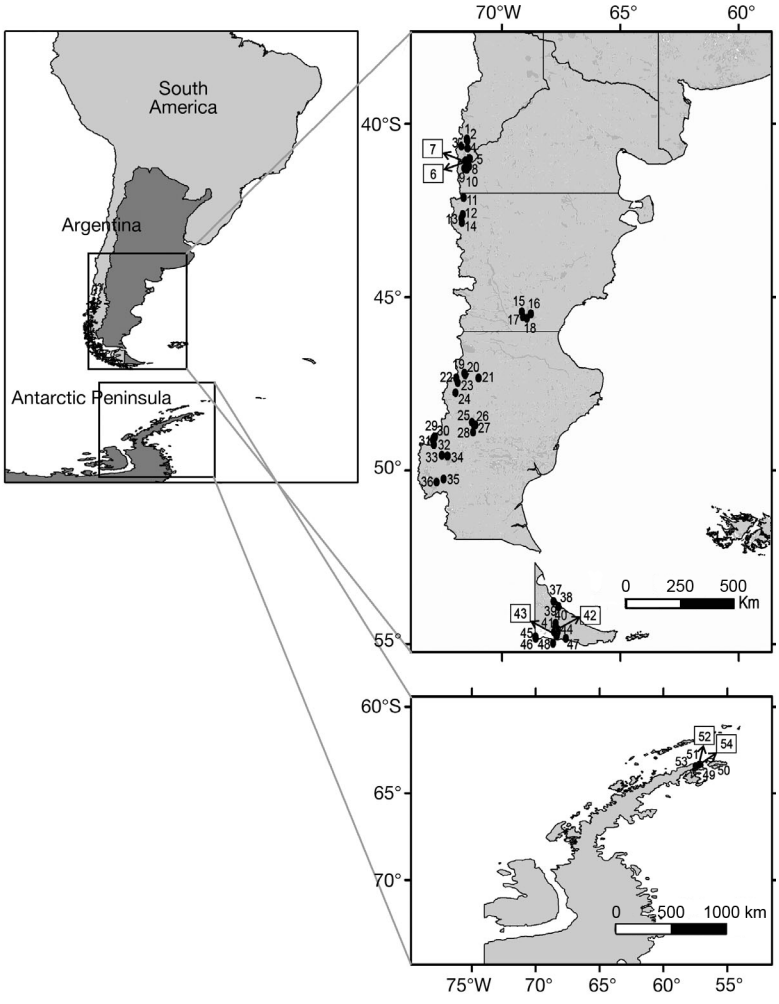

Fig. 1. Location of 54 water bodies along a north-south transect in Patagonia (Argentina) and the Antarctic Peninsula that were studied to determine the presence, abundance and bacterivory rates of *Pseudopedinella* spp. See Table S2 in the Supplement for detailed information on each lake. The 6 lakes selected for grazing experiments (Nos. 6, 7, 42, 43, 52 and 54) are indicated with a square and arrows

about all sampled lakes, including the main abiotic variables, is available in several studies (Izaguirre et al. 1998, 2003, Unrein et al. 2005, Callieri et al. 2007, Schiaffino et al. 2011, Gerea 2013, Saad et al. 2013).

Data were collected during five different campaigns: (1) 12 lakes from North Patagonia were sampled once between December 2001 and February 2002; (2) 2 shallow lakes from North Patagonia were sampled monthly between November 2009 and January 2011 (except in July and December 2010), at 4 different depths (0, 3, 6 and 8 m) on each date; (3) 22 water bodies from South Patagonia (continental) were sampled once in November 2007; (4) 11 water bodies from South Patagonia (Tierra del Fuego Island) were sampled once in November 2008, and 3 of these lakes and 1 additional lake were sampled again in January 2010. Finally, (5) the 6 Antarctic water bodies were sampled twice between January and February 2004. Detailed information about each water body is presented in Table S2 in the Supplement at www.int-res.com/articles/suppl/a076p219_supp.pdf.

Sampling data

In the different campaigns three general types of water bodies were sampled: deep lakes (DL), shallow lakes (SL) and temporary ponds (P). For deep lakes, integrated samples were collected within the epilimnetic layers from the surface down to 5 m, whereas samples of shallow lakes and temporary ponds were obtained from about 30 cm below the surface. For each water body we determined *in situ* conductivity, pH, dissolved oxygen (DO) and water temperature using standard instruments (Saad et al. 2013). Samples collected in each water body were transferred to acid-washed and pre-rinsed plastic bottles, and were transported to the laboratory in darkness. The concentration of nutrients, i.e. ammonium-N (NH_4), nitrate-N (NO_3), nitrite-N(NO_2^-) and soluble reactive phosphorus (SRP), phytoplanktonic chlorophyll *a* (chl *a*) and dissolved organic carbon (DOC) were analyzed following the methods already described in Saad et al. (2013). Dissolved inorganic nitrogen (DIN) was defined as the sum of nitrate, nitrite and ammonium.

Samples for *Pseudopedinella* identification and quantification were fixed with ice-cold filtered glutaraldehyde 10% (final concentration 1%). Between 15 and 40 ml of the fixed sampled were filtered through a 0.8 µm polycarbonate black filter (Millipore) stained with 5 µg ml^{-1} (final concentration) of 4′,6-diamidino-2-phenylindole (DAPI) according to Porter & Feig (1980). The filters were mounted over microscope slides using immersion oil for fluorescence microscopy and were stored at −20°C. The samples were inspected under 1000× magnification using an epifluorescence microscope (Olympus BX50), equipped with an HBO 50 lamp and a filter set for blue light, green light and UV excitation. Abundances of *Pseudopedinella* as well as of other autotrophic, MF and heterotrophic (HF) nanoplanktonic

organisms were determined. For further explanation of the classification of autotrophic and mixotrophic phytoplankton taxa see the description of 'Grazing experiments' below. We used these results to compare the relative abundance of *Pseudopedinella* in relation to different variables.

Three different analyses and a series of grazing experiments were performed on different samples as described below; further are provided in Table S2 in the Supplement.

