

# Plankton in waters adjacent to the Laje de Santos state marine conservation park, Brazil: spatio-temporal distribution surveys\*

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## ABSTRACT

The coastal marine plankton plays a major role in ecosystem functioning by linking pelagic and benthonic environments through energy fluxes. Understanding the dynamic of planktonic organisms is also crucial for conservation and management purposes. Plankton was sampled at ten sites in the waters of the PEMLS and the adjacent area, on four different occasions through 2013 and 2015 in order to identify key planktonic groups and protocols for long-term monitoring. Ninety taxa of zooplanktonic organisms were found with holoplanktonic copepods and cladocerans dominating samples. Zooplankton biomass, mortality and taxonomic composition varied both in space and time. Surface chlorophyll-a concentrations varied spatio-temporally. A protocol for monitoring the plankton of the waters in and adjacent to the PEMLS is suggested based on biomass and mortality of zooplankton and biomass of phytoplankton using periodically in situ calibrated ocean color satellite imagery.

**DESCRIPTORS:** Marine Protected Area, Plankton Composition, Conservation, Laje de Santos, Monitoring.

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## RESUMO

O plâncton marinho costeiro é uma peça fundamental no funcionamento do ecossistema, conectando os ambientes pelágico e bentônico em fluxos de material e energia. A dinâmica dos organismos planctônicos, ou seja, suas composições e abundâncias no tempo e espaço, é uma ferramenta importante para práticas de conservação e manejo. Em quatro ocasiões entre 2013 e 2015, amostragens discretas de plâncton foram realizadas em dez pontos em e ao redor do PEMLS, com o objetivo de identificar grupos importantes e estabelecer protocolos para monitoramento a longo prazo. Foram encontrados 90 táxons zooplancônicos, sendo copépodes e cladóceros os grupos dominantes, como esperado. A biomassa, mortalidade e composição taxonômica do zooplâncton variaram entre os locais e entre as amostragens. As concentrações de clorofila-a superficial também variaram espaço-temporalmente e ilustram a limitação de amostragens discretas para algumas das variáveis testadas. Os resultados sugerem um protocolo de monitoramento do plâncton do PEMLS baseado na biomassa e mortalidade do zooplâncton. Já a biomassa do fitoplâncton pode ser estimada por análises in vivo de amostras de água do mar e imagens de satélite.

**Descritores:** Área de Proteção Marinha, Composição de Plâncton, Conservação, Laje de Santos, Monitoramento.

## INTRODUCTION

Marine Protected Areas (MPAs) are important conservation tools for maintaining marine ecosystems, which are being crescent altered by human impacts. The ultimate goal in designing and implementing MPAs is to create a network of protected areas that are connected through the active and passive dispersal of the organisms inhabiting those areas (GRORUD-COLVERT et al., 2014). Planktonic communities can affect biogeochemical cycles and the coupling of the benthic-pelagic system (KAMBURSKA; FONDA-UMANI, 2009). Changes in abundance and or composition of plankton (i.e., their dynamics) will impact pelagic production and affect the material and energy fluxes to nektonic and benthonic species (LESLIE et al., 2005; ROOHI et al., 2010). In addition, the drift of planktonic larvae may supply invasive species to both benthic and pelagic systems (WONHAM et al., 2001; OLENINA et al., 2010). Plankton is, therefore, a fundamental model group for multidisciplinary projects on ecosystem functioning, with important implications for the management and conservation of marine habitats. Recently, the scientific community started using whole plankton approaches to better describe temporal change in pelagic systems (e.g. ROMAGNAN et. al, 2015). Nonetheless, it is necessary to define key species and groups for a given environment.

Plankton communities are important to a better understand of bioinvasion, the benthic-pelagic coupling and the influence on benthic communities, as environmental bioindicators and for fisheries resources from local to regional scales. Previous oceanographic studies undertaken on the southeastern Brazilian coast have provided some information leading to an initial understanding of plankton by explaining circulation patterns and water mass distribution (MIRANDA; CASTRO-FILHO, 1989). Some studies have focused on how oceanographic processes can affect the pelagic food web through distribution patterns, composition and abundance of phytoplankton (BRANDINI, 1988), zooplankton (LOPES et al., 2006) and fishes (ANSANO et al., 1991; KATSURAGAWA; MATSUURA, 1992; KATSURAGAWA; EKAU, 2003), showing that physical oceanic features are responsible for structuring pelagic and benthonic communities. This region is affected by cold fronts, meteorological systems that change the physical forcings, wave height and larval transport on scales varying from days to weeks (MAZZUCO et al., 2015).

The understanding of plankton community and dynamics is a valuable tool for a link among scientific

knowledge, management and conservation. Here, a preliminary multidisciplinary observation was undertaken in the Laje de Santos Marine State Park (PEMLS) region, located in the southeastern Brazilian coast to aid on the design of future protocols and observations for improving the management and conservation of the park. The PEMLS is located near the port of Santos, the biggest in South America and which thus plays a central role in propagating bioinvasion. Despite the economic, social and environmental importance of this region, the biodiversity and spatial-temporal planktonic dynamic is still poorly known, as studies on the plankton of this region focused on specific taxons (e.g. MATSUURA et al., 1980, LUIZ et al., 2009). There are no systematic studies on plankton composition and dynamics in the PEMLS providing biological data for investigation into the link between plankton and the benthic, pelagic, physical or chemical environments, nor that serve to support management decisions. In this study, we sampled the plankton in the waters in and adjacent to the PEMLS on four different occasions in order to identify key groups and protocols for long-term monitoring. We intend to present a first set of data regarding composition, mortality, biomass of zooplankton and composition and biomass of phytoplankton such as will help managers and analysts to create standard conservation protocols.

