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Biodiversidade e distribuição de larvas de invertebrados da plataforma Sudeste-Sul do Brasil (21–34 ºS), com ênfase em larvas de Decapoda

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"**Biodiversidade e distribuição de larvas de invertebrados da plataforma Sudeste-Sul do Brasil (21-34°S), com ênfase em larvas de Decapoda**"

Por

Manoela Costa Brandão

Tese julgada e aprovada em sua forma final pelos membros titulares da Banca Examinadora (030/PPGECO/2015) do Programa de Pós-Graduação em Ecologia - UFSC.

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RESUMO

A maioria dos invertebrados marinhos produz larvas planctônicas, que permanecem desde minutos até meses no plâncton. Larvas de crustáceos decápodes frequentemente representam uma grande porcentagem das comunidades planctônicas em regiões neríticas. As características ambientais locais são um dos principais fatores que estruturam as comunidades planctônicas. Um dos principais desafios para os ecólogos marinhos é compreender em que medida as assembleias de larvas são influenciadas por fatores físicos e biológicos em diferentes cenários ambientais. No capítulo 1 foi testada a hipótese de que zonas frontais influenciam a distribuição da comunidade de larvas de invertebrados bentônicos ao longo da plataforma continental Sudeste-Sul do Brasil (entre 21 ºS e 34 ºS). Os resultados mostraram que a abundância e composição do meroplâncton são influenciadas pela distância da costa, frentes costeiras, de plataforma e estuarinas, além da ressurgência da Água Central do Atlântico Sul. Decapoda foi o grupo meroplanctônico mais frequente e recebeu maior destaque e resolução taxonômica na descrição da comunidade, originando o capítulo 2. O principal objetivo do capítulo 2 foi identificar os processos oceanográficos que estruturam a comunidade de larvas de decápodes ao longo da plataforma Sudeste-Sul do Brasil. Foi verificado que existe um acoplamento entre a abundância de larvas e a concentração de clorofila, especialmente para larvas de espécies bentônicas. Associações específicas de larvas foram observadas nos cenários ambientais mais relevantes: ambientes costeiros, ambientes oceânicos, e ambientes com predominância de escoamento continental. Dentre as larvas de decápodes bentônicos, caranguejos braquiúros apresentaram maior abundância e diversidade, especialmente próximo à costa. Com o objetivo de contribuir para os avanços na identificação molecular de larvas de decápodes, foi elaborado o capítulo 3. Nesse estudo, Brachyura foi utilizado como o primeiro grupo para investigar a viabilidade de identificação das larvas por *DNA barcoding*, em um arquipélago costeiro (27 ºS) do Sudeste-Sul do Brasil. A maior parte das larvas foi identificada com sucesso em nível específico, indicando que o uso dessa metodologia em trabalhos futuros de meso e larga escala pode contribuir para a identificação precisa e rápida de larvas de decápodes.

Palavras-chave: meroplâncton, holoplâncton, larvas de Decapoda, gradiente costa-oceano, plataforma continental, identificação, Brachyura, *DNA barcoding*.

ABSTRACT

Most marine invertebrates produce planktonic larvae, which may spend from minutes to months in the plankton. Decapod crustacean larvae often form a large portion of the planktonic community in neritic regions. The local environmental characteristics are one of the main factors which structure the planktonic communities. One of the major challenges for marine ecologists is to understand to which extent larval assemblages are influenced by physical and biological factors under different environmental scenarios. In chapter 1 we tested the hypothesis that frontal zones affect the distribution of the benthic invertebrate larval community along the South Brazil Shelf (between 21 °S and 34 °S). The results showed that the abundance and composition of the meroplankton are influenced by the distance from the coast, coastal, shelf and estuarine fronts, in addition to the upwelling of the South Atlantic Central Water. Decapod was the most frequent meroplanktonic group and received more emphasis and taxonomic resolution in the community description, originating chapter 2. The main goal of chapter 2 was to identify the oceanographic processes that structure the decapod larval community along the South Brazil Shelf. A coupling between chlorophyll concentration and larval abundance, mainly of early larvae of benthic species, was observed. Specific assemblages of larvae were observed in the most relevant environmental scenarios: coastal environments, offshore environments, and areas highly influenced by the continental runoff. Among benthic decapod larvae, brachyuran crabs presented the highest abundance and diversity, especially near the coast. In order to contribute to advances in the molecular identification of decapod larvae we developed chapter 3. In this study, Brachyura was used as the first group to investigate the feasibility of using DNA barcoding for larval identification in a coastal archipelago (27 ºS) of the South Brazil Shelf. The majority of the larvae were successfully identified into species, indicating that the use of this method in future mesoscale and large-scale studies, may contribute to precise and fast identification of decapod larvae.