Analyses of samples

Morphology of *Pseudopedinella*

To analyze the morphology and size of *Pseudopedinella* cells, we randomly selected 14 out of the 30 water bodies where this algae was present. About 40 pictures were obtained from the DAPI-stained sample. The images were analyzed with the software Image Pro Plus 4.5. Between 20 and 49 individuals were measured per lake (avg. 30). The cellular volume (μm^3) was estimated for each individual by applying the geometric models proposed by Sun & Liu (2003). An average value of the cellular volume was obtained for each lake. The algal biomass was estimated using the formula presented by Menden-Deuer & Lessard (2000), where algal biomass (pg C $cell^{-1}$) = 0.216 × (algal volume [μm^3 $cell^{-1}$])$^{0.939}$.

Presence and abundance of *Pseudopedinella*

To determine the variables that constrain the presence and abundance of *Pseudopedinella*, we used all the data, in order to cover as many different limnological conditions as possible. For this purpose, we considered the 54 sampled water bodies. For the analysis, we considered 43 aquatic systems which were sampled once, and 11 which were sampled twice. Thus, the analysis included a total of 65 (43+22) samples. Among the water bodies sampled twice, we included the 2 spring data sets (November 2009 and November 2010) of the 2 shallow North Patagonian lakes which were sampled during an annual period (Campaign 2; Table S2 in the Supplement).

Vertical and temporal variation of *Pseudopedinella*

With the aim of analyzing variations in the abundance of *Pseudopedinella* with time and depth the complete dataset of the longest campaign (Campaign 2) was considered. The 2 sampled water bodies, Morenito and Escondido North, are neighboring oligotrophic shallow lakes from North Patagonia. Both share roughly similar abiotic features but present different DOC concentrations and underwater light conditions (M. Gerea et al. unpubl. data).

Grazing experiments

Six lakes in which *Pseudopedinella* was present were selected to analyze its grazing activity and impact on the bacterial community: SL Morenito, SL Escondido North (Nahuel Huapi National Park), DL Escondido South (Tierra del Fuego National Park), SL Victoria, SL Esperanza, and SL Encantado. A total of 14 grazing experiments were performed using fluorescently labeled bacteria (FLB) as a tracer. FLB were prepared from a culture of *Brevundimonas diminuta* (syn. *Pseudomonas diminuta*) following the technique described in Unrein et al. (2007). *B. diminuta* has previously been used to prepare FLB because of their relatively small size (average size: 0.33 µm wide, 0.76 µm length, 0.065 μm^3 volume) (Unrein et al. 2007, Izaguirre et al. 2012), which is comparable to that of the indigenous bacteria in the studied lakes (0.26 to 0.33 µm wide and 0.48 to 0.56 µm length, 0.031 to 0.056 μm^3 volume). For each experiment we collected 5 l of lake water in an acid washed plastic bottle thoroughly rinsed with distilled water. Water samples were gently filtered through a zooplankton mesh to reduce the presence of large predators (e.g. copepods, cladocerans) in the sample. Experiments were run in triplicate. In those performed in Antarctica and in South Patagonia, bottles were incubated *in situ* 30 cm beneath the water surface. Due to the logistical impossibility of incubating the bottles *in situ*, the experiments performed in North Patagonia were placed in an incubation chamber at a fixed light intensity of 100 µmol photons m^{-2} s^{-1} and at the *in situ* photoperiod and temperature conditions. The mean irradiance in the water column over the year averaged 151 µmol photons m^{-2} s^{-1} (range: 35 to 253 µmol photons m^{-2} s^{-1}) in SL Escondido North and 192 µmol photons m^{-2} s^{-1} (range: 47 to 394 µmol photons m^{-2} s^{-1}) in SL Morenito (Gerea 2013). The concentration of tracer (FLB) used corresponded to about 20% of the natural abundance of the heterotrophic bacteria. Experimental samples were taken at three different times. The first extraction (T0) was immediately after the addition of the tracer, while the frequency of the following extractions (T1 and T2) varied in each set

of experiments depending on the environment condition (mainly water temperature). Preliminary experiments in each environment revealed that ingestion rates were linear for the first 2 h in Antarctic lakes and for the first 45 min in Patagonian waters. Therefore, the frequency of subsampling in Antarctica was 1 h, while in Patagonian experiments it was between 15 and 20 min. From each replicate bottle, 100 ml were fixed with the same volume of pre-filtered cold-glutaraldehyde at 4% of concentration, to avoid the egestion of the ingested prey (Sanders et al. 1989).

Depending on the experiment, between 30 and 80 ml of each fixed experimental samples T0, T1 and T2 were filtered through a 0.8-µm pore size polycarbonate black filter (Millipore). In addition, a smaller volume (about 20 ml) of T0 fixed samples was filtered over 0.2 µm filters. All samples were stained with DAPI following the technique described above. Filters mounted on slides were stored at −20°C until processing. Samples were inspected at 1000× magnification under an epifluorescence microscope. Filters of 0.2 µm pore size were used to estimate bacteria and FLB initial abundance. Protist abundances (autotrophic algae, MF and HF) and ingested FLB (presence of FLB inside digestive vacuoles of MF and HF) were quantified on the 0.8-µm pore size filters. HFs were identified by their morphology, by the presence of a nucleus under UV excitation, and by the absence of red fluorescence (chlorophyll) under blue light excitation. Autotrophic algae, *Pseudopedinella* spp. and other MF were identified by their morphology and by the red color of chlorophyll autofluorescence under blue light excitation. Tracer particles (FLBs, appearing green) within each individual were also quantified at the same time. Between 50 and 80 cells of each group of bacterivores (i.e. *Pseudopedinella*, other MF and HF) were inspected in each sample to determine the ingestion rate. We classified the phytoplankton taxa either as autotrophic or mixotrophic depending on their phagotrophic capability, according to published data based on grazing experiments (Tranvik et al. 1989, Jones 1994, Queimaliños 2002) and to the results of our experiments with FLBs. Within 'other MF', we identified different genera of chrysophytes (e.g. *Dinobryon* and *Ochromonas*), cryptophytes, dinoflagellates and haptophytes; however, for the purpose of the present work they were all clustered into a single group. The identification of these taxa by epifluorescence is not always straightforward, in particular for smaller organisms. Therefore, in those cases where the identification of MF was not clear, and the specimen did not contain FLBs, the flagellate was considered to be an autotroph. This decision probably led to an underestimation of the abundance of 'other MF'; nevertheless, the calculation of impact of total mixotrophs is not affected by this bias.