## MATERIAL AND METHODS

### STUDY AREA

Sampling was carried out in waters in and adjacent to the Laje de Santos Marine State Park (PEMLS), located off Santos, São Paulo State, Brazil. The park is situated 42 km from the coast and its proximity to urban, industrial and port activities has reinforced the need for marine conservation. The park, the first marine park in São Paulo State, was created in 1993. Ten sites in the area both in and surrounding PEMLS were previously determined (Figure 1). Sites 1 to 4 are located outside the park. Sites 1, 2 are located near to rocky platforms, similar to the Laje of Santos, in proximity with estuaries and the Port of Santos, thus having a higher anthropic influence. Site 3 is also near a rocky platform, but far away from human discharges. Site 4 was selected because it receives the dragged material from the Port of Santos and it is equidistant of the Laje of Santos and the coastline. Sites 5 to 10 were randomly selected within the limits of the PEMLS by all the groups from the MAPELMS project.

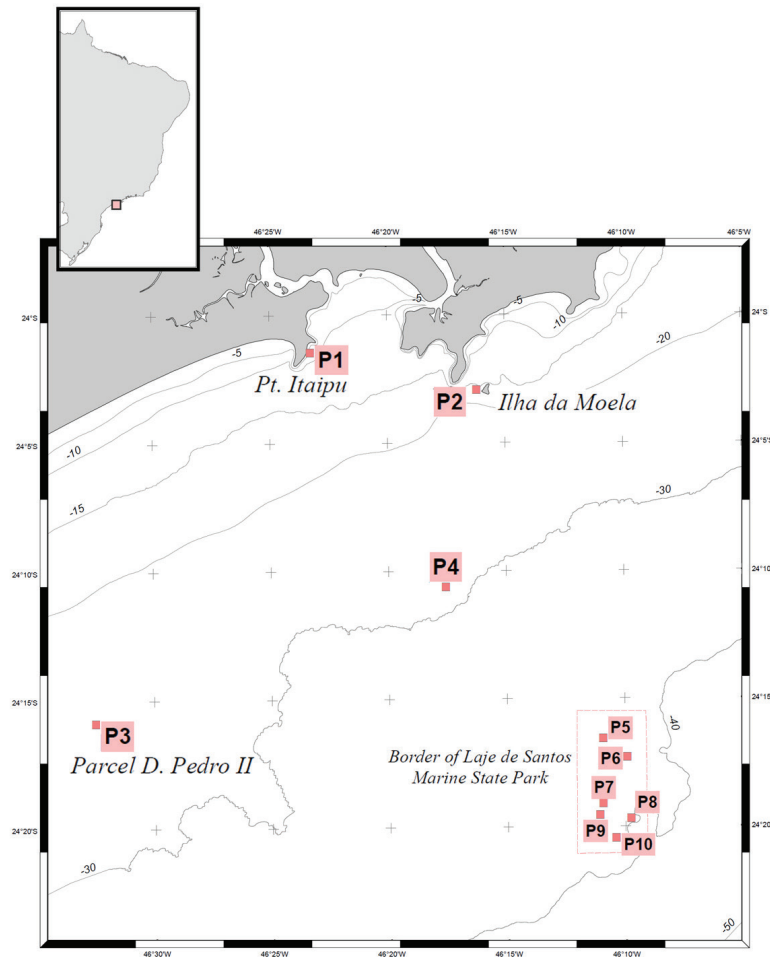


Figure 1. Map of the study area. Sites 1 to 10 are highlighted.

*SAMPLING*

Four sampling cruises were conducted during spring/2013, summer/2014, winter/2014 and summer/2015 at 10 sites in waters both inside and adjacent to the PEMLS. For zooplankton samples for density and diversity, three horizontal plankton tows were run at the surface and the bottom for each area, during 3 minutes using a 200µm-mesh net with an attached flowmeter (Sea-gear Corporation, model MF315). Samples were preserved in alcohol 70% and aliquots (1/8) were analyzed under the stereomicroscope. Zooplankton was identified to the lowest taxonomic level. Zooplankton density was calculated based on filtered sea water volume during tows.

Zooplankton total biomass and mortality were investigated from qualitative vertical tows with 3 tows per site for

each variable. Total zooplankton biomass was evaluated by sample volume displacement after 48h of decantation. Mortality was estimated by adding 1.5 ml of neutral red per 1L of concentrated zooplankton sample. Neutral red is a vital stain that stains bright red the live zooplankton whereas dead ones are unstained. Samples were stained for 15 min and preserved in formalin 4% in the fridge.

Phytoplanktonic biomass was estimated by collecting water at the surface, mid water and bottom using Van Dorn bottles at the 10 sites in waters in and adjacent to the PEMLS, with three replicates at each site. Two replicates were used for *in vivo* fluorescence analyses, the other replicate was immediately filtered (Watman GF/F filters) and extracted in acetone solution 90% and dimethyl-sulfate oxide (6:4 by volume). Extract fluorescence was read in a Turner Designs model Trilogy fluorimeter by the Welschmeyer method WELSCHMEYER (1994).

