

Differences in photosynthetic pigment signatures between phytoneuston and phytoplankton communities in a coastal lagoon of Baja California

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Abstract In order to understand the relationships between the dynamics of phytoplankton populations in the surface microlayer (MIL) and in the water column below (SSW), this study used high-performance liquid chromatography-derived pigment markers in samples from a coastal lagoon of Baja California (Estero de Punta Banda, EPB) under summer (October 2003) and winter (December 2003) conditions. Photosynthetic pigment signatures of phytoplankton at the air–sea interface (phytoneuston) and subsurface measurements were related to bottom-up (temperature, salinity, nutrient concentrations) and top-down factors (zooplankton abundance). Slicks and scum layers were observed in the inner part of the lagoon and coincided with greater stratification of layers just below the sea surface and lower wind intensities. In general, spatial variability in pigment markers and ancillary data was very high and resulted in non-significant differences between MIL and subsurface samples when different regions of EPB or sampling dates were compared. However, different patterns were found between pigments and environ-

mental factors of MIL and SSW samples when the relative numbers of stations with positive and negative differences ($\Delta X = X_{\text{MIL}} - X_{\text{SSW}}$) were computed. For each survey, pigment markers of phytoneuston and phytoplankton samples were not necessarily correlated. Further analysis revealed that those markers (19'-butanoyloxyfucoxanthin, prasinoloxanthin, divinylchlorophyll *a*) corresponded to picophytoplankton groups (haptophyte, prasinophyte, and prochlorophyte). On both dates, the MIL was enriched in 19'-hexanoyloxyfucoxanthin (a marker for a type 4 haptophyte) and fucoxanthin (marker for bacillariophytes, haptophytes, and crysophytes) and depleted in peridinin (marker for dinophytes). Different zooplankton grazers accumulated in the MIL (loricate tintinnids) and in SSW (copepod nauplii).

Introduction

Characterization of phytoplankton assemblages in coastal waters has been a valuable tool to detect disturbance of biogeochemical cycles due to anthropogenic influences such as eutrophication (Rocha et al. 2002). In the same way, modifications to phytoneuston, the phytoplankton communities living within the top millimeters of the sea surface (microlayer) (GESAMP 1995), may provide some insight on human impacts in coastal embayments (Zaitsev 1992). Since photosynthetic activity of phytoneuston populations may alter air–sea gas fluxes (Frew et al. 2004), differences in autotrophic components of the microlayer (MIL) can potentially affect regional geochemical budgets especially in inshore waters where the

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variability is greatest. Additionally, variations in phytoneuston assemblages have a particular significance for higher trophic levels since survival rates of numerous types of invertebrate larvae (e.g., crab zoea) depend on microalga availability in the MIL (GESAMP 1995). This is more evident near the coast where a greater coupling between benthic and planktonic ecosystems prevails. In general, microflagellates and pennate diatoms have been reported as the main phytoneuston groups in coastal waters (Hardy 1982; De Souza and Chretiennot-Dinet 1984; Hardy and Apts 1989). Most taxonomic studies of the MIL have been based on microscopic observations or spectrophotometric measurements of pigments (Manzi et al. 1977; Hardy and Apts 1984; Ignatiades 1990). However, these methods have two important drawbacks (1) small flagellates ($<5\ \mu\text{m}$) often suffer breakage when preserved and cannot be identified using optical microscopy (Jeffrey et al. 1997); and (2) spectrophotometric determinations are affected by phaeopigments and require relatively larger volumes of sample than chromatographic analysis (Mantoura et al. 1997). The use of high-performance liquid chromatography (HPLC) may overcome these difficulties even though this approach has not previously been applied to characterize phytoneuston communities. Most investigations of phytoplankton composition using HPLC have been carried out for SSW samples in coastal and oceanic waters (Schlüter et al. 2000; Zapata et al. 2004). Since phytoneuston communities are formed not only by autochthonous populations but also by transient populations derived from SSW (Carlson 1982), the characterization of subsurface phytoplankton pigment markers is essential to understand the dynamics of the phytoplankton populations living in the MIL.

The objective of this study was to analyze differences in HPLC-derived pigment markers between phytoneuston and subsurface phytoplankton assemblages in a coastal temperate lagoon of the southern California current system (Estero de Punta Banda, EPB) in summer (October) and winter (December) conditions. Concentrations of photosynthetic pigments were determined for two regions of EPB (outer and inner) and related to temporal variations in environmental variables (temperature, salinity, nutrients, and abundance of zooplankton grazers) along the lagoon. We hypothesized that MIL and subsurface phytoplankton communities would have more pronounced differences in the inner part of the lagoon where seagrass prairies and associated epiphytic microalgae are well developed (Montes-Hugo 2001).

Materials and methods

Study area

Estero de Punta Banda is the most northern coastal lagoon of Baja California and the nearest to the Mexico–USA border (Fig. 1). Water turbidity along the lagoon defines two distinct regions characterized by low (outer part) and high (inner) underwater light attenuation (Montes-Hugo et al. 2003). The construction of a dike in the inner region of EPB (Fig. 1) has not significantly altered the general circulation and the