Keywords: meroplankton, holoplankton, Decapoda larvae, cost-ocean gradient, continental shelf, identification, Brachyura, DNA barcoding.

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INTRODUÇÃO GERAL

O ciclo de vida da maior parte dos invertebrados marinhos inclui uma fase larval planctônica de vida livre, que difere dos adultos em tamanho, forma, habitat, nutrição e/ou mobilidade, podendo ser planctotrófica (se alimentam de outros organismos planctônicos) ou lecitotrófica (absorvem nutrientes presentes no vitelo) (Young, 2001). Dentre as espécies de invertebrados marinhos que apresentam uma fase larval, aproximadamente 85% apresentam uma fase planctotrófica longa (de semanas a meses no plâncton), 5% apresentam uma fase planctotrófica curta (de horas a dias no plâncton), enquanto 10% produzem larvas lecitotróficas (Thorson, 1950).

A presença de uma fase larval planctônica durante o ciclo de vida aumenta significativamente o potencial de dispersão, de colonização de novos habitats, e a conectividade genética entre populações, especialmente para espécies bentônicas sésseis. Por outro lado, as larvas estão sujeitas a uma grande pressão de predação enquanto permanecem no plâncton, além de correrem o risco de serem transportadas para locais com condições ambientais desfavoráveis (Pechenik, 1999).

A ecologia larval tem sido frequentemente apontada como fundamental para entender os processos que influenciam as populações, comunidades e ecossistemas marinhos (Webb et al., 2006), já que o fornecimento de larvas é um dos principais fatores que influenciam a estabilidade e os padrões de distribuição das populações bentônicas (Young, 2001).

Identificar padrões que estruturam a distribuição e a abundância das espécies, bem como as interações físicas, químicas e biológicas que determinam essas distribuições e abundâncias, constituem questões centrais em ecologia de comunidades (Ricklefs, 1987; Vellend, 2010). Ricklefs & Miller (1999) definem comunidade como uma associação de populações biológicas, e assembleia como um conjunto de grupos taxonomicamente relacionados ocorrendo na mesma região.

Comunidades planctônicas são frequentemente estruturadas em assembleias relacionadas com características ambientais locais (Munk et al., 2003). Sendo que um dos principais desafios para os ecólogos marinhos é entender o quanto as assembleias de larvas são influenciadas por componentes biológicos e/ou físicos em diferentes cenários ambientais (Hidalgo et al., 2014). A distribuição das larvas de invertebrados é influenciada por características físico-químicas (como temperatura, salinidade, nutrientes e correntes marinhas) e biológicas (como produtividade primária e secundária) presentes no ambiente

pelágico, que compreende a coluna de água das zonas neríticas e oceânicas.

Plataformas continentais, que consistem na zona de transição entre as zonas nerítica e oceânica, incluem a presença de diversas massas de água com características físicas e químicas distintas e, portanto, uma série de zonas frontais. Frentes são definidas como a manifestação na superfície ou subsuperfície do encontro de duas massas de água ou de processos oceanográficos de origens contrastantes, cuja formação pode ser favorecida pela interação de características topográficas, hidrodinâmicas e oceanográficas (Acha et al., 2015). A presença de frentes influencia a diversidade e a distribuição espacial das comunidades planctônicas devido ao acúmulo de nutrientes, produção primária ou secundária, e retenção larval (Munk et al., 2003). Além disso, zonas frontais são conhecidas como regiões com maior diversidade biológica, devido à convergência de espécies que habitam diferentes massas de água (Acha et al., 2004).

A região Sudeste-Sul da plataforma continental brasileira, compreendida entre Cabo de São Tomé (21 ºS) e Chuí (34 ºS), é influenciada por diferentes processos oceanográficos que aumentam significativamente a disponibilidade de nutrientes nas camadas superficiais, em contraste com a maior parte da margem continental brasileira, que é considerada predominantemente oligotrófica devido à influência da Água Tropical (AT) associada à Corrente do Brasil (CB) (Brandini, 2006).