Spatial distribution of surface chlorophyll-a was investigated with ocean color images derived from the MODIS/Aqua sensor and ocean color algorithm OC3 (O'REILLY et al., 1998). Images from October 10, 2013; January 28, 2014; June 30, 2014 and January 17, 2015 were processed for level zero (L0) to level L2, using SEADAS version 7 and the atmospheric correction MUMM proposed by RUDDICK et al. (2000). The absolute chlorophyll values observed in the images should not be considered quantitatively (see CARVALHO et al., 2014) but help illustrate the large spatial variability of phytoplankton biomass in the region at a given time. It is important to keep in mind that these images are snap shots of minutes when the satellites pass over a given area.

In addition, phytoplankton diversity for organisms larger than 20  $\mu\text{m}$  was evaluated from sites 7, 8 and 10 of spring/2013 through vertical tows with 20  $\mu\text{m}$  mesh size. Total filtered volume was estimated from net mouth area and tow depth. Organisms were counted and identified to the lowest taxonomic level under an Olympus (mod. CKX41) inverted microscope. Harmful species were identified using the UNESCO Taxonomic Reference List (<http://www.marinespecies.org/hab/index.php>). Uthermol chambers were used to settle 2 ml of sample and cells were counted under an inverted microscope up to 400 individuals to normalize the occurrence of species.

### STATISTICAL ANALYSES

Zooplankton density, biomass and mortality data were analyzed according to a two-way analysis of variance with factors "time" (fixed, 4 levels: spring/2013, summer/2014, winter/2014 and summer/2015) and "site" (fixed, sites 1 to 10). Depth was not considered for these analyses, summing up 6 replicates for each factor combination. Data were transformed to natural log of (x+1) when homoscedasticity was not achieved. *A posteriori* comparisons were run using the SNK (Student–Newman–Keuls) test.

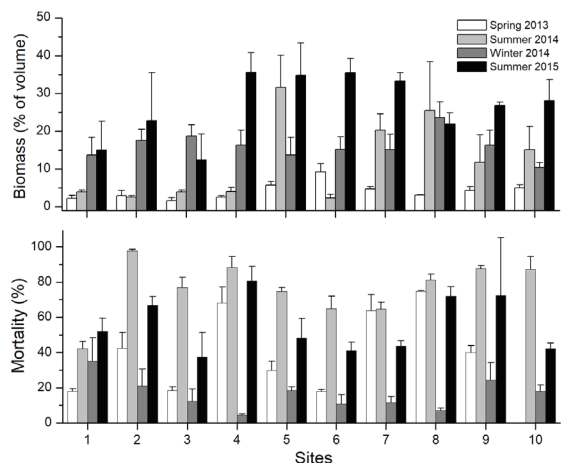
A PERMANOVA was run to investigate zooplankton composition using the same factors described above. The Bray-Curtis distance after 999 permutations was used. The taxonomic level used was class, since it was highly represented in our samples (16 classes). Classes found in only one sample (Tentaculata and Crinoidea) were removed from the analyses. The SIMPER test was used to detect the main classes underlying the formation of clusters and data were plotted on an nMDS. Box plots were used to show phytoplankton the biomass variation on each cruise.

## RESULTS

### ZOOPLANKTON

Zooplankton biomass and mortality varied spatial and temporally (Table 1). Biomass was lowest in spring/2013 and highest in summer/2015. Considering the spatial variation within the area covered by each cruise, no variation in biomass was observed among sites in spring/2013 and winter/2014. During the summer/2014, the highest values of biomass were observed at sites 5 and 8 and during summer/2015, the lowest value was obtained at site 3 (SNK test,  $p < 0.05$ ). Large temporal variation in biomass of zooplankton was detected in each site (Figure 2). Mortality was highest on both summer periods (2014 and 2015) with similar patterns among sites. Lower mortality values were detected in spring/2013 and winter/2014 (SNK test,  $p < 0.05$ ). Similar to biomass fluctuation, mortality of zooplankton also varied through time within sampling sites (Figure 2).

We found 90 taxa of zooplanktonic organisms belonging to Phyla Annelida, Arthropoda, Briozoa, Chaetognatha, Chordata, Cnidaria, Ctenophora, Echinodermata, Mollusca, Nematoda, Heliozoa, Ciliophora, Myozoa, Radiozoa and Foraminifera (Appendix 1). In general, all development stages, including eggs, larvae and adults, were found. The holoplanktonic copepods and cladocerans dominated all samples.



**Figure 2.** - Mean biomass and mortality of zooplankton at sites during the sampling events. Error bars represent standard error.

The relative abundance of the copepods was high in all cruises, totaling 78, 34, 50 and 67% during spring/2013, summer/2014, winter/2014 and summer/2015, respectively. Copepod density varied both spatially and temporally (Table

2). They occurred in all areas during the four sampling events, but the densities observed spring/2013 and summer/2014 were lower than those in winter/2014 and summer/2015. No differences were found among sites in spring/2013, but great variability in copepod density was detected during the other sampling events (Figure 3, (SNK test,  $p < 0.05$ ).

Cladocerans occurred on all the cruises with relative abundances corresponding to 5, 5, 19 and 21% for the four sampling events, respectively. The most abundant species was *Penilia avirostris* (Crustacea: Branchiopoda), with varying spatial and temporal distribution (Table 2). The highest density of *P. avirostris* occurred in winter/2014 and the lowest during spring/2013 (SNK test,  $p < 0.05$ ). Summer periods showed higher densities in sites outside the PEMLS (sites 1 to 4) while lower values were observed in the remaining sites (5 to 10). During the winter of 2014, when higher densities of *P. avirostris* were detected, these cladocerans dominated sites in the PELMS (sites 5 to 10; Figure 4).