For each group of bacterivores, ingestion rates (FLB cell^{-1} h^{-1}) were calculated over the regression slope of the average number of prey ingested per individual and time. Ingestion rate was considered significant when the 95% confidence intervals of initial and final time did not overlap (Rice 1988 cited in Havskum & Riemann 1996). Clearance rates (CR; nl cell^{-1} h^{-1}) were calculated by dividing the ingestion rate by the FLB concentration. Cell-specific grazing rates (CSGR; bacteria cell^{-1} h^{-1}) were estimated by multiplying CR by the bacterial concentration, assuming that native bacteria and FLB were grazed upon at the same rates. The grazing impact (GI; bacteria ml^{-1} d^{-1}) by each group of bacterivores was estimated by multiplying their CSGR by their abundance (cells ml^{-1}). Total GI was estimated as the sum of the GI by all bacterivores in the sample. Bacterial turnover rates (% d^{-1}) were estimated by expressing the GI (bacteria ml^{-1} d^{-1}) as a percentage of the corresponding bacterial abundance (bacteria ml^{-1}). The cell-specific ingestion rate, as percentage of the cell carbon per day (% d^{-1}), was calculated for *Pseudopedinella* by dividing its CSGRs expressed in biomass of bacteria (mg C bacteria cell^{-1} d^{-1}) by the mean flagellate biomass (mg C cell^{-1}). We assumed a bacterial carbon content of 0.02 pg C cell^{-1} (Lee & Fuhrman 1987).

Statistical analyses

The Kruskal-Wallis (K-W) test was used to analyze variations of *Pseudopedinella* cellular volume among lakes. The relationship between *Pseudopedinella* abundance and biological and physical environmental variables was assessed by means of Pearson correlations; *Pseudopedinella* abundance was compared among deep lakes, shallow lakes and temporary ponds using K-W analysis. Regression analyses were employed to assess the relationship between the total and relative abundance of *Pseudopedinella* and the various physico-chemical variables. One-way ANOVA was applied to compare variations in the vertical distribution of *Pseudopedinella* in each of the two lakes sampled during an annual period. Regression analyses were applied to describe the relation between CSGR and biotic and abiotic variables. A multiple regression model was generated to

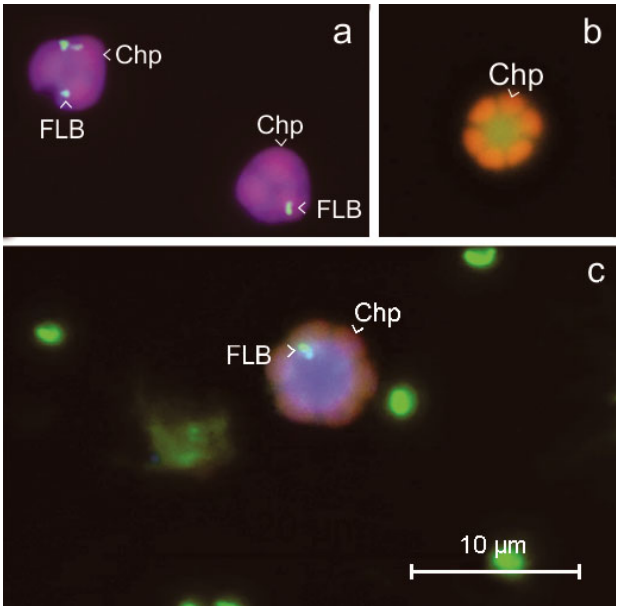

Fig. 2. *Pseudopedinella* spp. with (a) 3 chloroplasts (Chp) and (b,c) with 6 chloroplasts. Panels (a) and (c) show individuals with one or more fluorescently labeled bacteria (FLB) inside digestive vacuoles. Each of the images in panels (a) and (c) is an overlay of 2 pictures, one taken under UV excitation (to see the DNA stained with DAPI) and the other under blue light (to see the chlorophyll in red, and the FLB stained with DTAF in green); the image in panel (b) was taken under blue light excitation

identify the most relevant variables explaining CSGR. Variables were log-transformed when the normality assumption was not met.

RESULTS

Morphology

Cells were always found solitary and free-living, and were observed from the anterior view. *Pseudopedinella* was unambiguously determined based on the characteristic radial symmetry and the absence of anterior tentacles, even though the stalk was never observed (Fig. 2). Cells with 3 (Fig. 2a) and 6 (Fig. 2b,c) peripheral chloroplasts could also be observed, probably indicating that more than 1 species was present in the studied lakes. Considering that the present work was focused on the genus, we did not discriminate among different species of *Pseudopedinella*, and hereinafter this group will be referred to as *Pseudopedinella* spp. In our samples, cell diameter ranged from 3.4 to 7.0 µm, and cellular volume from 31 to 183 µm^3. The K-W analysis indicated significant variations of the cellular volume of *Pseudopedinella* spp. among the studied lakes ($p < 0.001$; Fig. 3).