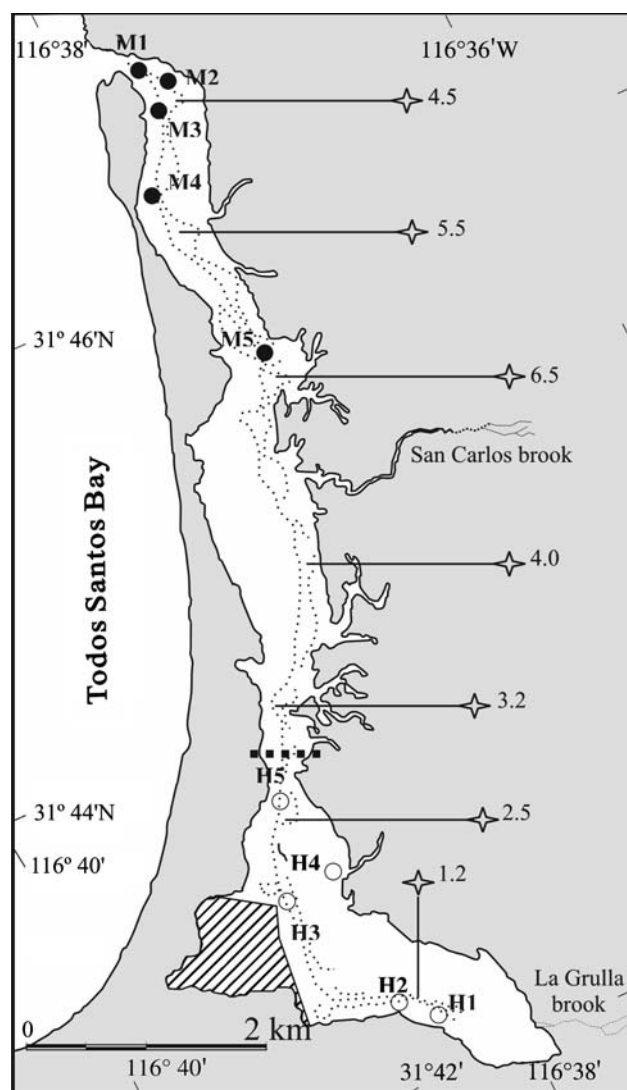


Fig. 1 Study area. Outer region (filled circles, M1–M5) and inner region (open circles, H1–H5) stations are separated with a thick broken line. Main channel is indicated with a dotted line. Stars represent average bottom depths (m) of the channel based on mean tidal level. In the southwest corner (dashed area) there is a zone affected by an artificial dike built in the early 1980s. Planned construction inside the dike was not done

biological functioning of the lagoon (Ibarra-Obando and Escofet 1987). In general, EPB is a negative estuary except during “El Niño” events (Galindo-Bect et al. 1999). EPB has a shallow water column (mean bottom depth ~1.48 m) and is characterized by strong semi-diurnal tidal currents (up to 1 m s^{-1}) (Pritchard et al. 1978). In coastal lagoons of northwest Baja California, mixing by tidal currents tends to homogenize the water column especially in the inner part of water bodies (Lara-Lara et al. 1980). Regarding the present status of biological communities and water quality parameters, EPB is within the range of the less ecologically impacted temperate water bodies of the Mexican Pacific coast (Ibarra-Obando et al. 2000). Extensive salt marshes, mud-flats, and beds of *Zostera marina*, are the main ecological subsystems of EPB (Ibarra-Obando and Poumian-Tapia 1991).

Unlike light, the influences of temperature, salinity, and nutrient concentrations are of secondary importance in phytoplankton pigment concentrations of EPB (Montes-Hugo et al. 2004). However, the significance of these environmental factors on phytoneuston composition and dynamics has not been evaluated in this embayment. Chlorophyll *a* and nutrient concentrations have a very patchy distribution along EPB (Acosta-Ruiz and Alvarez-Borrego 1974; Galindo-Bect et al. 1999). However, maximum chlorophyll values for different periods of the year are generally observed at the lagoon’s mouth, and vice versa for nutrient concentrations (Camacho-Ibar and Alvarez-Borrego 1988; Montes-Hugo 2001).

Field measurements

Microlayer and SSW samples (0.5 m below the sea surface) were collected using a boat during 2, 4 October, and 2–3 December 2003 under spring tide conditions. Care was taken to avoid wave disruption of surface slicks due to boat splashing. Surveys were performed in the morning (0930–1130 hours, inner part) and afternoon hours (1230–1430 hours, outer part) at high tide. Ten sampling locations were selected throughout the lagoon (Fig. 1) to encompass the distinct patchiness of sea surface slicks (De Souza and Chretiennot-Dinet 1984). MIL samples for temperature, salinity, nutrients, pigments, microphytoplankton, and zooplankton grazers were obtained from the top of the sea surface layer by immersing a polypropylene box obliquely (bottom first) and allowing the water to flow in over the lip until the box was nearly full (Hardy 1973). This sampler proved to be very efficient ($\sim 300 \text{ ml min}^{-1}$) to collect relatively large volumes (100–500 ml) of MIL samples needed for HPLC

determinations. Hardy (1973) found that a thin sea surface layer (~4 mm thick) is generally well sampled using a similar sampler. The boundary between MIL and SSW is not well defined due to turbulent and diffusive processes (Schimpf et al. 2004). A fine measurement of MIL thickness is parameter-dependent and would require advanced techniques (e.g., laser-induced fluorescence) which were beyond the scope of the present study.

Subsurface water samples for temperature, salinity, nutrients, pigments, microphytoplankton, and zooplankton grazers were obtained with acid-washed Nalgene bottles (2 l) at 0.5 m. These bottles were opened and closed in situ. To avoid the effects of ambient light on pigments, subsamples were immediately placed in Nalgene dark bottles (500 ml). Temperature and salinity were measured by pumping a discrete volume through the conductimetric cell and thermo-coupled electrode of a CTD (SeaBird Inc.). Nutrient samples ($\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ , PO_4^{3-} , and H_4SiO_4) were filtered in the field through 1- μm pore size membranes (Whatman GF/F glass-fiber, 25 mm, 0.7- μm nominal pore size), and transported to Ensenada in dry ice to be processed. Nutrient analysis was done manually in triplicate following Strickland and Parsons (1972). Pigment samples were filtered in the field through Whatman GF/F glass-fiber filters, placed in plastic filter-holders, and kept frozen in liquid nitrogen (2 weeks) until HPLC analysis. Samples for quantifying microphytoplankton groups (bacillariophyte, dinophyte, and euglenophyte) and zooplankton grazers (copepod nauplii and loricate tintinnids) were preserved with Lugol’s solution and concentrated over 24 h using 100-ml settling chambers. Identification and counting of microphytoplankton and zooplankton grazers were performed with a Zeiss inverted microscope (Utermöhl 1958) at 100–400 \times magnification. Dominant microphytoplankters were defined as those species contributing >50% of total abundance in a sample.

Given that wind events and wave activity can disrupt surface slicks, wind velocity and direction were measured with a manual anemometer 2 m above the sea surface, and wave height was visually estimated during all observations. Tidal range values were obtained from the Mexican tides forecast database (Servicio de predicción de mareas, CICESE, Ensenada).