A boom of heliozoans was observed in summer/2014, corresponding to 55% of sampled planktonic organisms concentrated at sites 6, 7, 9 and 10. They were absent in spring/2013 and summer/2015 and appeared in low relative abundance (0.6%) in winter/2014 (Appendix 1).

Zooplankton composition, in taxonomic level of class, varied between sampling events and sites (Table 3). Pair-wise comparisons indicated distinct compositions at sites 4, 6, 7 and 10 during each sampling event. No sites showed similar composition throughout the sampling events. Site 5 showed similar zooplankton composition for summer of 2014 and 2015. Despite great variability, zooplankton composition was similar on all sampling events and SIMPER results indicated Maxillopoda (85, 84, 56, 69%) and Branchiopoda (5, 10, 20 and 25%) as the major contributors to the formation of the groups on each event, respectively.

**Table 1.** ANOVA results for zooplankton biomass and mortality during the four cruises at the 10 sampling sites in or near the PEMLS. Significant values in bold.

Source of variation	Biomass				Mortality			
	M.S.	d.f.	F	p	M.S.	d.f.	F	p
Cruise	2619.7	3	37.66	<b>&lt;0.001</b>	17506.8	3	54.70	<b>&lt;0.001</b>
Site	201.5	9	2.90	<b>0.005</b>	1326.1	9	4.14	<b>&lt;0.001</b>
Cr x Si	135.4	27	1.95	<b>0.012</b>	876.2	27	2.74	<b>&lt;0.001</b>
Error	69.6	80			320.1	80		
	C = 0.1776; $p < 0.05$				C = 0.2509; $p < 0.01$			

**Table 2.** ANOVA results for copepods and *Penilia avirostris* densities during the four cruises at the 10 sampling sites in or near the PEMLS. Significant values in bold.

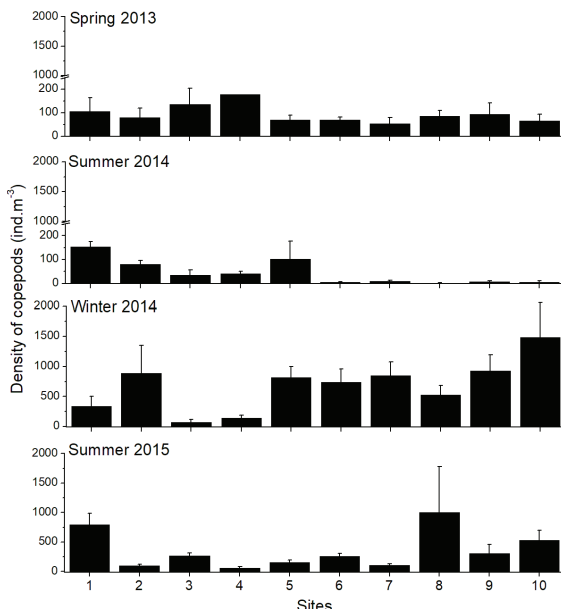
Source of variation	Copepods				<i>Penilia avirostris</i>			
	M.S.	d.f.	F	p	M.S.	d.f.	F	p
Cruise	151.20	3	94.47	<b>&lt;0.001</b>	156.77	3	146.56	<b>&lt;0.001</b>
Site	4.83	9	3.02	<b>0.002</b>	9.47	9	8.85	<b>&lt;0.001</b>
Cr x Si	9.84	27	6.15	<b>&lt;0.001</b>	9.15	27	8.55	<b>&lt;0.001</b>
Error	1.60	200			1.07	200		
	C = 0.1035; $p < 0.05$				C = 0.1223; $p < 0.01$			

**Appendix 1.** Relative abundance of zooplankton sampled at the 10 sites in the adjacent waters to the PEMLS on the four sampling events (C1: spring/2013; C2: summer/2014; C3: winter/2014 and C4: summer/2015).