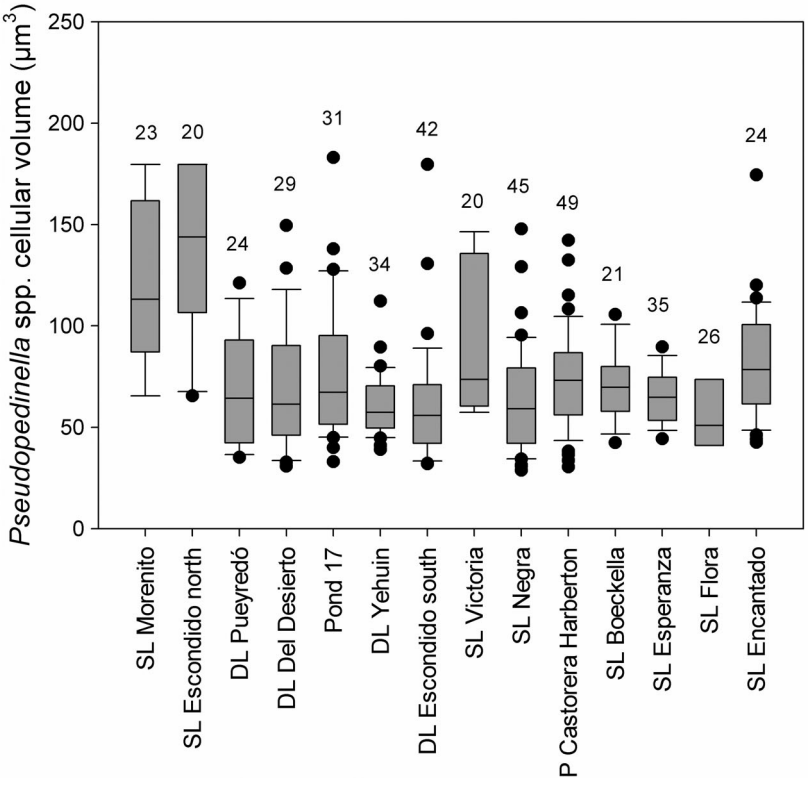

Fig. 3. Cellular volume of *Pseudopedinella* spp. in a subset of the studied lakes, ordered according to increasing latitude from left to right. Numbers above the boxplots correspond to the number of cells measured for each lake. Boxes indicate 25th and 75th percentiles; lines indicate median; whiskers indicate 10th and 90th percentiles; circles indicate outliers. P: pond; SL: shallow lake; DL: deep lake

Presence and distribution

Pseudopedinella spp. were found in 30 out of 54 lakes regardless of latitude. Their abundances ranged between 10 and 461 cell ml^{-1}, although an exceptionally high abundance (1409 cell ml^{-1}) was observed in 1 Antarctic lake (SL Encantado). The abundance of *Pseudopedinella* spp. did not differ between shallow and deep lakes, though it was significantly higher than in tem-

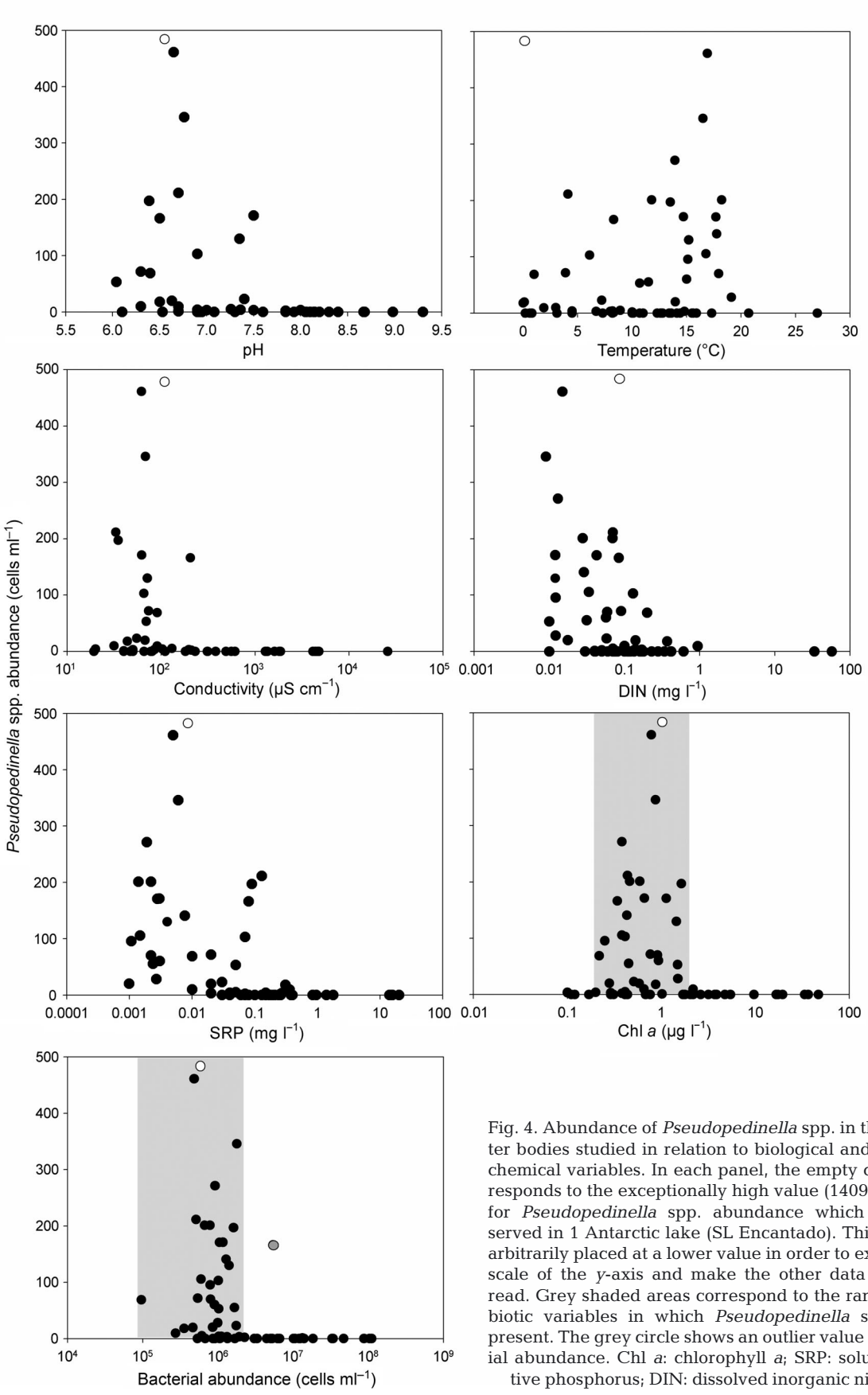

Fig. 4. Abundance of *Pseudopedinella* spp. in the 54 water bodies studied in relation to biological and physicochemical variables. In each panel, the empty circle corresponds to the exceptionally high value (1409 cell ml^{-1}) for *Pseudopedinella* spp. abundance which was observed in 1 Antarctic lake (SL Encantado). This circle is arbitrarily placed at a lower value in order to expand the scale of the *y*-axis and make the other data easier to read. Grey shaded areas correspond to the range of the biotic variables in which *Pseudopedinella* spp. were present. The grey circle shows an outlier value of bacterial abundance. Chl *a*: chlorophyll *a*; SRP: soluble reactive phosphorus; DIN: dissolved inorganic nitrogen

