



Spatiotemporal variability and trophic relations of bacterioplankton and phytoplankton in Cuban oceanic waters

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ABSTRACT.—Studying the temporal variability of lower trophic levels allows a better understanding of the type and degree of coupling that exists in the pelagic ecosystem in the Caribbean region. The aim of the present study was to characterize the structure and functioning of the <20 μm planktonic fractions in oligotrophic oceanic waters of Cuba and their relation with environmental parameters, as well as the role played by heterotrophic components in the microbial loop in the pelagic ecosystem. The distribution of picoplanktonic and nanoplanktonic biomass in the oceanic waters of Cuba did not differ significantly among years in the same season. The highest biomass values were found in the northern vs the southern region, which may be related to coastal upwelling, as well as to the contributions of nutrients and organic matter from coastal ecosystems. Furthermore, the biomass of the heterotrophic component was much larger than that of the autotrophic component, confirming the role played by small (<20 μm) heterotrophic organisms in the microbial loop. The present findings may be useful for tracking future variation in bacterioplankton and phytoplankton in the Caribbean region.

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The oligotrophic zones of oceans are characterized by the predominance of microbial trophic webs, where recycling processes are important because of their high complexity, fast transfer rates, and the coexistence of multiple trophic compartments within the same size fraction (Mouriño et al. 2005, Pavés and González 2008, Fuhrman et al. 2015). In some areas, the heterotrophic/autotrophic biomass ratio has been reported to be higher than in more productive systems, and a significant percentage of the organic material circulates through the heterotrophic compartment (Vargas and González 2004, Aristegui and Montero 2005).

Knowing the distribution and functional relations of the planktonic fractions in the pelagic ecosystem allows assessment of the zones where organic matter synthesis and degradation occurs (Nagata 2000, Kirchman 2000, Fuhrman et al. 2015). The autotrophic fraction represents one of the sources that contribute dissolved organic matter to the systems. In turn, this biomass is used by heterotrophic picoplankton. The heterotrophic fraction of nanoplankton exerts control by predation on autotrophic and bacterial populations. In addition, this fraction takes part in the decomposition of organic matter and serves as a potential food source for the microzooplankton

filter feeders (Christaki et al. 2000, Naustvoll 2000, García-Pámanes and Lara-Lara 2001, Verity et al. 2002, Froneman et al. 2004, Calbet and Landry 2004, Lugioyo et al. 2007, Linacre et al. 2015).

The distribution of planktonic fractions is closely related to hydrodynamics. It is well known that oceanic waters adjacent to Cuba are characterized by cyclonic and anticyclonic eddies (Victoria and Penié 1998, Gutiérrez et al. 1998, Richardson and Schoeman 2004, Mitrani et al. 2006). In winter, vertical mixing processes in the water column are favored, while in summer, greater stratification occurs (Rodas et al. 2007, Arriaza et al. 2008).

The aim of the present study was to characterize: (1) the structure and functioning of $<20\ \mu\text{m}$ planktonic fractions in Cuban oligotrophic oceanic waters and their relation with environmental parameters, and (2) the role played by heterotrophic components in the functioning of microbial loop in the pelagic ecosystem.

MATERIALS AND METHODS

The oceanic waters adjacent to Cuba extend from Cape San Antonio to the area surrounding Punta de Maisí and from the slope of the insular shelf to the legal limit of Exclusive Economic Zone, including the oceanic waters of Yucatán Strait and part of the Gulf of Mexico, Florida Straits, San Nicolas and Old Bahamas channels, and the Winward Passage (Victoria and Penié 1998) (Fig. 1).

Between 1989 and 2005, 134 stations were sampled: 69 in southern and 65 in northern oceanic waters of Cuba (Fig. 1). In the south, three research cruises were conducted in the summer months (May–June 1989, July–August 1989, and August 2005) and three in winter (February–March 1989, January–February 1990, and March 2005). In the north, four cruises were conducted in the summer (September 1991 and 1996, July–August 2003, and August 2005) and two in the winter (February 1997 and March 1999). Samples were taken at the surface level (0 m), thermocline, and 150 m using 5-L Nansen oceanographic bottles.

