Catarina da Rocha Marcolin

**Plankton and particle biomass size spectra
on the Southwest Atlantic: Case studies in
tropical and subtropical areas**

Thesis submitted to the
Oceanographic Institute of the
University of São Paulo, in
partial fulfillment of the
requirements for the degree of
Doctor of Sciences in Biological
Oceanography.

Supervisor: Dr. Rubens M. Lopes

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Corrected Version

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Certified in __/__/____, by

Supervisor: _____

Dr. Rubens Mendes Lopes

_____ Grade

Committee member: _____

Dr.

_____ Grade

Committee member: _____

Dr.

_____ Grade

Committee member: _____

Dr.

_____ Grade

Committee member: _____

Dr.

_____ Grade

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Abstract

This thesis is centered on the application of the Normalized Biomass Size Spectra (NBSS) theory to the study of plankton systems in shelf and oceanic areas of the tropical and subtropical Southwest Atlantic. I evaluated NBSS parameters over different environmental settings and their utility as proxies for system in Brazilian waters. The LOPC and the ZooScan are recently developed optical systems to automatically detect and measure plankton and particle size distributions *in situ* and in laboratory, respectively. I present two case studies: the first deals with the spatial variability over the Abrolhos Bank and adjacent oceanic areas in tropical latitudes, and the second focuses on temporal variability of plankton communities along a 5-year time series on a fixed station on the inner shelf at a subtropical location (Ubatuba, São Paulo). The data sets consisted of vertical profiles obtained with a LOPC and plankton preserved samples collected with a 200- μ m-mesh net. I observed in both data sets the accumulation of small particles (< 1 mm) above and within the pycnocline. NBSS slopes and intercepts were significantly different according to the contrasting environmental conditions observed in both areas; higher intercepts and steeper slopes were associated with higher productivity. The results highlight: i) water-column stratification as a key feature driving particle and plankton vertical distribution, ii) NBSS parameters as indicators of different environmental settings, and iii) that the cold and nutrient-rich South Atlantic Central Water (SACW) have an important role structuring the zooplankton size distributions over the Abrolhos Bank and vicinities and off Ubatuba. The NBSS parameters associated with information on plankton composition and distributions provided important information to evaluate the influence of

oceanographic forcing on plankton dynamics in distinct ecosystems of the Southwest Atlantic.

Key-words: NBSS, plankton, stratification, productivity, Southwest Atlantic, Brazil

RESUMO

O tema central desta tese é a aplicação da teoria do espectro de biomassa normalizado (NBSS, em inglês) no estudo de sistemas planctônicos em áreas costeiras e oceânicas, no Atlântico Sudoeste tropical e subtropical. Eu avaliei os parâmetros do NBSS em diferentes situações ambientais na Plataforma Continental Brasileira e a utilidade desses índices para inferir sobre produtividade. O LOPC e o ZooScan são sistemas ópticos recentemente desenvolvidos para detectar e mensurar a distribuição de tamanhos de partículas e zooplâncton *in situ* e em laboratório, respectivamente. Eu apresento dois estudos de caso: o primeiro lida com a variabilidade espacial sobre o banco de Abrolhos e áreas adjacentes oceânicas em latitudes tropicais, enquanto o segundo enfatiza a variabilidade temporal de comunidades planctônicas ao longo de 5 anos numa estação fixa na plataforma interna em uma localidade subtropical (Ubatuba, SP). O conjunto de dados consistiu de perfis verticais obtidos com o LOPC e com rede de plâncton, malha de 200- μ m. Eu observei o acúmulo de partículas < 1 mm na picnoclina e acima desta de forma consistente. Ambos inclinação e intercepto das retas ajustadas ao NBSS responderam às condições ambientais contrastantes em ambas as áreas; associamos interceptos maiores e inclinações mais negativas com maior produtividade do plâncton. Os principais resultados indicam: i) que a estratificação é um fator chave na distribuição vertical de partículas e do plâncton, ii) os parâmetros do NBSS como indicadores de diferentes condições ambientais e iii) que a Água Central do Atlântico Sul (ACAS, fria e rica em nutrientes) tem um importante papel na estruturação da distribuição de tamanhos acima do Banco de Abrolhos e proximidades e ao largo de Ubatuba. Os parâmetros do NBSS, associados com informações sobre a composição taxonômica e distribuição do

plâncton foram importantes para avaliar a influência de feições oceanográficas sobre a dinâmica do mesozooplâncton em diferentes ecossistemas no Atlântico Sudoeste.

Palavras-chave: NBSS, plâncton, estratificação, produtividade, Atlântico Sudoeste, Brasil.

Overview

1 Overview

The technology used to obtain biological oceanographic data is being developed extremely fast in the last decades, including plankton-observing systems. Measuring plankton size and abundance is a time consuming task and devices such as the Laser Optical Particle Counter (LOPC) and the ZooScan are examples of useful tools providing quick and reliable estimates of plankton size and abundance. As a key feature of aquatic ecosystems, seston and plankton size distribution can be analyzed within the scope of the Normalized Biomass Size Spectra (NBSS) concept. The main focus of this thesis is on the NBSS approach as a tool to analyze spatial and temporal variability of seston and plankton communities on Brazilian waters, in response to oceanographic forcing.

In chapter 1 I present a brief overview on the NBSS theory, followed by a summary of recently developed technologies capable of estimating NBSS metrics both *in situ* and in the laboratory using automatic or semi-automatic methods. In Chapters 2 and 3 I report on two case studies based on the NBSS approach; these chapters are formatted as manuscripts for publication. In Chapter 2 I described the spatial variability of plankton and particle size spectra on the Abrolhos Bank and vicinities, and associated NBSS parameters with ecological properties of this tropical ecosystem. In Chapter 3 I focused on the size spectra variability along a 5-year time series at a fixed station on the inner shelf off Ubatuba, subtropical Brazil. Finally, an annex to this thesis provides details on data treatment with the ZooScan and the LOPC; the two instruments applied in the present study, and on the size spectra calculations.

1.1 Plankton biomass size spectrum

Size is an important parameter for characterizing plankton communities because of its direct influence on physiological rates (Ikeda 1974, Platt and Denman 1978). For instance, photosynthetic efficiency and growth rates are governed to a large extent by phytoplankton cell size. Because of their high surface-to-volume ratio, small cells harvest light and nutrients more efficiently and, therefore, have higher growth rates than large cells (Geider *et al.* 1986), which is an advantage under low light and low nutrients conditions. On the other hand, large phytoplankton cells have an advantage under high irradiance (Cermeño *et al.* 2005) and high nutrient levels (Maguer *et al.* 2009).

Size also governs a wide range of ecological processes and interactions (Bautista and Harris 1992). A striking example is the predator-prey body size relationship, since predators usually feed on prey smaller than themselves (Cohen *et al.* 1993, Hansen *et al.* 1994), although exceptions were described in planktonic trophic webs (Landry *et al.* 1958).

Sheldon *et al.* (1972) were the first to identify regular patterns on the size structure of different pelagic communities. Since then, theoretical models have explored the biomass flow from small to large organisms in pelagic systems (Kerr 1974, Platt and Denman 1978). In addition, size-based indices have been widely used, for example: i) to contrast the structure of plankton communities from different inland lakes with varying productivity (Sprules and Munawar 1986); ii) in models to test the response of plankton size structure to perturbations in the environment (Gin *et al.* 1998); iii) to predict trophic transfer efficiency and mean predator-prey body-mass ratios in

marine food webs (Jennings et al. 2002), and iv) to evaluate the impact of mesoscale physical processes on food web dynamics (Basedow et al. 2010). The application of such indices have now a solid empirical foundation (Krupica et al. 2012).

The biomass size spectrum is the distribution of biomass frequencies grouped by individual weights, normalized by the bin size also estimated in weight (Zhou, 2006), with units in m^{-3} .

$$\text{Biomass spectrum} = \frac{\text{biomass } (\mu\text{g C m}^{-3}) \text{ in the size interval } (\Delta w)}{\text{size interval } \mu\text{g C } (\Delta w)}$$

The Normalized Biomass Size Spectrum (NBSS) is plotted on a log-log scale as in Figure 1-1. The theory of the NBSS (Kerr and Dickie 2001) relates the slope of a linear regression fitted to the spectrum to biomass production rates, energy transfer efficiency and predator-prey interactions. Zhou (2006) argues that the NBSS slope is determined by the number of trophic levels and by energy assimilation efficiency. Hence, the NBSS slope provides relevant information about trophic structure and is an inherent property of plankton communities. The NBSS intercept is determined by the abundance of organisms in the lower range of the size spectra, and is associated with system productivity (Cyr and Peters, 1996; Bianchi, 2000).

Figure 1-1 - Log Normalized Biomass Size Spectrum distribution. The NBSS parameters are the slope (a) and intercept (b) of the linear regression.

Kerr and Dickie (2001) argue that in different environments, with distinct dominant species, similar abundance domes may exist on the biomass spectra, meaning that such structures develop independently of community composition. Therefore, ecosystems should consist of functional species groups, where the trophic function is conserved through the species that find the opportunity to develop during the different succession stages of a community.

In the last 20 years, the biomass size spectra and derived indices have been used for different purposes: (i) to estimate population dynamics parameters, including *in situ* weight-specific growth rates and intrinsic growth rates (Zhou and Huntley 1997); (ii) as an indicator of nutrient enrichment in estuaries, where slopes would reflect bottom-up and top-down effects (Moore and Suthers 2006, Stock et al. 2008); (iii) to evaluate plankton community structure in relation to water types, and examine transport and exchange of different populations between

coastal and oceanic waters (Zhou *et al.* 2009), and (iv) in models to explain the influence of coupling between predator and detritivore communities (Blanchard *et al.* 2009).

Plankton productivity is usually estimated by time-consuming methods (Zhou 2006), which may be severely biased in communities with numerous species and trophic links. In tropical and subtropical plankton communities the size spectra is wide-ranging and include many trophic levels (Hopcroft *et al.* 1998), causing difficulties in the estimation of primary production and grazing impact because of trophic cascade effects (Calbet and Saiz 2013).

In this sense, the NBSS is an important parameter to be considered in the study of plankton communities because it integrates a set of biological responses to environmental factors and inter- and intraspecific relationships (Ye *et al.* 2013). On the other hand, Petchey and Belgrano (2010) argue that treating organisms as particles differing only in size can be often controversial, and not well integrated with taxonomy-focused research. Plankton communities are highly variable systems, and integrating taxonomy with size-based approaches is crucial to understand their dynamics.

1.2 Recent technologies applied to plankton and seston characterization

Wiebe and Benfield (2003) presented an extensive review on the evolution of techniques and instrumentation applied to plankton abundance and distribution studies, from the Hensen net in the late 19th century to multiple sensor systems and remotely operated vehicles into the 21st century. More recently Benfield *et al.* (2007) summarized

the progresses achieved in the development of plankton imaging systems over the last decades, as exemplified in Table 1-1.

Despite the unquestionable technological advances of plankton detection systems, it is still challenging to estimate particle and plankton assemblages with sufficient accuracy, resolution and regularity to understand their variability (Checkley *et al.* 2008). In addition, most imaging systems mentioned in Table 1-1 requires the use of automated or semi-automated classification software, which represents another significant challenge. Plankton organisms are morphologically diverse both in phylogenetic and in ontogenetic terms, their size span through orders of magnitude, body composition vary from hard-shelled species to extremely fragile forms, and constraints related to sensor resolution and animal orientation brings even more complexity to the task (Benfield *et al.* 2007). Nevertheless, although the taxonomic resolution will undoubtedly vary within and among groups, it is now possible to achieve species-level identification using certain benchtop and *in situ* systems, as well as to accelerate processing of plankton samples, and to detect seston (including fragile aggregates) and plankton with improved spatial and temporal resolution.

Table 1-1 Summary of recently developed optical devices and its application. LB: Lab-based; IS: In-situ.

Instrument	Laboratory-based devices	Size range	Reference
FlowCAM	A flow cytometer and microscope to analyze discrete and pumped samples in the phyto- and microzooplankton size range	3-300 μm	Sieracki et al. (1998)
ZooScan	Water-proof scanner for preserved samples that allows semi-automatic mesozooplankton counting and identification	>300 μm	Grosjean et al. (2004)
FlowCytobot and Imaging FlowCytobot	A submersible flow cytometer that uses fluorescence and light scattering signals from a laser beam. Latest: 10-100 μm .	~1-10 μm	Olson et al. (2003); Olson and Sosik (2007)
Instrument	In-situ devices	Size range	Reference
In situ CritterCam	Camera system for capturing videos of organisms.	5 μm - 5 cm	Strickler et al. (1997)
HOLOCAM	Holographic recording: obtains 3-dimensional images with a 10- μm resolution	1 μm - 1000 μm	Craig et al. (2000)
SIPPER	Shadowed Image Particle Profiler and Evaluation Recorder. High-speed digital cameras for sizes ranging from	200 μm - several cm	Samson et al. (2001)
ZOOVIS	Zooplankton Visualization System. High-speed digital.	200 μm - several cm	Benfield et al. (2003)
LOPC	Laser Optical Particle Counter. Provides continuous real-time information on mesozooplankton size and abundance.	100 μm - 35 mm	Herman et al. (2004)
LAPIS	Large-Area Plankton Imaging System. Video-imaging of macrozooplankton	0.5 cm - 1m	Madin et al. (2006)
ISIIS	In Situ Ichthyoplankton Imaging System. Digital line scan camera, with a 68- μm resolution, coupled to environmental sensors, such as CTD and fluorometer	1 mm - 12 cm	Cowen and Guigand (2008)
Fido- ϕ	Two-dimensional planar laser imaging system, mounted on a free-falling platform, to quantify properties of large fluorescent particles, obtaining images every 10-30 cm	~20 μm - 2 cm	Franks and Jaffe (2008)

The Laser Optical Particle Counter (LOPC, Brooke Ocean Technologies, Dartmouth, Nova Scotia) has been used to estimate seston abundance and size *in situ*, with special emphasis on copepods (Checkley *et al.* 2008, Herman and Harvey 2006) and marine snow (Petrik *et al.* 2013). The ZooScan extracts images from preserved plankton samples and a semi-automatic recognition software provides identification, counting and sizing information for particles larger than 0.3 mm (Grosjean *et al.* 2004).

The combined use of LOPC and ZooScan have the potential to improve our understanding on how physico-chemical factors, induced by the seasonality of current systems, are related to changes in the size spectra, diversity and energy flow through zooplankton communities (Schultes and Lopes 2009).

2 Plankton and seston size spectra estimated by the LOPC and ZooScan in the Abrolhos Bank ecosystem (SE Atlantic)

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2.1 Abstract

The biomass size spectrum provides valuable information about the functioning of plankton systems. We evaluated hydrographic and bathymetric influences on biomass size spectra and on vertical distributions of plankton and seston above the Abrolhos Bank and in adjacent oceanic areas off Eastern Brazil. We used both *in situ* Laser Optical Particle Counter (LOPC) and preserved plankton samples analyzed with a ZooScan system to determine seston and plankton abundances, size distributions, and biomasses. Shelf stations, including those on the Abrolhos Bank, had higher particle concentrations and mesozooplankton biomasses than the vertically stratified oceanic stations. The latter were influenced by cold, nutrient-rich South Atlantic Central Water (SACW) present below the mixed layer, particularly toward the south of the study area. Small particles (< 1 mm) were more abundant above and within the pycnocline, whereas large particles (> 1 mm) had a more heterogeneous vertical distribution, but were more abundant above the pycnocline, especially at the oceanic stations. Calanoid

copepods usually dominated the mesozooplankton biomass spectra, but were accompanied by cyclopoids, appendicularians, and ostracods, the latter being particularly abundant during nighttime stations on the Abrolhos Bank. Both LOPC and ZooScan data showed significant differences in NBSS slopes and intercepts between shelf and oceanic stations. The higher intercepts and steeper slopes over the shelf are characteristic of higher productivity. The shallower slopes and presence of more biomass in larger particles indicate a more important contribution of large organisms and higher energy transfer efficiencies at the open ocean stations. Our results highlight the importance of the Abrolhos Bank for pelagic production in an otherwise oligotrophic ocean.

Key words: mesozooplankton, particles, coral reefs, vertical distribution, spatial distribution, biomass, NBSS, Abrolhos Bank.