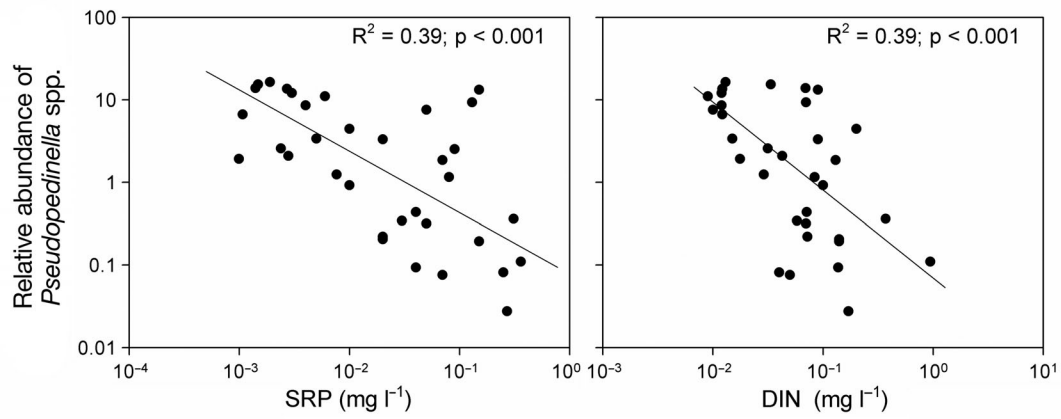

Fig. 5. Relative abundance of *Pseudopedinella* spp. among total phagotrophic nanoflagellates (mixotrophic and heterotrophic flagellates) in relation to soluble reactive phosphorus (SRP) and dissolved inorganic nitrogen (DIN) availability in the studied lakes

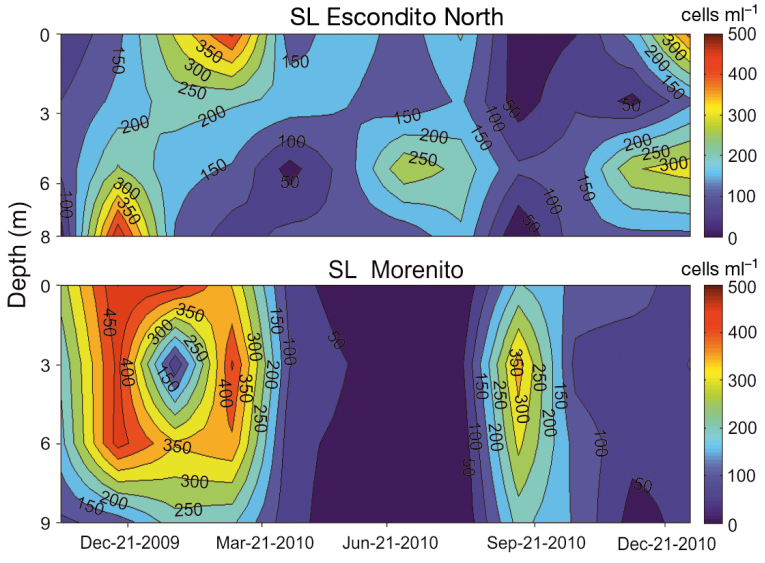

Fig. 6. Vertical and temporal distribution of *Pseudopedinella* spp. abundance in the shallow lakes (SL) Escondido North (No. 7 in Fig. 1) and Morenito (No. 6), North Patagonia

porary ponds (K-W test: H = 15.711, p < 0.001, Dunn's posterior test: p < 0.05). Abundance was negatively correlated with pH (N = 54, ρ = −0.438, p = 0.028). The highest abundances were observed at slightly acid water (pH values ~6.5), while this alga was absent at pH values >7.5 (Fig. 4). *Pseudopedinella* spp. were found over a wide range of temperature from 0 to 20°C, and at relatively low conductivity (usually <90 µS cm^{-1}, and never >210 µS cm^{-1}; Fig. 4). Regression analyses showed that *Pseudopedinella* spp. abundance was negatively related to DIN concentration (R^2 = 0.12, p = 0.039) and SRP concentration (R^2 = 0.22, p = 0.004). *Pseudopedinella* spp. were absent in lakes with concentrations of chl *a* >2.2 µg l^{-1} and bacterial abundances >1.7 × 10^6 cell ml^{-1}, except for 1 lake where bacterial abundance reached 5.4 × 10^6 cells ml^{-1} (Fig. 4). The abundance of *Pseudopedinella* spp. was negatively correlated with bacterial abundance (N = 54, ρ = −0.29, p = 0.019). Overall, these results showed that this genus was exclusively found in oligotrophic environments.

In those lakes in which *Pseudopedinella* spp. was present, it represented between 1 and 23% (avg. 6.7%) of the nanophytoplankton (2 to 20 µm in size), taking into account all autotrophic and mixotrophic species. Considering the total phagotrophic nanoflagellates (MF + HF; Table S3 in the Supplement at www.int-res.com/articles/suppl/a076p219_supp.pdf), *Pseudopedinella* spp. represented on average 4.7% of total abundance, ranging from 1.4 to 16.4%. Their relative abundance tended to decrease when dissolved nutrients (SRP and DIN) increased (N = 34; SRP: R^2 = 0.39, p < 0.001; DIN: R^2 = 0.39, p < 0.001) (Fig. 5).

