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Estimating phytoplankton size-fractionated primary production in the northwestern Iberian upwelling: Is mixotrophy relevant in pigmented nanoplankton?

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ABSTRACT

Primary production of micro- (PP_{micro}), nano- (PP_{nano}) and picophytoplankton (PP_{pico}) in the NW Iberian margin were estimated by combining biomarker pigments to derive class-specific chlorophyll concentration and published class-specific photophysiological variables for large oceanic scales (Uitz et al., 2008). The accuracy of this approach was assessed comparing the predicted total primary production ($PP_p = PP_{micro} + PP_{nano} + PP_{pico}$) with the measured total primary production (PP_m). Despite the general agreement, PP_p overestimated PP_m when mixing in the water column was important. Therefore, the photophysiological variables originally derived from stratified and oligotrophic zones with strong influence of photoacclimation in the water column were re-evaluated to incorporate the particular conditions usually found in coastal upwelling systems, characterized by higher homogenization of the water column and lesser importance of photoacclimation. With this new fractionation we estimated the export capacity (*f*-ratio = new production) of the microbial plankton community, which can be assimilated to the fraction of primary production due to microphytoplankton. The NW Iberian margin showed *f*-ratios varying between the highest values recorded for coastal upwelling systems ($f > 0.75$) and the low values usually found in oligotrophic oceanic areas ($f < 0.1$). This size-fractionated primary production combined with phytoplankton size-fractionated biomass to obtain turnover rates allowed us to infer the existence of mixotrophy within nanophytoplankton. The occurrence of this type of nutrition was indirectly verified by comparing carbon fixation with estimates of gross primary production based on the metabolic theory of ecology. Realistic values of the photosynthetic quotient ($PQ = 1.78 \pm 0.17$; mol O_2 mol C^{-1}) were only obtained when heterotrophic nutrition of nanophytoplankton was considered.

Introduction

Matter and energy flows in oceanic pelagic food webs are strongly dependent on the phytoplankton size-classes prevailing at a given place and time. With dominance of picophytoplankton the carbon fixed by photosynthesis is mainly channeled through the microbial loop and so recycled within the microbial plankton realm (Pomeroy, 1974; Azam et al., 1982). The contrasting situation occurs under diatom dominance, when a significant fraction of the carbon fixed by phytoplankton is available to fuel higher trophic levels or be exported out of the pelagic environment (Goldman, 1988; Cushing, 1989).

Coastal upwelling systems have been traditionally viewed as zones where diatoms dominate in the phytoplankton community (Chavez et al., 1991; Tilstone et al., 2000). However, research conducted in the NW Iberian margin (Crespo et al., 2011; Espinoza-González et al., 2012) and in other coastal upwelling areas (Iriarte and González, 2004; Böttjer and Morales, 2007) clearly showed that small phytoplankton (<20 μm, pico- and nanophytoplankton) is present in these regions as a permanent background where diatoms thrive in response to upwelling events. This condition has led to first hypothesize and later demonstrate that the microbial food web in coastal upwelling zones is basically multivorous (Legendre and Rassoulzadegan, 1995; Vargas et al., 2007; Teixeira et al., 2011). The microbial loop (based on pico- and nanoplankton) would occur as a permanent feature to which the diatom food web is added during upwelling episodes (Barber and Hiscock, 2006; Teixeira et al., 2011; Espinoza-González et al., 2012). Consequently, carbon fixation should take place in the three phytoplankton size-classes (pico- nano- and microphytoplankton), with

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picophytoplankton and nanophytoplankton fixing carbon continuously and microphytoplankton doing it sporadically in response to upwelling events.

Although phytoplankton photosynthesis constitutes the main supply of matter and energy to the marine microbial food web, studies specifically planned to determine the relative importance of primary production in each of the three phytoplankton size fractions in coastal upwelling systems are scarce and limited to specific moments (Joint et al., 2001; Teira et al., 2001; Iriarte and González, 2004). Studies covering a complete seasonal cycle are still fewer (Bode et al., 1994; Wilkerson et al., 2000) and they do not consider the three phytoplankton fractions. In this paper we attempt to fill this gap in our knowledge by estimating the size-fractionated primary production at the continental shelf of the NW Iberian margin throughout a seasonal cycle.

The approach used here to estimate size-fractionated primary production is based on the photophysiological parameterization firstly proposed by Claustre et al. (2005) and later improved by Uitz et al. (2008). This parameterization needs the chlorophyll concentration in the three phytoplankton fractions, which were derived from the phytoplankton pigment composition (Rodríguez et al., 2006) using seven accessory pigments as taxonomic biomarkers to estimate the fraction of total chlorophyll concentration ascribed to each phytoplankton size-class (Vidussi et al., 2001; Uitz et al., 2006). This chlorophyll fractionation was later combined with the class-specific photophysiological properties provided by Uitz et al. (2008) to estimate size-fractionated and total primary production. The accuracy of this procedure was validated by comparing the resulting total primary production to the total primary production estimated from photophysiological variables actually measured in the region.

