**Antarctic Affairs. Vol 1 (2015) 1-8**
ISSN: 2451-7755 (Print) - Journal homepage: www.antarticaffairs.org


# Species Composition and Photoacclimation of Marine Antarctic Benthic Diatom Species.

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## ABSTRACT

*Antarctic benthic diatom communities from coastal areas are exposed to drastic seasonal changes of light intensity and duration, and persist during the long polar winter at extreme low light intensities as food supply for the overwintering Antarctic fauna. However, due to the characteristics of these communities, there are few studies on the species composition and their low light photo-acclimation. In this study, the taxonomic species composition and their relative abundance of the benthic diatom community at different sites and depths at Bahía Covadonga (Bernardo O'Higgins Station) (63°19'15"S, 57°53'55"W) were assessed. The photosynthetic response of cultured species of the genus Navicula to different light intensities and their recovery capacity were determined by using a Modulated Pulse fluorometer (Water PAM) to register their Chlorophyll fluorescence after exposing the cells to different light intensities. The benthic diatoms community had a high species richness and diversity, with a characteristic and specific distribution among the different sites and depths analyzed. All species studied, from different depths showed low light photo acclimation and photoinhibition to light intensities >100 µMol Photons $cm^{-2}$ $sec^{-1}$. Nitzschia species showed a higher tolerance and recovery capacity to high light intensities than those from the genus Navicula. The results suggest that this characteristic photosynthetic response may be related to the different distribution of the abundance of species over different illumination status on the sites and depths where they were collected. Nitzschia species could cope in environments exposed to higher radiation, with higher recovery ability than those from the genus Navicula. The possible relationship of these observations with motility and other cellular properties of diatom species is discussed.*

## Keywords

Antarctic benthic diatoms, photosynthetic response, photoacclimation, photoinhibition, recovery, Navicula, Nitzschia.

## INTRODUCTION

Benthic diatoms are responsible for an important percentage (40-50%) of marine coastal primary production (Falkowski, 1997; Underwood, 1999). In particular, on Antarctic coastal areas benthic diatoms are exposed to drastic seasonal variations of radiation intensity and duration. During the long Polar winter they subsist under the marine ice layer, at extreme low light intensity (1 to 10 % incident light) (Robinson, 1995). Thus, they constitute the "winter forage" in the base of the coastal food web for a wide range of overwintering organisms such as small mollusks, crustaceans, and krill, as well as their consumers. In general, microalgae photoacclimation to high light consists of coordinated physiologic and chemical composition adjustments that equilibrate light acquisition and the use of $CO_2$ fixation and other metabolic process. This response is particularly importan t in avoiding Photosystem II damage, the reduction of microalgae light harvesting, the display of energy dissipation mechanisms, deviating excess energy and displaying a photoprotection response (Arrigo et al 2010). On the other hand, when exposed to low light, photoacclimation increases the ability of light harvesting, and the reduction of energy use on cellular maintenance processes and growth.

Photoacclimation on Antarctic oceanic phytoplankton diatoms such as Phaeocystis antarctica y Fragilariopsis cylindrus have been extensively studied (Robinson, 1995). However, many of the characteristics of coastal benthic diatoms, such as their species composition, distribution, and response to the fluctuations of light intensity and their tolerance and recovery capacity when exposed to high irradiance, have been studied less and remain misunderstood. The aim of this study is to assess the diatom species composition of the benthic community living in the coastal area of Bahia Covadonga, (Bernardo O'Higgins Antarctic Station), and to determine the characteristics of the photosynthetic response and recovery capacity of isolated and cultured species from the genus Navicula in reaction to different light intensities.