2.2 Introduction

Since the pioneering work of Sheldon et al. (1972), plankton systems have often been described on the basis of organism size and biomass spectra, as these estimates provide useful information about ecosystem structure (Quinones, 1994), and have the potential to reveal ecological interactions not so easily depicted with conventional taxonomic approaches (Krupica et al., 2012). The theory of the Normalized Biomass Size Spectrum (NBSS; Kerr and Dickie, 2001) associates the intercepts and slopes of a linear regression fitted to the spectrum with biomass production rates, energy transfer efficiencies and predator - prey interactions (Zhou, 2006). The NBSS and other size-based indices have been used in theoretical models to

explore coupling between predator and detritivore communities (Stock et al., 2008; Blanchard et al., 2009). Empirical methods have used the NBSS to estimate population dynamics parameters (Zhou and Huntley, 1997), to evaluate the effects of nutrient enrichment in estuaries (Moore and Suthers, 2006), to determine the effects of fisheries on coral reefs (Graham et al., 2005), to detect spatial and temporal trends in plankton communities (Krupica et al., 2012), and to describe size-structured food webs (Jennings and Mackinson, 2003).

Marine ecosystem structure is tightly connected to both bottom-up and top-down control mechanisms that have consequences for organism size structure (Gilabert 2001; Suthers et al., 2006). Hydrographic features, such as presence of a pycnocline or nutrient input from deep waters or estuaries, are typical examples of physical features providing bottom-up control. The effects of such processes should be observable on zooplankton biomass size spectra. For instance, Finlay et al. (2007) found that bottom-up effects were strong drivers of zooplankton size structure in lakes.

With the development of optical instrumentation having the capability for high-quality image acquisition or particle counting and sizing, plankton size and biomass can now be more quickly estimated both *in situ* and in the laboratory (Benfield et al., 2007; Stemmann and Boss, 2012). One example is the Laser Optical Particle Counter, which generates higher size-resolved information on particle abundance - detecting particles over the size range of 100 μm -35 mm reported Equivalent Spherical Diameter (ESD) - than its previous generation, the OPC, which detected particles over a size range of $\sim$ 250 μm -25 mm (Herman et al., 2004). Recently developed analytical tools

have provided means to distinguish large zooplankton (> 1 mm) from marine snow for LOPC data using particle opacity and size (Checkley et al., 2008; Jackson and Checkley, 2011). Optical measurements may, therefore, deliver continuous *in situ* data of particle distributions with more accuracy, resolution, and regularity than previous methods (Boyd and Trull, 2007; Guidi et al. 2008).

The data from particle counters and *in situ* imaging devices can be compared with zooplankton abundance and biomass measurements obtained from plankton tows (Herman and Harvey, 2006; González-Quirós and Checkley, 2006; Schultes and Lopes, 2009; Schultes et al., 2013). Image analysis of net samples provides a quick way to obtain size and biomass distributions of zooplankton. For example, the benchtop Zooscan can generate plankton size distributions for a large number of preserved samples (Gorsky et al., 2010). Schultes and Lopes (2009) found that the comparison between LOPC and scanned net samples can be difficult in shallow waters over the Abrolhos Bank due to high detrital particle loads there. This is expected because the LOPC is not able to distinguish between detritus and organisms, particularly for objects smaller than 2 mm, and because of the different responses to detritus of the two sampling methods.

Differences between the LOPC tunnel area and the mouth of the net lead to sampling biases (Schultes and Lopes 2009). For example, large organisms can avoid the LOPC tunnel, but not the net opening, and small organisms and fragile particles can be detected by the LOPC, but are not retained by the nets. Despite these differences, the size structure, *i.e.* NBSS, is a conservative and informative characteristic of planktonic communities that is independent of taxonomy (Kerr and Dickie, 2001). Studies

including size spectra from both, LOPC and net plankton estimates with image analysis, are rare in the literature (e.g. Schultes and Lopes, 2009; Schultes et al., 2013). We will test if LOPC and ZooScan NBSS parameters are comparable, providing the same responses to different environments. If they do, the LOPC can provide a valuable monitoring tool because of its ability to generate a large amount of interpretable data very quickly.

The Abrolhos Bank is the largest extension of the narrow Eastern Brazilian continental shelf, extending up to 220 km into the ocean. The bank harbors the world's largest contiguous rhodolith bed, covering about 20,900 km² (Amado-Filho et al., 2012), is the largest coral reef system of the South Atlantic, and supports an exceptionally high biodiversity (Leão, 1996; Werner et al., 2000). South of Abrolhos Bank, seamounts are found along the Vitoria-Trindade Ridge. The productive waters of the bank exert a profound influence on the economic and social dynamics of the area, especially on fishing and tourism (Costa et al., 2003).

The plankton community structure of the Abrolhos Bank and vicinity is poorly known. Previous studies have provided descriptions of the composition, abundance, and biomass distribution of both phyto- and zooplankton (Valentin and Monteiro-Ribas 1993, Brandini et al., 1997; Ekau, 1999; Knoppers et al., 1999; Susini-Ribeiro, 1999), as well as on phytoplankton production (Gaeta et al., 1999), but no information on plankton size structure is available. In fact, plankton communities are often neglected in coral reef studies worldwide, although they represent a significant food source for reef-building corals (Porter, 1976; Ferrier-Pagès et al., 2003) and associated biota, especially reef fish (Russell, 1983).

In this paper, we evaluate hydrographical and bathymetric influences on the plankton composition and size spectra of the Abrolhos Bank and adjacent oceanic areas using a combination of in situ and laboratory-based approaches to determine seston and plankton abundance and biomass. We hypothesize that shelf and oceanic areas have contrasting plankton and particle size structures, and use NBSS intercepts and slopes as our working tools. We also test for differences between shelf and oceanic stations with respect to their mesozooplankton and particle biomass.

2.3 Material and Methods

2.3.1 The study region

The observations analyzed here were made as a part of the PROABROLHOS winter expedition, conducted off the coast of Brazil in July - August 2007 (Figure 2-1). Sampling covered oceanographic settings ranging from shallow, turbid, near-shore waters to oligotrophic "blue waters" over the continental slope during day or night. Stations were categorized as either "shelf" or "oceanic" (see Figure 2-1, oceanic stations inside squares) based on the distance from the coast and the local depth (shelf: from 19 m - station 1A - to 78 m - station 8C; oceanic from 474 m - station 3E - to 4032 m - station 1E). There was a total of 56 stations, with 51 sampled using the LOPC and 46 sampled with plankton nets.

Figure 2-1: Station map showing the location of the Abrolhos Bank and a simplified scheme of the currents in the region (A, see also Soutelino et al., 2013) and the location of LOPC, ZooScan, and both LOPC and ZooScan (L+Z) stations (B). RCE: Royal Charlotte Eddy; AE: Abrolhos Eddy. The boxes in B indicate oceanic stations. Abrolhos Bank and vicinities; July - August 2007. All subsequent figures refer to the same sampling area and period.

2.3.2 LOPC and zooplankton sampling

The LOPC was the main sampling tool of this study. An LOPC660 model with a size detection range of 0.1 – 35 mm was installed inside a conical-cylindrical, 200- μ m mesh-sized ring net (diameter 60 cm) with an interoperable Micro-CTD (AML Microsystems). A calibrated flowmeter (General Oceanics; Mechanical Digital Flowmeter Model 2030) was installed on the T-frame where LOPC was mounted, outside the LOPC tunnel and within the net ring, in accord with the LOPC user manual. This apparatus was towed as

close to the bottom as possible, but no deeper than ~200 m. Average LOPC tow speed was 0.77 m s^{-1} (± 0.13 std).

The net samples were recovered immediately and fixed in formaldehyde (4% final concentration) buffered with sodium tetraborate for subsequent scanning and image analysis. Further details on LOPC sampling are presented in Schultes and Lopes (2009). Fluorescence was estimated with a fluorometer, which was deployed coupled to a CTD (Falmouth Scientific Inc.). The Brunt-Väisälä frequency (N^2) distributions were calculated from salinity, temperature and depth profiles using the Matlab™ seawater toolbox (Morgan, 1994). The N^2 frequency is proportional to the square root of the density gradient and is often used as a measure of seawater density stratification.

2.3.3 Plankton processing with the Zooscan

Preserved zooplankton samples were scanned with the Zooscan system (Grosjean et al. 2004) at a resolution of 2400 dpi, following the scanning protocol described in Schultes and Lopes (2009). A fraction of each plankton sample (1/8 - 1/128) was isolated with a Motoda splitter and analyzed after being scanned in duplicate. Images were processed with *Zooprocess*. The lower recognition size limit of particles was set to 250 μm reported equivalent spherical diameter (ESD).

The *Zooprocess* software isolates images, known as vignettes, of individual objects. For each vignette, a range of parameters relating to size, grey level and form were stored in a result file (*.pid), which was subsequently loaded into *Plankton Identifier* (Gorsky et al. 2010). Vignettes were classified using the Random Forest algorithm in *Plankton Identifier* (Version 1.3.0). Cross

validation of the learning set yielded variable recall values, e.g. 0.93 (Cyclopoida), 0.88 (Appendicularia), 0.86 (Thaliacea), 0.85 (Ostracoda), 0.77 (Chaetognatha), 0.73 (Calanoida), 0.72 (Poecilostomatoida), and 0.45 (detritus, such as: sand/silt, aggregates, exuvia, fecal pellets and undetermined organic debris). Results were then manually validated to correct for residual misclassification among the taxonomic categories presented in here. The validated results were processed to remove noise, inorganic fibers, scanning artifacts, and vignettes with multiple organisms. Typically, there were fewer than 5% of the vignettes with multiple organisms for a sample.

Biomass was estimated for each organism from its volume, assuming that all was shaped as prolate spheroids with an axis ratio of 3:1 and a density of 1 mg mm^{-3} . Normalized biomass size spectra (NBSS) were then calculated by summing the accumulated biomass as a function of size for the size bins provided by the LOPC post-processing software (Schultes and Lopes, 2009). The slopes and intercepts were calculated by fitting a linear regression to each spectrum using log-transformed data. The values for the lower and upper size ranges of each spectrum were removed to compensate for the low sampling efficiency of the net at these boundaries. Hopcroft et al. (2001) estimated that a 200- μm net is more efficient for sampling organisms from 450 and 1400 μm prosome length in tropical regions. Taking that into account, we included only size classes ranging from the peak of the distribution up to the first empty class for the calculation of NBSS slopes and intercepts.

2.3.4 LOPC calculations of NBSS distributions

The LOPC and the AML Micro-CTD data were processed with the LOPC post-processing routine using the exact sample counts (LOPC_PostPro, available at www.alexherman.com) to obtain data with a vertical resolution of 1 m, cutting-off the upper 2 m depth to minimize false counts of air bubbles near the surface. We calculated the log normalized biomass size spectra (NBSS) for each sample as $NBSS = \Delta M / \Delta m$, where ΔM is the total particle mass in a size region and Δm is the difference in mass of the largest and smallest possible particles for that region. The slopes and intercepts were calculated by fitting a linear regression to each spectrum. The same criteria were used to calculate NBSS slopes and intercepts for LOPC samples as for those from Zooscan. Data was adjusted for differences in sampling volume between LOPC and Zooscan. More details in section 6.3.

2.3.5 Data treatment and statistical analysis

After checking for the normality and homoscedasticity of the NBSS parameters, Wilcoxon or Student's t-test were used to test for differences in NBSS slopes and intercepts, calculated with both LOPC and ZooScan, according to the station categories. All tests were performed using the package "pgirmess" in the statistical environment R (R Development Core Team, 2011, Giraudoux, 2012). For data manipulation and plotting we used "lattice" (Sarkar, 2008) and "gtools" (Warnes, 2013).

The volume spectrum (nVd , where n is the number spectrum, V is particle volume, and d is diameter) describes the distribution of particle volume normalized so that the area under a curve is proportional to the

integrated particle volume when plotted against the logarithm of the particle diameter (ESD). We computed it as in Checkley et al. (2008). We calculated the vertically integrated total particle volume per unit area ($\text{cm}^3 \text{m}^{-2}$) after first determining the total particle concentration per volume through the water column ($\text{cm}^3 \text{m}^{-3}$) by integrating nVd with respect to log of diameter; we then integrated the result with respect to depth. We used this vertically-integrated particle volume to describe spatial differences between shelf and oceanic stations. We dropped samples from the 0-2 m depth range to minimize false counts of gas bubbles. We also checked this total particle volume distribution for normality and homoscedasticity; total volume values were log-transformed to achieve a normal distribution and a Student t-test was used to test differences between station categories.

Although previous authors have been able to distinguish marine snow from LOPC data using particle opacity characteristics (Checkley et al., 2008; Jackson and Checkley, 2011), we did not have enough samples to perform the analysis with statistical accuracy.

2.4 Results

2.4.1 Environmental variables

Temperature and salinity were both vertically homogeneous through the water column in the shelf stations, while in the oceanic stations, it sharply decreased below ~100 m (Figure 2-2). The average temperature was higher at shelf than oceanic stations (Table 1) because of the greater tow depths (up to 200 m) at oceanic stations, which included colder deep water. Higher values of the N^2 frequency distribution were present at the oceanic stations

than at the shelf, a result of the greater stratification of the water column offshore. Mean fluorescence was significantly higher ($p < 0.01$) on the shelf, with highest values above the Abrolhos Bank, at stations 4B (mean = 0.79), 4A (mean = 0.57) and 5B (mean = 0.59).

Figure 2-2: Vertical profiles of temperature ($^{\circ}\text{C}$) and salinity, averaged for shelf (A) and oceanic (B) stations, as defined in the text. Note the different scales for the depth axes.

Table 2-1: Descriptive statistics of environmental variables and Normalized Biomass Size Spectra (NBSS) parameters in the Shelf and Oceanic Stations. Abrolhos Bank and vicinities. July - August 2007.

	Shelf		Oceanic	
	Mean $\pm$ std	range	mean $\pm$ std	range
Temperature	24.48 $\pm$ 0.61	22.12 / 25.81	23.20 $\pm$ 2.23	15.51 / 26.02
Salinity	37.32 $\pm$ 0.22	36.54 / 37.66	35.93 $\pm$ 0.95	34.50 / 37.61
N ² $\times 10^{-4}$	0.19 $\pm$ 0.65	-1.3 / 11	0.41 $\pm$ 0.5	-0.74 / 4.8
Fluorescence	0.42 $\pm$ 0.15	0.12 / 0.95	0.14 $\pm$ 0.08	0.01 / 0.55
NBSS				
Intercept LOPC	4.81 $\pm$ 0.49	3.86 / 5.62	3.76 $\pm$ 0.26	3.41 / 4.23
Intercept ZooScan	4.36 $\pm$ 0.43	3.72 / 5.61	3.58 $\pm$ 0.37	2.91 / 4.21
Slope LOPC	-0.96 $\pm$ 0.15	-1.25 / -0.74	-0.86 $\pm$ 0.06	-0.95 / -0.75
Slope ZooScan	-1.01 $\pm$ 0.20	-1.40 / -0.67	-0.91 $\pm$ 0.19	-1.41 / -0.61

2.4.2 Particle and mesozooplankton spatial distribution

The shelf stations had significantly greater ($p = 0.05$) total particle volumes ($108.2 \pm 75.6 \text{ cm}^3 \text{ m}^{-2}$) than oceanic stations ($70.4 \pm 74.3 \text{ cm}^3 \text{ m}^{-2}$; Figure 2-3A; LOPC data). There appeared to be a north-south gradient in the shelf total particle volume, with higher values on the Abrolhos Bank than at the southernmost coastal stations. Larger particles ($> 1 \text{ mm}$) dominated biomass at the southernmost shelf stations (from Station 6A on), in the region where the oceanic influence is greater (Figure 2-3A), as well as at the oceanic stations. Mesozooplankton biomass was greater at the shelf stations, both on the Abrolhos Bank and at the southernmost region, particularly during nighttime observations (Figure 2-3B; ZooScan data).

Diel variations were not as evident for total particle volume estimates. Mean mesozooplankton biomass over the shelf ranged from 3.1 to 591.9 mg m⁻³ (mean = 162.9 ± 139.5 mg m⁻³), while mean biomass at the oceanic stations ranged lower, from 1.2 to 219.2 mg m⁻³ (mean = 57.3 ± 56.3 mg m⁻³). Mesozooplankton biomass per unit area was also significantly greater ($p < 0.01$) over the shelf than offshore. Calanoids were the dominant group at the majority of stations (Figure 2-3C). However, the highest biomass values were at stations 7C and 8B (above the Abrolhos Bank) and were associated with high concentrations of ostracods during nighttime. Ostracods were as much as 72% of the total biomass there. Appendicularians were abundant at some of the stations furthest from the coast and those above seamounts (3D and 10E). The dominance of calanoids was striking on the shelf, while the oceanic stations also included important contributions in all size classes from other groups, especially the cyclopoids (see section 2.4.4).