Pigment analysis by HPLC

Filtered samples were extracted with 90% cold acetone HPLC grade (1 ml) during mechanical disruption of the cells with an ultrasonic probe (Beat-Beater,

Biospec Inc.). Extraction of chilled acetone extracts was repeated in three cycles of 30 s using vials covered with aluminum foil to avoid pigment degradation due to ambient light. Between cycles each sample was refrigerated (-20°C) in the dark. Acetone extracts were clarified twice by centrifugation for 2 min at 2,000g. After pellet elimination, 400 μl were ultra-centrifuged for 5 min at 15,000g at low temperature (4°C). A final volume of 200 μl was stored refrigerated, and two aliquots (20 μl) of this volume were injected into the HPLC column after adding 60 μl of 28 mM buffer TBAA (tetrabutyl ammonium acetate, $\text{pH} = 6.5$).

Pigment quantification was done following basic protocols of Van Heukelem and Thomas (2001) with modifications in the solvent delivery profile (%B, min): 5%, 0 min; 5%, 5 min; 95%, 22 min; 95%, 27 min; 5%, 30 min. The HPLC instrument was a Shimadzu AV-10 series equipped with a Zorbax Eclipse XDB-C8 column (4.6×150 mm, 3.5- μm diameter particles). The HPLC loop was rinsed for 10 min between samples and care was taken to avoid bubbles during each HPLC run. Chromatographic separation was carried out under thermostatic conditions (60°C) and a constant flow rate of 1 ml min^{-1} . The HPLC setting used cannot differentiate chlorophyll *c1* from chlorophyll *c2*, and lutein from zeaxanthin (Van Heukelem and Thomas 2001).

Photosynthetic pigment signatures (chlorophyll *c3*, peridinin, 19'-butanoyloxyfucoxanthin (19'-BF), fucoxanthin, neoxanthin, prasinoxanthin, violaxanthin, 19'-hexanoyloxyfucoxanthin (19'-HF), alloxanthin, chlorophyll *b*, and divinyl-*chl a* (DV-*chl a*) were calibrated with commercial pigment standards (DHK, Inc., Sweden), or with pigments isolated and purified from phytoplankton cultures by thin layer chromatography and HPLC. Concentrations of standards were verified by UV-Vis spectrophotometry at the wavelength of the maximum absorption using literature values of extinction coefficients (Jeffrey et al. 1997). Pigment concentration was considered not detectable when the area under a peak was $<1\%$.

Statistical analysis

For each variable (temperature, salinity, nutrients, pigments, microphytoplankton, and zooplankton grazers), differences between MIL and SSW values were evaluated with two tests. First, linear regression was computed between MIL and SSW samples ($\hat{X}_{\text{MIL}} = AX_{\text{SSW}} + B$) for each region of the lagoon and for each survey (October and December). The null hypotheses (regression slope $A = 1$ and intercept $B = 0$) were analyzed in each case with a Student's *t* test

(Sokal and Rohlf 1995). Regression slopes greater or less than 1, and intercept values greater or less than zero, implied MIL enrichment or depletion with respect to subsurface samples, respectively. It is important to make clear that a high coefficient of determination (r^2) for the regression is not indicative of any enrichment of the MIL and on the other hand, a low r^2 is not necessarily indicative of the absence of enrichment of the MIL. For example, we could have a very low r^2 , which would indicate no correlation between MIL and SSW values, and yet have all data points in the dispersion diagram above the 45° line, and this would be a case of enrichment of the MIL with respect to SSW. Thus, in order to have a more complete analysis we need to apply the second method which consists of comparisons between phytoneston and phytoplankton variables performed for each region by testing the regional average of the $X_{\text{MIL}} - X_{\text{SSW}}$ differences (ΔX) against the null hypothesis $\Delta X = 0$. Enrichment factors (concentration or abundance ratios between MIL and SSW samples) were used to describe the accumulation of phytoplankton cells, pigments, and zooplankton organisms in the MIL.

Results

Environmental variables

Dry weather conditions (precipitation ~ 120 mm year^{-1} , Edgard Pavia, Servicio de Meteorología, CICESE) characterized the 2003 surveys (non-El Niño year). October surveys were characterized by relatively clear skies ($<50\%$ overcast) with respect to December surveys (totally overcast). Before the December surveys, it rained and some freshwater input was evident in the inner region of EPB (not measured) (average salinity decreased by >1 in December with respect to that of October, Table 1). In October, wind speed was greatest (up to 7 m s^{-1}) in the outer region of EPB while in December maximum wind intensities (up to 4 m s^{-1}) were measured in the middle portion of the lagoon (locations M4 and M5, Fig. 1, <http://www.iod.ucsd.edu/~mmontes/>). In both surveys, calm waters characterized the inner region of EPB and brown bands of floating material (slicks and scums) were visible during ebb tide. Although average wind speed during sampling was similar in both surveys (~ 2.5 m s^{-1}), maximum wind speeds were observed in October. In both surveys, greatest wave heights created by northwesterly winds at different locations throughout EPB were 0.75 m. These large waves provided energy for strong mixing of the water column in this shallow lagoon.

Table 1 Variability of environmental data along the Estero Punta Banda

		WS	WH	T	S	NIT	NH ₄	PO ₄	H ₄ SiO ₄	
October	M	MIL	5.40 (1.03)	0.48 (0.17)	20.32 (0.35)	33.28 (0.41)	0.40 (0.21)	4.79 (1.19)	0.65 (0.11)	6.58 (1.26)
		SSW			20.26(0.44)	33.36 (0.36)	0.41 (0.19)	5.19 (1.35)	1.15 (0.38)	7.14 (0.92)
		% $\Delta X+$			60	40	20	60	40	60
	H	MIL	2.80 (1.24)	0.19 (0.14)	21.77 (0.44)	33.82 (0.53)	1.30 (0.34)	7.27 (1.00)	1.18 (0.27)	11.00 (1.30)
		SSW			20.95 (0.69)	34.68 (0.47)	1.11 (0.22)	6.51 (0.50)	1.19 (0.26)	10.82 (1.57)
		% $\Delta X+$			80	20	60	60	40	40
December	M	MIL	1.90 (0.67)	0.15 (0.07)	15.32 (0.42)	33.20 (0.35)	0.36 (0.12)	1.98 (0.64)	0.53 (0.07)	4.76 (1.07)
		SSW			15.43 (0.36)	33.58 (0.22)	0.39 (0.14)	2.24 (0.69)	0.58 (0.15)	4.61 (1.19)
		% $\Delta X+$			20	40	40	60	60	80
	H	MIL	2.60 (0.66)	0.42 (0.16)	15.18 (0.34)	32.46 (0.38)	1.24 (0.42)	3.77 (0.71)	1.04 (0.33)	14.04 (5.89)
		SSW			15.20 (0.36)	33.16 (0.50)	1.36 (0.58)	2.45 (0.50)	0.84 (0.16)	10.22 (2.35)
		% $\Delta X+$			40	20	60	80	60	60