Dentre os principais processos que aumentam a produtividade biológica na região destacam-se a ressurgência da Água Central do Atlântico Sul (ACAS) até a superfície, observada com frequência em Cabo de São Tomé (21 °S), Cabo Frio (23 °S), e Cabo de Santa Marta Grande (28 °S) (Castro & Miranda, 1998; Möller et al., 2008; Campos et al., 2013); e o aporte continental, influenciado por descargas de água doce de diversos estuários (Braga & Niencheski, 2006), com destaque para o complexo estuarino do Rio da Prata (21–34 ºS) e da Lagoa dos Patos (32 ºS) (Acha et al., 2004). Além disso, a região está sujeita a instabilidades de mesoescala, como vórtices (Pereira et al., 2009) e meandros da CB (Lorenzzetti et al., 2009), que acarretam variações físicas e podem estar associados a núcleos com concentrações elevadas de nutrientes, influenciando, dessa forma, os padrões de distribuição de organismos planctônicos (Brandini, 2006).

O desenvolvimento larval de diversas espécies bentônicas ocorre entre a zona nerítica e a quebra da plataforma continental, onde as larvas de crustáceos decápodes frequentemente são dominantes (e.g.

Schwamborn et al., 1999; González-Gordillo & Rodríguez, 2003). As larvas de decápodes apresentam uma grande importância ecológica, devido à abundância, diversidade e diferentes papéis ocupados nas cadeias alimentares.

Na região da plataforma continental Sudeste-Sul brasileira a maior parte dos estudos a respeito da estrutura da comunidade de larvas de invertebrados e sua interação com processos hidrológicos foram conduzidos em regiões estuarinas (e.g. Veloso & Valentin, 1993; Schwamborn & Bonecker, 1996) ou em regiões sujeitas a eventos de ressurgência. No sistema de ressurgência de Cabo Frio, alta abundância do meroplâncton foi associada a eventos de ressurgência (Yoshinaga et al., 2010). Além disso, larvas de bivalves e cirripédios apresentaram uma forte sazonalidade e um acoplamento com a biomassa fitoplanctônica principalmente na primavera, ao longo de uma série temporal de 15 anos (Fernandes et al., 2012). No Cabo de Santa Marta Grande a distribuição de paralarvas de cefalópodes mostrou uma relação com intrusões da ACAS na superfície (Vidal et al., 2010). Em relação à distribuição da comunidade de larvas de decápodes, a maior parte dos estudos também está concentrada em regiões estuarinas, como na entrada da Baía de Guanabara (22 ºS) (Fernandes et al., 2002), na Baía da Babitonga (26 ºS) (Marafon-Almeida, 2009), e de larvas de camarões peneídeos na desembocadura da Lagoa dos Patos (32 ºS) (Calazans, 2002). Estudos que englobem uma grade amostral da região costeira à oceânica são raros e muitos permanecem na literatura cinza, como os desenvolvidos na plataforma continental de Santa Catarina e Paraná (Ballabio, 2011) e em dois perfis latitudinais ente o Brasil e a África (20 ºS e 30 ºS) (Marafon-Almeida, 2015).

Por outro lado, a estrutura da comunidade holoplântonica já foi estudada em maior detalhe na região, sendo que os principais resultados incluem a diminuição da abundância ao longo do gradiente costa-oceano (e.g. Lopes et al., 2006; Resgalla Jr., 2011); e aumentos da abundância localizados sob a plataforma intermediária, geralmente associados a intrusões da ACAS na superfície (e.g. Valentin, 1984; Brandini et al., 2014).

Um dos principais fatores responsáveis pelo maior conhecimento das comunidades holoplanctônicas em detrimento das comunidades meroplanctônicas no mundo é a dificuldade de identificação das larvas, um dos principais gargalos encontrados até hoje, mesmo quando a morfologia dos adultos permite a diagnose inequívoca em nível específico. Sendo assim larvas de espécies comuns são frequentemente

difíceis ou impossíveis de identificar, com base somente na morfologia (Burton, 2009).

A identificação de larvas de decápodes tem sido tradicionalmente baseada em descrições a partir do desenvolvimento em laboratório, provenientes de fêmeas ovígeras de espécies conhecidas. Contudo, o cultivo de larvas através da série completa de desenvolvimento é extremamente trabalhoso e desafiador, particularmente para grupos com muitos estágios larvais, como muitos caranguejos da infraordem Brachyura (e.g. Stuck & Truesdale, 1988; Cuesta et al., 2011). Caranguejos braquiúros constituem aproximadamente 50% das 15.000 espécies viventes de decápodes (De Grave et al., 2009), das quais aproximadamente 370 foram registradas ao longo da costa brasileira (Melo, 1996). Brachyura é o grupo com maior diversidade entre os decápodes, e maior abundância entre os decápodes bentônicos nas regiões neríticas, tanto na fase larval quanto adulta (e.g. Shanks et al., 2003; Boos et al., 2012). Como era de se esperar, na costa e na plataforma continental do Sudeste-Sul do Brasil eles representam um dos grupos mais diversos e abundantes da fauna de invertebrados bentônicos (e.g. Amaral & Rossi-Wongtschowski, 2004; Capítoli & Bemvenuti, 2004) e um importante recurso pesqueiro, representado principalmente pelos caranguejos-de-profundidade, do gênero *Chaceon* (Athiê & Rossi-Wongtschowski, 2004).