Figure 2-3: Spatial distribution of total particle volume per unit area from LOPC data ($\text{cm}^3 \text{ m}^{-2}$) (A), mesozooplankton total biomass estimated using the ZooScan (mg m^{-3}) (B), and relative contribution (%) of the main mesozooplankton taxa to total biomass (C). Stations with "grey moon" symbols were sampled during the night.

In the southernmost stations, large organisms such as salps and chaetognaths were the main contributors to the biomass of the system, leading to high total biomass values there (Figure 2-4). Because of their large sizes, these organisms were important contributors to total biomass even when present at low numerical abundances (e.g. stations 7B, 7C, 8B, 10C and 11A).

Figure 2-4: Spatial distribution of biomass for chaetognaths (purple triangles) and thaliaceans (green triangles) estimated using the ZooScan (mg m^{-3}).

Vertical profiles at shelf stations (Figure 2-5) show two general patterns: i) small particles had greater biomass than large particles at 71% of the stations; exceptions were 6 (1BC, 5D, 6BC, 8C) out of 21 stations; ii) particles were, in general, homogenously distributed throughout the water column. Small particles increased slightly towards the bottom. This was the case at 8 out of 21 stations, *i.e.* 38%. These stations were 1A, 2A, 4A, 5C, 6A, 7AB, 8B, which are mostly coastal and very shallow. Re-suspension of sediment there could explain this increase. Profiles obtained at three offshore stations over seamounts (3D, 8D, 10E) are presented separately in Figure 2-5 and show no obvious tendency.

Figure 2-5: Vertical profiles of particles < 1 mm and > 1 mm over the shelf. Last row represents stations above seamounts, despite their low depth ranges these were classified as oceanic stations since they are far from the coast. Black and green lines represent N^2 frequency 10^{-4} s^{-1} and chlorophyll fluorescence distributions, respectively (black and green axes on top).

Figure 2-6: Vertical profiles of particles < 1 mm and > 1 mm at the oceanic stations. For seamounts see Figure 2-5. Black and green lines represent N^2 frequency 10^{-4} s^{-1} and chlorophyll fluorescence distributions, respectively (black and green axes on top).

Vertical profiles at oceanic stations (Figure 2-6) show three general patterns: i) small and large particles were equally important in terms of biomass; ii) small particles decreased below the mixed layer at 19 out of 28 stations, *i.e.* 68%, except for stations 1E, 7E, 9CD, 11CE, 12BCD most of which are situated in the area southeast of Abrolhos bank; iii) large particles had higher biomass in the mixed layer than below the pycnocline at 22 out of 28 stations, *i.e.* 79%. Exceptions (1DE, 2BC, 3E, 10C) were mostly located to the north of Abrolhos Bank. Note that most of particle biomass vertical distributions were in phase with, or above, fluorescence and N^2 frequency peaks, especially for < 1 mm particles.

The average of the vertical nVd size distribution is shown in Figure 2-7 and 2-8. The mixed layer (as shown by the small values of the N^2 frequency), extended to the bottom of the sampling ranges over the shelf. Phytoplankton concentrations, as described by fluorescence, were high, especially above the Abrolhos Bank (Figure 2-5), with largest values in the 15-30 m depth range (Figure 2-7). The average particle maximum determined with the LOPC nVd distribution occurred between 10-30 m; particles ranging from approximately 0.35 to 1.0 mm were the main contributors to total particle volume. Large (> 1 mm) particles were sporadically distributed in the water column.

At oceanic stations the pycnocline depth varied widely from 50 m to more than 100 m (data not shown). Fluorescence was lower in the mixed layer close to the surface, increasing at 40-130 m depth; it did peak above the pycnocline, decreasing deeper (Figure 2-8). Particles < 1 mm were most abundant between 15 and 60 m depth, and were less abundant than over the shelf (Figure 2-7, Figure 2-8).

Particles > 1 mm were present primarily in the upper 80 m and accounted for a large fraction of the total particle volume. Despite the greater numeric abundance of smaller-sized particles, larger particles were an important component of total particle biomass at the oceanic stations. The vertical distribution of particles < 1 mm was associated with both N^2 and fluorescence distributions, with higher concentrations above the pycnocline and in regions where fluorescence was higher.

Figure 2-7: Shelf station distribution of the particle normalized volume spectra ($nVds$) calculated from the LOPC observations; average (top panel) and standard deviation (middle panel) of nVd , as well as number of particles in each size range (bottom panel), are shown. The black line in the top panel indicates the average Brunt-Väisälä frequency; the white line indicates the average chlorophyll fluorescence. Size ranges shown as having 10 particles in lower panel actually have 10 or more. There is greater statistical confidence in concentrations calculated using larger numbers.

Figure 2-8: Oceanic station distribution of the particle normalized volume spectra (nVd s) calculated from the LOPC observations; nVd average (top panel) and standard deviations (middle panel), as well as number of particles in each bin (bottom panel), are shown. The black line in the top panel indicates the average Brunt-Väisälä frequency; the white line represents the average chlorophyll fluorescence. Size ranges shown as having 10 particles in lower panel actually have 10 or more.

2.4.3 NBSS in relation to the distance from the coast

The shapes and parameters of the NBSS calculated from data collected by both LOPC and ZooScan were different at shelf and oceanic stations (Figure 2-9 and Figure 2-10). Shelf stations had significantly larger intercepts (LOPC: $p < 0.01$; ZooScan: $p < 0.01$) than oceanic stations (see also Table 1), consistent with the shelf stations being more productive (Figure 2-10). On the other hand, the flatter slopes (LOPC: $p < 0.01$; ZooScan: $p < 0.01$) at the oceanic stations are consistent with a greater contribution by larger organisms relative to small mesozooplankton.

Figure 2-9: Normalized Biomass Size Spectra (NBSS) estimated using the LOPC (A) and using preserved net samples analyzed with the Zooscan (B), for shelf stations. Bottom axes show the particle biomasses, while top axes show the equivalent diameters.

Figure 2-10: Mean Normalized Biomass Size Spectra NBSS slopes (A, B) and intercepts (C, D) calculated using data from ZooScan (A and C) and LOPC (B and D) for shelf and oceanic stations. The Wilcoxon test detected significant differences between the two domains for all comparisons ($p < 0.05$).

The decrease in biomass for organisms smaller than 450 μm and resulting dome shape of the NBSS estimated by the ZooScan (Figure 2-11) is consistent with the lower sampling efficiency of the 200 μm -mesh net at the smaller sizes. The higher NBSS intercepts calculated for the LOPC data could result from the sampling of additional particles, i.e. marine snow, a result of the inability of the LOPC to distinguish between living and non-living objects, as well as the tendency of nets to disrupt fragile detrital particles. Nevertheless, the higher biomass measured with the LOPC at the lower end of the size spectrum also results from the ability of the LOPC to detect small zooplankton down to size of 100 μm ESD (Herman et al. 2004), a size fraction that is generally sampled poorly by conventional nets but that can be a dominant component of zooplankton biomass (Hopcroft et al. 2001).

2.4.4 Mesozooplankton biomass size spectra

The size distributions of the major zooplankton taxa illustrate their contributions to the NBSS profile (Figure 2-11). At the shelf stations, maxima of copepod and ostracod biomass size distributions were in phase with the NBSS peaks (around 0.5 mm); ostracods had another peak in a larger size class (around 0.85 mm). At the oceanic stations some groups, such as copepods, thaliaceans, and chaetognaths, had a broader size distribution without gaps in the upper end of the spectra. Therefore, the shallower slopes in the open ocean and steeper slopes over the shelf are the result of both lower abundance of the smaller and higher abundance of the larger organisms in the offshore stations and by differences in the ranges of size distribution for the main taxonomic groups.

Figure 2-11: Mesozooplankton Normalized Biomass Size Spectra (NBSS) superimposed on the average abundance size distributions (colored lines) of the main mesozooplankton taxa determined with the ZooScan (right axis). Shelf domain (A) and oceanic stations (B). Size distributions [Equivalent Spherical Diameter (ESD), in mm] depicted on the upper axis. ost: Ostracoda; cal: Calanoida; cyc: Cyclopoida; poe: Poecilostomatoida; app: Appendicularia; tha: Thaliacea; cha: Chaetognatha; Det: Detritus. Bottom axes show the log of particle biomasses, while top axes show the equivalent diameters.

Although the detritus size distribution is also superimposed with the NBSS on Figure 2-11, it was not included in the NBSS calculations derived from ZooScan data (grey circles, Figure 2-11) because detrital material is not properly collected by nets (González-Quiróz and Checkley, 2006). However, we can make a spatial comparison assuming that the relative biases on detritus abundance estimates were the same in all samples. This results in shelf stations having almost one order of magnitude more detritus than the oceanic stations (Figure 2-11).

2.5 Discussion

2.5.1 The regional spatial structure of particle distribution

The vertical structure of oceanographic properties was an important factor for the vertical distribution of small particles in the Abrolhos region, since <1 mm particles were more abundant in the mixed layer. The fluorescence peaks found within or above the pycnocline suggest the influence of thermohaline stratification on the phytoplankton distribution, consistent with the findings of Susini-Ribeiro et al. (2013). These associations between discontinuities in the water density, indicated by the high values of the N^2 frequency, and the high concentrations of phytoplankton and sestonic particles have been reported often in other coastal and oceanic regions (e.g. Lampitt et al., 1993; MacIntyre et al., 1995; Sullivan et al., 2010), but remain virtually unstudied off the tropical and subtropical SW Atlantic.

Despite the variability associated with the size distribution of particles in the Abrolhos Bank and vicinity, the average nVd distribution provided a general

picture of the particle vertical structure at shelf and oceanic stations. The higher particle concentration on the shelf and higher contribution of larger particles to total biomass offshore are consistent with the steeper slopes and higher NBSS intercepts at the shelf relative to oceanic stations. The total particle volume decline contrasts with the increase in mesozooplankton biomass at the shelf stations toward the south, suggesting that there is a latitudinal gradient in the proportional distribution of living and non-living particles, with a higher detrital input over the bank than on the southern shelf, again consistent with the findings of Susini-Ribeiro et al. (2013). Although detrital abundance estimated by the ZooScan is not accurate in absolute numbers, it can provide additional useful information to compare shelf and oceanic stations. Because the LOPC samples include detritus, distributions calculated from LOPC data should have larger values for NBSS intercepts than those calculated using ZooScan data over the shelf, as has been observed in earlier analyses (Schultes and Lopes 2009). Both, in the study of Schultes and Lopes (2009) and in the present analysis, the estimates from the two approaches were closer at oceanic stations, implying that most particles detected by the LOPC offshore were zooplankton (Figure 2-8 and Figure 2-9).

The Abrolhos Bank and the Vitoria-Trindade Ridge form a topographical barrier to the Brazil Current (BC). In this region, eddies and cyclonic vortices, such as the Vitória Eddy (VE) (Schmid et al., 1995), develop along the eastern and southern shelf edges and promote upwelling or bottom intrusions of the cold and nutrient-rich South Atlantic Central Water (SACW). This water mass is known to increase phytoplankton production on the Brazilian shelf and is important as a natural eutrophication agent for the

mixing layer (Saldanha-Corrêa and Gíanesella, 2004). Gaeta et al. (1999) detected low nitrate concentration, chlorophyll biomass and primary productivity on the Abrolhos Bank and proposed that high pelagic productivity was restricted to the VE periphery. In our study, chlorophyll fluorescence and mesozooplankton biomass maxima had a more extensive distribution across the Abrolhos Bank and the southernmost shelf than they predicted and coincided with microphytoplankton spatial variations during the same cruise (Susini-Ribeiro et al., 2013).

Sediment resuspension and advection is effective on the inner shelf adjacent to the Caravelas estuarine system (Schettini et al., 2013), where the along-shelf momentum associated to wind and bottom stresses leads to strong north-northeastward currents in winter (Teixeira et al., 2013). This is probably the cause for the high total particle abundance recorded in the < 1 mm size range by the LOPC in station 4A, with no corresponding zooplankton peak.

Other forces should be associated with the development of the populations of larger organisms at the southernmost stations and offshore. For example, SACW seasonal intrusions in the southeast Brazil stimulate blooms of salps and higher contributions of larger organisms (Lopes et al., 1999; Resgalla Jr et al., 2001). The offshore and southernmost areas are more likely to be influenced by SACW in deeper depths, below the pycnocline, when bottom intrusions are induced by the BC interaction with the Abrolhos Bank topography and/or by the VE. Since the nutrients are provided in "pulses" during these intrusions, large phytoplankton has an advantage (Susini-Ribeiro et al., 2013) and populations of larger zooplankton are more likely to be developed, as we observed in the southern section of the study area, explaining the higher

biomass of chaetognaths and thaliaceans (Figure 2-4). We suggest that SACW bottom intrusions are triggering the population increase of these large organisms in the southern part of the Bank and in the southernmost oceanic stations.

2.5.2 Benthopelagic coupling

Benthopelagic coupling at the time of our study appeared to be strong. Benthic algal species and tychoplankton were an important component of the phytoplankton composition at the Abrolhos Bank stations (Susini-Ribeiro et al., 2013). Furthermore, crab larvae in the water column were all for reef-associated species (Koettker and Lopes, 2013). Although decapods biomass was usually < 5% of total mesozooplankton biomass, our data set shows the importance of other benthic and demersal species for mesozooplankton biomass over Abrolhos Bank (Figure 2-3C). Because of their larger average size, ostracods provided more biomass, with fewer numbers, to the pelagic system than in regions on the Abrolhos Bank where copepods dominated. The large number of ostracods in the plankton tows above the bank are probably bottom-dwelling species, which inhabit the bank region. Male ostracods produce bioluminescence as a display toward receptive bottom-dwelling females, and in specific habitats, such as in reef and seagrass systems, males synchronize in swarms after twilight (Morin, 1986). Most of the Abrolhos Bank stations with higher abundance of ostracods were sampled during the night ("moon" symbol in Figure 3A), and many of those ostracods were carrying eggs (data not shown), suggesting that these peaks could have originated from a reproduction-driven behavior. Therefore, an important source of the pelagic biomass above the Abrolhos Bank must be provided by

demersal species, further illustrating the importance of the reef system to the local pelagic productivity.

2.5.3 Size distributions and NBSS parameters

This is the first work to describe the vertical particle distribution and biomass size spectra in the Abrolhos Bank and vicinity, and one of the few to deal with mesozooplankton community structure in the area, examples of the latter being Valentin and Monteiro-Ribas (1993), Ekau (1999) and Knoppers et al. (1999). LOPC studies usually describe high latitude or temperate environments dominated by one or few large zooplankton species (Herman and Harvey 2006, Zhou et al, 2009; Basedow et al., 2010; Basedow et al., 2013). At our low latitude sites, there were two domains: the continental shelf, including the area above the bank, and the oceanic blue waters. The shelf domain was dominated by small (< 1 mm) calanoid copepods, which alternated in dominance with ostracods over the bank. High particle and plankton biomass, and significantly steeper NBSS slopes indicated a relatively larger contribution of smaller particles to biomass.

In the oceanic domain, appendicularians and larger copepods, chaetognaths and salps were relatively more important, leading to flatter NBSS slopes. Cyclopoid copepods were also more abundant offshore than in the well-mixed waters of the Abrolhos inner shelf. Schultes et al. (2013) found that cyclopoids increased in abundance from coast to ocean along a transect on the Eastern Atlantic continental shelf, and suggested a better performance of the typical ambush-feeding behavior of cyclopoids (Saiz et al., 2003) in less turbulent, stratified offshore waters. In addition, cyclopoid copepods efficiently feed on small,

flagellate phytoplankton species and microzooplankton, typical of the oligotrophic conditions in the oceanic domain (Paffenhoefer, 1998). Likewise, appendicularians are able to tap the nano- and picophytoplankton pool (Scheinberg et al. 2005) in oligotrophic waters.

Susini-Ribeiro (1999) has suggested a high degree of development of the microbial food web in the Abrolhos Bank area, with higher contribution of picoplankton relative to nano- and microplankton. They reported higher plankton biomass above the Abrolhos Bank and in the southern section of the continental shelf, and showed that phototrophic organisms dominated on the bank while heterotrophs were relatively more important in the open ocean. Analysis of microphytoplankton communities (Susini-Ribeiro et al. 2013) reported that large-celled species, particularly dinoflagellates and diatoms, occurred offshore as a response to SACW intrusion. All their observations are in good agreement with our results for the size and taxonomic composition of mesozooplankton found in the study area.