## MATERIALS AND METHODS

**Sampling site**

Sampling was performed by scuba divers at two depths in the Covadonga Bay and several other sites around the Bernardo O'Higgins Antarctic Station (63°19'15"S, 57°53'55"W) during the summer of 2009 (Figure 1A). The sites were denominated Ice Front, in contact with the high tide (IF); Intertidal (IT), and Benthos (10-20 m) as detailed on Figure 1B. Pieces of colored ice, or small stones samples that were collected in plastic 50 mL Falcon tubes were taken to the station lab. There, the cells were collected by thawing the ice samples, and by scraping the stones. Cells were concentrated through filtration with a 11 µm nylon mesh, previously filtered with a 100 µm mesh for discarding the higher size fraction of the samples. Microalgae were recovered via 50 ml culture flasks in f/2- culture medium (Guillard and Ryther 1962) and were incubated at 4° C with natural illumination. For taxonomic identification, 5 ml samples were prepared with 50% ethanol, that were later oxidized with 30% Hydrogen peroxide and washed in distilled water (Battarbee, 1986). Samples were then mounted with NAPHRAX® and observed under phase contrast microscopes (Zeiss) at 100X magnification. Previous taxonomic studies were used to identify the species and genus level. (Round et al. 1990, Witkowski et al. 2000, Al-Handal 2008). Some species were confirmed by SEM (data not shown).

**Species isolation**

Benthic diatom pecies were isolated by taking one cell with the tip of an enlarged Pasteur pipette, and after 3 washing steps with sterile culture medium, were deposited on microplate wells (Nunc™), and kept in culture at 4ºC, at 5 - 50 µMol Photons $m^{-2}$ $s^{-1}$, and a photoperiod of 14:10h D: L, (dark :Light) until growth was observed. The procedure was repeated many times on positive isolates in order to attain clonal cultures. When this was achieved, cultured species were identified, and kept in culture for experimental procedures. The isolated species used in this study were Navicula perminuta, isolated from Benthos samples (20 m), Navicula cancellata, taken from Intertidal samples, and sp1 (N. pusilla, not confirmed) and sp2 (not determined), isolated from samples taken from Ice Fronts (IF) and Intertidal (IT), respectively (Figure 2).

**Photosynthetic parameters**

To assess the photosynthetic condition of cells exposed to different light treatments, light response curves were performed using a Pulse Amplitude Modulated fluorometer (Water-PAM; Walz GmbH, Effeltrich, Germany). Thus, a 3 ml aliquot of cells from each species culture (in triplicate), were incubated for 5 hours under different light intensities: 0,5; 5; 10; 100 and 200 µMol Photons m-2 s-1, at 4ºC. Then, the cells from each treatment were individually transferred to a quartz cuvette, and minimum fluorescence (F0) was recorded. Upon application of a saturating light (>3,000 µMol photons m-2 s-1) pulse of 0.6 sec, maximum fluorescence (Fm) was recorded for each sample. With these parameters Fv / Fm, or Effective Quantum Yield ($ø_{II}$), was calculated. This is a measure of the Photosystem II efficiency, and is obtained from the equation (Fm – F0)/ Fm. From these values, the maximum rate of Electron Transport (rETRmax), the light utilization efficiency (Ek), and the saturating light coefficient (Ek), were calculated (Schreiber 2004). After 30 minutes of incubation of the samples in darkness for their photosystem recovery, the Chlorophyll fluorescence was measured again, and the recovery rate of the species was expressed as the percentage of the initial values of $ø_{II}$ at the different light intensities.

## RESULTS

**Benthic diatoms species composition**

From 30 field samples examined, up to 51 pennate diatoms taxa from Bahía Covadonga were determined. Dominant genus were Navicula, Amphora, Cocconeis, and Gomphonemopsis. The Benthic community revealed a characteristic taxonomic composition of the different sites and depths analyzed that included few dominant common species. For instance, the small size species Navicula perminuta (7 - 12 µm) was found all over the sampling sites and depths and was the most abundant species (Table 1). However, Navicula perminuta showed higher abundance in benthos, than Intertidal sites, and Ice Front samples in a proportion 3:2:1, respectively (data not shown).