Biomass of planktonic organisms $<20\ \mu\text{m}$ [picoplankton (HP), autotrophic nanoplankton (AN), and heterotrophic nanoplankton (HN)] was determined using epifluorescence microscopy for counts, which were then used to calculate biovolume (Hobbie et al. 1977, Caron 1983). Next, the Sherr and Sherr (1984) criterion was used to express the biomass in carbon units for the picoplankton and nanoplankton fractions.

The estimation of consumption rate among the different trophic categories was conducted following Peter (1994), as reported by Vargas and González (2004). Heterotrophic nanoplankton and microzooplankton were considered predators, while total picoplankton and nanoplankton were assumed to be prey. For the calculation, the average values of concentration, biovolume, and temperature were used. Samples were collected at stations with two environmental conditions: (1) oceanic waters with shelf influence (South, $n = 27$; and North, $n = 19$), and (2) strictly oceanic waters (South, $n = 40$; and North, $n = 35$).

Dissolved oxygen (DO), nitrates and nitrites (NOX), and inorganic phosphate (PO_4) concentrations were determined according to IOC-UNESCO (1983) and FAO (1975). Salinity and temperature were measured with a multi-parameter sensor (Hanna HI 9828) and chlorophyll concentration a spectrophotometric method (SCOR-UNESCO 1966).

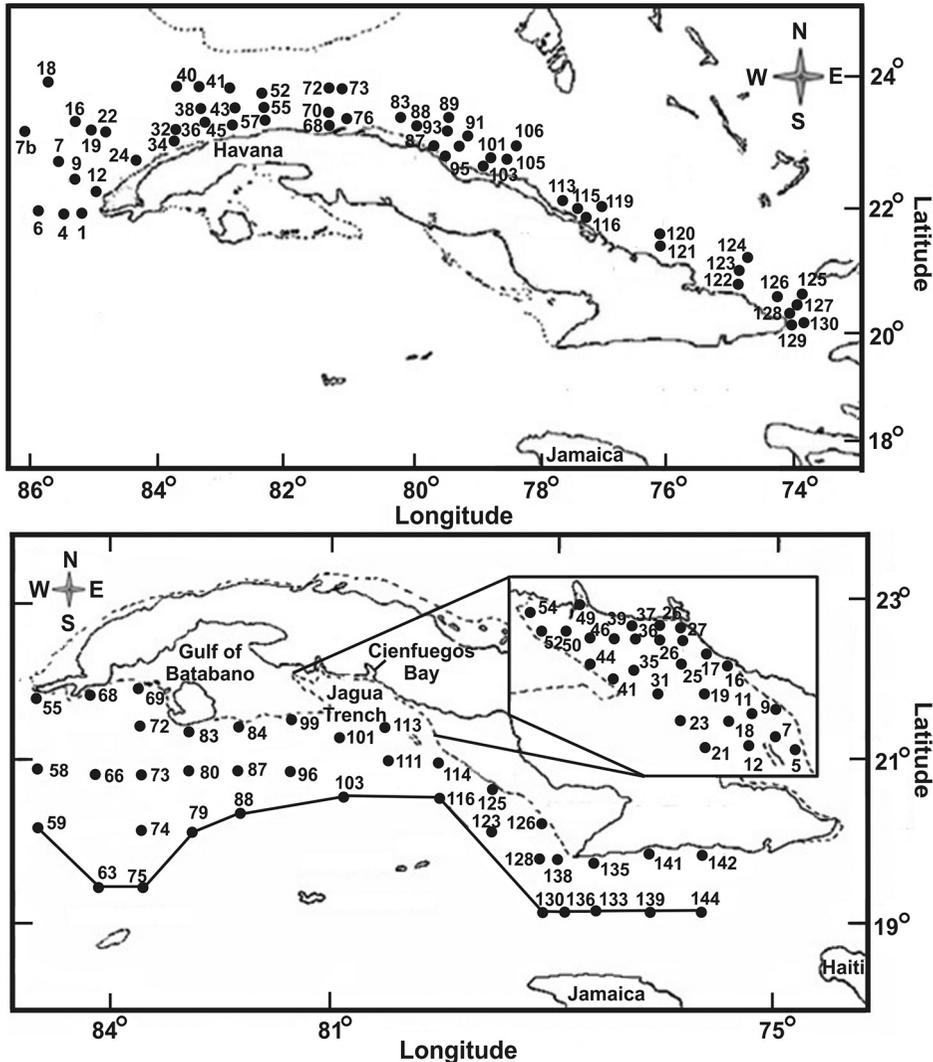


Figure 1. Location of sampling stations in the oceanic waters to the south and north of Cuba.