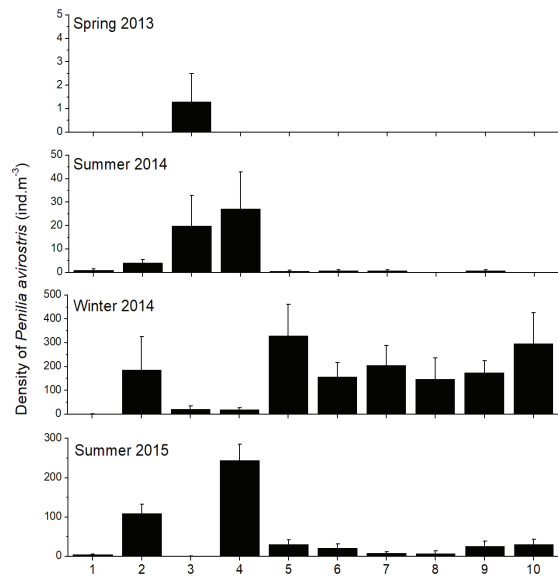
Kingdom	Phylum	Class	Order	Family	Genus	Species	% of individuals											
							C1	C2	C3	C4								
Animalia	Annelida	Polychaeta		Syllidae		Larva	0,052	0,000	0,000	0,035								
							0,001	0,000	0,002	0,000								
							0,001	0,000	0,000	0,000								
	Branchiopoda	(Cladocera)	Diplostraca		Podonidae	<i>Pseudevadne</i>	<i>P. tergestina</i>	1,122	0,000	0,189	0,000							
								3,692	0,000	0,000	0,000							
								0,000	0,029	8,896	2,295							
								Daphniidae	<i>Daphnia</i>	0,000	0,023	0,034	0,000					
										0,556	0,202	0,000	0,000					
								Sididae	<i>Penilia</i>	<i>P. avirostris</i>	0,008	0,000	0,000	0,000				
											0,000	5,230	10,039	18,789				
								Malacostraca		Amphipoda		Hyperiididae	<i>Hyperia</i>		0,850	0,000	0,000	0,000
															0,000	0,000	0,065	0,024
															0,000	0,009	0,000	0,000
	Gammaridae	<i>Gammarus</i>	0,000	0,000	0,036	0,016												
			0,008	0,000	0,000	0,004												
	Decapoda (Anomura)						Larva								0,000	0,000	0,005	0,000
															0,029	0,000	0,005	0,000
	Decapoda	Luciferidae	<i>Lucifer</i>				<i>L. typus</i>								0,000	0,085	0,009	0,000
															0,000	0,167	0,138	0,016
	Mysida	Mysidae													0,065	0,003	0,000	0,035
	40,183	13,012	3,524	1,945														
	Maxillopoda (Copepoda)		Calanoida		Poecilostomatoida	Corycaeidae	<i>Corycaeus</i>	37,330	20,674	38,554	60,248							
								0,000	0,325	7,466	4,633							
								Clausidiidae	<i>Hemicyclops</i>	0,000	0,000	0,144	0,000					
										0,029	0,000	1,141	0,531					
								Peltidiidae	<i>Clytemnestra</i>	<i>C. scutellata</i>	0,573	0,000	0,000	0,000				
											1,273	0,000	0,092	0,000				
								Maxillopoda (Cirripedia)						Nauplii	0,0162	0	0,568	0,110
															0,016	0,067	0,142	0,483
								Ostracoda	Halocyprida						0,023	1,057	0,086	0,000
															0,006	0,000	0,000	0,000
								Zoea	0,296	0,727	0,336	0,725						
Nauplii								3,171	0,094	0,916	0,534							
Other larvae								0,307	0,003	0,000	0,008							
Egg								0,787	0,164	0,000	0,000							
Briozoa							0,009	0,000	0,000	0,000								
Chaetognatha							0,078	0,489	3,545	1,185								

Kingdom	Phylum	Class	Order	Family	Genus	Species	C1	C2	C3	C4			
Animalia	Chordata (Tunicata)	Appendicularia						1,356	0,000	0,000	0,000		
				Oikopleuridae	<i>Oikopleura</i>		0,518	0,006	12,470	3,886			
		Thaliacea	Doliolida	Doliolidae	<i>Doliolum</i>		0,000	0,012	0,000	0,000			
			Salpida	Salpidae	<i>Thalia</i>	<i>T. democratica</i>	0,000	0,006	4,995	1,128			
	Chordata (Cephalochordata)						Larva	0,012	0,000	0,000	0,000		
							Egg	1,225	0,140	0,147	1,513		
	Chordata (Vertebrata)	Pisces					Larva	0,006	0,064	0,018	0,071		
							Juvenile	0,000	0,000	0,002	0,000		
									0,001	0,023	0,000	0,000	
	Cnidaria							0,008	0,530	0,002	0,000		
								0,002	0,009	0,025	0,000		
						Abylidae	<i>Abylopsis</i>	<i>A. eschscholtzi</i>	0,000	0,000	0,041	0,012	
							<i>Bassia</i>	<i>B. bassensis</i>	0,000	0,000	0,032	0,000	
								0,002	0,000	0,007	0,000		
								0,000	0,000	0,009	0,000		
						Diphyidae			0,000	0,000	0,005	0,000	
							<i>Chelophyes</i>	<i>C. appendiculata</i>	0,005	0,000	0,523	0,151	
								0,000	0,000	0,047	0,000		
						Trachymedusae	Rhopalomedusidae	<i>Aglaura</i>	<i>A. hemistoma</i>	0,000	0,000	0,020	0,000
							Geryoniidae	<i>Liriope</i>	<i>L. tetraphylla</i>	0,000	0,000	1,578	0,035
								0,000	0,307	0,000	0,004		
								0,001	0,000	0,000	0,000		
						Narcomedusae	Aeginidae	<i>Solmundella</i>	<i>S. bitentaculata</i>	0,000	0,000	0,016	0,000
						Anthoathecata	Hydractiniidae	<i>Podocoryne</i>		0,000	0,000	0,235	0,000
								0,000	0,000	0,023	0,000		
							Actinula larva	0,030	0,000	0,000	0,000		
	Ctenophora	Tentaculata	Lobata	Bolinopsidae	<i>Mnemiopsis</i>			0,000	0,000	0,009	0,000		
							0,000	0,000	0,005	0,000			
	Echinodermata	Asterozoa						Bipinnaria larva	0,000	0,000	0,000	0,020	
								Pluteus larva	0,140	0,000	0,000	0,024	
							0,073	0,088	0,271	0,397			
							1,263	0,000	0,000	0,000			
							0,000	0,243	0,000	0,000			
						0,002	0,000	0,000	0,000				
Mollusca	Bivalvia					Creseidae	<i>Creseis</i>	0,000	0,009	0,000	0,000		
						Creseidae	<i>Creseis</i>	<i>C. acicula</i>	0,000	0,000	1,610	0,063	
	Gastropoda	Thecosomata					Limacinidae	<i>Limacina</i>	0,000	0,006	0,016	0,000	
									0,000	0,000	0,007	0,020	
								0,001	0,000	0,000	0,000		
						Littorinimorpha	Carinariidae		0,000	0,000	1,346	1,014	