M and H are outer (mouth) and inner regions (head), respectively. For each comparison, for microlayer (MIL) and subsurface (SSW), regional average and percentage of samples with enrichment (% $\Delta X+$ = proportion of observations where $(X_{MIL} - X_{SSW} > 0) \times 100$) are summarized; one standard error is indicated between parentheses

MIL microlayer, SSW subsurface water, WS wind speed (m s^{-1}), WH wave height (m), T water temperature ($^{\circ}\text{C}$), S salinity, NIT nitrate + nitrite (μM), NH₄ ammonia (μM), PO₄ phosphate (μM), H₄SiO₄ silicate (μM)

Although, maximum tidal range was similar for both surveys (~ 1.47 m), different tidal conditions characterized mouth and head locations of EPB in December (ebb-transition in the inner part, flood in the outer part) while they were similar in October (flood tides).

In general, spatial variability of MIL–SSW differences of all studied variables was large and in most cases resulted in non-significant differences between MIL and subsurface samples when regions were compared by both methods mentioned above (Tables 1, 2, 3, 4). Since we have very few degrees of freedom (4), it was not possible to apply a non-parametric sign test to the ΔX values. Therefore, the percentage of number of locations with positive ΔX values (% $\Delta X+$) were computed to grossly estimate a ‘probability’ of enrichment of the MIL with respect to subsurface samples. Water temperature measurements showed significant thermal stratification (Δ temperature was up to $\sim 2.5^{\circ}\text{C}$) during October survey (% $\Delta X+$ was 80 for the inner region) (Table 1). Salinity values were generally lower in MIL with respect to SSW samples in both surveys especially in the inner region of EPB (% $\Delta X+$ = 20).

Enrichment and depletion of nutrient concentrations in MIL samples varied with the type of nutrient, location, and sampling date. In the inner region of EPB, inorganic nitrogenous compounds (nitrate + nitrite) did not present significant differences between the MIL and SSW during both sampling dates ($P > 0.05$, Student t test, Table 2). However, a higher frequency of MIL enrichment was noticed in the inner part of the lagoon (% $\Delta X+$ = 60, Table 1). Ammonia was preferentially concentrated in the MIL in all regional comparisons (% $\Delta X+$ \geq 60) (ΔNH_4^+ up to $5.4 \mu\text{M}$).

Unlike the other nutrients, phosphate was significantly enriched in MIL samples with respect to those of SSW in the December survey and in the outer region ($B = 0.27$ with $P = 0.011$, Table 2). Higher phosphate concentrations in MIL samples were more frequently observed in December (% $\Delta X+$ = 60) (ΔPO_4^{3-} up to $0.9 \mu\text{M}$). Silicate enrichment in the MIL was detected mainly in the December survey (% $\Delta X+$ \geq 60) ($\Delta\text{H}_4\text{SiO}_4$ up to $17.8 \mu\text{M}$) possibly due to freshwater input.

Phytoplankton pigment signatures

Chlorophyll $c3$, 19'-butanoyloxyfucoxanthin, prasinoxanthin, violaxanthin, 19'-hexanoyloxyfucoxanthin, and divinyl-chlorophyll a for MIL and SSW samples did not show any significant correlation, the other pigment markers had significant correlations in some cases (Table 2). Only fucoxanthin and chl b had significant differences between phytoneuston and phytoplankton (depletion of MIL with respect to SSW) and it was in December and in the outer region of EPB, all the other pigments did not show significant differences with the regressions (Table 2). In most cases, standing stocks of phytoneuston and phytoplankton as estimated from chlorophyll a did not co-varied and the regressions showed no significant differences between MIL and SSW samples (Table 2).

Chlorophyll a had a relatively high frequency of enrichment in the MIL of the outer region of EPB in October (% $\Delta X+$ = 80, Δ chlorophyll a up to $2.5 \mu\text{g l}^{-1}$) and in the MIL of the inner region in December (% $\Delta X+$ = 60, Δ chlorophyll a up to $0.6 \mu\text{g l}^{-1}$) (Table 3). Chlorophyll $c3$, a pigment marker of type 2b

Table 2 Summary of statistical results for microlayer versus subsurface parameters