Other abundant species were Cocconeis costata, Cocconeis orbicularis, Navicula directa, Gomphonemopsis obscura, Pseudogomphonema kamtschaticum, Amphora gourdonii, Gomphonemopsis obscura, Planothidium hauckianum and Pseudogomphonema kamtschaticum. Less abundant large size diatoms species such as Trachyneis aspera and Pleurosigma obscura were found in Benthos sites at 10 and 20 m. The highest values of specific richness and diversity index (H') were recorded in the Intertidal zone (IT), and the lowest was found in Ice Front samples (IF) (Table 2).

**Photosynthetic response**

As shown in Figure 3, all species analyzed showed higher efficiency of photosystem II at lower light intensities that reflects their low light photoacclimation. They were photoinhibited at higher light intensity values (200 µMol Photons $m^{-2}s^{1}$). However, at 0,5 µMol Photons $m^{-2}$ $s^{-1}$, the species of the genus Navicula showed lower values of Effective Quantum yield (øII) (0,4 - 0,5) than the species of the genus  (0,6 - 0,7), and the decay of the initial values with the increase of light intensity was more pronounced. Photoinhibition of Navicula species was observed at lower light intensity, compared to that of Nitzschia species (100 and 200 µMol Photons m-2 s-1, respectively). Consequently, Nitzschia species have shown a higher capacity to recover their photosynthetic performance after 30 minutes in darkness, compared to Navicula species. As shown in Figure 4, Nitzschia species had 70-80% of the initial Effective quantum yield (øII) and Navicula species (30-40%), at 100 µMol Photons m-2 s-1.

Values of photosynthetic efficiency (∂) and ETRmax of Navicula species were significantly inferior to those of, ($p< 0,05$). However, the saturating light coefficient Ek was similar in all of the species (Table 3).

## DISCUSSION

The benthic diatoms community in Bahia Covadonga contained abundant pennate diatoms with a wide array of species. These species showed a particular and different distribution among the distinct sites and depths studied, from Ice Fronts contacting the high tides, to Benthos (20 m depth). The identified diatoms are characteristic of the Antarctic benthos. The most abundant diatoms found through this research coincide also with the large proportion identified in microalgae communities in other Antarctic coastal areas (Cibic et al 2007, Salleh 2011). The species composition data is similar to that found in other studies in Potter Cove, King George Island (Zacher, 2007, Wulff et al 2008; Al-Handal 2010). In particular, Navicula perminuta was described as the most abundant and widely distributed in all the sampling sites studied (Zacher, 2007).

Although all the species studied were low light acclimated, showing photoinhibition at light intensities over 100 µMol Photons $m^{-2}$ $s^{-1}$, species of the genus Navícula displayed different and characteristic photosynthetic responses to increasing light intensities. Navicula perminuta and N. cancellata showed minor photosystem II efficiency (øII) than  species, this difference was more noticeable at higher light intensity values (> 50 µMol Photons m-2 s-1). The same was observed for Navicula species photosynthetic efficiency (∂), and ERTmax compared to  sp1 and  sp2 (Table 3).

Navicula species also showed an inferior dark recovery capacity after the treatments at high light, suggesting some extent of photosystem II damage. This high light tolerance difference may be related to their variation in the distribution of species abundances among the sites and depths studied. Thus, Navicula cancellata was isolated from samples collected in the Intertidal zone, and the Navicula perminuta culture used in this study was isolated from benthos sample. Species of the genus were distributed in areas more exposed to environmental radiation, such as the Ice Fronts, and were rarely found in the Intertidal and benthos samples. These results suggest that these different responses are related to the specific habitats where the species were more abundant. According to this, Nitzschia species could cope with and tolerate more illuminated environments, with a higher photosystem

II recovery ability than Navicula species. Differences in the capacity to cope with changes in the environmental light intensity on diatom species from different habitats have been extensively studied (Van Leeuwe 2005, Lavaud 2007) and the plasticity of the photosynthetic systems of diatoms from Antarctic phytoplankton and ice have been compared (Petrou 2011a, Mangoni 2009). Moreover, the specific sensitivity to changes in salinity and temperature that links the photosynthetic capacity and ecological niche have been characterized (Petrou 2011b). On the other hand, differences in the photosynthetic efficiency of the photosystem II, and their recovery capacity to UV and PAR radiation, have been observed in benthic diatoms (Wulff et al 2008).