Materials and methods

Sampling

A station located on the NW Iberian shelf (150 m depth) in front of the Ría de Vigo (42°07.8′N, 9°10.2′W) was visited weekly from 15 May 2001 to 24 April 2002 on board R/V 'Mytilus' (Fig. 1). Sampling took place with a conductivity–temperature–depth (CTD) probe (SBE 9/11) fitted with a fluorometer and attached to a rosette equipped with 12 PVC Niskin bottles. Seawater samples to determine nitrate and chlorophyll *a* (chl *a*) concentrations were collected from the CTD upcasts at 7–8 depths in the water column from surface to bottom. Samples to characterize the size-structure and photophysiology of the phytoplankton community were also taken at several depths within the photic layer. These sampling depths were selected after inspecting the fluorescence profiles to ensure that the subsurface chlorophyll maximum was sampled when present. The spectral light field at sea surface and in the water column was also determined following the approach detailed by Lorenzo et al. (2004), which allows estimating the transmittance at the air–sea interface and the scalar spectral irradiance at each hour and depth in the water column.

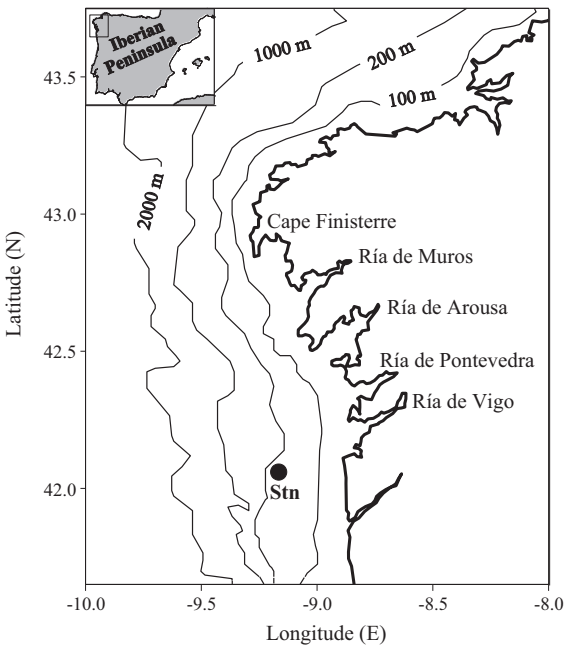

Fig. 1. The NW Iberian margin showing the position of the sampled station. The location of the 4 Rías Baixas (Vigo, Pontevedra, Arousa and Muros) is also shown.

Nitrate and chlorophyll

Nitrate concentrations (μmol kg^{-1}) were determined by segmented flow analysis according to Hansen and Grasshoff (1983). For chl *a*, seawater volumes of 100–250 ml were filtered through 25 mm Whatman GF/F filters using low vacuum. The filters were then frozen at −20 °C before pigments were extracted in 90% acetone over 24 h in the dark at 4 °C. Chl *a* concentration (mg m^{-3}) was determined by fluorometry in a Turner Designs fluorometer calibrated with pure chl *a* (Sigma Chemical).

Size-fractionated phytoplankton biomass

Samples to determine phytoplankton biomass and community size-structure were collected from 4 to 5 depths within the photic layer, which varied between 27 and 88 m (Fig. 3D). Epifluorescence microscopy was used to identify pico- (<2 μm) and nanophytoplankton (2–20 μm) in samples of 10 ml. These samples were fixed with buffered 0.2 μm filtered formaldehyde and then filtered through 0.2 μm black Millipore-Isopore filters placed on top of 0.45 μm Millipore backing filters. Excitation with blue light was used to enumerate autotrophic organisms that were identified by yellow (for the case of *Synechococcus*-type cyanobacteria) and red autofluorescence. This technique does not provide a correct identification of *Prochlorococcus*. However, *Prochlorococcus* is only present in the region at very low abundance during short time periods in autumn, when seasonal upwelling–downwelling transition occurs and oceanic waters are advected over the shelf (Rodríguez et al., 2006). This means that *Prochlorococcus* does not constitute an important part of the total phytoplankton biomass in this upwelling region. Dimensions were measured and cell volumes were calculated assuming a spherical shape or by approximation to the nearest geometrical shape (Hillebrand et al., 1999). Cell carbon was estimated according to Bratbak and Dundas (1984) for *Synechococcus*, Verity et al. (1992) for pico- and nanoflagellates and Strathmann, (1967) for small (<20 μm) naked dinoflagellates.

Microphytoplankton (>20 μm) was determined in samples of 100 ml preserved in Lugol's iodine. The samples were sedimented in composite sedimentation chambers and observed with an inverted microscope. The small species were enumerated from two transects scanned at 400× and 200×, while the whole slide was scanned at 100× to count the larger species. Phototrophic species of dinoflagellates, flagellates and ciliates were discriminated following Lessard and Swift (1986) and also using our historical records of epifluorescence microscopy of fresh samples. All organisms with chloroplasts were assumed to be phototrophic. Biovolumes were estimated following Hillebrand et al. (1999) and cell carbon was calculated according to Strathmann (1967) for diatoms and dinoflagellates, Verity et al. (1992) for flagellates and Putt and