Variable		Region	A	P	B	P	r ²	n
T	October	M	0.75 (0.14)*	0.148	5.19 (2.88)	0.146	0.90	5
		H	0.43 (0.28)		1.28 (5.81)		0.45	5
	December	M	1.15 (0.07)**	0.098	-2.37 (1.15)	0.108	0.99	5
		H	0.82 (0.27)		2.70 (4.09)		0.76	5
S	October	M	0.67 (0.55)		1.08 (1.84)		0.33	5
		H	0.81 (0.42)		3.29(1.45)		0.60	5
	December	M	-0.69 (0.85)		6.63 (2.85)		0.18	5
		H	0.52 (0.32)		1.52 (1.07)		0.47	5
NIT	October	M	1.09 (0.07)**	0.373	-0.05 (-0.04)	0.372	0.99	5
		H	0.68 (0.78)		0.55 (0.94)		0.20	5
	December	M	0.80 (0.21)*	0.403	0.05 (0.102)	0.649	0.82	5
		H	0.46 (0.32)		0.61 (0.58)		0.41	5
NH ₄	October	M	0.55 (0.40)		1.92 (2.34)		0.39	5
		H	-0.98 (1.00)		1.37 (6.61)		0.24	5
	December	M	0.85 (0.21)*	0.515	0.08 (0.56)	0.893	0.84	5
		H	0.31 (0.81)		3.00 (2.13)		0.05	5
PO ₄	October	M	-0.09 (0.16)		0.76 (0.22)		0.11	5
		H	0.91 (0.29)		0.10 (0.37)		0.77	5
	December	M	0.44 (0.10)*	0.005*	0.27 (0.06)*	0.011*	0.86	5
		H	1.68 (0.62)		-0.37 (0.56)		0.71	5
H ₄ SiO ₄	October	M	1.04 (0.51)		-0.84 (3.77)		0.58	5
		H	0.62 (0.32)		4.32 (3.61)		0.55	5
	December	M	0.84 (0.18)*	0.424	0.85 (0.92)	0.408	0.88	5
		H	2.19 (0.71)		-8.33 (7.99)		0.76	5
chl a	October	M	1.03 (0.45)		0.50 (1.02)		0.64	5
		H	0.59 (0.32)		0.21 (0.18)		0.53	5
	December	M	0.75 (0.25)		0.84 (0.23)		0.75	5
		H	1.20 (0.32)*		0.07 (0.32)		0.82	5
chl c3	October	M	12.1 (19.4)		1.62 (5.02)		0.12	4
		H	0.03 (0.03)		0.03 (0.03)		0.54	3
	December	M	-0.03 (0.67)		0.08 (0.04)		0.01	4
		H	-0.11 (0.20)		0.05 (0.04)		0.09	3
Peridinin	October	M	0.66 (0.28)	0.406	0.35 (0.98)	0.552	0.65	5
		H	1.26 (0.28)*		-2.34 (3.61)		0.87	5
	December	M	0.88 (0.18)*	0.541	6.14 (32.3)	0.858	0.88	5
		H	0.33 (0.49)		4.44 (8.39)		0.14	5
19'-BF	October	M	-0.02 (0.06)		3.54 (4.06)		0.03	3
		H	-1.18 (1.17)		40.9 (18.0)		0.25	4
	December	M	0.23 (0.51)		5.45 (8.76)		0.06	3
		H	16.5 (6.34)		-22.1 (66.1)		0.69	3
Fucoxanthin	October	M	0.90 (0.10)**	0.374	19.9 (13.9)	0.226	0.96	5
		H	-0.19 (0.44)		64.9(21.5)		0.06	5
	December	M	0.49 (0.13)*	0.017*	12.2 (5.27)	0.082	0.82	5
		H	1.15 (0.17)**		-35.0 (105.2)		0.94	5
Neoxanthin	October	M	1.61 (1.68)		11.7 (33.9)		0.23	5
		H	1.10 (0.32)*		23.7 (64.3)		0.80	5
	December	M	0.99 (0.03)**	0.756	-0.25 (0.67)	0.728	0.99	3
		H	0.46 (1.01)		37.1 (58.3)		0.07	5
Prasincoxanthin	October	M	0.01 (0.10)		65.1 (17.3)		0.24	5
		H	0.12 (0.01)		29.4 (10.0)		0.35	5
	December	M	-0.12 (0.36)		5.55 (5.36)		0.04	4
		H	-0.08 (0.29)		20.7 (11.2)		0.02	5
Violaxanthin	October	M	-0.09 (0.25)		20.8 (12.1)		0.05	4
		H	0.79 (0.29)		-1.94 (6.29)		0.71	4
	December	M-H	0.10 (0.13)		0.82 (0.79)		0.08	4
		H	-8.28 (13.5)		0.07 (0.04)		0.11	5
19'-HF	October	M	-8.28 (13.5)		23.8 (14.3)		0.07	5
		H	-27.5 (59.1)		20.2 (13.3)		0.16	6
Alloxanthin	December	M-H	0.75 (0.60)		24.8 (23.2)		0.16	5
		M	0.50 (0.67)		28.1 (50.1)		0.76	5
	October	M	0.50 (0.67)		24.8 (23.2)		0.16	5
		H	0.69 (0.23)		28.1 (50.1)		0.76	5
	December	M	1.07 (0.25)*	0.793	-0.56 (2.29)	0.819	0.86	5
		H	1.17 (0.21)*		-9.2 (14.1)		0.549	0.91

Table 2 continued

Variable		Region	A	P	B	P	r ²	n
chl <i>b</i>	October	M	0.62 (0.19)		119.4 (55.1)		0.78	5
		H	0.62 (0.18)		44.9 (48.8)		0.79	5
	December	M	0.71 (0.07)**	0.014*	1.65 (6.85)	0.821	0.97	4
		H	0.46 (0.71)		116.0 (97.3)		0.12	5
DV-chl <i>a</i>	October	M-H	-0.36 (1.23)		0.01 (0.01)		0.01	3
Diat	October	M	0.57 (1.04)		4.55 (5.38)		0.09	5
		H	0.49 (0.21)		2.02 (1.61)		0.64	5
	December	M	1.45 (1.06)		2.71 (15.8)		0.39	5
		H	0.20 (1.17)		50.0 (62.1)		0.02	5
Dino	October	M	0.21 (0.20)	<0.001**	56.6 (37.8)	0.167	0.27	5
		H	0.40 (0.01)*		0.54 (0.32)		0.85	5
	December	M	0.66 (1.68)		47.9 (46.9)		0.05	5
		H	0.48 (3.80)		12.3 (14.8)		0.01	5
Eug	October	M	-0.02 (0.19)		3.32 (1.42)		0.01	5
		H	0.97 (1.26)		0.30 (0.46)		0.17	5
	December	M	2.98 (1.06)		-1.88 (3.10)		0.73	5
		H	0.11 (0.86)		5.13 (5.10)		0.01	5
Tin	October	M	0.37 (0.95)		3.06 (2.46)		0.05	5
		H	-0.42 (0.92)		6.54 (2.99)		0.07	5
	December	M	0.16 (0.71)		5.48 (4.53)		0.02	5
		H	2.27 (2.18)		-11.6 (53.8)		0.27	5
Cope	October	M	-0.24 (0.27)		0.26 (0.25)		0.21	5
		H	0.35 (0.51)		0.33 (0.35)		0.14	5
	December	M	0.49 (0.02)**	<0.001**	0.005 (0.02)	0.816	0.95	5
		H	0.16 (0.19)		0.05 (0.05)		0.19	5

M and H are outer and inner regions of EPB, respectively. Between parentheses is indicated one standard error. Determination coefficient (r^2) is calculated for each curve. Number of observations (n) is less than ten when photosynthetic pigments are not detectable (nd). The level of statistical significance of t Student comparison (P) between coefficient A and 1, and between B and 0 is indicated when linear regression slope is not zero

The linear fitting between microlayer and subsurface data is $\hat{X}_{\text{MIL}} = AX_{\text{SSW}} + B$