Many other characteristics may have an effect on the photoacclimation response of benthic diatoms and are relevant to consider in further studies. Factors such as cellular motility, volume and shape may play a role in the ability of diatom species to cope with a changing environmental light. In support of this idea, a recent study on benthic diatoms from intertidal sediments (Barnett 2015) has shown, for the first time, a relationship between the photoprotective capacity and the motility of the diatom cells. Motile diatom species had a lower tolerance rank to high light than non-motile species. This is due to the fact that a photoprotective mechanism allows diatom cells to evade the effects of high light exposition.

It is important to note that in the present study, the more sensitive to high light intensity species, both Navicula cancellata and Navicula perminuta, are motile. On the contrary, the more tolerant species sp1 and N. sp2, are non-motile (data not shown). These observations of the cultures open an interesting perspective for further studies in order to better understand the capability of diatom species to cope with a changing environmental light, and to persist in Antarctic low light environments.

## CONCLUSIONS

The benthic Antarctic diatoms of Bahia Covadonga showed a distribution of relative abundance, richness and diversity, which is characteristic of the different sites analyzed: Ice front, intertidal, and benthos (10- 20m), with few abundant species found in common. The benthic Antarctic diatoms species of genus Navicula were photoacclimated to low light. However, Nitzschia species could cope with and tolerate more illuminated environments, with a higher recovery capacity than Navicula species. These results showed that the species of the genus Navicula and Nitzschia studied displayed differences in their photosynthetic response plasticity.

## AKNOWLEDGEMENTS

To Katherina Petrou and Ernesto Molina (UTS, Sydney, Australia) for their support and advice on photosynthesis response experiments and calculations. To Carolina Díaz, Claudio Rivas, Alvaro Palma, Angie Díaz and Luis Henríquez, for their collaboration on sampling, transport and identification of samples. To the 2009 crew at Antarctic Station Bernardo O'Higgins. Grant INACH- T11-08.