Data processing was performed using Statistica Version 6.0 software (StatSoft, Inc., 1984–2001) and MapInfo Professional 6.5 (© MapInfo Corporation 1985–2001). Mann-Whitney non-parametric tests (Siegel 1974) and Student's *t*-tests (Zar 1996), for the comparison of mean values, were used to examine for temporal and zone-specific differences. To reveal relations between the biomass of planktonic fractions and abiotic (PO_4 , $\text{NO}_2 + \text{NO}_3$) and biotic (autotrophic and heterotrophic picoplankton, nanoplankton and microzooplankton) factors, Pearson's correlation coefficients were used. An alpha of 0.05 was used to declare statistical significant. The spatial representation of biomass of the plankton fractions was performed using value ranges according to the scale proposed by Frontier (1981).

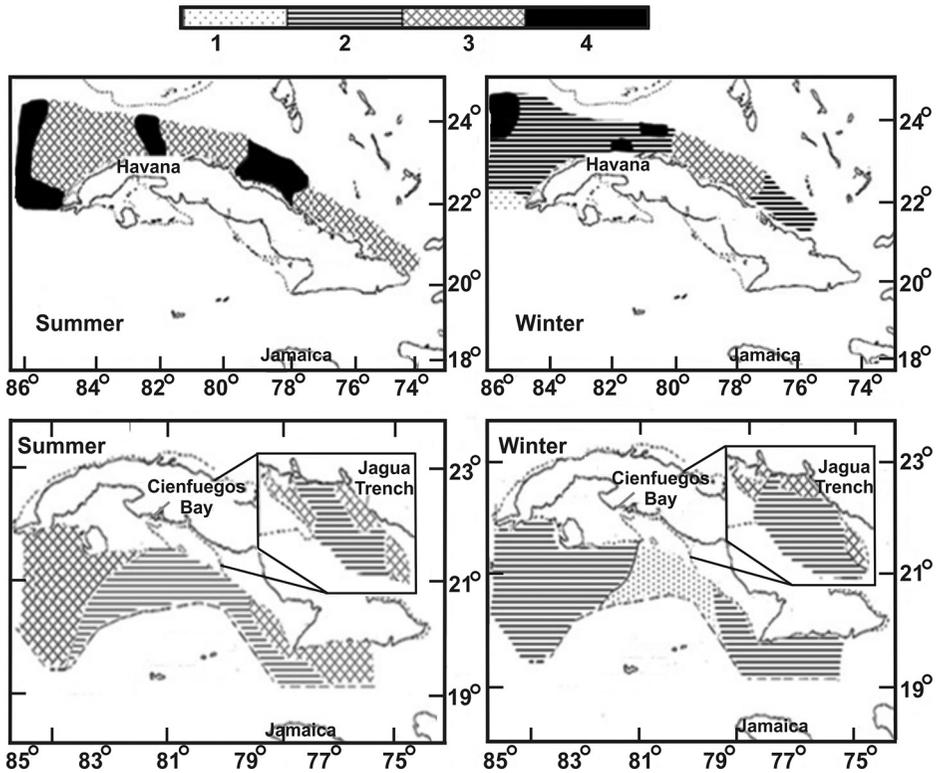


Figure 2. Summer and winter distribution of the picoplanktonic biomass in oceanic waters of Cuba. Upper bar indicates picoplanktonic biomass (mgC m^{-3}) as follows: 1 (1.7–5.1), 2 (5.1–13.6), 3 (13.6–30.6), 4 (30.6–62.9).

RESULTS

In the oceanic waters of Cuba, no significant differences were found in picoplankton and nanoplankton biomass for the same season among the years analyzed (picoplankton: $U = 28.0$, $P = 0.096$, $n = 134$; and nanoplankton: $U = 35.5$, $P = 0.273$, $n = 134$).

