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An evaluation of the application of CHEMTAX to Antarctic coastal pigment data

Wendy A. Kozlowski [a,*], Douglas Deutschman [b], Irene Garibotti [c], Charles Trees [d], Maria Vernet [a]

[a] Integrative Oceanography Division, Scripps Institution of Oceanography, Mail Code 0218, La Jolla, CA 92093-0218, USA
[b] Department of Biology, San Diego State University, 5500 Campanile Drive, San Diego, CA 92182, USA
[c] Instituto Argentino de Nivología, Glaciología y Ciencias Ambientales, C.C. 330, 5500 Mendoza, Argentina
[d] NATO Undersea Research Center, La Spezia, Italy

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ABSTRACT

Presented is an evaluation of the application of CHEMTAX (CHEMical TAXonomy) to Antarctic coastal pigments collected along the western Antarctic Peninsula (wAP). Overall analytical error is $<20\%$ for all pigments involved in the analysis. CHEMTAX was stable within a range of input pigment ratios; data were analyzed in three bins based on light depths, with each year's data run independently. Results were validated by comparison to those from CHEMTAX methods that included randomized error, feedback loops and additional diagnostic pigments. Blooms during mid-summer (chlorophyll *a* concentrations $>5\ \mu g\ L^{-1}$) were dominated primarily by either diatoms or cryptomonads. Mixed flagellates can also be abundant and *Pheaocystis* spp. and prasinophytes are frequently present in low concentrations. Comparison with microscopy shows CHEMTAX to give superior results in identifying *Pheaocystis* spp. with favorable results for other groups. This analysis shows CHEMTAX to be a reliable and stable tool for providing estimations of the main phytoplankton taxa in wAP waters based on long-term data collected during a 12-year time series.

1. Introduction

Common methods for estimation of phytoplankton abundance and composition include microscopy (e.g., Gomi et al., 2007), flow cytometry (e.g., Smith et al., 2007; Sosik and Olson, 2007), genetic analysis (e.g., Medlin et al., 2006; Countway and Caron, 2006) and several versions of methods that can be categorized as chemotaxonomic, i.e., groupings based on the presence of chemical markers. The latter category includes the use of single or multiple pigment markers and multiple linear regression analysis to determine contribution by various groups to the total chlorophyll *a* (chl_*a*) pool (Gieskes et al., 1988; Peeken, 1997), the application of inverse methods to develop a least-squares solution to a matrix algorithm (Tarantola, 1987; Letelier et al., 1993), and an iterative method of matrix factorization for determination of algal class abundance (Mackey et al., 1996).

Composition and distribution of phytoplankton communities can be documented using high performance liquid chromatography (HPLC) analysis of photosynthetic pigments (Jeffrey, 1980). Chemotaxonomic methods, based on the presence of characteristic pigments, mostly carotenoids, in algal phyla, are an extension of this approach (Gieskes and Kraay, 1986). The CHEMTAX (CHEMical TAXonomy) method has been widely used to determine phytoplankton composition, including in the Southern Ocean (e.g., Wright and van den Enden, 2000). Along with benefits such as having the ability to be reprocessed if needed, CHEMTAX has provided a means of estimating phytoplankton composition in datasets that might not have otherwise been analyzed for more traditional means of phytoplankton enumeration. CHEMTAX results have correlated strongly with those from microscopy, and in some instances have revealed the presence of groups not detected with traditional enumeration methods (e.g., cryptophytes, Wright et al., 1996; Havskum et al., 2004). Primary concerns regarding use of chemotaxonomic methods center around non-unique pigment markers (Schlüter and Møhlenberg, 2003; Zapata et al., 2004) and potential fluctuations of the pigment ratios both at a species and at a cellular level under various physiological stressors (Jeffrey, 1981; Goericke and Montoya, 1998; DiTullio et al., 2007). However, with appropriate precautions, awareness of impacts and implications of physiological stressors, and some knowledge of potential populations within a sample region (Irigoien et al., 2004), CHEMTAX is considered a viable method for determination of phytoplankton composition (Mackey et al., 1998).

* Corresponding author. Tel./fax: +1 607 533 0107.
E-mail addresses: wkoz@ucsd.edu (W.A. Kozlowski), ddeutschman@sciences.sdsu.edu (D. Deutschman), ireneg@lab.cricyt.edu.ar (I. Garibotti), trees@nurc.nato.int (C. Trees), mvernet@ucsd.edu (M. Vernet).

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Although many studies have examined variability of Antarctic phytoplankton structure and dynamics, our understanding of the large-scale geographic and long-term temporal variability remains weak. Previous studies examining phytoplankton composition and distribution have primarily focused on short term, limited scale sampling. The Palmer Long Term Ecological Research (Pal LTER) project has as its focus the marine ecosystem of the western Antarctic Peninsula (wAP). Pal LTER has been collecting data in this region since 1991, providing a temporally and spatially extensive dataset for better understanding of the ecology of the area. HPLC analysis was chosen at the project's onset as a combined approach to taxonomy and optical studies related to phytoplankton under the premise that pigment analysis provides high consistency between users and laboratories with low variability and high reproducibility (Hooker et al., 2005), requires less time and labor than microscopy, and is a lower cost alternative to genetic studies and flow cytometry. However, the long-term nature of the Pal LTER data brings challenges not usually present in analysis of single cruises. Variability in methods and instruments as well as the large size of the dataset make the development of a consistent method for determination of phytoplankton composition particularly imperative.