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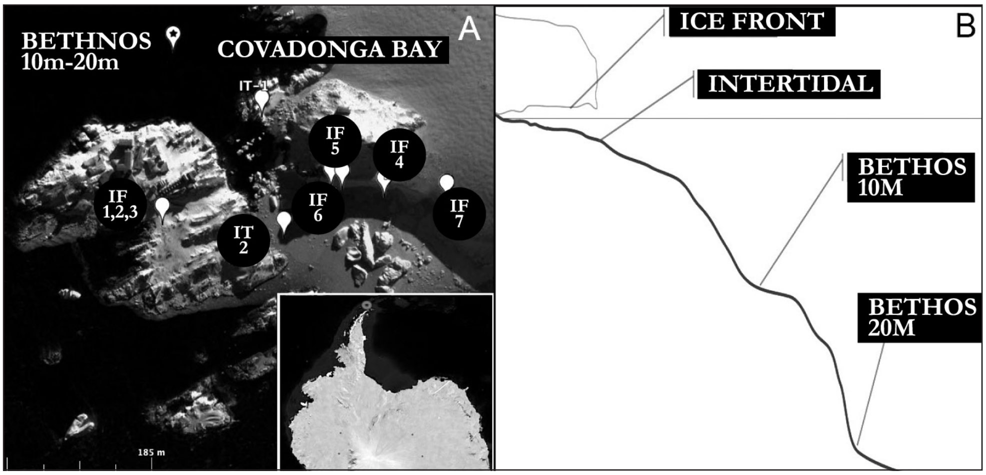
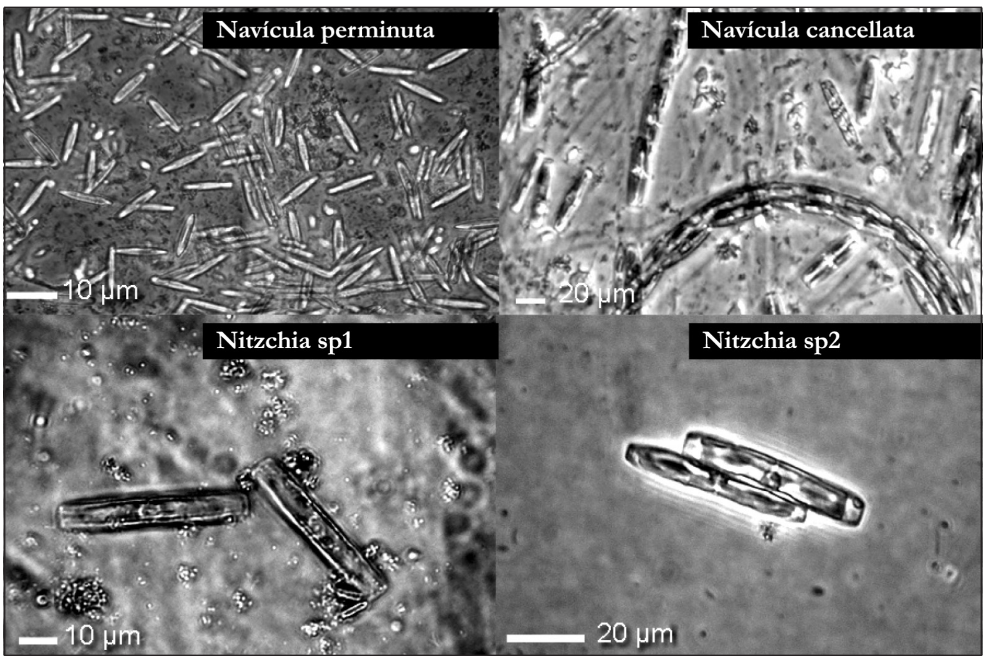
FIGURE 1



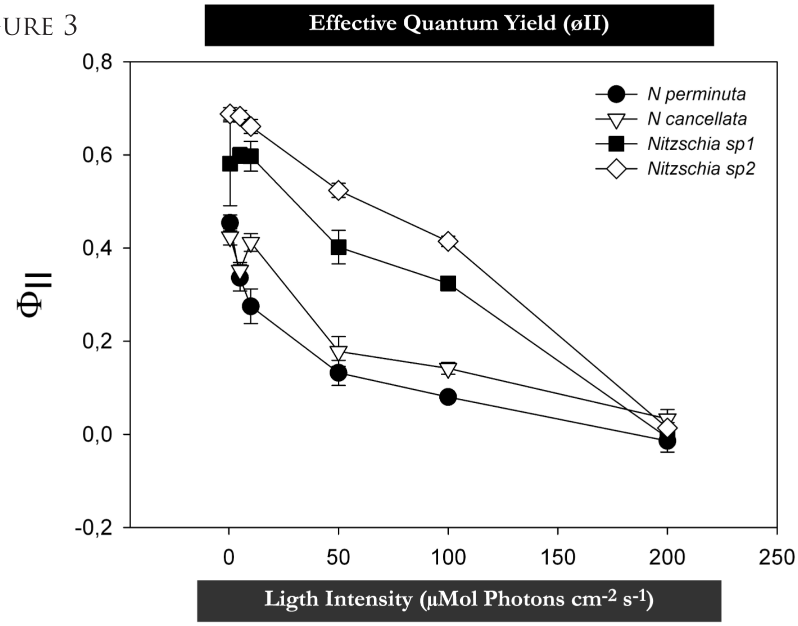

*A. Sampilng sites in Bahía Covadonga, Antarctic Station Bernardo O'Higgins IF: Ice Front; IT: Intertidal.*
*B. Detail of sampling sites and depths.*