Kingdom	Phylum	Class	Order	Family	Genus	Species	C1	C2	C3	C4
	Nematoda						0,000	0,006	0,000	0,000
	Heliozoa						0,000	54,569	0,571	0,000
Chromista	Ciliophora	Oligotrichea	Choreotrichida	Strobilidiidae	<i>Strobilidium</i>		0,000	0,000	0,000	0,020
			Tintinnina				2,387	0,000	0,000	0,000
	Ciliophora	Oligohymenophorea	Sessilida	Zoothamniidae	<i>Zoothamnium</i>		0,000	0,003	0,007	0,004
Chromista	Myzozoa (Dinoflagellata)	Dinophyceae	Gonyaulacales	Ceratiaceae	<i>Ceratium</i>		0,494	0,000	0,000	0,000
							0,066	0,000	0,000	0,000
							0,002	0,000	0,005	0,000
	Radiozoa	Acantharia					0,172	0,015	0,000	0,000
(Rhizaria)	Foraminifera	Globobulimina	Rotaliida	Globigerinidae	<i>Globigerina</i>		0,000	0,000	0,014	0,000
Others							0,051	0,398	0,049	0,000



**Figure 3.** Mean density of copepods at sites during the sampling events. Error bars represent standard error.



**Figure 4.** Mean density of *Penilia avirostris* at sites during the four sampling events. Error bars represent standard error.

*PHYTOPLANKTON*

The survey during spring/2013 on sites 7, 8 and 10 for organisms larger than 20 μm, revealed a total of 139 phytoplanktonic taxa were Diatomacea dominated samples (Appendix 2). In general, the abundance of phytoplankton

cells per sample volume was higher at sites 7 (n = 597) and 10 (n = 412) than at site 8 (n = 148). *Coscinodiscos* was dominant at site 7, while at site 8 *Coscinodiscos* and *Chaetoceros cf. didymus* were the most abundant. At site 10, the cyanobacteria *Trichodesmium* occurred in greater abundance (Appendix 2).



**Appendix 2.** Abundance of phytoplankton (cells.L-1) sampled at the sites 7, 8 and 10 in the adjacent waters to the PEMLS in the spring of 2013.

Taxa	7	8	10
<b>Cyanobacteria</b>			
<i>Anabaena</i> sp01	774		
<i>Trichodesmium</i> sp01			6524
<b>Coccolithophore</b>			
Coccolithophore ni		16	
<b>Diatoms</b>			
<i>Actinoptychos senarius</i>	32		
<i>Asteromphalus</i> sp01		16	
<i>Bacteriastrum delicatulum</i>		346	
<i>Bacteriastrum hyalinum</i>		165	
<i>Bacteriastrum</i> sp01	48		
cf <i>Grammatophora</i> 01		16	
cf <i>Pleurosigma</i> 01	48		
cf <i>Pseudo-nitzschia</i> 01		66	
cf <i>Schröderella</i> 01	48		
cf <i>Skeletonema</i> 01		330	
cf <i>Thalassiosira</i> 01	1097		
cf <i>Thalassiosira</i> 01			315
<i>Chaetoceros cf decipiens</i>		214	
<i>Chaetoceros cf didymus</i>		1219	
<i>Chaetoceros coarctatus</i>			49
<i>Chaetoceros messanensis</i>		791	
<i>Chaetoceros</i> sp01	16	82	
<i>Chaetoceros</i> sp02		49	
<i>Chaetoceros</i> sp03		33	
<i>Climacodium frauenfeldianum</i>	16		
<i>Coscinodiscus cf alboranii</i>			24
<i>Coscinodiscus cf centralis</i>		33	
<i>Coscinodiscus cf concinnus</i>			24
<i>Coscinodiscus gigas</i>	161	16	
<i>Coscinodiscus</i> sp01	5612	1203	388
<i>Cyclotella</i> sp01	16		
<i>Delphineis</i> sp01	1677	115	
<i>Detonula</i> sp01	274	49	
<i>Diploneis</i> sp01	65	99	24
<b>Diatom</b>			
<i>Fragilariopsis doliolos</i>	919	313	
<i>Grammatophora cf adriatica</i>	65		
<i>Grammatophora</i> sp01			97
<i>Guinardia flacida</i>	32	132	
<i>Guinardia</i> sp01	16		
<i>Guinardia striata</i>		214	
<i>Haslea</i> sp01		16	24
<i>Hemiaulus hauckii</i>		16	
<i>Hemiaulus membranaceae</i>	355	66	
<i>Hemiaulus sinensis</i>	145	16	243
<i>Hemiaulus</i> sp01	16		
<i>Hemidiscus cuneiformis</i>			24
<i>Hemidiscus</i> sp01	16	82	
<b>Taxa</b>	<b>7</b>	<b>8</b>	<b>10</b>
<i>Leptocylindrus minimus</i>	97		
<i>Lioloma pacificum</i>	161	33	
<i>Meuniera membranaceae</i>	661	363	24
<i>Navicula cf septentrionalis</i>			97
<i>Nitzschia cf lorenziana</i>	16	16	
<i>Nitzschia membranaceae</i>	16	16	
<i>Odontela sinensis</i>	32		
<i>Palmeria</i> sp01	32		
<i>Paralia sulcata</i>	32	148	
Pennate ni01	32		
<i>Pleurosigma</i> sp01	32	49	
<i>Pleurosigma</i> sp02		33	
<i>Pseudo-nitzschia</i> sp01		49	
<i>Pseudoeunotia doliolos</i>			121
<i>Rhizosolenia cf fragilissima</i>		115	
<i>Rhizosolenia cf pugens</i>		115	
<i>Rhizosolenia cf setigera</i>	81		
<i>Rhizosolenia robusta</i>	48	115	
<i>Rhizosolenia</i> sp01		82	
<i>Rhizosolenia</i> sp02			24
<i>Stephanopyxis turris</i>		16	
<i>Thalassionema nitzschoides</i>	419	412	146
<b>Diatom</b>			
<i>Thalassionema</i> sp01	32		
<i>Thalassionema</i> sp02	32		
<i>Thalassionema</i> sp03	32		
Thalassionemataceae		16	
<i>Thalassiosira cf deliculata</i>		16	
<i>Thalassiosira concaviuscula</i>	677	379	
<i>Thalassiosira rotula</i>			24
<i>Thalassiosira</i> sp02	113	428	315
<i>Thalassiosira</i> sp03	532	66	170
<i>Thalassiosira</i> sp04	16		
<i>Thalassiothrix frauenfeldi</i>			49
<b>Dinoflagellate</b>			
<i>Alexandrium cf fraterculus</i>			267
<i>Alexandrium</i> sp01	113		
<i>Alexandrium</i> sp02		49	
<i>Ceratium azoricum</i>	97	33	
<i>Ceratium cf horridum</i>		33	
<i>Ceratium cf vultur</i>	16		
<i>Ceratium furca</i>	355	82	146
<i>Ceratium fusus</i>	16		73
<i>Ceratium horridum</i>	65		73
<i>Ceratium inflatum</i>	32		
<i>Ceratium macroceros</i>	16		
<i>Ceratium</i> sp01	48		
<i>Ceratium teres</i>		16	
<i>Ceratium trichoceros</i>	32		
<i>Ceratium tripos</i>	194	33	
cf <i>Gambierdiscus toxicus</i>			24
cf <i>Prorocentrum</i> 01	32		
cf <i>Prorocentrum</i> 02	16		
cf <i>Pyrophacus</i> 01	81		