In oceanic waters during the summer, the picoplanktonic biomass averaged 37 (SE 12) mg C m^{-3} in the north and 16 (SE 8) mg C m^{-3} in the south. During winter, picoplanktonic biomass ranged between 15.3 (SE 5.1) and 12 (SE 5.3) mg C m^{-3} in north and south oceanic waters, respectively (Fig. 2). The biomass values of picoplankton were significantly higher in summer than winter values ($U = 106.00$, $n = 97$, $P < 0.0001$) (Fig. 2).

During summer, three zones of high biomass for picoplankton were identified, the northwestern area (49 mg C m^{-3}), north of Havana (42 mg C m^{-3}), and the oceanic waters bordering the Sabana-Camagüey Archipelago (SCA) (55 mg C m^{-3}). During winter, the highest biomass values were located in three specific zones of the western region between Cape San Antonio and Matanzas ($30.6\text{--}62.9 \text{ mg C m}^{-3}$). In the south, the biomass varied between 5.1 and 30.6 mg C m^{-3} , and the highest values were found in the southwestern and southeastern regions, and to the west and east of Jagua Trench ($13.6\text{--}30.6 \text{ mg C m}^{-3}$) (Fig. 2).

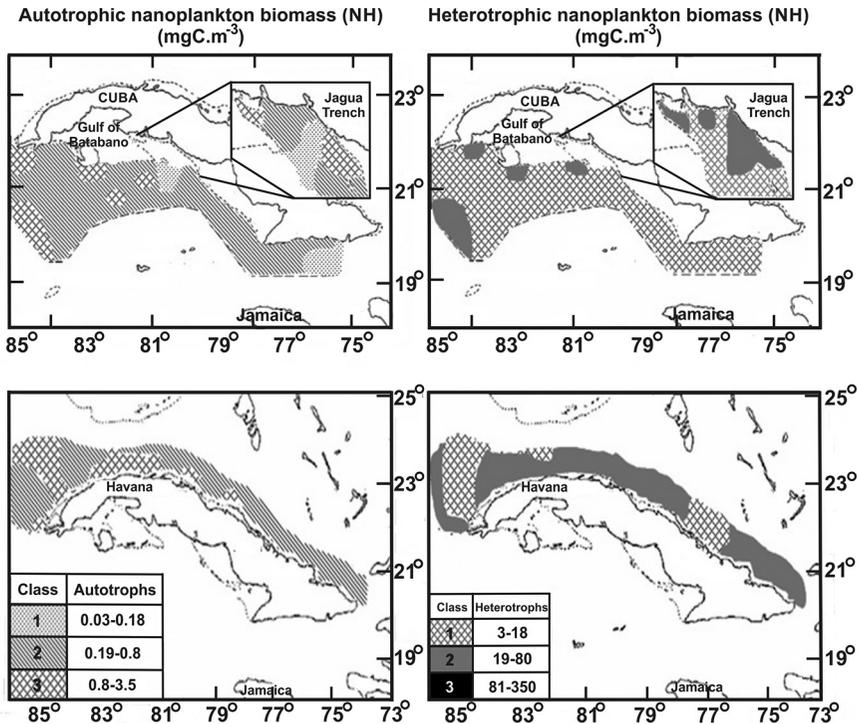


Figure 3. Horizontal distribution of the biomass of the autotrophic and heterotrophic nanoplankton in the oceanic waters adjacent to Cuba in summer. The range of values is based on the scale proposed by Frontier (1981).

During summer, the oceanic waters south of Cuba had AN biomass values that varied between 0.03 and 1.7 mg C m⁻³, with a mean of 0.51 (SE 0.24) mg C m⁻³, whereas the HN biomass ranged between 4.8 and 62.4 mg C m⁻³ with a mean of 14.4 (SE 4.2) mg C m⁻³.

AN and HN biomass had similar distribution patterns with higher values located in areas adjacent to the southwestern platform, in western oceanic waters to the east and west of Jagua Trench and to the south of Cienfuegos Bay (Fig. 3). Jagua Trench region (JT) was characterized by significantly higher values of nanoplankton biomass than the other oceanic waters examined ($U = 262.00$, $n = 65$, $P = 0.0006$).