FIGURE 2



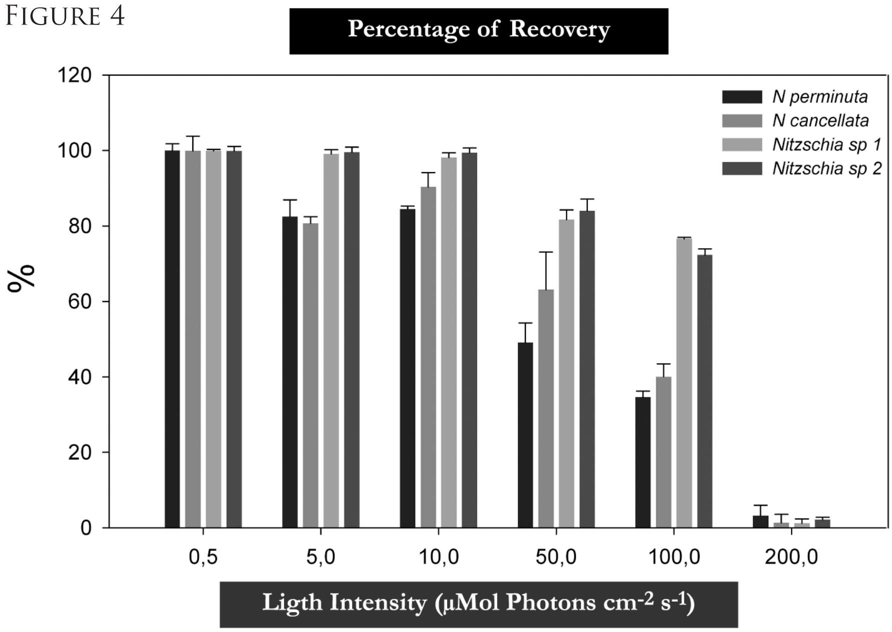

*Isolated species in culture analyzed in this study-.*

FIGURE 3




*Photosynthetic response. Effecive Quantum Yield (øII) of Navicula perminuta (black circles), Navicula cancellata (clear triangles) Nitzschia sp1 (black squares) y Nitzschia sp2 (clear diamonds) to different light intensities.*

FIGURE 4




*Dark Recovery capacity. Percentage of recovery of the photosynthetic response to different light intensity, after 30 min. of dark incubation.*

#Table 1: *Most abundant benthic diatom species identified in Bahia Covadonga.*

| ICE FRONT | INTERTIDAL | BENTHOS |
|---|---|---|
| Pinnularia krookii<br>Navicula perminuta<br>Nitzschia pusilla<br>Nitzschia spp.<br>Navicula glaciei<br>Odontella litigiosa<br>Synedra kerguelensis<br>Cocconeis orbicularis<br>Pseudogomphonema kamtschaticum | Navicula perminuta<br>Navicula gracilis<br>Gomphonemopsis obscura<br>Planothidium hauckianum<br>Navicula directa | Navicula perminuta<br>Cocconeis imperatrix<br>Cocconeis costata<br>Opephora olsenii<br>Amphora gourdonii<br>Navicula directa<br>Navicula cancellata |

#TABLE 2: *Richness and diversity index of benthic diatoms at Bahia Covadonga.*

| | RICHNESS | DIVERSITY (H') |
|---|---|---|
| ICE FRONT | 9 | 1,7 |
| INTERTIDAL | 13 | 2,4 |
| BENTHOS | 10,1 | 1,3 |

#TABLE 3: *Photosynthetic parameters of species from genus Navicula and Nitzschia.*

| SPECIES | Photosynthetic Parameters | | |
|---|---|---|---|
| | ETRM | ALPHA | EK |
| NAVICULA PERMINUTA | 7,9 | 0,3 | 26,3 |
| NAVICULA CANCELLATA | 12,5 | 0,4 | 34,9 |
| NITZSCHIA SP 1 | 53,3 | 0,5 | 104,7 |
| NITZSCHIA SP 2 | 34,3 | 1,3 | 27,0 |

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