T temperature ($^{\circ}\text{C}$), S salinity (ups), NIT nitrate + nitrite concentration (μM), NH_4 ammonia concentration (μM), PO_4 phosphate concentration (μM), H_4SiO_4 silicate concentration (μM); concentration of HPLC pigments markers ($\mu\text{g l}^{-1}$) 10^3 , 19-BF $19\text{-butanoxyfucoxanthin}$, 19-HF $19\text{-hexanoxyfucoxanthin}$, $\text{DV-chl } a$, divinyl-chlorophyll a , $\text{chl } a$, b and c are chlorophyll a , b and c , respectively, Diat diatom abundance (cells ml^{-1}), Dino dinoflagellate abundance (cells ml^{-1}), Eug euglenophyte abundance (cells ml^{-1}), Tin abundance of loricate tintinnids (ind. ml^{-1}), Cope abundance of copepod nauplii (ind. ml^{-1})

*Significant differences at 95% confidence level when A is different from 0

**Significant differences at 99% confidence level when A is different from 0

and 4 haptophytes and crysophytes, had a greater proportion of samples with positive (MIL enrichment) Δ chlorophyll $c3$ values for the outer than for the inner part of the lagoon in both surveys. Peridinin, a pigment marker of dinophytes, had consistently higher values in subsurface samples than in MIL samples (Δ peridinin up to $-0.3 \mu\text{g l}^{-1}$) ($\% \Delta X+$ was 40 in all cases, Table 3).

In the outer region during both surveys, 19-BF , a pigment marker of type 2b and 4 haptophytes, crysophytes, and pelagophytes, had depletion in most of the MIL samples ($\% \Delta X+$ down to 0), and in the inner part of EPB there was essentially no difference between MIL and SSW ($\% \Delta X+$ = 0.50 and 0.75, Table 3). Fucoxanthin, a pigment marker of bacillariophytes, haptophytes, and crysophytes, generally had higher concentrations in the MIL than in subsurface samples (up to fourfold) in both surveys. However, in the outer

region and in December, a significant fucoxanthin depletion of MIL samples was detected with regression analysis (Table 2). Fucoxanthin was enriched in MIL samples particularly in the inner part of EPB in October ($\% \Delta X+$ up to 80, Table 3). Neoxanthin, a pigment marker for euglenophytes and chlorophytes, had a pattern comparable to that for fucoxanthin but a large fraction of the values measured for the inner part of the lagoon in December evidenced depletion (down to one-third) in the MIL with respect to values for subsurface samples (Table 3).

Prasinocanthin, a pigment marker of prasinophytes, was preferentially accumulated in the MIL of the outer part of EPB in October ($\% \Delta X+$ = 80, Δ prasinocanthin $\sim 2.8 \mu\text{g l}^{-1}$, Table 3) and vice versa in December ($\% \Delta X+$ = 25) In the inner part of EPB, the $\% \Delta X+$ did not show any particular tendency for enrichment nor

Table 3 Variability of photosynthetic pigment signatures of phytoplankton along the Estero Punta Banda

	chl <i>a</i>	chl <i>c</i>	Per	19'-BF	Fuco	Neo	Pras	Viol	19'-HF	Allo	chl <i>b</i>	DV-chl
Oct M	2.43 (0.84)	6.23 (5.57)	217.45 (85.26)	14.28 (NC)	122.64 (35.90)	39.68 (16.94)	74.85 (13.97)	29.44 (5.73)	535.88 (241.26)	39.32 (12.27)	277.15 (47.90)	30.10 (4.82)
SSW	1.87 (0.65)	0.29 (0.12)	277.05 (104.25)	67.48 (39.39)	114.03 (39.12)	17.38 (5.08)	124.40 (84.47)	40.18 (20.01)	24.75 (10.79)	28.89 (9.74)	252.83 (67.75)	3.51 (NC)
% $\Delta X+$	80	75	40	0	60	60	80	50	80	60	60	67
H MIL	0.50 (0.09)	0.09 (0.04)	11.30 (4.69)	53.39 (15.73)	56.43 (8.06)	21.37 (6.63)	39.63 (6.55)	17.60 (10.02)	18.88 (8.58)	15.47 (5.04)	179.30 (54.20)	11.48 (NC)
SSW	0.50 (0.11)	1.68 (1.61)	10.85 (3.48)	12.69 (10.77)	43.99 (10.18)	17.27 (5.40)	84.22 (32.00)	19.60 (8.10)	17.84 (8.09)	18.28 (6.34)	216.85 (78.03)	8.22 (0.36)
% $\Delta X+$	40	33	40	75	80	80	40	20	60	20	20	50
Dec M	0.69 (0.18)	0.09 (0.03)	125.25 (51.61)	20.50 (7.90)	29.95 (4.69)	36.75 (11.57)	10.02 (1.76)	3.29 (NC)	32.87 (NC)	7.56 (2.85)	72.56 (20.85)	NC
SSW	0.82 (0.20)	0.09 (0.05)	135.84 (55.32)	20.09 (6.84)	36.42 (8.73)	37.95 (10.37)	15.91 (3.13)	10.41 (7.19)	41.17 (NC)	7.61 (2.47)	216.85 (29.50)	NC
% $\Delta X+$	20	60	40	33	50	50	25	50	50	20	0	NC
H MIL	1.12 (0.33)	0.17 (NC)	15.16 (4.98)	208.27 (200.54)	422.97 (280.39)	62.24 (17.94)	18.28 (5.99)	3.92 (2.51)	54.08 (14.18)	55.30 (22.68)	171.85 (42.62)	NC
SSW	0.87 (0.25)	0.19 (0.15)	13.95 (5.09)	16.01 (3.91)	397.83 (235.94)	54.34 (9.94)	30.12 (11.54)	8.20 (NC)	18.89 (8.52)	55.27 (18.54)	216.85 (14.32)	NC
% $\Delta X+$	60	25	40	50	60	40	60	50	100	20	60	NC

M and H are outer (mouth) and inner regions (head), respectively. For each comparison, regional average (first row), standard error (second row) and percentage of samples with enrichment ($\% \Delta X+ = \#(X_{MIL} - X_{SSW} > 0) \times 100$) are summarized