In oceanic waters to the north, AN biomass in summer ranged from 0.2 to 0.8 mg C m⁻³. The highest values were found in the western zone between Cabañas and Matanzas Bays, and in two areas of the Sabana-Camagüey Archipelago (Fig. 3). During winter, the biomass distribution pattern of NA was heterogeneous; values were higher than those in summer, and the maxima were found in the area bordering Matanzas Bay.

During summer, HN biomass had a predominance of values between 19 and 67 mg C m⁻³ in oceanic waters north of Cuba (Fig. 3). During winter, biomass values were higher than during summer, with a mean of 110 (SE 87) mg C m⁻³ and with maxima occurring in the section between Nuevitás and Gibara. The western zone was characterized by an area of low biomass values during summer (3–18 mg C m⁻³), which increased by two orders of magnitude by winter.

Table 1. Estimated values of ingestion rate (cells plankton hr^{-1}) for different trophic categories, obtained at stations with different environmental conditions.

Location and depth	Predator/prey trophic relations		
	Heterotrophic nanoplankton/picoplankton	Microzooplankton/Picoplankton	Total microzooplankton/Nanoplankton
Oceanic waters north of Cuba			
Stations			
0 m	20–38	7,275–8,603	267–718
Thermocline	32–57	6,002–8,362	358–774
150 m	43–67	4,941–5,493	96–238
Stations with shelf influence			
0 m	19–27	2,850–3,492	607–687
Thermocline	34–44	1,659–1,974	253–459
150 m	25–53	3,505–4,106	201–326
Oceanic waters south of Cuba			
Stations			
0 m	16–24	687–983	83–101
Thermocline	6–20	992–1,072	41–86
150 m	10–26	2,638–2,930	72–98
Stations with shelf influence			
0 m	33–47	2,890–3,179	22–43
Thermocline	3–13	1,290–1,388	81–103
150 m	4–12	1,471–1,631	23–35

In oceanic waters to the north, the biomass values of the nanoplankton fraction were significantly lower in summer than in winter ($U = 426.00$, $n = 97$, $P = 0.00002$) and significantly higher with respect to the south ($U = 3533$, $n = 203$, $P = 0.0014$).

Given the uncertainty in the magnitude of carbon fluxes in oligotrophic oceans, another important aspect to understand the trophic relations among planktonic fractions is the analysis of consumption. In the oceanic waters south of Cuba, estimates of the ingestion rate (IR) in the water column from 0 to 150 m suggested that at oceanic stations with shelf influence, as well for strictly oceanic stations, the HN can ingest from 7 to 47 cells picoplankton hr^{-1} , and microzooplankton ingests between 687 and 3179 cells picoplankton hr^{-1} and between 22 and 103 cells nanoplankton hr^{-1} (Table 1).

At oceanic stations with shelf influence, the maximum values of the ingestion rate for the HN and for the microzooplankton on picoplankton were obtained at surface level (33–47 cells picoplankton hr^{-1} and 2890–3179 cells picoplankton hr^{-1} , respectively). At the thermocline, the microzooplankton IR on nanoplankton had maximum values (81–103 cells picoplankton hr^{-1}) (Table 1).

At oceanic stations, predation pressure of heterotrophic nanoplankton and microzooplankton on picoplankton was 16–26 cells picoplankton hr^{-1} and 2638–2930 cells picoplankton hr^{-1} , respectively, at a depth of 150 m. At the surface (0 m), nanoplankton was subjected to increased grazing pressure by the microzooplankton (83–101 cells picoplankton hr^{-1}) (Table 1).

In oceanic waters to the north, IR was higher with respect to the waters to the south. HN consumed between 19 and 67 cells picoplankton hr^{-1} , while microzooplankton

Table 2. Relationships between planktonic fractions and abiotic factors in Cuban oceanic waters. r is the Pearson correlation coefficient. Non-significant correlations are not shown in the table.