Appendix 2 cont.

Taxa	7	8	10
cf <i>Triposolenia</i> 01			24
Cyst	161		
<i>Dinophysis acuminata</i>	16		
<i>Dinophysis caudata</i>	48	49	73
<i>Dinoflagellate</i>			
<i>Gonyaulax</i> sp01			49
<i>Gonyaulax</i> sp02			24
<i>Gymnodiniales</i>	32	16	
<i>Ornithocercos</i> sp01	16		
<i>Peridinales</i>	226	33	
<i>Peridinium</i> cf <i>quarnerense</i>			121
<i>Peridinium</i> cf <i>steinii</i>	210	16	170
<i>Phalacroma rotundatum</i>			49
<i>Podolampas bipes</i>			24
<i>Podolampas</i> sp01	81	33	
<i>Prorocentrum</i> cf <i>balticum</i>	113	33	
<i>Prorocentrum</i> cf <i>ermaginatum</i>	16		
<i>Prorocentrum</i> cf <i>magnum</i>		16	
<i>Prorocentrum</i> cf <i>minimum</i>	16		
<i>Prorocentrum compressum</i>	403	33	146
<i>Prorocentrum micans</i>			49
<i>Prorocentrum</i> sp01	16		
<i>Protoperidinium</i> cf <i>oblongum</i>	113		
<i>Protoperidinium</i> cf <i>obtusum</i>	16		
<i>Protoperidinium</i> cf <i>pentagonum</i>	65		
<i>Protoperidinium crassipens</i>	48		
<i>Protoperidinium divergens</i>	16		73
<i>Protoperidinium grande</i>			24
<i>Protoperidinium oblongum</i>		33	
<i>Protoperidinium ovatum</i>			24
<i>Protoperidinium pentagonum</i>		16	
<i>Protoperidinium steinii</i>	145		
<i>Protoperidinium</i> sp01		16	
<i>Pyrocystis lunula</i>	48	16	24
<i>Pyrophacus</i> sp01			49
<i>Scrippsiella</i> cf <i>trochoidea</i>			49
Protozooplankton			
<i>Ebria</i> sp01			24
<i>Hermesinium</i> sp01	258	99	315
<i>Vorticella</i> sp01	274		

Phytoplanktonic biomass varied among sampling events and the highest variation was observed during summer/2014 (Figure 5). The surface chlorophyll-a concentration attained higher values close to the shore, and the concentration decreased with distance from the coast (Figure 6), as expected. We observed relatively high values of chlorophyll-a (above 5mg.m<sup>-3</sup>) in October 2013 and June 2014, coinciding with the first (spring/2013) and the third (winter/2014) sampling events, respectively.