Vertical and temporal distribution of abundance

Pseudopedinella spp. were present during all the studied year in SL Escondido North and SL Morenito. The latter has lower DOC concentration and higher transparency than SL Escondido North. No clear vertical variation in *Pseudopedinella* spp. abundance was found in either of the lakes, as a consequence of the permanent mixing of the water column of these polymictic lakes (1-way ANOVA, SL Escondido North: F = 0.831, p = 0.484; SL Morenito: F = 0.381,

p = 0.767) (Fig. 6). The temporal variation of cell abundance showed 2 peaks recorded in summer and spring in both lakes (Fig. 6). Temporal variation in *Pseudopedinella* spp. abundance was positively related with temperature (N = 24, ρ = 0.446; p = 0.029), with the maximum abundance at temperature values between 16 and 20°C, and was negatively affected by DIN concentrations (N = 24, ρ = −0.447; p = 0.028). In this regard, minimum values of *Pseudopedinella* spp. abundance were observed at the maximum values of DIN.

Bacterivory rates

Fourteen grazing experiments were performed in 6 oligotrophic water bodies along the latitudinal transect. *Pseudopedinella* spp. ingested FLB at a significant rate in all of them (Fig. 2a,c). The CSGR was 6.74 bacteria cell^{-1} h^{-1} (Table 1), and the lowest values were recorded in Antarctic lakes, probably because of the low temperature in these environments. CSGR and temperature fitted to a hyperbolic function in which the CSGR steeply increased with water temperature, up to about 6°C (Fig. 7), while above this value it fluctuated around the average CSGR (2.83 bacteria cell^{-1} h^{-1}; Table 1). Interestingly, CSGR decreased significantly together with the increase in DIN concentration (N = 14, R^2 = 0.53, p = 0.003; Fig. 7), and increased with bacterial abundance (N = 14, R^2 = 0.61, p = 0.001; Fig. 7). Stepwise multiple regressions found bacterial abundance to be the only significant variable, and it explained the 61% (p < 0.001) of the variability in CSGR.

The CR was variable among lakes and averaged 3.28 nl cell^{-1} h^{-1} (Table 1). This parameter was negatively related to bacterial abun-

Table 1. Grazing parameters of *Pseudopedinella* spp. measured in each lake. Numbers following the name of the lake in the left hand column correspond to those shown in Fig. 1. SL: shallow lake; DL: deep lake; CSGR: cell-specific grazing rate; % grazing impact: percentage of grazing impact of *Pseudopedinella* spp. with respect to total grazing; bact.: bacteria

Region	Water body (number)	Sampling date	*Pseudopedinella* spp. abundance (cells ml^{-1})	CSGR (bact. cell^{-1} h^{-1})	Clearance rate (nl cell^{-1} h^{-1})	Grazing impact (bact. ml^{-1} d^{-1})	Bacterial turnover (% bact. d^{-1})	Cell-specific grazing rate in % cell C content (% d^{-1})	% grazing impact
North Patagonia	SL Morenito (6)	May 2010	14	2.99	2.86	4.4×10^2	0.04	6.9	1
		Aug 2010	151	4.88	5.76	1.9×10^3	2.30	11.1	22
		Oct 2010	22	1.41	1.86	6.7×10^2	0.09	3.3	2
		Jan 2011	60	4.13	3.95	6.1×10^3	0.58	9.6	13
	SL Escondido North (7)	May 2010	80	6.74	4.33	1.6×10^4	1.01	15.6	12
		Aug 2010	89	2.64	6.19	6.0×10^3	1.37	6.1	24
		Oct 2010	80	1.90	2.57	3.6×10^3	0.47	4.4	11
		Jan 2011	95	4.25	3.89	8.5×10^3	0.78	9.9	10
South Patagonia (Tierra del Fuego Island)	DL Escondido South (42)	Jan 2010	53	4.32	4.22	5.5×10^3	0.5	2.0	2
	SL Victoria (43)	Jan 2010	197	3.24	2.02	1.5×10^4	0.9	2.0	6
Antarctic Peninsula (Hope Bay)	SL Esperanza (52)	Jan 2004	69	0.26	2.67	4.2×10^2	0.44	1.1	-
		Feb 2004	211	1.50	2.93	7.6×10^3	1.48	6.6	-
	SL Encantado (54)	Feb 2004	20	0.41	0.89	1.9×10^2	0.04	1.2	-
		Mar 2004	1409	0.96	1.84	3.3×10^3	6.21	3.5	-
		Average	182	2.83	3.28	8.7×10^3	1.16	6.0	10
		SD	358	1.88	1.53	9.2×10^3	1.58	4.3	8
		Minimum	14	0.26	0.89	1.9×10^2	0.04	1.1	1
		Maximum	1409	6.74	6.19	3.3×10^4	6.21	15.6	24