Nutrient availability, as well as depth of the pycnocline, which influences turbulence and mixing intensity, appear to control the size and diversity of the microphytoplankton, which are the first links of the food web (Susini-Ribeiro et al. 2013). The assemblages of these organisms will reflect through the organisms developing in the chain. In our study, the higher NBSS intercepts at the shelf stations, including the Abrolhos Bank, imply high production within the smaller mesozooplankton size range and the steeper slopes suggest a less efficient biomass transfer to large size classes. The primary production is hence transferred to small calanoid copepods and, ultimately, to the benthic communities. The shallower NBSS slopes and lower intercepts at oceanic stations, in

addition to their lower chlorophyll fluorescence, are consistent with a less productive system having higher energy transfer efficiency and biomass recycling (Zhou, 2006) and a larger number of trophic levels in the mesozooplankton and micronekton size range. These differences in the zooplankton size spectra between shelf and offshore areas can be explained as a consequence of different energy sources and food availability for the first elements of the food web.

2.5.4 NBSS theory applied

The association between the parameters of the linear regression fitted to the NBSS and the ecological features of planktonic systems (e.g. productivity, trophic structure) has been widely addressed in the literature. Sprules and Munawar (1986) in inland lakes and Iriarte and González (2004) off Chile found steeper NBSS slopes in oligotrophic systems compared to productive areas. They showed that in less productive systems a significant proportion of the primary productivity was provided by microbial food webs. These patterns should be a recurrent feature because small cells are favored in oligotrophic conditions due to their higher surface:volume ratio, which enables a more efficient nutrient assimilation. During upwelling events, larger cells have an advantage when turbulence is high and nutrients are available in pulses (Falkowski and Oliver, 2007). The consequence would be shallower slopes, which indeed are observed in association with high production rates during upwelling events (Iriarte and González, 2004; San Martín et al., 2006).

On the other hand, Zhou et al. (2009) have argued that a high potential productivity is indicated by steeper

slopes because of higher proportion of herbivorous zooplankton in the system (Zhou and Huntley, 1997; Zhou, 2006). Jennings and Warr (2003) studied the trophic structure of fish communities, and observed that low predator:prey body size ratios were characteristic of trophic webs with higher number of links. Jennings and Mackinson (2003) associated steeper NBSS slopes with those low ratios, which led them to suggest that such slopes indicate more stable environments. Hence, both shallower and steeper slopes have been considered as indicative of higher pelagic productivity, and steeper slopes have been associated with higher ecosystem stability. Therefore, we still need to be careful about using NBSS parameters as a proxy for system productivity or stability without previous information about the ecosystem. Different size ranges were used to calculate the NBSS for the above-mentioned studies (Table 2-2). For example, Zhou et al. (2009) estimated the NBSS for zooplankton $> 250 \mu\text{m}$ while Iriarte and González (2004) and San Martín et al. (2006) worked with a much smaller size spectra ($> 1 \mu\text{m}$), and Jennings and Mackinson (2003) investigated fish-sized biomass spectra.

Table 2-2: Comparison of mesozooplankton Normalized Biomass Size Spectra (NBSS) slopes and size ranges at different locations. Expanded from Matsuno et al (2012). Abrolhos Bank and vicinities. July - August 2007.

Location/region	Unit	Size range (mm)	Slope	References
Chile - cold-nutrient-rich conditions	Carbon	0.7 / 21	-0.44	Iriarte and Gonz��les (2004)
Gulf of St Lawrence (open water)	Biovolume	0.25 / 2	-0.47	Herman and Harvey (2006)
Barents Sea	Biovolume	0.25 / 14	-0.63	Basedow et al (2010)
NW Mediterranean (protected area)	Wet weight	20 / 1200	-0.65	Macpherson et al (2002)
Bay of Biscay (oceanic stations)	Carbon	0.27 / 1.7	-0.71 / -0.64	Sourisseau and Carlotti (2006)
Tasman Sea	Biovolume	0.11 / 3.3	-0.69	Baird et al (2008)
Ushant Tidal Front (stratified side/offshore)	Carbon	0.3 / 2	-0.73	Schultes et al (2013)
Chukchi Sea (less productive)	Biovolume	0.25 / 5	-0.86	Matsuno et al (2012)
Gulf of St Lawrence (estuary)	Biovolume	0.25 / 2	-0.90	Herman and Harvey (2006)
Western Antarctic Peninsula (Fall)	Biovolume	0.25 / 14	-0.92	Zhou et al (2009)
Chile - El Ni��o, oligotrophic conditions	Carbon	0.7 / 21	-0.93	Iriarte and Gonz��les (2004)
Coral Sea	Biovolume	0.11 / 3.3	-0.97	Baird et al (2008)
Southwest Coral Sea	Wet mass	0.25 / 2.5	-1.00	Suthers et al (2006)
Bay of Biscay (coastal stations)	Carbon	0.27 / 1.7	-1.05 / -0.91	Sourisseau and Carlotti (2006)
NW Mediterranean (unprotected area)	Wet weight	20 / 1200	-1.04	Macpherson et al (2002)
Scotia Sea (spring)	Carbon	small phyto to large schyphomedusae	-1.09	Tarling et al (2012)
North Iberian Shelf	Carbon	0.25 / 17	-1.11	Nogueira et al (2004)

Chukchi Sea (more productive)	Biovolume	0.25 / 5	-1.11	Matsuno et al (2012)
North Pacific Ocean	Carbon	0.18 / 4.0	-1.13	Rodriguez and Mullin (1986)
Northwest Atlantic Ocean	Carbon	0.07 / 8.0	-1.14	Quinones et al (2003)
Ushant Tidal Front (front)	Carbon	0.3 / 2	-1.15	Schultes et al (2013)
California Current	Carbon	0.2 / 3.3	-1.43	Huntley et al (1995)
Ushant Tidal Front (mixed side/nearshore)	Carbon	0.3 / 2	-1.76	Schultes et al (2013)
Western Antarctic Peninsula (Summer)	Biovolume	0.25 / 14	-1.80	Zhou et al (2009)
Australian Estuary	Wet mass	0.25 / 1.6	-1.89	Moore and Suthers (2006)
California Bight	Biovolume	0.025 / 4.0	-2.30	Napp et al (1993)
Brazilian Continental Shelf (Coastal Stations and Abrolhos Bank)	Biovolume - ZooScan	0.25 / 8	-1.01	This study
	Biovolume - LOPC	0.1 / 5	-1.25	This study
Brazilian Continental Shelf (Oceanic Stations)	Biovolume - ZooScan	0.25 / 8	-0.91	This study
	Biovolume - LOPC	0.1 / 5	-0.86	This study

We believe that integrated studies covering simultaneously a wide range of sizes and trophic levels are necessary to clarify whether NBSS-derived metrics and their interpretations are universally applicable to the aquatic ecosystem. This is a difficult task as many methodological challenges are involved, such as that sampling efficiency must remain close to 100% across size groups and trophic levels to avoid unrealistic domes and gaps along the size spectra, and must adequately depict the variable spatial and temporal scales in life cycle strategies from bacteria to fish. This is still beyond our existing observational

capabilities, but recent developments in automated systems for ocean biology studies (Karlson and Lopes, 2009, Stemmann and Boss, 2012) indicate that such approach is not that far in the horizon.

2.5.5 Conclusions

This study provided data on plankton and particle size spectra distribution and composition for an extensive shelf and oceanic area off Eastern Brazil (16 - 22°S) during winter conditions. Vertical profiles with an optical particle counter followed by image analysis of preserved net samples showed that the pelagic trophic structure in the area is influenced by water column stratification in the offshore domain, and by changes in water circulation in response to the complex seabed topography on the wide continental shelf of Abrolhos Bank.

An important finding of this study was the observation of high particle and plankton biomass associated with steep slopes and high intercepts of the Normalized Biomass Size Spectrum (NBSS) over the bank. Because estuarine influence on the Abrolhos coral reef ecosystem was minimal during the course of our observations (Schettini et al., 2013; Koettker and Lopes, 2013), the increased pelagic productivity and suspended particle concentration over the bank probably resulted from intense benthic-pelagic coupling. Low particle biomass, shallow slopes and low intercepts were found in the vertically stratified oceanic domain influenced by nutrient-rich waters below the mixed layer. These results, in combination with the significant contribution of large organisms to total biomass, mainly in the southernmost stations influenced by eddies and bottom intrusions, indicate high

energy transfer efficiencies through the oceanic plankton food web. In addition, the relationship between water density stratification and particle abundance was apparent, as both small- (< 1 mm) and large-sized (> 1 mm) organisms were concentrated within or above the pycnocline in offshore areas.

Both LOPC and ZooScan were useful tools to provide rapid information on plankton size distribution and NBSS parameters. Although the instruments have different size detection limits and deal with different sampling efficiencies in the lower and upper ends of the size spectra, both detected similar patterns, including the significant differences in the NBSS slopes and intercepts between shelf and oceanic stations. Further studies with combined use of both instruments, as well as with additional sensors and platforms, can help to enhance our mechanistic understanding of the dynamics and ecological role of plankton and seston in aquatic environments.

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3 Seasonal variability of plankton and seston vertical structure off Ubatuba, Brazil, with emphasis on the biomass size spectra

3.1 Abstract

Recently developed optical systems are valuable tools to automatically detect and measure plankton size distributions, accelerating the analysis of large data sets. We evaluated how the seasonal intrusion of the South Atlantic Central Water (SACW) influences plankton biomass size spectra (NBSS) and particles size distributions on a 5-year time series in subtropical Brazil. We collected monthly samples in a fixed station in the inner part of the Southeast Brazilian Continental Shelf, off Ubatuba, using a Laser Optical Plankton Counter (LOPC) for *in situ* particle analysis, and a ZooScan to estimate metrics from plankton images obtained from scanned samples. A 200- μ m-mesh closing net was vertically towed, sampling two different strata of the water column, considering the pycnocline position, when it was detected. We found evidence of increase in mesozooplankton productivity associated with the intrusion phase, when nitrate and Chlorophyll *a* increased. Particle distribution (0.10-1.00 mm ESD) was correlated with the Brünt-Väisälä frequency distribution, accumulating above the pycnocline during intrusions. In winter, cold fronts and stronger winds eroded the pycnocline, and resuspension probably led particles to accumulate close to the bottom. Significantly steeper NBSS slopes and higher intercepts during SACW intrusions indicated changes in the plankton trophic structure related to bottom-up controls.

Key words: Time series, mesozooplankton, size structure, NBSS, South Atlantic.

3.2 Introduction

Size has emerged as an important parameter to characterize plankton communities because it directly influences physiological rates (Ikeda 1974, Platt and Denman 1978), and has a strong correlation with a wide variety of processes and ecological interactions (Bautista and Harris 1992), including primary and secondary production (Boudreau *et al.* 1991). For instance, predator-prey relationships are usually governed by size traits since most predators feed on smaller prey (Cohen *et al.* 1993, Hansen *et al.* 1994), although exceptions were described in planktonic trophic webs (Landry *et al.* 1958).

Size-based indices have been widely used (Gin *et al.* 1998, Jennings *et al.* 2002, Shin *et al.* 2005, Sprules and Goyke 1994) and are potentially applicable to reveal patterns otherwise non-conceivable through conventional taxonomic approaches (Krupica *et al.* 2012). One example is the Normalized Biomass Size Spectra (NBSS) theory (Kerr and Dickie 2001), which associates the parameters of the linear regression fitted to the spectra with biomass production rates, energy transfer efficiency and predator-prey interactions. Zhou (2006) argues that the number of trophic levels and the energy assimilation efficiency determines the NBSS slope. In this sense, the NBSS slope provides information on the trophic structure and is an inherent property of plankton systems. Furthermore, the intercept is determined by the abundance of the smaller size bins and is associated with system productivity (Cyr and Peters, 1996; Bianchi 2000).

The estimation of plankton productivity by means of traditional methods is a daunting task in ecosystems with a large number of species and trophic links (Zhou 2006). This is the case of tropical and subtropical communities with

wide size spectra along many trophic positions (Hopcroft et al. 1998). The NBSS approach is useful in such condition, as well as to study communities from different regions and environments where differences in taxonomic composition hampers traditional comparisons (Hopcroft et al. 2001). Therefore, the NBSS integrates a set of populational responses to environmental factors and intra- and interspecific relationships.

The seston component of marine pelagic systems is linked to organic matter fluxes along trophic webs (Angeler et al. 2007). Despite its importance, few studies focus on seston dynamics because the non-living biogenic portion (e.g., aggregates, abandoned houses of appendicularians and pteropods mucous nets), is fragile and not adequately retained by nets (González-Quirós and Checkley 2006), demanding the use of non-destructive techniques for quantification (Checkley et al. 2008). Optical equipments such as the Laser Optical Particle Counter (LOPC, Brooke Ocean Technologies, Dartmouth, Nova Scotia) have been used to estimate seston abundance *in situ*, with especial interest on copepods (Checkley et al. 2008, Herman and Harvey 2006). The data sets provided by the LOPC allow size-based analyses and the use of advanced mathematics to express populational processes of particles and plankton (Edwardsen et al. 2002, Platt and Denman 1978, Zhou and Huntley 1997).

On the other hand, the ZooScan provides images from sampled particles and organisms. Images are analyzed in a semi-automatic process to obtain plankton size distributions and varied levels of taxonomic resolution. The combined use of the LOPC and the ZooScan has provided useful information on zooplankton size spectra, diversity

and food-web energetics (Schultes and Lopes 2009; Marcolin et al., 2013).

Plankton diversity and abundance vary to a large extent within seasonal, annual, and decadal time scales, especially on continental shelf ecosystems. Climatic and oceanographic factors have been widely described as the main bottom-up drivers of such variability (D'Alcalà et al. 2004). Improved sampling strategies of both zooplankton assemblages and oceanographic parameters should increment our current understanding of how and why plankton communities vary through time (Lopes 2007). Multiannual time series are a robust tool to rebuild zooplankton seasonal cycles and their associated environmental factors (D'Alcalà et al. 2004, Licandro and Ibanez 2000). Few studies have described biomass size spectra variations through time (e.g. Gin et al. 1999; Huete-Ortega et al. 2009; Roy et al. 2010), despite their potential to reveal changes in food web structure and dynamics.

Although long-term time-series are available for temperate regions (D'Alcalà et al. 2004, Fevre-Lehoerff et al. 1995, Gailhard et al. 2002, Hrbáček et al. 2003, Licandro and Ibanez 2000, Pitois and Fox 2006), they are still relatively rare in tropical and subtropical systems, especially in the southern hemisphere (Ayón and Swartzaman 2008). Moreover, most of plankton time-series evaluate changes in species composition and abundance without taking into account the variability in plankton size structure. Mackas et al. (2012) argue that cross-region comparisons for changes in the size spectrum among zooplankton communities are still an unresolved issue requiring further investigation.

Zooplankton studies off the northern coast of São Paulo have mainly focused on: (i) taxonomical aspects and

spatio-temporal variations (Lopes *et al.* 2006, Pinese 1982, Silva 2011, Vega-Pérez 1993), (ii) diel vertical migration (Milstein 1979, Moreira *et al.* 2007), (iii) copepod reproduction and secondary production (Melo Júnior 2009, Miyashita *et al.* 2009), (iv) population dynamics and reproduction of cladocerans and appendicularians (Miyashita 2010), and (v) temporal coupling between phytoplankton and meroplankton (Fernandes *et al.* 2012). Only the studies conducted by Melo Júnior (2009), Miyashita *et al.* (2010) and Fernandes *et al.* (2012) included multiannual time series.

The seasonal intrusion of the cold and nutrient-rich South Atlantic Central Water (SACW) is a relevant oceanographic feature off Southeast Brazil, as it enhances biological productivity (Metzler *et al.* 1997, Saldanha-Corrêa and Giancesella 2004), including zooplankton secondary production (Lopes *et al.* 1999). In this study we test the hypothesis that SACW seasonal intrusions lead to changes in zooplankton biomass and size distributions, as depicted by NBSS parameters. We also evaluate the influence of bottom intrusions on the vertical distribution of zooplankton and particles, and discuss the implications of our results in a context of food web structure.

3.3 Material and Methods

3.3.1 Study area

The Southeast Brazilian Continental Shelf (SBCS) extends from São Tomé Cape (22°S) in Rio de Janeiro, to Santa Marta Cape in Santa Catarina. The SBCS characteristics are typical of mid-latitude shelves (Castro 1996). The SBCS extends for 1,100 km with a total area of 150.000 km², and the shelf break depth varies from 120 to

180 m (Zembruski 1979). The Brazil Current (BC) is the most important oceanic feature of the South Atlantic western edge (Rossi-Wongtschowski and Madureira 2006).