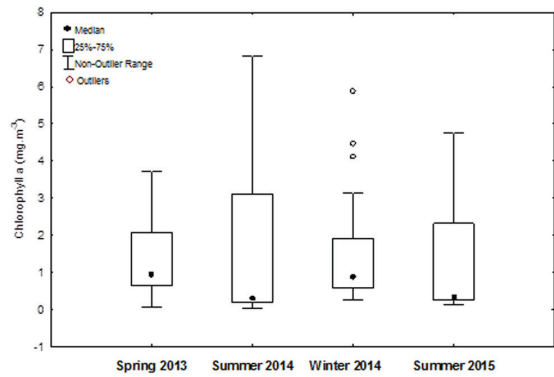


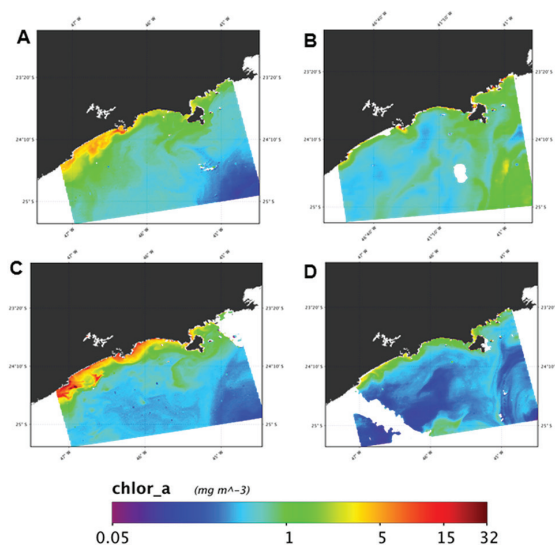
Figure 5. Variation in chlorophyll a from phytoplankton of the PEMLS during the sampling events.

DISCUSSION

Plankton in the PEMLS showed high diversity and spatio-temporal variability. Spatially, much variation was observed in biomass and mortality rates and no local interferences seem to affect these variables. Considering the importance of a wide monitoring programme for a MPA with a protocol with fast results in case of environmental impact, the biomass and mortality of zooplankton served as good indicators for monitoring temporal plankton dynamics, due to the easy feasibility and temporal changes being higher during the summer sampling events (2014 and 2015). Although it is unclear which drivers would be influencing such variation, we can notice that the higher variability in the summer occurred at the same time of the highest variability in the phytoplanktonic biomass. Here we present initial data for this MPA, and it is important to indicate as a support for the design of a specific long term programme to understand the dynamics and integration of the planktonic system and environmental drivers factors.

Our results present a great biodiversity in this area and some potential groups to be used as indicators of the plankton dynamics. In this case, it is important to consider the extremes groups: the most abundant, and the most variable ones. Diatomacea dominated the phytoplankton samples while Copepods (Crustacea: Maxillopoda) and cladocerans (Crustacea: Branchiopoda) dominated throughout the sampling cruises, as had occurred in other studies undertaken in Brazilian coastal waters (DOMINGOS-NUNES; REGALLA JR., 2012; LOPES, 2007; REGALLA JR., 2011). Copepods and cladocerans high densities in all sites and seasons suggest that these crustaceans may be an

important indicator of physical conditions in areas in and adjacent to the PEMLS. Cladocerans distribution, specifically, can indicate the role of water masses (as stated, e.g., by MUXAGATA; MONTÚ, 1999) as important factors in zooplankton distribution for management questions. Among the cladocerans, *Penilia avirostris* dominated in the samples. Peaks during summer and autumn have been reported for this species in temperate areas (CALBET et al., 2001). However, we found higher densities during the winter/2014. As the main components of zooplankton, Copepods and Cladocerans are potential indicators for the zooplankton dynamics and the focus on their population dynamics will be an important tool for monitoring the pelagic system at this region.



**Figure 6.** Spatial distribution of surface chlorophyll in the inner and middle continental shelf off São Paulo State (A) October 10, 2013; (B) January 28, 2014; (C) June 30, 2014 and (D) January 17, 2015.

However, it is important to highlight the importance of the less abundant groups and those with larger variability. In this case, such groups would indicate changes in the pelagic system that deserves attention of the management of the area. Here, we presented initial data to start to understand such dynamics. The bloom observed for heliozoans may be explained by the existence of an intermittent planktonic stage for these organisms, forming blooms during the hotter months (GIERE, 2009). Their restricted spatial and temporal distributions, encompassing just four sites during one sampling event (summer/2014), reinforce

the bloom explanation. A new bloom was expected in the following summer (2015), but we did not observe it. Based on the first observations, it is indicated for the further long term programme to monitor this group in order to evaluate their link with climatic drivers or also, changes in food web dynamics.

There is great spatial heterogeneity in the pelagic environment, seeing that organisms are patchily distributed (VALIELA, 1995). Patches are formed by both physical processes in the water column, such as Langmuir circulation cells or internal waves (SHANKS, 1995), and biological processes like synchronized larval release (EPIFANIO, 2003; STEVENS, 2003; PETRONE et al., 2005), vertical migration, predator avoidance, feeding and reproduction (FOLT; BURNS, 1999). In this way, even frequently replicated sampling may not answer specific questions, but general patterns can be found.

Marine plankton has been suggested as a key to identifying changes in marine ecosystems, especially those related to climate issues (HAYS et al., 2005). We present here specific data on the spatio-temporal dynamics of plankton in this MPA as a preliminary basis for the drawing up of plans for the monitoring and management of this area. Based on this first evaluation, we suggest a simple and quick protocol for the monitoring based on the biomass and mortality of zooplankton and the biomass of phytoplankton using periodically in situ calibrated ocean color satellite imagery.

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