Variables	r	P
Picoplankton vs nanoplankton	0.47	<0.05
Picoplankton vs microzooplankton	0.33	<0.05
Picoplankton vs $\text{NO}_2 + \text{NO}_3$	0.61	<0.05
pH vs heterotrophic nanoplankton	0.33	<0.05
pH vs autotrophic nanoplankton	0.35	<0.05
pH vs $\text{NO}_2 + \text{NO}_3$	0.31	<0.05
Picoplankton vs dissolved oxygen	-0.41	<0.05
Autotrophic nanoplankton vs heterotrophic nanoplankton	-0.39	<0.05
Autotrophic nanoplankton vs chlorophyll a	0.34	<0.05
Autotrophic nanoplankton vs microzooplankton	0.52	<0.05
Autotrophic nanoplankton vs PO_4	0.47	<0.05
Heterotrophic nanoplankton vs $\text{P}_{\text{O}4}$	0.32	<0.05

between 1659 and 8603 cells picoplankton hr^{-1} and between 116 and 774 cells picoplankton hr^{-1} (Table 1).

At oceanic stations with shelf influence, an increase was observed in the estimated values of the ingestion rate of heterotrophic nanoplankton on picoplankton, starting from the thermocline (34–44 cells picoplankton hr^{-1}). Toward the bottom of the photic layer (150 m), values of 25–53 cells picoplankton hr^{-1} were observed, and microzooplankton predation pressure was higher (3505–4106 picoplankton hr^{-1}) (Table 1).

At oceanic stations, IR estimates had a trend similar to that obtained at the stations influenced by shelf, with an increase in values that begins at the thermocline, with a HN consumption of 32–57 cells picoplankton hr^{-1} (thermocline) and 43–67 cells picoplankton hr^{-1} (150 m). However, the microzooplankton predation pressure had an inverse trend, with higher values at the surface (8275–8603 cells picoplankton hr^{-1}) decreasing towards 150 m (4941–5493 cells picoplankton hr^{-1}). Microzooplankton IR on nanoplankton was estimated to be 658–774 cells picoplankton hr^{-1} at the thermocline (Table 1).

Positive correlations emerged between microzooplankton and nitrate concentrations, and picoplankton and nanoplankton biomass (Table 2). Heterotrophic picoplankton was positively correlated with heterotrophic nanoplankton, autotrophic nanoplankton, and nitrate concentration. In addition, a negative correlation was found between picoplankton biomass and dissolved oxygen (Table 2). The biomass of autotrophic nanoplankton had a negative correlation with heterotrophic nanoplankton, and a positive correlation with chlorophyll a and microzooplankton biomass. In addition, HN and AN correlated positively with phosphate (Table 2).

Phosphate and nitrate + nitrite nitrogen concentrations in the oceanic waters to the north and south differed significantly. In the north, the mean values of phosphate concentration were 0.194 (SE 0.14) $\mu\text{mol L}^{-1}$, and in the south, 0.126 (SE 0.08) $\mu\text{mol L}^{-1}$ ($F_{1,206} = 18.35$, $P = 0.00003$). The mean concentration of nitrate + nitrite nitrogen was five times higher in the north [0.61 (SE 0.46) $\mu\text{mol L}^{-1}$] than in the south [0.11 (SE 0.1) $\mu\text{mol L}^{-1}$] ($F_{1,206} = 47.04$; $P < 0.0001$) (Fig. 4).