The inner portion of the SBCS is mainly influenced by the Coastal Water (CW) and the South Atlantic Central Water (SACW). CW is resultant from the mixing between the warm and salty (temperature $> 20^{\circ}\text{C}$; salinity > 36.4) Tropical Water (TW) carried by the BC, and fresh water from continental origin. CW has the lowest salinity (32.2 - 35.4) and highest temperature values of the SBCS ($> 20^{\circ}\text{C}$), with vertically homogenous characteristics due to wind mixing. The SACW is colder (temperature $< 20^{\circ}\text{C}$), less salty (salinity < 36.4), and nutrient-rich; nitrate levels $> 6\ \mu\text{M}$ (Saldanha-Corrêa and Giancesella 2004).

According to Stramma and England (1999), in the northern part of the Subantarctic Zone, a light type of the Subantarctic Mode Water forms the SACW. Its formation occurs in the confluence zone of the Brazil and Falkland currents. The SACW is transported within the South Atlantic Current toward Africa. In there, one part of SACW passes into the Indian Ocean toward the south of the Agulhas Current. The other part contributes to the Benguela Current and is transported toward the Brazilian Shelf by the South Equatorial Current. SACW is carried toward the equator with both the North Brazil Current and the North Brazil Undercurrent (Stramma and England 1999).

Toward the south-southwest, SACW takes its pathway occupying the depth range of 200-500 m along the continental slope (Castro and Miranda 1998). The large-scale high-pressure center of the South Atlantic promotes the prevalence of E-NE winds mainly from mid-Spring to late summer, with a large component parallel to the coast, which promotes the transport of TW oceanward compensated by SACW

transport on the opposite direction. As a result, SACW reaches the inner portion of the shelf off Ubatuba and causes water column stratification (Castro *et al.* 1987, Mascarenhas *et al.* 1971). TW mainly occupies the outer area of the SBCS. During winter, S-SW winds are more frequent and intense due to increased polar cold fronts, which intensify vertical mixing; CW predominates and TW usually spreads more effectively onto the mid-shelf (Castro *et al.* 1987).

SACW intrusions in the SBCS are probably associated with upwelling events off Cabo Frio (RJ) and to meanders and eddies of the Brazil Current (BC), in addition to changes in the wind patterns (Lopes 2006). The striking presence of meanders and eddies in the BC is mainly induced by topographical features (Cunha 2008, Mascarenhas *et al.* 1971).

3.3.2 Sampling strategy

Monthly samples were taken from July 2007 to June 2012 at a fixed station (23°36'S, 44°58'W) on the inner shelf of the Southeast Brazilian Continental Shelf (SBCS) (Figure 3-1). A Laser Optical Particle Counter (LOPC) was vertically towed coupled to a Micro-CTD (AML Microsystems) from 2-3 m above the bottom to the surface. Vertical profiles of chlorophyll fluorescence ($\mu\text{g L}^{-1}$) were also obtained from a Free-Falling Optical Profiler, or Profiler II (Satlantic: <http://satlantic.com/profiler>). Plankton samples were collected with a closing 200- μm mesh sized net. Vertical tows were also performed from 2-3 m above the bottom to the basis of the pycnocline, when it was detected, and from there to the surface. When no pycnocline was detected two layers of equal depth range were sampled,

using mid-depth as a reference. The samples were preserved with 4% buffered formalin diluted in seawater.

Figure 3-1: Fixed station of the ANTARES program in the coastal area of Ubatuba, São Paulo.

3.3.3 Chlorophyll a and nitrate

Seawater samples were collected at 5-m depth intervals, from the surface to ~38 m depth, using an 11-L Niskin bottle. Approximately 2 L of seawater were filtered through Whatman® GF/F filters; pigments were extracted for 24 h using 90% acetone, and analyzed by fluorimetry (Welschmeyer 1994) to obtain chlorophyll a concentrations. For nitrate analysis, 300 mL of seawater were filtered through Whatman® GF/F fiberglass filters and analyzed in the spectrophotometer HITACHI® U-1100, according to methods described in Grasshoff (1976), adapted by Paranhos (1996).

Nitrate and Chlorophyll *a* data sets were provided by the Primary Production Laboratory from IOUSP.

3.3.4 Water column stratification indices and wind speed

We estimated the Brunt-Väisälä frequency (N^2) from salinity and temperature vertical profiles, obtained from the microCTD, using the Matlab® *seawater* toolbox. The N^2 is a measure of water column stability and is often used as a stratification index (e.g. Falkowski and Oliver, 2007; Huete-Ortega et al., 2010). To identify discontinuities in the water column, we calculated the standard deviation of the Brunt-Väisälä frequency distributions, referred hereafter as σN^2 .

We obtained Reanalysis wind speed measurements from the US National Oceanic and Atmospheric Administration web site (NCEP Reanalysis Data, 2007-2012). We extracted daily measurements from the u and v components of the wind speed; u is the zonal velocity, refers to the horizontal wind towards east, whereas v is the meridional velocity, which is the horizontal wind towards north. The data grid comprised latitudes from 17.5°S to 30°S, and longitudes from 47.5°W to 35°W.

3.3.5 LOPC metrics

We explained the procedures to obtain vertical profiles of particles, number and volume spectra from raw LOPC data sets in details in sections 6.2 and 6.2.1, respectively.

3.3.6 Plankton processing and image analysis

Since larger organisms in a plankton sample are usually less abundant (Hopcroft et al. 2001), we sieved each sample through a 500- μm mesh and took aliquots of both smaller (<500 μm) and larger (>500 μm) size classes using a Motoda splitter (Omori and Ikeda 1984). Typically we processed aliquots of 1/32 and 1/16 for the small and large size classes, respectively.

We scanned the preserved aliquots with a ZooScan to obtain digital images of plankton samples, and both aliquots - small and large - were used for abundance and biomass size distributions estimates, according to the following formula:

$$\text{Total abundance} = \frac{n_s * sp_s + n_L * sp_L}{\text{Filtered volume'}}$$

where n_s = number of organisms in the small aliquot, sp_s = splitting factor of the small aliquot, n_L = number of organisms in the large aliquot, sp_L = splitting factor of the large aliquot.

The image processing was the same as described in section 2.3.3, except that the lower recognition size limit of particles was set to 300 μm equivalent spherical diameter (ESD). More details on scanning and imaging processing with the ZooScan are described in section 6.1.

3.3.7 Biomass estimates and NBSS

We estimated biomass data for each taxonomic group as carbon weight using published regression equations that relate size, area or volume to weight (Table 3-1). Such approach has been successfully applied in other studies on the SBCS (Oliveira 2009b) and elsewhere (Bell and Hopcroft

2008, Irigoien *et al.* 2009). We calculated the Normalized Biomass Size Spectra (NBSS) dividing the accumulated biomass of each size class by the weight of the class (see section 6.3). In this chapter we used carbon biomass for the zooplankton biomass distributions and both biovolume and carbon biomass to calculate the NBSS. For the biovolume calculations we assumed that a zooplankton has the shape of a prolate spheroid, with an aspect ratio of 1:3, using the formula

$$\frac{4}{9} \pi r^3$$

We calculated the seston NBSS considering the LOPC residual and Gaussian distributions (see section 6.3), in addition to the total distribution. We estimated NBSS slopes and intercepts for each sample by adjusting the data to a linear regression model.

Table 3-1: Regression equations used to estimate carbon weight (μgC) from size for different taxonomic groups. A: area; L: length; V: volume defined as $4/3 * \pi * (\text{feret}/2)^3$; L_{TR} : trunk length. References: Uye (1982), Beers (1966), Sato et al. (2001), Gibson and Paffenhöfer (2002), Henjes (2004), and Lehetette and Hernández-León (2006).

Group	Equation	
Foraminiferida	$V \text{ cm}^3 * 89$	Henjes (2004)
Bryozoa	$43.38 * A \text{ mm}^2^{1.54} * 0.432$	
Echinodermatha	$43.38 * A \text{ mm}^2^{1.54} * 0.432$	
Copepoda	$45.25 * A \text{ mm}^2^{1.59} * 0.455$	
Cladocera	$43.97 * A \text{ mm}^2^{1.52} * 0.481$	Uye (1982) e Lehetette and Hernández-León (2006)
Polychaeta	$43.38 * A \text{ mm}^2^{1.54} * 0.299$	
Ostracoda	$99.46 * A \text{ mm}^2^{1.28} * 0.369$	
Mollusca	$43.38 * A \text{ mm}^2^{1.54} * 0.227$	
Peracarida	$49.58 * A \text{ mm}^2^{1.48} * 0.407$	Lehetette and Hernández-León (2006) e Davis and Wiebe (1985)
Chaetognatha	$23.45 * A \text{ mm}^2^{1.19} * 0.283$	
Decapoda and Euphausiacea	$49.58 * A \text{ mm}^2^{1.48} * 0.407$	Lehetette and Hernández-León (2006)
Siphonophora	$43.17 * A \text{ mm}^2^{1.02} * 0.109$	Beers (1966)
Other Cnidaria	$43.17 * A \text{ mm}^2^{1.02} * 0.072$	Lehetette and Hernández-León (2006)
Thaliacea	$0.3396 * A \text{ mm}^2^{2.642}$	Gibson and Paffenhöfer (2002)
Appendicularia	$2.62 * 10^{-8} * L_{\text{TR}} \mu\text{m}^{2.83}$	Sato et al., (2001)

3.4 Results

3.4.1 Temporal variability

3.4.1.1 Wind regime

Regarding wind vectors, a negative u component means that wind is blowing from east to west, whereas a negative v component means that the wind is blowing from north to south (Figure 3-2). Wind direction in this study is expressed in terms of the direction from which it originates.

Figure 3-2 Sketch of wind speed components and their directions.

Wind speed components u and v had a similar temporal variability with more negative values found from September through February. The u component was more intense than the v component during the whole period (Figure 3-3), since it had more negative values, and the predominant direction was E-NE. The u component assumed positive values only during the winter, especially in the month of June, when the direction was mostly NW-SW.

Figure 3-3 Wind speed u and v components in the vicinities of the Antares fixed station. The grid used for average encompassed latitudes from 17.5°S to 30°S , and longitudes from 47.5°W to 35°W .

3.4.1.2 Hydrography and Zooplankton

We used temperature and salinity signatures to detect SACW intrusions. When SACW was not detected TW, CW or both dominated the water column; this will be further referred the TW/CW domain. We observed SACW intrusions from September/October through April, *i.e.* from mid-spring, through summer into mid-fall. The depth of the SACW temperature signature cut-off (20°C), as well as the 18°C temperature depth, provides an estimate of the vertical extent of cooler water in the water column during intrusions (Figure 3-4). SACW intrusions were apparently stronger in the last months of 2011 and beginning of 2012; they were first detected in September, then occupied the whole water column between October and November and reached up to 3-10 m depth until April. On the other hand, the early-2010 summer probably experienced the weakest influence from bottom intrusions. August 2007, October and November 2011 had the lowest salinity levels, almost reaching values < 33 .

Figure 3-4 Temporal distribution of the depth at which the temperatures 18 and 20°C were detected. The black lines in the bottom axis indicate months when SACW intrusion was detected.

The SACW intrusion phase coincides with peaks in the distribution of nitrate, Chlorophyll a, mesozooplanton abundance and biomass (Figure 3-5). Nitrate and Chlorophyll a were higher from August/September through April/March in the lower layer, showing that during SACW bottom intrusions there is a nutrient enrichment below the pycnocline, whereas in the upper layer low concentrations were typical during the entire period.

Zooplankton abundance and carbon biomass estimated from scanned samples were higher from September/October through April, especially in the upper layer (Figure 3-5). Carbon biomass was also higher during intrusions. Highest values were observed during January and February 2012, and November 2007, in the upper layer; in the lower layer, highest values were recorded in November 2010, and March 2010 and 2012. These peaks in biomass occurred when calanoids (including the large species *Calanoides carinatus*), poecilostomatoids, *Penilia avirostris*, and/or chaetognaths attained peak abundances (see section 3.4.1.3).

Figure 3-5 Temporal distribution of temperature (A), salinity (B), Chlorophyll a (C), nitrate (D), and mesozooplankton total abundance (E) and carbon biomass (F) in the upper (black lines) and lower (gray lines) layers. Black lines in the bottom axis indicate months when SACW intrusion was detected.

Figure 3-6 Temporal distribution of σ_N^2 and particle volume in the Gaussian and residual distributions, in the upper (A) and lower (B) layer. The left vertical axis refers to σ_N^2 , whereas the right axis refers to particle volume. Black lines in the bottom axis indicate months when SACW intrusion was detected.

3.4.1.3 Zooplankton community structure

The most important taxonomic groups in terms of abundance and/or biomass were calanoids, poecilostomatoids (families Corycaeidae and Oncaeidae), cyclopoids (mainly *Oithona* spp.), the cladoceran *Penilia avirostris*, and Appendicularia, Thaliacea and Chaetognatha. The temporal distribution of the main taxonomic groups is shown in Figure 3-7 and Figure 3-8.

Copepods and *P. avirostris* were the most numerous taxa. Abundance and biomass were usually larger in the upper than in the lower layer. Biomass peaks in the lower layer were detected for thaliaceans and chaetognaths. From August 2010 through March 2011, calanoids and poecilostomatoids also had larger biomass in the lower layer.

Calanoids were three times more abundant in January and February 2012 than whenever else through the sampling period. Poecilostomatoids were more abundant during the summers of 2007 and 2012, and during spring 2008 in the upper layer. In the lower layer, higher biomass occurred in the summer, especially in December 2009. *Penilia avirostris* was also more abundant in warmer months in the upper layer, especially in November 2007, January 2009, and April 2012.

Chaetognaths were important in terms of biomass because of their large size, and were among the few groups reaching higher abundance in the lower layer. Chaetognaths excelled in December 2009 and in the year of 2010, in March, and from October through January 2011. Appendicularians were more abundant in the upper layer during the entire period, except in July 2010, and January 2011. They were more abundant along 2010, and had a peak in March 2008. The largest appendicularian abundance and biomass values were detected from March to May.

Figure 3-7: Temporal variability in the abundance of the main zooplankton groups from July 2007 to June 2012, in the upper and lower layers. Note the different abundance scales. Black lines in the bottom axis indicate months when SACW intrusion was detected.

Figure 3-8 Temporal variability of carbon biomass in the main zooplankton groups from July 2007 to June 2012, in the upper and lower layers. Note the different scales. Black lines in the bottom axis indicate months when SACW intrusion was detected.

Calanoids were dominant in terms of relative abundance and biomass during most of the time series, in both depth layers (Figure 3-9). However, *P. avirostris* usually outnumbered calanoids during warmer months in the upper layer; during the summer of 2008 and 2010 *P. avirostris* also dominated in the lower layer, followed by poecilostomatoids (Figure 3-9). *Calanoides carinatus* occurred usually associated with SACW bottom intrusions, providing an important contribution to total biomass because of its large size (data not shown) despite of a low abundance. During intrusions, *P. avirostris*, *C. carinatus*, other calanoids and thaliaceans were important in terms of relative abundance and biomass (Figure 3-10).

Poecilostomatoids, chaetognaths and appendicularians were more abundant during the TW/CW regime especially in the lower layer (Figure 3-9). In terms of relative biomass, poecilostomatoids, chaetognaths and oithonids were more important during the TW/CW phase than during SACW intrusions (Figure 3-10), except during the salp abundance and biomass peak in June 2011. In this month an abrupt increase in the proportional abundance of salps occurred in the lower layer, from 19-m down to 39-m depth. Salps comprised 54% of the total zooplankton abundance and 41% of total carbon biomass during June 2011 (Figure 3-10). At the same time, LOPC profiles detected near-bottom particle peaks (see section 3.4.2, Figure 3-14).

Figure 3-9: Proportional abundance contribution of the main zooplankton groups from July 2007 to June 2012, in the upper and lower layer. Cal: Calanoida; Poe: Poecilostomatoida; App: Appendicularia; Tha: Thaliacea; Oit: Oithonidae; Pav: *Penilia avirostris*; Chae: Chaetognatha; Oth: Others.

Although numerically abundant, poecilostomatoids were less important in terms of biomass because of their small size (mean = 0.47 mm ESD). On the other hand, chaetognaths were less abundant, but had a larger contribution to total biomass, also due to their individual size (mean = 1.43 mm ESD). Calanoids (mean = 0.58 mm ESD) and *P. avirostris* (mean = 0.58 mm ESD) were as important in abundance as in biomass because of their high concentration during the entire sampling period.