Oct October, Dec December, MIL microlayer, SSW subsurface water, chl *a* chlorophyll *a* ($\mu\text{g l}^{-1}$), chl *c* chlorophyll *c* ($\mu\text{g l}^{-1}$), *Per* peridinin ($\mu\text{g l}^{-1}$), *19'-BF* 19'-butanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$), *Fuco* fucoxanthin ($\mu\text{g l}^{-1}$), *Neo* neoxanthin ($\mu\text{g l}^{-1}$), *Pras* prasinoxanthin ($\mu\text{g l}^{-1}$), *Viol* violaxanthin ($\mu\text{g l}^{-1}$), *19'-HF* 19'-hexanoyloxyfucoxanthin ($\mu\text{g l}^{-1}$), *Allo* alloxanthin ($\mu\text{g l}^{-1}$), *DV-chl* Divinyl-chlorophyll *a* ($\mu\text{g l}^{-1}$), *10*³, *NC* unable to compute standard error

depletion of prasinoxanthin in MIL samples, in both surveys. Violaxanthin, a pigment marker of raphidophytes, had depletion in MIL samples with respect to those of SSW in the inner region and in October, in all other cases there was neither a tendency for enrichment nor for depletion. 19'-HF, a pigment marker of type 4 haptophytes, had large percentages of $\Delta X+$ for the outer region in October and for the inner region in December ($\% \Delta X+$ equal to 100 and 80, respectively, $\Delta HF -0.45 \mu\text{g l}^{-1}$, enrichment up to >20-fold), in the other two occasions there was no particular tendency for enrichment. In most cases, alloxanthin, a pigment marker of cryptophytes, had higher concentrations in the subsurface layer (MIL depletion up to threefold) except in October in the outer part of EPB (Table 3). As it was mentioned above, in the outer region and in December, a significant chl *b* depletion of MIL samples was detected with regression analysis (Table 2). With the percentage of $\Delta X+$, chlorophyll *b*, a pigment marker of prasinophytes, chlorophytes, and euglenophytes also showed depletion in MIL samples in October in the inner part of EPB, and in December in the outer part (Table 3). DV-chl *a*, a pigment marker of prochlorophytes, was only detected in October and highest concentrations were measured in MIL samples (Table 3).

Diatoms were more abundant in the MIL than in subsurface samples in the outer region of EPB in December ($\% \Delta X+ = 80$, Δ diatom up to $29 \times 10^3 \text{ cells l}^{-1}$, Table 4). In the other cases, diatom abundances neither showed any tendency for enrichment nor depletion in the MIL. Dinoflagellates were less abundant in the MIL than in the subsurface samples throughout the lagoon in October (Δ dinoflagellates up to $-278 \times 10^3 \text{ cells l}^{-1}$ in the inner part), and vice versa in December ($\% \Delta X+$ up to 100, Table 4). During the whole study, *Prorocentrum micans* was the most abundant dinoflagellate in the MIL while *Lingulodinium polyedrum* was the most numerous in SSW. In general, pennate diatoms (e.g., *Cocconeis* sp., *Nitzschia* sp., *Amphora* sp., *Navicula* sp.) were more frequently observed in MIL than in SSW samples, and this difference was enhanced in December in the inner region of EPB. Accumulation of pennate diatoms in the MIL was generally associated with copious amounts of macroalgae and seagrass debris, cyanobacteria filaments, and crab larvae. A common finding on both sampling dates was the higher densities of euglenophytes in MIL than in SSW samples ($\% \Delta X+$ up to 100, Δ euglenophyte up to $11 \times 10^3 \text{ cells l}^{-1}$, Table 4) with an exception of the inner part in December where MIL samples were depleted of euglenophytes. Abundances of all phytoplankton groups in MIL and SSW

samples were not significantly correlated (Table 2), except dinoflagellates (inner region in October, Table 2). Also, the abundance of zooplankton grazers (loricate tintinnids and copepod nauplii) in MIL and SSW samples were uncorrelated in most of the cases but in December in the outer region (Table 2). In general, loricate tintinnids were often more abundant in the MIL (% $\Delta X+$ up to 80, Δ tintinnids up to 115.7×10^3 ind. l^{-1} , highest enrichment factor ~ 4.5 -fold), with the exception of the inner part of EPB in December (Table 4). Copepod nauplii densities tended to be higher in subsurface samples (% $\Delta X+$ down to 25, Δ copepods down to $-1,349$ ind. l^{-1} , and highest depletion in the MIL approximately ninefold in the outer part, Table 4).

Discussion

Despite the renewed scientific interest in MIL studies (Momzikoff et al. 2004; Agogu e et al. 2005; Obernosterer et al. 2005), characterization of phytoplankton groups living in the upper millimeters of the sea surface has received less attention. In the present study, vertical microstructure differences between MIL and subsurface phytoplankton assemblages were described for the first time using HPLC chemotaxonomic markers in a temperate coastal environment of the southern California Current System. Although the magnitude of these differences is expected to vary depending on sampling techniques (e.g., glass plate) (Hardy and Apts 1989), the general pigment enrichment/depletion trends observed for the MIL on both sampling dates may be realistic.

An important finding of this work was that photosynthetic pigment signatures in MIL and SSW samples were not necessarily correlated. That was the case for the pigment signatures of phytoplankton classes with relatively small sizes (picophytoplankton, $<5 \mu m$) and flagellates (19'-HF and 19'-BF of haptophytes, prasin-oxanthin of prasinophytes, and DV-chl *a* of phrochlorophytes). Consequently, for these specific phytoplankton classes, accumulation and depletion of cells in the MIL were not controlled by continuous upward transport of SSW cells due to physical factors (gradient producers) including eddy diffusivity, bubble transport, and density variations near the sea surface (Agogu e et al. 2005). Instead, differences in biological factors such as phytoplankton motility, differential autotrophic growth, and zooplankton grazing between MIL and SSW samples could have had a greater influence on MIL–SSW pigment distributions of small flagellates (Hardy 1982). Joux et al. (2006) studied the microbial structure in the sea surface MIL of coastal waters of the Mediterranean Sea and found that unlike other parameters (e.g., chl *a*, bacteria abundance), abundances of autotrophic nanoflagellates of MIL and SSW samples were not correlated. In this work, all photosynthetic pigment signatures of SSW samples were also detected in MIL samples, showing that phytoplankton assemblages in the MIL were originally derived from subsurface samples. Although qualitatively similar in terms of pigment type, MIL and SSW phytoplankton communities usually had different proportions of pigment markers. Therefore, it is reasonable to think of phytoneuston communities as a 'different combination' of subsurface phytoplankton groups. Analysis of environmental variables (temper-

Table 4 Variability of microphytoplankton and zooplankton abundances along the Estero Punta Banda