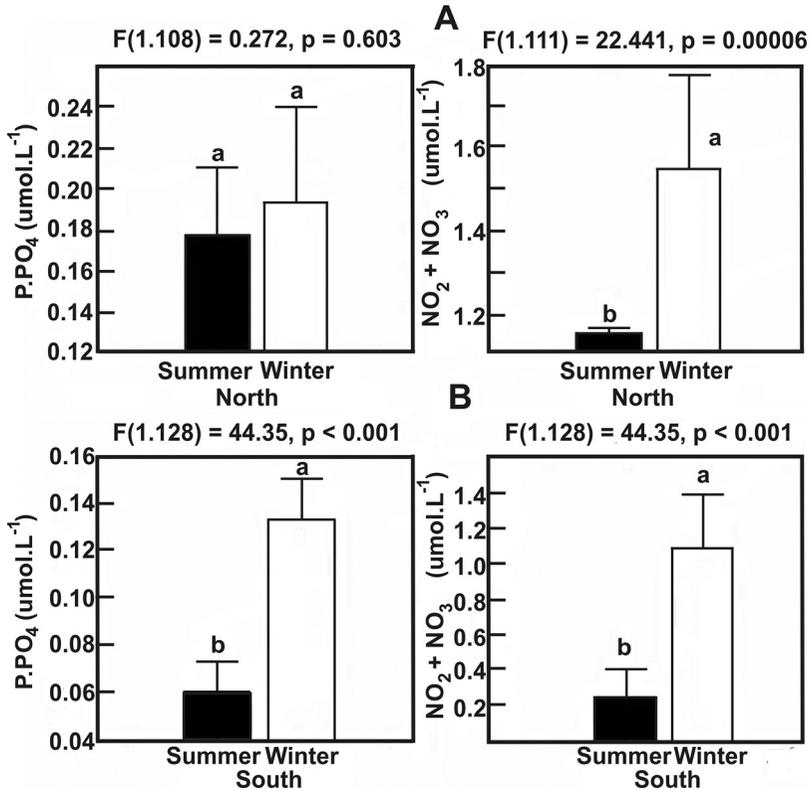


Figure 4. Variation of the concentration of phosphate and nitrate + nitrite in the oceanic waters adjacent to Cuba. North: $n = 82$, South: $n = 126$.

DISCUSSION

In general, the distribution patterns of picoplankton and nanoplankton biomass in the oceanic waters of Cuba are related to anticyclonic and permanent cyclonic eddies (García 1991, Gutiérrez et al. 1998, Richardson and Schoeman 2004). According to Arriaza et al. (2008), the spatiotemporal permanence of these complex dynamics is due to the relation of oceanic circulation with the submarine topography and thermal characteristics that influence water stratification (Rodas et al. 2007).

In the southwestern and northwestern regions and to the east and west of Jagua Trench, this dynamic complex favors vertical mixing and sustained nutrient input from deep enriched layers, as well as the contribution of enriched waters from neighboring shelves, which are conducive to horizontal and vertical nutrient gradients that stimulate the response of small planktonic organisms (Arístegui and Montero 2005).

Similar results have been reported by Linacre et al. (2015), who found that the main mechanism distributing picoplankton carbon standing stocks is related to the regional mesoscale dynamics in the Gulf of Mexico: anticyclonic and cyclonic eddies aggregate, disperse, and vertically transport the picoplankton carbon biomass within the mixed layer of this oligotrophic open-ocean environment. The biomass peaks in deeper layers of the euphotic zone may be sustained by nitrogen inputs to the lower euphotic zone provided by physical mechanisms, such as deep winter

mixing, pumping of deep water by cyclonic eddies, eddy interaction with continental slopes, and surface waters mixing by hurricane activity.

In the north, the biomass values for planktonic fractions were higher than in south, and heterotrophic components were the greater than autotrophic components. In this area, due to its narrow submarine shelf, nutrient enrichment is reinforced by the movement of Ekman currents (Arriaza et al. 2008), which favor coastal upwelling and stimulate biological production (Baisre 2004).

In other studies, several heterotrophic eukaryotic picoplankton groups have been identified in the ocean. The studies carried out by Fuhrman (1992), Baines et al. (1994), and Buck et al. (1996) confirmed that, in oligotrophic waters, the small sized-heterotrophic components occurred at greater or equivalent biomass to that of the autotrophic components. In the North Sea, Medlin et al. (2006) found a prevalence of the non-photosynthetic component of this fraction, which is consistent with López-García et al (2001), Moon-van der Staay et al. (2001), Paffenhöfer et al. (2003), and Massana et al. (2004) for other regions.

The positive correlation between heterotrophic picoplankton concentration and heterotrophic nanoplankton biomass is related to the reduced doubling time of picoplankton, which masks the regulatory function of heterotrophic nanoplankton (Madigan et al. 2000). Sieburth and Davis (1982) found a positive correlation between picoplankton biomass and heterotrophic nanoplankton in the Sargasso Sea and the Caribbean Sea, and suggested that this relation is common in oligotrophic waters.