Figure 3-10: Proportional carbon biomass contribution of the main zooplankton groups from July 2007 to June 2012, in the upper and lower layer. Cal: Calanoida; Poe: Poecilostomatoida; App: Appendicularia; Tha: Thaliacea; Oit: Oithonidae; Pav: *Penilia avirostris*; Chae: Chaetognatha; Oth: Others.

3.4.2 Particle vertical distribution and stratification

Temperature and salinity profiles were averaged according to the SACW intrusion phase or the TW/CW domain and are shown in Figure 3-11. Note the 5°C differences in the temperature from the surface to near-bottom during the intrusions, whereas during the TW/CW domain, temperature was homogeneously distributed through the water column. During both phases, salinity is lower in the surface and increases toward deeper depths, however it remains lower than 36 during SACW intrusions, but attains more than 36 when TW/CW dominates.

Figure 3-11 Vertical profiles of average temperature and salinity during SACW intrusion phase and during the TW/CW domain in the Antares fixed station.

To evaluate the influence of stratification on the vertical distribution of particle size structure, we plotted particle biomass in different size classes superimposed with N^2 over depth; the data was averaged according to the TW/CW domain (Figure 3-12) and the SACW intrusion phase (Figure 3-13).

Figure 3-12: Average vertical profiles of particle biomass in $\mu\text{g m}^{-3}$ for different size classes, average N^2 in s^{-1} and average chlorophyll fluorescence in $\mu\text{g L}^{-1}$. CW and/or TW domain. Note different scales. Size classes in mm are indicated inside plots.

As seen before, during TW/CW domain we did not observe water column stratification, and particle vertical distribution was homogeneous in all size ranges. Chlorophyll fluorescence was homogeneously distributed as well.

During SACW intrusions particle biomass was higher above the pycnocline in the upper 15-m depth range, irrespective of size class. Particle biomass peaks were in phase with N^2 peaks. Particle distribution in the 0.10 - 0.25 mm size range was coincident with the shallowest N^2 peaks, at 3 and 6 m depth; particle distribution in the 0.25 - 0.50 mm size range was in phase with all three N^2 peaks, at 3, 6 and 11 m depth. Particle distribution in the

0.50 - 2.00 mm size range followed the shallowest and the deepest N^2 peaks, at 3 and 11 m depth, respectively. Particle biomass and N^2 distributions during SACW intrusions were linearly correlated for particles ranging from 0.10 to 1.00 mm (Table 3-2). For particles > 1 mm, the p-value of the correlation was 0.02, but the R^2 value was too low (0.15). If we consider the number of samples during the intrusion phase ($n = 24$), the R^2 critical value should be > 0.40 to be considered significant. During the non-intrusion phase correlations were not significant. The subsurface chlorophyll maxima (below 15-m depth) were in phase with N^2 oscillations within the same depth range, but the shallowest N^2 peaks had no effect on fluorescence.

Figure 3-13: Average vertical profiles of particle biomass in $\mu\text{g m}^{-3}$ for different size classes, average N^2 in s^{-1} and average chlorophyll fluorescence in $\mu\text{g L}^{-1}$. SACW intrusion period. Note different scales. Size classes in mm are indicated inside plots. Blue arrows indicate where peaks of N^2 maxima were in phase with peaks of particle biomass.

Table 3-2: Correlation between average vertical profiles of particle biomass - zooplankton and aggregates - for different size classes and average N^2 , according to the dominant water mass SACW or CW/TW. R^2 and p-values are shown.

Particle size class	SACW		CW/TW	
	R^2 / p-value		R^2 / p-value	
0.10-0.25 mm	0.48	p < 0.01	< 0.1	p > 0.05
0.25-0.35 mm	0.49	p < 0.01	< 0.1	p > 0.05
0.35-0.50 mm	0.47	p < 0.01	< 0.1	p > 0.05
0.50-1.00 mm	0.41	p < 0.01	< 0.1	p > 0.05
1.00-2.00 mm	0.15	p = 0.02	< 0.1	p > 0.05

Zooplankton total abundance and biomass were also similarly associated with water column stratification in the upper layer. Although the linear fit for biomass was marginally significant, the total abundance and biomass of zooplankton in shallower depths were larger when σN^2 was higher. These results indicate that both particles and organisms accumulate above the pycnocline.

Table 3-3: Correlation between σN^2 and zooplankton total abundance and biomass in the upper layer. Monthly averages.

Correlation	R^2	p-value	Significance
Abundance upper	0.36	0.04	*
Biomass upper	0.32	0.05	Marginally significant

We observed a recurrent feature in June, in 2010, 2011 and 2012 when a large biomass of particles in the range of 0.10 - 1.00 mm occurred below 25-m depth (Figure

3-14). In those months SACW was retreated to the outer shelf, and no pycnoline was observed.

Figure 3-14: N^2 frequency (red line, s^{-1}), Chlorophyll fluorescence (green line, $\mu\text{g L}^{-1}$) and particle vertical distribution in different size bins, in mm (coloured symbols) in June 2010, 2011 and 2012. No fluorescence data is available for June 2010.

Although in June 2011 thaliaceans dominated the zooplankton community, the salp average size in this month was 3.8 mm feret diameter and 2.2 mm ESD, whereas the LOPC detected strong signals in the size range from 0.35 to 1 mm ESD. The net mesh size - 200 μm - efficiently captures organisms from 0.45 mm (Hopcroft *et al.* 2001) and the ZooScan detection limit is 0.30 mm. Therefore, the particles detected by the LOPC are probably not mesozooplankton. These could be aggregates, which are not well preserved in plankton samples, and/or sinking fecal pellets that accumulate close to the bottom, since there was no stratification, or that were resuspended from the bottom.

3.4.3 Size structure

The temporal distribution of the NBSS slopes calculated for mesozooplankton and particles is plotted in Figure 3-15. Steeper slopes were mainly found during the SACW intrusion phase, in the upper layer. The steepest values were found in January and March 2009, the former associated with a peak of *P. avirostris* and the latter again with *P. avirostris* in addition to small calanoids, including *T. turbinata* and poecilostomatoids. In the lower layer the steepest slope occurred in March 2010, when chaetognaths and calanoids were dominant. The winter of 2008 and 2010, and summer of 2009 and 2012 had the flattest and the steepest slopes, respectively.

The Gaussian and residual NBSS slopes varied widely through time. The steepest values in the Gaussian and residual distributions occurred in June 2009 and June 2012, respectively. Shallowest slopes were observed for the residual distribution during the winter of 2007, 2008 and 2009. However, winter samplings returned the steepest slopes in subsequent years.

Figure 3-15: Temporal distribution of NBSS slopes estimated with the ZooScan (upper panel) for the upper and lower layers, and with the LOPC (lower panel) for the Gaussian and residual distributions.

The organisms that mainly contributed to the NBSS outline are shown in Figure 3-16 and Figure 3-17. Calanoids biomass had two peaks, one in the small size range during SACW intrusions, especially in the upper layer (Figure 3-16), and a second peak in the upper end of the spectrum during TW/CW domain, which was related to large individuals of the genera *Labidocera*, *Eucalanus* and *Euchaeta* (Figure 3-17). In contrast, *Temora stylifera* had a peak in the small size range during TW/CW domain, and in the upper end of the spectrum during SACW intrusions (Figure 3-17). The peaks in the biomass size distribution of *T. turbinata*

alternated with those from *T. stylifera*, and the biomass size spectrum of these congeneric species overlapped only during SACW intrusions, in the lower layer. *Calanoides carinatus* occurred sporadically during TW/CW domain, but had a broader mass distribution, especially in the upper end of the spectrum, during SACW intrusions (Figure 3-17).

Oithonids and poecilostomatoids were more important during SACW intrusions. *Penilia avirostris* mass distribution was more important in the upper layers, also especially during SACW intrusions. Despite its large size, thaliaceans have a lower contribution in terms of carbon biomass, except in the lower layer, during TW/CW, when high densities occurred in June 2011. Even in this occasion, the carbon mass distribution was narrow. Nevertheless, chaetognaths and cnidarians have a broader distribution towards the upper end of the spectra during SACW intrusions.

Figure 3-16: ZooScan-derived NBSS for different taxonomic groups in the upper and lower layer, during SACW intrusion and during TW/CW dominance. Cal: Calanoida; Oit: Oithonidae; Poe: Poecilostomatoida; Pav: *Penilia avirostris*; Tha: Thaliacea; Chae: Chaetognatha; Cni: Cnidaria.

Figure 3-17: ZooScan-derived NBSS for selected copepod taxa in the upper and lower layer, during SACW intrusion and during TW/CW dominance. Cari: *Calanoides carinatus*; Ttur: *Temora turbinata*; Tsty: *T. stylifera*; LCal: Large calanoids including *Labidocera* sp., *Eucalanus* sp. and *Euchaeta* sp.

3.4.4 Statistical analysis

3.4.4.1 Wind speed vs hydrography

The monthly averages of the *u* and *v* components were significantly correlated with nitrate and Chlorophyll *a* concentrations in the lower layer; the *u* component also correlated with total zooplankton abundance and total zooplankton biomass in the upper layer (Table 3-4). When wind speed averages of 10 days prior to sampling were considered, the *u* component also had significant correlations with zooplankton abundance and biomass. For this component, monthly averages provided higher R^2 values. Considering the *v* component, there were correlations in the 10-day average with nitrate, Chlorophyll *a* and zooplankton abundance; monthly averages were correlated with Chlorophyll *a* and zooplankton biomass. These linear correlations had negative slopes, meaning that higher concentrations of Chlorophyll *a*, nitrate, and zooplankton abundance and biomass were associated with more negative values of the wind speed component, *i.e.* NE winds.

Table 3-4: Correlations between wind speed measurements and the variables nitrate, Chlorophyll a, and zooplankton total abundance and biomass. Monthly averages. The grid for the wind data comprised latitudes from 17.5°S to 30°S, and longitudes from 47.5°W to 35°W.

Variable	Layer	Wind component	p-value	R ²	Significance
Temperature	Lower	u component monthly average	< 0.01	0.75	*
Nitrate	Lower		< 0.01	0.74	*
Chlorophyll a	Lower		< 0.01	0.70	*
Abundance	Upper		0.03	0.40	*
Biomass	Upper		0.03	0.38	*
Temperature	Lower	v component monthly average	0.05	0.34	*
Nitrate	Lower		0.04	0.35	*
Chlorophyll a	Lower		0.05	0.34	*
Abundance	Upper		0.21	0.15	
Biomass	Upper		0.13	0.21	
Nitrate	Lower	u component - 10 days before sampling average	< 0.01	0.73	*
Chlorophyll a	Lower		< 0.01	0.56	*
Abundance	Upper		0.02	0.43	*
Biomass	Upper		0.02	0.43	*
Nitrate	Lower	v component - 10 days before sampling average	0.07	0.30	
Chlorophyll a	Lower		0.03	0.38	*
Abundance	Upper		0.05	0.33	marginally significant
Biomass	Upper		0.04	0.37	*

3.4.4.2 Differences between SACW intrusions and TW/CW domain

Differences in σ_N^2 , and in the u and v wind speed components between seasons with SACW intrusions and during TW/CW domain were significant (Table 3-5). During the intrusion phase there were higher σ_N^2 values, indicating

stronger stratification, and more negative values of the wind speed components, indicating stronger winds.

Table 3-5 Wind speed measurements, u and v component, and σ_N^2 , average and standard deviation according to SACW intrusions and TW/CW domain. p-values refer to the results of Wilcoxon rank sum tests.

	SACW	TW/CW	
	Median $\pm$ std	Median $\pm$ std	p-value
u component	-2.97 ± 1.07	-1.26 ± 1.40	<0.01
v component	-1.46 ± 1.11	-0.59 ± 1.59	<0.01
σ_N^2	$1.00 \times 10^{-3} \pm 0.6 \times 10^{-3}$	$0.26 \times 10^{-3} \pm 0.28 \times 10^{-3}$	<0.01

Mesozooplankton total abundance and total biomass were also higher during SACW intrusions then during TW/CW domain, but differences were only significant in the upper layer (Figure 3-18). In contrast, Chlorophyll a and nitrate were significantly higher in the lower layer during SACW intrusions ($p < 0.05$, Figure 3-19).

Figure 3-18: Median mesozooplankton abundance (A, B) and carbon biomass (C, D) in the upper layer and lower layers, according to the SACW intrusions phase and TW/CW domain. The asterisk indicates when differences were significant ($p < 0.05$, Mann-Whitney U-test); n represents the number of samples.

Figure 3-19: Median Chlorophyll a (A, B) and nitrate (C, D) concentrations in the upper layer and lower layers, according to the SACW intrusions and TW/CW domain. The asterisk indicates when differences were significant ($p < 0.05$, Mann-Whitney U-test); n represents the number of samples. Chla: Chlorophyll a.

Mesozooplankton NBSS intercepts - calculated in terms of carbon - covaried with total abundance ($p < 0.01$; $R^2 = 0.47$ and $R^2 = 0.59$ in the upper and lower layer, respectively) and total biomass ($p < 0.01$; $R^2 = 0.43$ and $R^2 = 0.49$ in the upper and lower layer, respectively). Therefore, intercepts and total production were linearly correlated.

During SACW intrusions mesozooplankton slopes were steeper than during TW/CW domain (Table 3-6). This was true for NBSS calculated in terms of both carbon and volume, for the upper and lower layer, and for the integrated water column. Differences were significant for carbon slopes in the upper layer, and marginally significant in the lower layer ($p = 0.04$ and $p = 0.09$, respectively). LOPC NBSS slopes were also steeper during SACW intrusions considering the residual distribution (Table 3-6). On the other hand, slopes were shallower during intrusions for the total and Gaussian distributions and the difference was marginally significant for the total distribution ($p = 0.09$).

Intercepts were higher during SACW intrusions for each of the ways we estimated NBSS: for mesozooplankton carbon and volume NBSS, for both layers and for the integrated water column, and for LOPC total, Gaussian and residual distributions (Table 3-6). Differences were significant for carbon NBSS in the upper layer (Figure 3-20) and for the integrated water column, and also for the LOPC residual distribution (Figure 3-21). Differences were marginally significant for mesozooplankton NBSS intercepts in terms of volume also in the upper layer and for the integrated water column ($p = 0.06$ and $p = 0.07$, respectively).

Table 3-6: Results of Wilcoxon rank sum tests performed with NBSS parameters in relation to SACW intrusions and TW/CW domain. Zooplankton NBSS parameters were calculated for the integrated water column, for the upper and lower layers, in terms of volume and carbon biomass. LOPC NBSS parameters were calculated for the residual, Gaussian and total distributions for the integrated water column. Median $\pm$ standard deviation.

		SACW	TW/CW	
		Upper Layer		p-value
ZooScan	Slope	-1.05 $\pm$ 0.41	-1.00 $\pm$ 0.23	0.42
Volume	Intercept	4.78 $\pm$ 0.89	4.48 $\pm$ 0.53	0.06
ZooScan	Slope	-1.43 $\pm$ 0.61	-1.25 $\pm$ 0.26	0.04
Cabon	Intercept	3.75 $\pm$ 0.65	3.34 $\pm$ 0.37	<0.01
Median size	ESD	618.43 $\pm$ 123.76	622.30 $\pm$ 75.41	0.36
		Lower Layer		
ZooScan	Slope	-1.12 $\pm$ 0.38	-1.06 $\pm$ 0.32	0.42
Volume	Intercept	4.43 $\pm$ 1.06	4.37 $\pm$ 0.90	0.33
ZooScan	Slope	-1.38 $\pm$ 0.47	-1.21 $\pm$ 0.36	0.09
Cabon	Intercept	3.61 $\pm$ 1.04	3.36 $\pm$ 0.87	0.31
Median size	ESD	634.82 $\pm$ 137.03	643.84 $\pm$ 174.88	0.81
Removing June 2011		634.82 $\pm$ 137.03	609.33 $\pm$ 82.31	0.57
Integrated		SACW	TW/CW	
ZooScan	Slope	-1.05 $\pm$ 0.35	-0.94 $\pm$ 0.31	0.47
Volume	Intercept	4.77 $\pm$ 0.79	4.47 $\pm$ 0.59	0.07
ZooScan	Slope	-1.34 $\pm$ 0.47	-1.28 $\pm$ 0.22	0.14
Carbon	Intercept	3.69 $\pm$ 0.61	3.37 $\pm$ 0.38	0.02
LOPC	Slope	-1.00 $\pm$ 0.09	-1.06 $\pm$ 0.22	0.09
Total	Intercept	5.54 $\pm$ 0.23	5.33 $\pm$ 0.63	0.33
LOPC	Slope	-1.67 $\pm$ 0.26	-1.80 $\pm$ 0.14	0.51
Gauss	Intercept	6.70 $\pm$ 0.75	6.42 $\pm$ 0.51	0.25
LOPC	Slope	-0.95 $\pm$ 0.10	-0.89 $\pm$ 0.16	0.25
Residual	Intercept	4.94 $\pm$ 0.26	4.52 $\pm$ 0.34	0.04

Figure 3-20. ZooScan-derived zooplankton NBSS Slopes (A, B) and intercepts (C, D) in the upper (A, C) and lower (B, D) layers; according to the dominant water mass, TW/CW or SACW. NBSS was calculated using carbon biomass. Significant differences are indicated by asterisks (*).