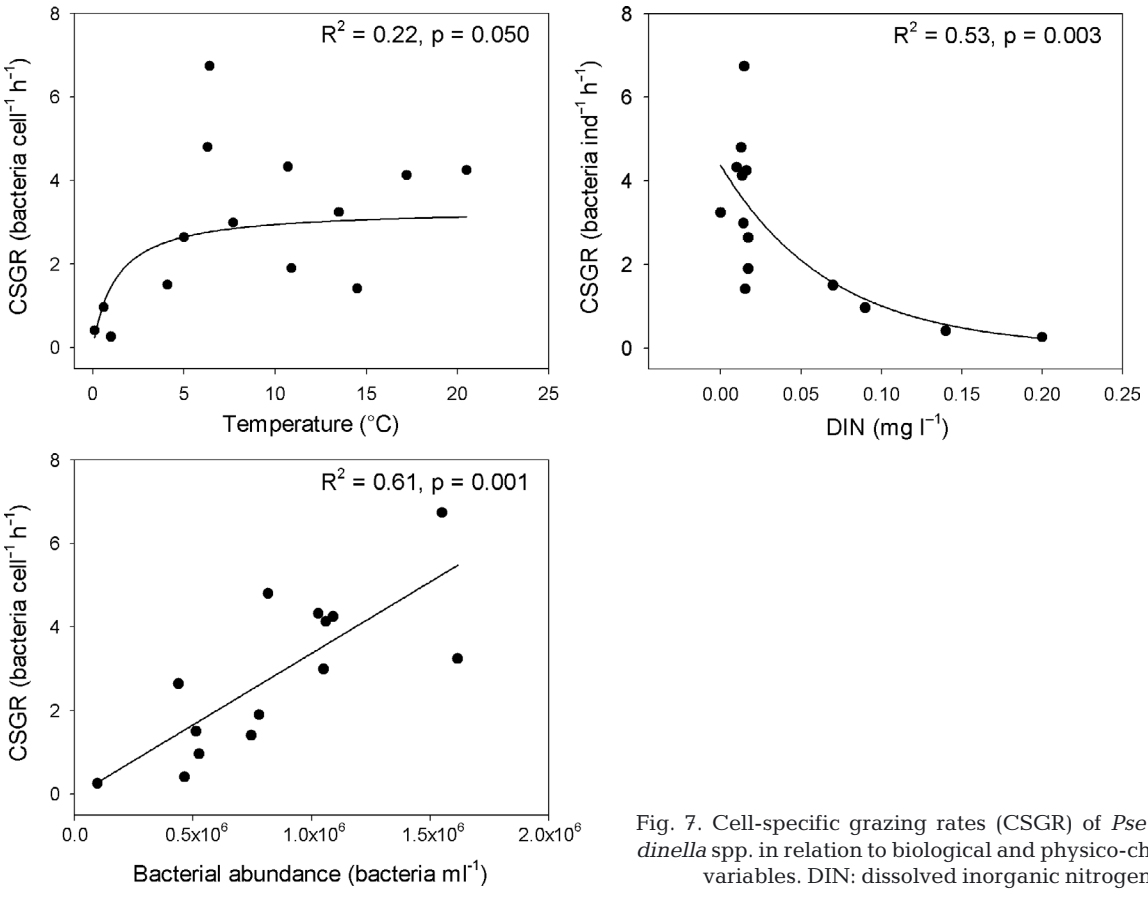

Fig. 7. Cell-specific grazing rates (CSGR) of *Pseudopedinella* spp. in relation to biological and physico-chemical variables. DIN: dissolved inorganic nitrogen

dance (N = 14, ρ = −0.475, p = 0.047). The CSGR expressed as percentage of the cellular carbon per day varied between 1.1 and 15.6% d^{-1} (Table 1).

This single genus removed up to 6.2% of the bacterial standing stock per day during periods of relatively high abundance (Table 1). *Pseudopedinella* spp. grazing caused an average impact on the bacterial community of 8.7×10^3 bacteria ml^{-1} d^{-1}. Total GI by phagotrophic flagellates (MF and HF) was estimated in 10 out of 14 experiments. Even though *Pseudopedinella* spp. never exceeded 20% of the abundance of total bacterivores (avg. 4.7%), in our experiments up to 24% of the total GI was due to this alga (Table 1).

DISCUSSION

The morphological analysis of *Pseudopedinella* along a broad spectrum of physico-chemical conditions showed a marked variability in cell size (i.e. cell diameter ranged between 3.4 and 7 µm). Such variability could be explained by the presence of more than 1 species in our samples. Supporting this hypothesis, individuals with 3 and 6 chloroplasts were observed. Seven species of *Pseudopedinella* are mentioned in the literature (Sekiguchi et al. 2003; Table S1 in the Supplement). However, based on Guiry & Guiry (2015), there are 3 confirmed species: *P. pyriformis* ('*pyriforme*', as the Holotype of the genus), *P. elastica* and *P. thomsenii*, while the confirmation of a fourth species of this genus (*P. erkensis*) is considered to require further investigation. The individuals with 3 chloroplasts correspond to *P. thomsenii* (Sekiguchi et al. 2003), which is the only species of this genus which possesses 3 chloroplasts (Thomsen 1988). The individuals with 6 chloroplasts could not be determined to a finer taxonomic detail, which requires observation of ultrastructural features. *P. elastica* and *P. erkensis* were previously mentioned in freshwater environments (Table S1 in the Supplement), so the specimens with 6 chloroplasts present in our samples could correspond to either of these species. Besides, phenotypic plasticity can also explain the observed variation in cell size, as can factors such as light (O'Farrell et al. 2007), nutrient availability (Li et al. 2000), temperature (Morabito et al. 2007) and grazing (De Hoyos et al. 1998), which

have been proven to influence the morphology of other algal groups.

The presence of *Pseudopedinella* spp. seems not to be constrained by water temperature, as we found specimens in the coldest Antarctic shallow lakes (close to 0°C) and the North Patagonian lakes where temperature reached up to 20°C in summer. In accordance with our results, *P. pyriformis* has been also found in a wide range of temperatures (between 2 and 19°C) in marine environments (Ostroff et al. 1980). Nevertheless, in our study, higher abundances were recorded in spring and summer in the annual survey (avg. 14.3°C, range 7 to 20°C), and between 15 and 20°C when all studied lakes were compared. For marine *P. pyriformis*, maximum *in situ* growth rate was observed at 15°C (Ostroff et al. 1980). Phagotrophic behaviour agreed with this pattern: CSGR increased with temperature between 0 and 6°C, and had little variation at higher temperatures.

In our samples, *Pseudopedinella* spp. were restricted to oligotrophic environments, with low chl *a* and nutrient concentrations, and low bacterial abundances. Moreover, the relative abundance of *Pseudopedinella* spp. was negatively related to nutrient concentration, suggesting also a preference for nutrient-poor waters. Even though this genus was sporadically found growing well in experiments with nutrient enrichments (Lagus et al. 2004), most records of *Pseudopedinella* in marine and freshwater environments correspond to nutrient-poor waters (Rosén 1981, Hearing 1984, De Hoyos et al. 1998, Olrik 1998, Hobbie et al. 2000, Unrein et al. 2005, 2014, Gerea et al. 2013). In contrast to our results, some long-term investigations carried out in the Baltic Sea found a positive relationship between the abundance of *Pseudopedinella* spp. and nitrogen concentration (Carstensen & Heiskanen 2007, Suikkanen et al. 2007, 2013). However, values of DIN measured in the Baltic Sea (usually <0.1 mg l^{-1}) fall within the range of values determined in our study, but are far less than the maximum DIN levels where *Pseudopedinella* spp. were present in our study (Fig. 4). Thus, although in some cases individual samples could be misleading, we found a great consistency between *Pseudopedinella* spp. distribution and the trophic condition of the sampled aquatic systems. We consider that the eventual sampling bias (i.e. only 1 sample per lake in most environments) is minimized because the intra-lake variability would be lower than the between-lake variability in this kind of study. If we had employed a different sampling strategy in our widespread survey (i.e. several samples in each system) we probably could have found differences in *Pseudopedinella* spp. abundances, but it can be expected that nutrient concentrations would have remained in the same range, meaning that the probability that the trophic condition of the lakes changes with depth or time is very low.