The specific goal of this study was to establish and apply to the Pal LTER HPLC dataset a robust means of determining phytoplankton composition. In this paper, we report the results of phytoplankton community composition in the wAP from an analysis of 12 years of pigment data using CHEMTAX. Methods used for CHEMTAX processing CHEMTAX are reviewed, evaluated and validated. Results for three years are compared to microscopic classification and possible impacts of variation in light regimes on CHEMTAX output are presented. Examples of the resolution in phytoplankton group distribution as estimated by CHEMTAX are included.

2. Methods

2.1. Study area and sampling regime

Data was collected as part of the Pal LTER project along the wAP, from 63.6°S to 68.2°S and from 64°W to 73°W, bordering the eastern boundary of the Bellingshausen Sea to the South (Fig. 1). The region is characterized by a glacially sculpted coastline containing a series of islands, bays and passages (Anderson, 1999), and is often divided into three sub-regions, coastal, shelf and slope, based on bathymetry and associated biological and physical dynamics (Martinson et al., 2008).

Pal LTER large-scale sampling stations are laid out on a grid system (Waters and Smith, 1992), with transects (''grid lines'') 100 km apart running roughly south-east (onshore) to north-west (offshore) across the Antarctic continental shelf, approximately perpendicular to the coast. ''Grid stations'' are spaced 20 km apart along those lines (Fig. 1b). Sampling used in this analysis was limited to five cardinal lines, 200–600, which cover the region from Southern end of Anvers Island (600 line) to Marguerite Bay (200 line), and included all cardinal grid stations as well as other off-grid stations (e.g., near Palmer Station on Anvers Island, stations north and inland of Renaud Island and stations in the vicinity of southern Adelaide Island in the mouth of Marguerite Bay) occupied during yearly sampling efforts.

Water collection was done as part of the Pal LTER annual austral summer cruises from 1995 to 2007. Stations were occupied in most years between the first week in January and the first week of February (Table 1). Sampling was carried out from the R/V *Polar Duke* in 1995–1997 and from the ASRV *Laurence M. Gould* from 1998 to 2007. Not all stations were sampled on all cruises due to ice, weather or scheduling issues. HPLC data from January 2002 is not available.

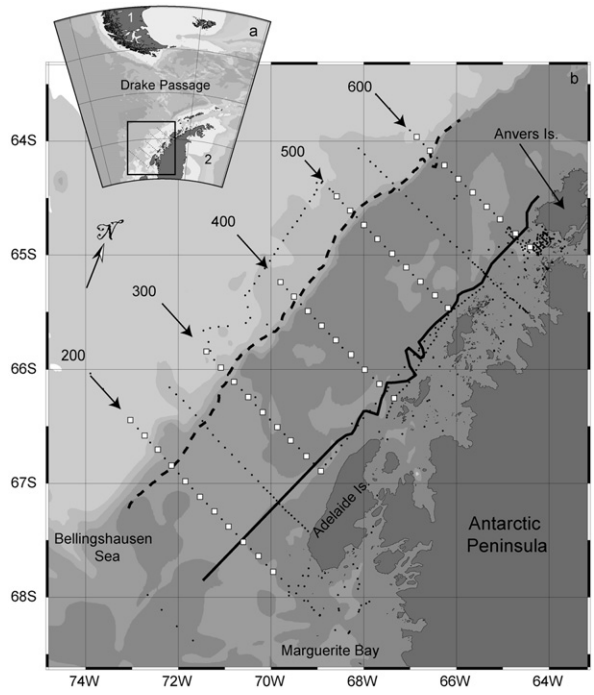

Fig. 1. Map of sampling region. Inset map (a) includes the southern tip of South America (1) and the Weddell Sea (2), and the black box marks the region blown up in main map (b). Cardinal lines 200–400 are marked and cardinal stations (sampled 8–12 years) are indicated by white boxes; stations visited fewer than seven years indicated by black circles. Solid and dotted black lines demark outer limits of shelf and slope regions, respectively, and the white box encloses the stations included in this analysis. Note inset map (a) is South Polar Orthographic projection while main map (b) is Mercator projection. Arrow indicates north for main map only.

Table 1
Palmer LTER cruise sampling summary. Palmer LTER cruise timing, duration and sampling stations included in this study, and total number of pigment samples used as input data to CHEMTAX for composition determination. Sampling at each station was extremely consistent, with a total of 4087 samples included in this analysis.

Year	Cruise dates	Cruise duration (days)	Stations (*n*)	Total samples (*n*)
1995	07 January–06 February	31	73	425
1996	08 January–10 February	34	65	384
1997	11 January–12 February	33	88	503
1998	28 January–13 February	17	30	155
1999	08 January–12 February	36	57	335
2000	09 January–26 January	18	43	231
2001	09 January–26 January	18	43	255
2003	05 January–02 February	29	64	382
2004	07 January–01 February	26	60	351
2005	04 January–01 February	29	55	332
2006	07 January–02 February	27	64	381
2007	07 January–04 February	29	59	353

2.2. Sampling and processing methods

At all stations sampled, water was collected in 10 L Go-Flo (1995–1997) or 12 L Niskin (1998–2007) bottles, at the surface and at depths corresponding to 50 ($\pm$6), 25 ($\pm$3), 10 ($\pm$2), 5 ($\pm$1) and 1 ($\pm$1) percent of surface photosynthetically available radiation (PAR, 400–700 nm). Light depths were determined immediately prior to the water collection using a Biospherical