Figure 3-21. LOPC-derived NBSS slopes and intercepts (A, C) and intercepts (B, D) for the residual (A, B) and Gaussian (C, D) distributions, according to the dominant water mass, TW/CW or SACW. Significant differences are indicated by asterisks (*).

As stratification became more intense, *i.e.* with high σ_N^2 values, mesozooplankton carbon NBSS intercepts were higher ($p < 0.01$; $R^2 = 0.17$) and slopes were steeper ($p = 0.02$; $R^2 = 0.13$) in the upper layer, although R^2 was too low for this relationship to be considered significant.

3.5 Discussion

In this study we tested the influence of the South Atlantic Central Water (SACW) on the vertical distribution and seasonal variability of mesozooplankton and particle abundance and biomass in a fixed station located on the SBCS. All physical and chemical variables that were investigated in this study, *i.e.* u and v wind components, Chlorophyll a , nitrate and σ_N^2 were important to distinguish the seasonal cycles of SACW intrusion and TW/CW domain. During SACW intrusions stratification and wind speed were stronger, and Chlorophyll a and nitrate concentrations were higher, as were particle and plankton abundance and carbon biomass.

SACW seasonal intrusions on the continental shelf result from changes in the wind speed direction (see section 3.3.1). Since wind speed components, Chlorophyll a , nitrate, abundance and carbon biomass were all associated with SACW intrusions, we expected that these variables also correlated to each other, at least to some extent. The u and v components of the wind speed were indeed linearly correlated with Chlorophyll a , nitrate, and mesozooplankton abundance and carbon biomass; σ_N^2 was correlated with mesozooplankton abundance and biomass; N^2 profiles correlated with the vertical distribution of particle biomass in the size range < 1 mm.

Interestingly, the SACW intrusions promoted increases in nitrate and Chlorophyll a in the lower layer, while the zooplankton biomass increased in the upper layer. Density discontinuities have been strongly associated with the accumulation of particles (MacIntyre *et al.* 1995), and the pycnocline has been described as the main cause of plankton patchiness (Hamner 1988). However, stratification should

not be a constraint to vertical migration of neither zooplankton nor phytoplankton. Laboratory experiments have demonstrated that small organisms, such as the dinoflagellate *Alexandrium tamarense*, can migrate vertically through a stratified water column ($\sim 10^{\circ}\text{C}$ of temperature range), and its migration is tightly connected to nitrogen availability (MacIntyre et al. 1997). This species only showed its nocturnal migrations when nitrate was depleted from the surface; this kind of behavior was also described for other species (e.g., Santos and Carreto, 1992).

Shulman et al. (2012) reported swimming speeds of 40 m per day for *Alexandrium* and *Dinophysis*. Therefore, it is plausible to assume that dinoflagellates are able to take up the surplus nitrate injected in the system during SACW bottom intrusions. In addition, the euphotic zone often reaches the bottom during SACW intrusions (Oliveira 2009a); hence even phytoplankton species with lower motility, such as diatoms, could also be favored by nutrient input during intrusions. As a consequence, the mesozooplankton community seems to be able to take an advantage of the increased primary production. The usual lower mesozooplankton concentrations in the bottom layer may also be reflecting diel vertical migrations. Considering that: i) zooplankton also vertically migrates searching for food and avoiding predators; ii) particles and zooplankton concentrated above the pycnocline, and iii) phytoplankton concentrated just below the pycnocline, we speculate that phytoplankton strategies to escape their predators are relatively more efficient than zooplankton strategies to find their prey.

3.5.1 Mesozooplankton temporal patterns

The strong association between SACW and mesozooplankton biomass confirm the importance of these intrusions to enhance local productivity (Metzler *et al.* 1997, Saldanha-Corrêa and Gíanesella 2004), especially of herbivorous mesozooplankton (Guenther *et al.* 2008), since we found typically herbivorous groups associated to intrusions (e.g. calanoids, including *Temora* spp. and *C. carinatus*, oithonids and *P. avirostris*). Katechakis *et al.* (2004) described *P. avirostris* as a passive filter-feeder that feeds efficiently under intermediate and high food concentrations; Turner (1986) found that oithonids feed on microzooplankton and large phytoplankton, and we observed that cyclopoids seem to have an advantage on stratified and less turbulent waters in another region of the Brazilian Continental Shelf (Chapter 2), probably because of its typical ambush-feeding mode (Saiz *et al.*, 2003).

The biomass size distribution of the two species of *Temora* was usually alternated, which suggests that the life cycle of these species are not synchronized, as pointed out by Cunha (2008). This could also reflect a competition for food resources, since there was an overlap in their distributions during SACW intrusions in the lower layer - high Chlorophyll *a*, high nitrate. Thus, when there is supposedly enough food for both species, they may develop similarly within the same size spectra. These are further evidences that SACW intrusions provide, in addition to nutrient enrichment, suitable food for the copepod development.

The winter in this region is associated with an increase of cold fronts; Tropical Water and/or Coastal Water were dominant, the pycnocline was virtually absent and vertical mixing likely increased, as reported by Castro

et al. (1987). The low nitrate and low Chlorophyll a concentrations indicate that food becomes limiting in this time of the year, at least for some organisms. Appendicularians, poecilostomatoids (oncaeids and corycaeids), and chaetognaths had a relatively more important role on the zooplankton community in the TW/CW domain, especially in the lower layer. Appendicularians are known to be well adapted to low food concentrations of oligotrophic waters (Scheinberg et al. 2005). The poecilostomatoid *Oncaea* was previously associated with abandoned appendicularian houses, feeding upon particles and organisms attached to the houses (Alldredge 1972); *Oncaea* was also positively correlated with particulate matter concentration (Paffenhöfer 1983). Finally, chaetognaths from the coast off Ubatuba were previously described to feed mainly on copepods, appendicularians, cladocerans, mollusk eggs and annelids; in the winter the most abundant groups in their guts were *Oncaea* spp. and appendicularians (Liang and Vega-pérez 1995). Therefore, during winter, when phytoplankton is probably not as available as during intrusion phases, other particles may become an important food source off Ubatuba, and even large organisms, such as chaetognaths and other gelatinous groups, can feed on particle-feeding organisms.

According to Madin (1974) salps are voracious non-selective filter-feeders able to collect particles from < 1 μm to 1 mm. *Thalia democratica* is the most common thaliacean species in the coast of Ubatuba and has extremely high growth rates and generation times (Heron 1972). These organisms were relatively more abundant during SACW intrusions, when particle volume was higher; however, there was a bloom of salps during June (winter), also accompanied by high particle concentrations close to the bottom.

3.5.2 Seston vertical size structure and stratification

The particle vertical distribution was strongly influenced by water column density discontinuities. Our results indicate that plankton thin layers develop under stratified conditions in the coastal region off Ubatuba. The evidence comes from the increased particle concentration in the < 1 mm size range within depth intervals typical of thin layers ($< 3 - 5$ m thick; Sullivan et al. 2010) associated with N^2 peaks during SACW intrusions. Plankton thin layers may extend horizontally over hundreds of meters to several kilometers (Moline et al. 2010), and persist for several days (McManus et al. 2007). Thin layers may be constituted of a variety of living and non-living material, including phytoplankton, zooplankton, marine snow, fecal pellets, as well as bacteria and virus (Rines et al. 2010). The concentration of organisms in thin layers is usually orders of magnitude higher than the adjacent depths (Cowles, 2003), and these biomass maxima impact the basis of the trophic web in coastal environments and influence on the success of foraging of fish larvae (Durham e Stocker 2012).

Although particles also accumulated in the mixed layer, there were differences on a finer vertical scale among distinct size classes. The mixed layer was not entirely mixed, since there were three main N^2 peaks, and particles with different sizes were distributed according to those peaks. Because chlorophyll fluorescence was only higher below the pycnocline, most particles that were retained in the initial 11-m depth were likely to be either microzooplankton or aggregates.

Lampitt et al. (1993), MacIntyre et al. (1995) and Petrik et al. (2013) also detected strong correlation between high concentrations of marine snow and density

discontinuities in the water column, identified by high N^2 values. MacIntyre et al. (1995) claimed that turbulence caused the particle accumulation, which would also cause higher accumulation of particulate material. In our study, particles > 1 mm were less influenced by σ_N^2 , thus other factors should be taken into account to describe their vertical distribution.

In contrast, when no pycnocline was observed, small particles, from 0.1 to 0.5 mm, tended to accumulate close to the bottom, especially during winter, in June 2010, 2011 and 2012. In those months SACW was restricted to the outer shelf, and because of an increase in the frequency of cold fronts and stronger winds, the pycnocline is eroded, and vertical mixing can increase (Castro et al. 1987). Since we found higher particle concentration close to the bottom, but no increase in mesozooplankton biomass or chlorophyll fluorescence, these particles are likely to be sinking marine snow, or resuspended aggregates. Even in June 2011, when there was a salp maximum, the particles detected by the LOPC did not correspond to the salp size range as collected with the nets. The particle size range from LOPC measurements was smaller than that of salps measured by the ZooScan. Although the size of transparent zooplankton may be underestimated by the LOPC (Herman et al. 2004), the particle concentration was much higher than salp abundance and the abundance of any other zooplankton group collected with the net. These results reinforce our interpretation that LOPC signals observed during June should be small non-living particles. The near-bottom LOPC signals could also be generated by salp fecal pellets, which sink relatively fast through the water column (Bruland and Silver, 1981).

We hypothesize that the positive average values in the u component, i.e. NW-SW winds, associated with the

detection of high particle loads close to the bottom could be used as an indicative of resuspension processes in this region.

3.5.3 NBSS and size structure temporal variability

The ZooScan-derived NBSS parameters were more similar to those from the LOPC residual fraction than those from the total particle distribution. Therefore, the separation of the LOPC data into Gaussian and residual distributions seems to be efficient to provide more consistent estimates for both particle and zooplankton size distributions.

Higher intercepts were previously associated with productivity increase by Sprules and Munawar (1986) and Iriarte and González (2004). The higher intercepts are consistent with the evidence of increasing productivity during SACW intrusions (see section 3.5.1). Saldanha-corrêa et al. (2004) also argue that the SACW acts as a natural eutrophication agent promoting the development of the autochthonous phytoplankton community. In addition, the steeper NBSS slopes could also be an indicative of higher productivity (Zhou et al. 2009), although this association can be controversial (Chapter 2, section 2.5.4).

The significant differences of ZooScan-derived NBSS carbon slopes and intercepts between the SACW intrusion and the TW/CW domain support the hypothesis that the plankton size structure and production change seasonally due to the influence of different climatological conditions. However, the lack of significant differences in the NBSS slopes derived from profiles and from ZooScan biovolume indicates the existence of strong variability that cannot be assigned to a particular cause based on the current evidence.

Steeper NBSS slopes were found in high productive areas closer to the coast in contrast with open ocean stations in the Abrolhos ecosystem (Chapter 2). However, we also expected that SACW bottom intrusions would produce an effect similar to the SACW upwelling that occurs in Cabo Frio: nutrients are injected in pulses, large phytoplankton (especially diatoms) is favored and then populations of large zooplankton have the chance to develop (Falkowski and Oliver 2007, Guenther *et al.* 2008). In upwelling conditions other authors have found that the slopes are in fact shallower than during subsidence phases (Iriarte and González, 2004; San Martin *et al.*, 2006). Possible causes for such apparent contradictions can be that i) the SACW seasonal influence off Ubatuba is not strong and frequent enough to produce such changes in the planktonic system, ii) there are other sources of variability acting on different scales, or iii) the NBSS parameters are not efficient to predict changes on this highly variable subtropical system.

Richardson (2008) argues that temperature is probably the most important physical variable structuring marine ecosystems. In a stratified water column with warmer surface temperature scenario, the surface water can be rapidly nutrient-depleted and the phytoplankton community is likely to be dominated by picoplankton and flagellates (Margalef 1997). On the other hand, under the SACW upwelling further north in Cabo Frio, when nutrients are injected in pulses, the phytoplankton community is largely dominated by diatoms (Guenther *et al.* 2008). Therefore, although SACW bottom intrusions inject nutrients in the system, as in an upwelling event, this water mass rarely reaches the surface on the inner shelf off Ubatuba. Furthermore, stratification is the rule during bottom intrusions. The steeper NBSS slopes indicate that small

organisms, more likely to feed on small phytoplankton, dominate the zooplankton community in the upper layer during intrusions.

The extent to which SACW intrusions influence the phytoplankton system in the inner shelf of the SBCS will depend, for example, on the SACW residence time and on light attenuation levels. Depending on the SACW residence time, there will be more or less nutrient available for phytoplankton. Depending on the light attenuation level, either diatoms or flagellates would dominate during SACW intrusion (Saldanha-Corrêa and Gíanesella 2004). The monthly sampling intervals may not have enough temporal resolution to allow a full understanding of how biomass size structure changes in relation to SACW intrusions, since stratification leading to nutrient depletion and light attenuation can be a key factor on phytoplankton community structure. On the other hand, the intense SACW intrusions in 2012, leading to peaks in nitrate, chlorophyll *a*, mesozooplankton and near-surface particle concentration, exemplify how bottom-up forces have a dramatic impact on the structure and dynamics of the pelagic ecosystem off Ubatuba in a multiannual scale. According to Mackas et al. (2012), both seasonal and multiannual variations in the bottom-up controls on primary and secondary production were apparent in the marine pelagic environments included in their literature review.

In terms of carbon biomass and size distributions, copepods were the most important group; during SACW intrusions most carbon mass was related to calanoids in the lower size range, except for *C. carinatus* and *T. stylifera*, which contributed to the upper end of the size spectra. Although chaetognaths and cnidarians had a broader distribution toward the upper end of the spectra, the

contributions of small copepods in the lower end were proportionately higher and slopes were steeper. On the other hand, the shallower slopes during the TW/CW domain can be attributed to large calanoids - *Labidocera* sp., *Euchaeta* sp. and *Eucalanus* sp. - in the upper end of the spectra, and to the lower contribution of small copepods and lower carbon content of thaliaceans, despite their large size.

Differences in the structure of the mesozooplankton community also point out to differences in the dominant feeding process, switching from a particle-feeding mode to an herbivorous mode during intrusions. Lopes et al. (1999) described SACW intrusions as the main controlling factor of the seasonal variability of copepods on the SBCS. However, other factors must be taken into account to explain the distribution of large calanoids and gelatinous groups associated with TW/CW domain.

3.6 Conclusions

The cycles of SACW intrusion and TW/CW domain were striking on the inner shelf off Ubatuba, influencing the mesozooplankton community structure and the vertical distribution of particles and zooplankton biomass. Although productivity was lower in the winter, particle feeding could be an important process structuring the food web, consisting an alternative food source for the zooplankton when phytoplankton concentrations are low.

The density structure of the water column cannot be neglected if one wants to understand the effects of SACW intrusions on plankton size structure off Ubatuba. More frequent sampling, focusing also on phytoplankton size

structure, would clarify the influence of SACW intrusions on the zooplankton biomass size spectra.

Despite the great number of studies about plankton time series recently published since 2005, some approaches are still unexplored, remaining gaps in the literature, such as comparisons between regions to test differences on the biomass size spectra (Mackas et al. 2012). Our study adds information on biomass size spectra changes in relation to SACW intrusions off Ubatuba, indicating future directions to understand the factors that regulate the trophic structure of the zooplankton community.

4 Concluding remarks

This thesis provided data on plankton and particle biomass size spectra distribution for an extensive shelf and oceanic area off Eastern Brazil and for a 5-year time series in a fixed station on the inner shelf off Ubatuba, in Southeastern Brazil.