			Diat	Dino	Eug	Tin	Cope
October	M	MIL	7.10 (2.48)	80.12 (30.78)	3.20 (1.06)	3.94 (0.92)	420 (143)
		SSW	4.47 (1.31)	109.87 (74.57)	3.94 (3.28)	2.36 (0.55)	739 (276)
		% $\Delta X+$	60	40	80	80	40
	H	MIL	4.50 (1.72)	1.52 (0.47)	0.61 (0.22)	5.23 (0.81)	537 (172)
		SSW	5.02 (2.80)	2.46 (1.09)	0.31 (0.09)	3.11 (0.49)	584 (179)
		% $\Delta X+$	40	0	80	80	40
December	M	MIL	22.58 (6.92)	59.86 (31.89)	6.08 (2.10)	6.29 (2.31)	95 (58)
		SSW	13.66 (2.96)	17.92 (10.67)	2.68 (0.60)	5.19 (1.87)	169 (121)
		% $\Delta X+$	80	100	100	60	25
	H	MIL	61.80 (37.35)	13.85 (7.83)	4.58 (2.55)	36.63 (28.03)	106 (46)
		SSW	38.70 (18.30)	3.07 (1.18)	4.83 (1.70)	21.24 (6.37)	156 (113)
		% $\Delta X+$	40	60	40	20	67

M and H outer (mouth) and inner region (head), respectively. For each comparison, regional average (first row), standard error (second row) and percentage of samples with enrichment ($\% \Delta X+ = X_{MIL} - X_{SSW} > 0$) $\times 100$) are summarized

MIL microlayer, SSW subsurface water, Diato diatom (cells ml^{-1}), Dino dinoflagellate (cells ml^{-1}), Eug euglenophyte (cells ml^{-1}), Tin loricate-tintinnids (ind. ml^{-1}), Cope copepod nauplii (ind. l^{-1})

ature, salinity, and nutrients) did not show a clear spatial or temporal relationship with the photosynthetic pigment markers. Part of this lack of relationship can be attributed to the patchy horizontal distribution of phytoneuston communities along the lagoon on both sampling dates. The most obvious example was the formation of slicks and scums in the inner part of EPB, especially in December. The development of these surface features coincided with relatively low wind intensities and higher stratification (negative Δ salinity and positive Δ temperature values) between MIL and SSW layers at the head of the lagoon. Further analysis of slicks/scums of the inner region of EPB (data not shown) shows that these features are rich in fucoxanthin (up to 25-fold enrichment) and represent an abundant flora of benthic pennate and epiphytic diatoms from seagrass beds. Nutrients did not seem to be limiting for phytoneuston growth since nutrient enrichments of the MIL (inner part of EPB in October) were not accompanied by chl *a* (index of biomass buildup) MIL enrichments.

For each pigment marker, the large spatial heterogeneity in MIL enrichment/depletion conditions in the EPB on both sampling dates made it impossible to detect MIL–SSW differences between different regions or sampling dates. However, some patterns were evident when the percentage of samples with positive ΔX values was calculated. In general, peridinin values measured in this study were higher in subsurface samples where *L. polyedrum* was the dominant dinophyte. Unlike most of the dinoflagellates inhabiting the MIL of EPB (e.g., *Prorocentrum* sp., *Protoperidinium* sp., *Gymnodium* sp.), *L. polyedrum* is autotrophic and explains the apparent discrepancy between the large abundance of dinoflagellates and lower peridinin concentrations found in MIL samples. The ubiquitous distribution of *L. polyedrum* in our study area is not surprising since recurrent blooms of this autotrophic dinoflagellate have been reported for the EPB entrance (Peña-Manjarrez et al. 2001). Specific accumulation of *L. polyedrum* in subsurface samples was possibly caused by more favorable growth conditions, such as higher pH (optimum growth at pH >8.5, Hinga 1992) below the MIL. Since the MIL of coastal waters typically has a lower pH (pH < 7.8) with respect to that of SSW (Lin et al. 1998), a lower survival of *L. polyedrum* near the sea surface might be expected. In a different way, *Prorocentrum micans*, the dominant dinoflagellate of MIL samples, can tolerate a wider pH range and has mixotrophic metabolism (Hinga 1992) which allows it to survive better inside the MIL. Higher abundances of *L. polyedrum* in subsurface samples also coincided with

higher copepod nauplii abundances supporting previous observations in EPB by Montes-Hugo et al. (2004) who suggested that photosynthetic dinoflagellates are actively consumed by copepod nauplii.

Considering % ΔX + values, fucoxanthin concentrations were commonly higher in MIL than in SSW samples. However, microscopic counts showed that diatoms were not always accumulated in the MIL (e.g., in the inner region of the lagoon on both sampling dates). This apparent discrepancy is due to the fact that fucoxanthin is also a pigment marker for haptophytes, which were present at the head of EPB on both dates as inferred from 19'-HF values. The frequent occurrence of pennate diatoms in MIL samples of EPB on both dates confirmed previous observations in other coastal water bodies (Hardy and Valett 1981; De Souza Lima and Chretiennot-Dinet 1984; Hardy and Apts 1989). In the present study, high densities of euglenoids were generally detected in MIL samples as reported by Hardy (1971) who found mid-day accumulations of euglenophytes in MIL samples of a temperate marine lagoon, and proposed positive phototaxis as the main mechanism. Overall, the 19'-HF pigment signature of haptophytes such as *Phaeocystis* sp., was often enriched in MIL samples with respect to SSW samples. This observation confirmed conclusions obtained from fucoxanthin MIL–SSW distributions and indicates the importance of this phytoplankton group as a conspicuous member of phytoneuston communities of EPB. Although pioneer studies provided evidence for the dominance of phytoflagellates in the MIL of coastal waters (Hardy and Valett 1981; Hardy and Apts 1984, 1989), these contributions did not identify those phytoneuston groups.

Bacillariophytes and haptophytes have a preference for MIL 'niches'. The reason for this is unknown and we can only speculate about some possible factors such as transport from seagrass beds (e.g., pennate diatoms, Hardy 1982) and high ammonia concentrations (e.g., haptophytes, Lewitus et al. 1998). Further investigations are necessary to answer these questions and to determine the effect of different climatic conditions on phytoneuston communities, such as the increased runoff during El Niño years. Due to the patchiness of phytoneuston assemblages on both sampling dates in the present study, the development of high-resolution sampling devices is recommended to better characterize this horizontal variability.

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