In the present study, the relationship between picoplankton, nanoplankton, and chlorophyll *a*, and the concentration of nitrates + nitrites, suggests that these nutrients favor the production of dissolved organic matter usable by the heterotrophic components (HP and HN). These components bring regenerated biogenic elements into the medium, which are used by autotrophic fractions, and contribute to the concentration of pigments and total primary productivity (Cuevas et al. 2009). The relationships found in our study among the fractions of picoplankton, nanoplankton, and nutrients are consistent with results from Caron et al. (1999) in the Sargasso Sea and Cuevas et al. (2004) off Chile.

That biomass of the heterotrophic components of picoplankton and nanoplankton was found to be higher by up to two orders of magnitude with respect to autotrophic biomass in Cuban oceanic waters suggests a fundamental role is played by heterotrophic organisms $<20 \mu\text{m}$ in the microbial loop. These organisms convert dissolved organic matter provided by autotrophs into biomass and transfer energy to the other trophic levels when consumed by protozoa and zooplankton (Ducklow 2000, Zoccarato et al. 2016). High heterotrophic biomass of organisms $<20 \mu\text{m}$ has also been reported for the tropical Pacific Ocean, where heterotrophic nanoplankton biomass was three times greater than that of autotrophs (Paffenhöfer et al. 2003).

In oligotrophic areas, the existence of an “inverted pyramid” has been observed, where the small heterotrophic components present a higher or equivalent biomass to that of the autotrophs (Buck et al. 1996). In these areas, the existence of communities in which the heterotrophic biomass exceeds that of the autotrophs is based on the high renewal rate of autotrophic fractions $<20 \mu\text{m}$. This high renewal rate is partly due to their high surface-to-volume ratio, which results in a high flow of dissolved organic matter, a high concentration of substrate available to be degraded, and a relative increase in respiratory processes associated with heterotrophic activity (del

Giorgio and Cole 2000). Cuevas et al. (2004), in an embayment off northern Chile, found that heterotrophic processes dominate over primary production.

The differences found in the contribution of trophic fractions $<200 \mu\text{m}$ in the oceanic waters of Cuba may be related to a lower availability of nutrients in the south. This occurs because picoplanktonic organisms rapidly assimilate small amounts of nutrients due to their greater surface-to-volume ratio, which generates the production of the organic substrate necessary to support the biomass of the other components of the microbial trophic web. In a microcosm of oligotrophic water taken from the eastern Mediterranean Sea, Tsiola et al. (2016) experimentally demonstrated that the addition of phosphorus (a limiting nutrient) had a positive effect on the growth of picoplankton populations, as did the combined effects of nitrogen and phosphorus on all planktonic fractions.

Collectively, the results obtained in the present study suggest that, in the south, substrate availability influences the metabolism of the picoplankton fraction and determines the response of the different trophic components in the system, suggesting bottom-up control. There is evidence of such a relationship between the synthesis of dissolved organic matter and its use by heterotrophic fractions (Troncoso et al. 2003, Cuevas et al. 2004). In oceanic waters to the north, the greater availability of nutrients and organic matter also stimulates the other fractions of the nanoplankton and microzooplankton; therefore, this availability of nutrients and organic matter favors predation on picoplankton, thus suggesting top-down control.

The ingestion rate estimates of the different trophic levels suggest that there is higher pressure of HN and microzooplankton predation on picoplankton in the north. These results are consistent with those obtained by Cuevas et al. (2004), who reported that under oligotrophic conditions, consumption increases due to the connection between the grazing activity of nanoplankton and microzooplankton, and the production of dissolved organic matter and regenerated inorganic nutrients. However, given the high complexity of microbial trophic relationships due to predation within the microzooplankton (Franzé and Modigh 2013), as well as of mixotrophy (Mitra et al. 2016), these results constitute only an approximate understanding of the carbon flows occurring in the oligotrophic oceanic waters of Cuba.

In summary, (1) the distribution of picoplanktonic and nanoplanktonic biomass in the oceanic waters of Cuba did not differ significantly among years in the same season, with the highest values found in the northern vs southern region; and (2) the functioning of the pelagic trophic web in Cuban oceanic waters is of the “inverted pyramid” type, with a heterotrophic biomass $<20 \mu\text{m}$, up to two orders of magnitude greater than the autotrophic biomass in both in winter and summer.

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