Significant differences on NBSS parameters, slopes and intercepts were found for the different environmental settings, and reflected changes on the plankton trophic structure. Higher intercepts were consistently associated with increased zooplankton biomass, while slopes varied in the two studied areas according to topographic (coastal/ocean gradient in Abrolhos) or seasonal (SACW intrusions or TW/CW dominance in Ubatuba) influences. We found steeper slopes associated with well-mixed, coastal and more productive areas on the Abrolhos Bank and vicinities. Considering temporal variability in the subtropical site, we found steeper slopes associated with stratified waters and more productive seasons. Table 4-1 summarizes the main trends in NBSS parameters according to different environmental settings.

As mentioned in section 2.5.4, both shallower and steeper slopes have been associated with higher pelagic productivity (Iriarte and González 2004; Zhou *et al.* 2009). Considering our findings in the previous chapters and the pertinent literature, we argue that NBSS parameters may provide relevant information to understand the structure and dynamics of pelagic ecosystems, although it is crucial that other data sources are concomitantly provided, such as taxonomic composition and water column thermohaline structure. NBSS slopes should be carefully interpreted, especially in regard to tropical and subtropical plankton

communities, which are extremely diversified leading to complex food web interactions.

Another consistent finding of this thesis was the relationship between water column stratification and particle abundance in the size range < 1 mm. Such correlation was found in both study areas, regardless of sampling period; particles were concentrated within or above the pycnocline when stratification occurred. Interestingly, vertical maxima in particle abundance and biomass were suggestive of the presence of plankton thin layers in certain situations. This deserves further detailed investigation, as thin layers are ecologically relevant oceanographic features shaping plankton systems in coastal seas (Sullivan et al. 2010; Durham and Stoecker, 2012).

Table 4-1 Summary of NBSS parameters over different environmental settings, considering spatial and temporal variability.

	Abrolhos Bank and vicinities		Ubatuba fixed station	
	Coastal	Oceanic	SACW intrusion	TW/CW domain
Density structure	Well-mixed	Stratified	Stratified	Well-mixed
Production	Higher	Lower	Higher	Lower
NBSS				
Intercepts	Higher	Lower	Higher	Lower
Slopes	Steeper	Shallower	Steeper	Shallower

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Appendix A

A brief account on sample
processing and analytical methods
with the ZooScan and the LOPC

6 Appendix A: A brief account on sample processing and analytical methods with the ZooScan and the LOPC

6.1 General procedures with the ZooScan

Preserved plankton samples were scanned with a ZooScan to obtain digital images of organisms and particles. We used the *Biotom* ZooScan model and the latest version of Zooprocess software at the time of analysis. Zooprocess is a specific ImageJ plugin (<http://rsb.info.nih.gov/ij/>) developed for plankton image analysis. The ZooScan image resolution was set to 2400 dpi, providing a 16-bit digital image. The subsequent text describes the main steps followed in this thesis from the preparation of preserved plankton samples to the final .txt file output with automatic classification results and size distributions. Detailed background on the ZooScan system can be found elsewhere (Gorsky *et al.* 2010, Grosjean *et al.* 2004).

1. Scanning a zooplankton sample with the ZooScan: main steps: A blank background image using distilled water was captured and processed every four scanned samples;
2. Each preserved plankton sample was sieved through a 40- μm mesh to remove formalin, and the sample was resuspended in distilled water;
3. Sample aliquots were taken using a Motoda splitter (Omori and Ikeda 1984). In certain cases, samples were sieved through a 500 μm -mesh to allow more efficient sampling of organisms through the entire size spectrum (see section 3.3.6).

4. The desired sample aliquot was poured on the ZooScan glass containing an appropriate sample holder (a plexiglass frame);
5. Organisms touching each other were manually separated on the scanning area and from the frame edges before scanning (Figure 6-1). Manual separation usually took ~15 min per aliquot;

Figure 6-1 - Manual separation of organisms and particles touching each other on the scanning area prior to aliquot digitalization.

6. The aliquot was scanned, the resulting image was normalized and the raw image was converted to full grey scale range;
7. Images were processed by subtracting the background and removing frame edges, in batch mode, during the night after the working day. An example of the image from a scanned sample, after this process, is shown in Figure 6-2;
8. Individual objects, *i.e.* vignettes, were extracted for Plankton Identifier software;

Figure 6-2 Plankton sample scanned with the ZooScan, after processing, before vignettes extraction.

9. A training set was built by sorting images into taxonomic groups, including categories for detritus, fibers and carcasses - extensive folder names, with spaces or parenthesis were avoided;

9.1. The training set consisted of an image dataset for each taxonomic group or particle category. As recommended by Gorsky et al. (2010), 200-300 vignettes were sorted by category. Some groups could be identified to the species level, such as the copepods *Calanoides carinatus*, *Temora turbinata* and *T. stylifera*, and the cnidarian *Aglaura hemistoma* (Figure 6-4);

9.2. Distinct training sets were used for each size class analyzed in each study area, because of the variable zooplankton composition.

- Different categories for ventral and lateral positions of the same taxon were also included;
10. A classifier and a confusion matrix were constructed; the classifier was cross-validated to evaluate the training set efficiency, then categories with low recall and high contamination were improved by manually moving displaced vignettes (Figure 6-3);
 11. The classifier was applied to predict objects classification, using the Random Forest algorithm;
 12. Image features considered in the classifier were the same recommended by Gorsky *et al.* (2010);
 13. Vignettes were extracted to folders according to prediction;
 14. All samples were manually validated using XnView;
 15. After validation, identifications were loaded from sorted vignettes; this procedure provided the analysis error as the percentage of images moved from their original folder, *i.e.* misclassified images;
 16. A new .txt file with the validated classification was created containing classification and metrics (in pixels) for each vignette. The size parameters were then converted from pixel to micrometer using the ratio 1.0 pixel: 10.58 μm , in accordance to the scanner resolution (Grosjean *et al.* 2004).

Error rate										
Values prediction										
Value	Recall	1-Precision		001_diatom_c	001_diatom_c_lat	00_badfocus	00_detritus	00_detri_esc	00_fiber	00_multiple
001_diatom_c	0.5600	0.4500	001_diatom_c	154	7	114	0	0	0	0
001_diatom_c_lat	0.7102	0.1300	001_diatom_c_lat	29	174	42	0	0	0	0
00_badfocus	0.7307	0.4632	00_badfocus	97	15	2711	53	15	101	18
00_detritus	0.2560	0.5152	00_detritus	0	1	167	64	0	2	0
00_detri_esc	0.0769	0.7500	00_detri_esc	0	2	38	0	5	0	0
00_fiber	0.2952	0.5350	00_fiber	0	0	209	5	0	93	0
			00_multiple	0	0	129	0	0	0	12

Figure 6-3 Confusion matrix after cross-validation in Plankton Identifier. Note how diatoms (001_diatom_c_lat), for example, had high recall value (71%), although they were misclassified as bad focus 42 times during the cross-validation.

Figure 6-4 - Vignettes extracted from samples scanned with the ZooScan and processed with ZooProcess. A: *Temora stylifera*, B: *Calanoides carinatus*, C: *Aglaura hemistoma*.

6.2 Processing LOPC raw data

The LOPC was mounted on a T-frame with an interoperable micro-CTD (AML Microsystems), as recommended in the user manual. The sampling tunnel area of our model is 70 x 70 mm, and the beam area (frontal cross-section) is 49 cm². The LOPC measurement principle is that the occluded particle cross-sectional area in the beam is measured relative to the area of the photodiode element detecting that occlusion (Herman *et al.* 2004). The digital size (DS) of a particle is the peak negative-going change in voltage sensed by each occluded diode (Checkley *et al.* 2008). Table 6-1 contains abbreviations used in this section.

Table 6-1: Notation. 1 ppm = 1 mm³L⁻¹ = 1 cm³m⁻³.

Symbol	Meaning
d	Particle diameter
d_{esd}	Particle equivalent spherical diameter
d_{od}	Particle occluded diameter (width)
DS	Digital size
ΔN	Concentration of particles within a size bin
Δd	Size bin width
n	Number spectrum
n_{agg}	n resulting from lognormal fitting
n_{res}	$n - n_{agg}$
V	Volume of an individual particle
nV	Volume spectrum as a function of d
nVd	nV times d
V_{tot}	Total particle volume
V_{agg}	Total volume of Gaussian fitted fraction
V_{res}	Total volume of residual fraction
y_g	Normal curve fit to nVd spectrum

The LOPC control software defines single-element particles (SEP) as those smaller than 1 mm and occluding only one or two photodiode elements, and multi-element particles (MEP) as those occluding three or more elements (Herman *et al.* 2004). SEPs DSs are stored as a frequency distribution every preset time interval, while for each MEP the occlusion start time and duration are recorded for each diode element in addition to its DS (Checkley *et al.* 2008). Calibration is performed by passing near-spherical beads with known size through the light beam and d_{esd} is computed as the diameter derived by equating the area of a circle to the area of a particle with irregular shape, such as a copepod (Herman *et al.* 2004).

The SEP equivalent spherical diameter (d_{esd}) was computed directly from the DS, as the diameter of an opaque sphere that attenuates the same amount of light. In the case of MEPs, d_{esd} was computed from a regression between DS and d_{esd} , after calibration, according to equation 1 from Checkley *et al.* (2008):

$$d_{esd} = 0.1806059 + 2.54589 \times 10^{-4} DS - 1.0988 \times 10^{-9} DS^2 + 9.54 \times 10^{-15} DS^3$$

The raw data files (.dat) provided by the LOPC (Figure 6-5) are structured as follows:

- 'L1' - 'L4' lines: represent the SEP counts through size bins. Each line has 32 bins, in 15 μm increments. Therefore, the first 6 bins of L1 should be zero, since the detection limit is 100 μm . SEPs range from 100 to 1920 μm ;
- 'L5' lines: The 5th value of L5 is the cumulative total transit time of particles in a certain period (3 s), and the 4th value of L5 is the total number of particles used in the summation. Mean transit time can

Particle abundance, volume and size distributions were calculated with 1-m depth intervals for each profile, and then averaged over depth ranges of plankton tows, following procedures developed in Matlab by Jackson (personal comm.), further adapted to the purposes of this thesis.

6.2.1 Number and volume spectrum calculations

For each profile, we calculated the number spectrum n , which is the rate of change in the number of organisms within a size range and is given by $n = -\frac{dN}{dd}$. For small size intervals Δd , we can approximate:

$$n \approx \frac{\Delta N}{\Delta d}$$

ΔN is the amount of organisms within a size bin and Δd is the size bin width: $\Delta d = \frac{2}{\left(\frac{d_{(i+1)}}{d_{(i)}}\right)+1} \cdot d_{(i)} \cdot \left(\frac{d_{(i+1)}}{d_{(i)}} - 1\right)$.

Particle counts were accumulated for every 3 or 5-m depth intervals, depending on the profile depth range, from the deepest depth to the shallowest limit of 2 m to avoid interference from bubbles. We calculated n for each depth interval, using logarithmic size class diameters where the upper value was 1.1447 times the lower value. This means that particle volumes at the upper size range of a bin were 1.5 times larger than at the lower size range (Petrik et al. 2013).

For spherical particles, particle volume is computed as $V = \frac{\pi}{6} d^3$. The volume spectrum nV is the particle volume distribution as a function of d . If the volume distribution is plotted against d , the area under the nV curve is

proportional to the total particle volume, given a certain size range (Jackson and Checkley 2011; Petrik et al. 2013). In this case it is difficult to distinguish the contribution of smaller particles since the large ones will have a disproportionate biovolume. Thus, plotting nV as a function of $\ln d$ stretches out regions with smaller d values and shrinks those with larger values, but also destroys the proportionality between the area under the curve and total particle volume. According to Petrik et al. (2013), multiplying the volume distribution by d to plot nVd as a function of $\ln d$ compensates for such distortion and the area under the curve becomes again proportional to the total volume. nVd is unitless, since n (cm^{-4}) is multiplied by V (cm^3) and d (cm).

Figure 6-6. Visualizing the same volumetric spectrum in different ways. Figure extracted from Petrik et al. (2013). (a) Volume spectrum, nV as a function of d_{esd} . (b) Volume spectrum, nV as a function of $\ln d_{\text{esd}}$. (c) Normalized volume spectrum nVd (ppm) as a function of $\ln d_{\text{esd}}$. The area under the curve is proportional to total particle volume in a certain size range for a and c, but not b.

The nVd spectra were fitted to normal curves, believed to describe marine snow, following procedures detailed by Petrik et al. (2013), who argue that the Gaussian fitting procedure isolates marine snow and aggregates from LOPC

profiles. Petrik et al. (2013) also claim that their procedure is more efficient to separate aggregates from zooplankton than the former technique using the d_{esd} to d_{od} ratio to distinguish snow- from zoo-like particles (Jackson and Checkley 2011).

The fitting function has the form

$$y_g = V_{agg} \frac{1}{\sigma\sqrt{2\pi}} e^{-(x-\mu)^2/2\sigma^2}$$

where $x = \ln d$, μ and σ describe the distribution peak and width, and V_{agg} is the total volume of particles covered by the distribution. The resulting number spectrum is thus

$$n_{agg} = \frac{y_g}{Vd}$$

and the residual distribution is the total distribution subtracted by the Gaussian distribution as:

$$n_{res}Vd = nVd - n_{agg}Vd$$

The residual distribution is believed to describe zooplankton. Full details on the fitting procedure are described in Petrik et al. (2013).

6.3 Calculating the NBSS

The logarithmic normalized biomass size spectra (NBSS) were calculated for zooplankton samples scanned with the ZooScan and separately for LOPC profiles. The NBSS is defined by the formula $NBSS = \Delta M / \Delta m$, where ΔM is the total particle mass in a size region and Δm is the difference in mass of the largest and smallest particles in that region. Size classes were built considering an exponential increase, as described in the previous section.

Mass can be expressed in terms of either volume or carbon weight. For the scanned samples, only organisms were used to compute particle mass, whereas for the LOPC profiles, both organisms and non-living particles were included. In Chapter 3, we also calculated NBSS for the Gaussian and residual distributions.

The values for the lower and upper size ranges of each spectrum should be removed to compensate for the low sampling efficiency of the net at those boundaries. Therefore, we only considered counts from the peak of the distribution to the first empty bin toward large particle sizes. Furthermore, the NBSS parameters, slope and intercept were calculated by fitting a linear regression to each spectrum. In Chapter 2, ZooScan NBSS were calculated in terms of particle volume, whereas in Chapter 3, NBSS were computed in terms of both volume and carbon. Also in Chapter 3, additional LOPC NBSS calculations were performed taking into account the Gaussian and residual distributions.

7 Appendix B - Glossary of terms

Plankton: organisms that drift in the water column and cannot swim against a current.

Zooplankton: heterotrophic planktonic organisms.

Phytoplankton: autotrophic planktonic organisms.

Tychoplankton: organisms that inhabit the ocean floor, but are eventually carried to the water column and found in plankton samples.

Seston: consists of both organisms (plankton) and non-living particles.

Aggregate: consists of particles, carcasses, detritus, fecal pellets, etc. Particle aggregation is an important process for the formation and transformation of organic and inorganic materials in the ocean. The physical processes that lead to particle aggregation are known as coagulation. More information can be found in Burd and Jackson (2009).

Marine snow: microscopic aggregates varying from millimeters to centimeters in size.

Vignettes: isolated images of individual objects (organisms).

Size bin: same as size class.

nVd : normalized volume spectrum. Describes the distribution of particle volume. When plotted against $\ln d$, the area under the curve is proportional to the integrated particle volume.

8 Appendix C - Acronyms

SBCS: Southeast Brazilian Continental Shelf

SACW: South Atlantic Central Water

TW: Tropical Water

CW: Costal Water

LOPC: Laser Optical Particle Counter

BV: Brunt-Väisälä frequency distribution

N^2 : Brunt-Väisälä frequency distribution

σN^2 : Standard deviation of the Brunt-Väisälä frequency distribution

Chla: Chlorophyll a

d : Particle diameter

d_{esd} : Particle equivalent spherical diameter

d_{od} : Particle occluded diameter (width)

DS: Digital size

ΔN : Concentration of particles within a size bin

Δd : Size bin width

n : Number spectrum

n_{agg} : n resulting from lognormal fitting

n_{res} : $n - n_{agg}$

V : Volume of an individual particle

nV : Volume spectrum as a function of d

nVd : nV times d

$n_{agg}Vd$: Normal curve fit to the nVd distribution, believed to describe aggregates.

$n_{res}Vd$: Residual nVd distribution, believed to describe zooplankton.

V_{tot} : Total particle volume

V_{agg} : Total volume of Gaussian fitted fraction

V_{res} : Total volume of residual fraction.

y_g : Normal curve fit to nVd spectrum.