In accordance with the preference of *Pseudopedinella* spp. for nutrient-poor environments, we observed that the CSGR was negatively related with the availability of dissolved inorganic nutrients. Many experiments with cultured and field mixotrophic flagellates demonstrate that grazing rates decrease after the addition of inorganic nutrients (Nygaard & Tobiesen 1993, Rothhaupt 1996a, Zubkov & Tarran 2008, Smalley et al. 2012); this is because nutrient assimilation in particulate form is energetically more costly than absorbing nutrients in dissolved inorganic form (Raven 1997). These results predict that an inverse relationship should be expected between dissolved inorganic nutrient concentrations and the *in situ* phagotrophic activity of mixotrophs, as was observed for *Pseudopedinella* spp. in our study. This also suggests that the growth of this alga depends on the mixotrophic nutrition in environments with low nitrogen and phosphorus concentrations. This assertion, although requiring confirmation by means of experimental assessments, supports the idea that phagotrophy is a way to supplement nutrients when these are limiting (Nygaard & Tobiesen 1993, Rothhaupt 1996a, Flöder et al. 2006, Carvalho & Granéli 2010). Vähätalo et al. (2011) proposed that *Pseudopedinella* is capable of growing autotrophically but grazes on bacteria under nutrient limiting conditions. The pattern observed here for *Pseudopedinella* spp. has also been recorded in some cryptophytes (Urabe et al. 2000, Unrein et al. 2014), haptophytes (Nygaard & Tobiesen 1993, Carvalho & Granéli 2010), prasinophytes (McKie-Krisberg et al. 2015), chrysophytes (Nygaard & Tobiesen 1993, Urabe et al. 2000) and dinoflagellates (Nygaard & Tobiesen 1993, Stoecker et al. 1997). This would partially explain the success of mixotrophs in oligotrophic systems that has been observed in natural environments as well as in experimental conditions (Olrik 1998, Hartmann et al. 2012, Unrein et al. 2014).

The values of CSGR (0.3 to 6.7 bacteria $cell^{-1}$ h^{-1}) obtained in our experiments are in line with the rates estimated by Havskum & Riemann (1996) in a marine system (1 to 2 bacteria $cell^{-1}$ h^{-1}) using the same methodology that we used. Previous research using radiolabeled (RLB) instead of fluorescently labeled bacteria (FLB) reported a CSGR of 18 bacteria $cell^{-1}$ h^{-1} for one strain of *Pseudopedinella* sp. (Nygaard & Tobiesen 1993). The available values of CR (Nygaard

& Tobiesen 1993, Havskum & Riemann 1996, the present study) range between 0.9 and 6.2 nl cell^{-1} h^{-1}. These rates of bacterivory are comparable to those observed for highly bacterivorous chrysophytes, like *Dinobryon* (see review by Unrein et al. 2010) and *Ochromonas* (Sanders et al. 1990, Epstein & Shiaris 1992, Callieri et al. 2006), and for HF of similar cell size (Laybourn-Parry & Marshall 2003, Jezbera et al. 2005, Pirlot et al. 2007, Unrein et al. 2007). Another indication of the relative importance of heterotrophic nutrition in *Pseudopedinella* spp. is the relatively high cell-specific ingestion rate, measured as percentage of cell carbon per day (between 1.1 and 15.6% d^{-1}). These values are similar to those reported for HF (Pernthaler et al. 1996, Unrein et al. 2007), chrysophytes (Izaguirre et al. 2012) and haptophytes (Unrein et al. 2014), and notably higher than those observed for the less bacterivorous cryptophytes (Unrein et al. 2014).

Interestingly, the increase of CSGR with the increment of bacterial abundance observed in this study for *Pseudopedinella* spp. corresponds to a functional response typical of heterotrophic protists (Fenchel 1982, Kiørboe et al. 2004). Similar patterns were observed for some highly heterotrophic chrysophytes like *Ochromonas* sp. and *Poterioochromonas malhamensis* (Rothhaupt 1996b, Rottberger et al. 2013). Overall, these results suggest that *Pseudopedinella* is a very active bacterivore and, according to the gradient spectrum proposed by Jones (1994) and the more recent review by Flynn et al. (2013), should be considered a highly heterotrophic photosynthetic protist.

Previous investigations mentioned that *Pseudopedinella* species often occur in low abundance (Havskum & Riemann 1996, Gerea et al. 2013). Coincidently, in all the studied water bodies in which *Pseudopedinella* spp. were present, their abundance rarely exceeded 400 cells ml^{-1}, and never dominated among phytoplankton (average 6.7%). However, this single genus accounted for up to 24% of total grazing impact, a result that highlights the significant impact that *Pseudopedinella* might have in oligotrophic waters. In this sense, it is important to underline that in a recent grazing experiment we also detected a high consumption of picocyanobacteria (*Synechococcus* sp.) by this genus in 2 lakes of North Patagonia (M. Gerea et al. unpubl. data). Grazing of picocyanobacteria by Dictyochophyceae was also observed in marine environments (Frías-López et al. 2009). These results support the idea that this group is a strong picoplanktonic grazer.

In summary, *Pseudopedinella* organisms were found mainly in oligotrophic environments and appeared to use phagotrophy to additionally acquire essential nutrients when these substances are limiting. In spite of their usually low abundance, their highly bacterivorous behavior resulted in a potentially high grazing impact on the bacterioplankton community, positioning this algae as a relevant component likely channelizing a relevant share of the C fluxes in some nutrient-poor environments.

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