A maioria dos caranguejos braquiúros passa pelas fases de zoé e megalopa durante o seu desenvolvimento larval. O número de estágios dentro da fase de zoé geralmente varia de dois (maior parte de majídeos) a oito (algumas portunídeos), podendo chegar até 12 (por exemplo, no gênero *Plagusia*), e é geralmente consistente dentro de uma família (Martin, 2014). Atualmente as informações a respeito da morfologia larval das espécies de braquiúros da costa brasileira estão compiladas em uma chave para a região (Koettker et al., 2012), porém a maior parte das espécies registradas não apresenta o período larval total nem parcialmente descrito (revisado em Koettker et al., 2012). Além disso, a similaridade morfológica entre espécies de alguns gêneros, como *Callinectes*, *Mithraculus* e *Mithrax*, impede a identificação larval em nível específico; sem contar com a plasticidade fenotípica encontrada entre as larvas amostradas no ambiente em relação às cultivadas em laboratório (Morgan, 2008), e os danos que algumas larvas sofrem durante a coleta.

Um esforço considerável tem sido feito no desenvolvimento e aprimoramento de ferramentas genéticas para identificação de espécies marinhas nos últimos 20 anos (revisado em Bucklin et al., 2011). *DNA*

barcoding tem sido a técnica molecular mais aplicada na identificação de larvas e ovos planctônicos (e.g. Webb et al., 2006; Gleason & Burton, 2012), usada inclusive para larvas de braquiúros (e.g. Pan et al., 2008; Pardo et al., 2009). A metodologia formalizada por Hebert et al. (2003) consiste na padronização da região do DNA a ser sequenciada pela comunidade científica, a fim de tornar efetivas as comparações entre os indivíduos sequenciados e, portanto, diagnose em nível de espécie, através da consulta a bancos de dados disponíveis on-line. Para os decápodes, regiões específicas de dois genes mitocondriais [citocromo c oxidase 1 (CO1) e grande subunidade ribossomal do RNA (16S rRNA)] são as mais utilizadas (Matzen da Silva et al., 2011). Uma das principais vantagens dessa metodologia é que a quantidade de DNA presente em um pequeno pedaço de tecido, como de uma larva de braquiúro, é suficiente para o sequenciamento da região padrão. Assim é fundamental haver a combinação da identificação de espécies através dos métodos morfológico e molecular (e.g. McManus & Katz, 2009; Pardo et al., 2009).

A maior parte dos estudos realizados com uso de ferramentas moleculares para identificação de larvas de caranguejo braquiúros até hoje focaram na detecção (e.g. Bilodeau et al., 1999; Ströher et al., 2011) e quantificação relativa (e.g. MaKinster et al., 1999; Pan et al., 2008) de uma única espécie. No entanto, estudos utilizando essa abordagem para identificar a comunidade de larvas de braquiúros de uma determinada área geográfica ainda são raros. No Brasil, o uso de *DNA barcoding* tem crescido desde a criação do Brazilian Barcode of Life (BrBOL - http://brbol.org/pt-br) em 2012, um consórcio brasileiro vinculada ao Barcode of Life (BOLD - http://www.barcodeoflife.org/) (Ratnasingham & Hebert, 2007), que tem como objetivos principais incentivar o uso da metodologia e concentrar seus resultados. Porém, a grande maioria dos registros no BrBOL são de espécies de insetos e peixes, deixando uma grande lacuna no conhecimento molecular sobre os decápodes, um dos grupos de metazoários mais abundantes e diversos da região nerítica brasileira.

A presente tese consiste em três capítulos. No primeiro capítulo, intitulado "*Meroplankton community structure across hydrographic fronts along the South Brazil Shelf*", foi analisada a influência das frentes oceanográficas na distribuição e abundância dos principais grupos meroplanctônicos da plataforma continental Sudeste-Sul do Brasil. Decapoda foi o grupo meroplanctônico mais frequente e recebeu maior destaque na descrição da comunidade e resolução taxonômica, originando o segundo capítulo. Intitulado "*Large-scale spatial*

variability of decapod and stomatopod larvae along the South Brazil Shelf", esse trabalho investigou a estrutura da comunidade de larvas de decápodes na região e a influência dos principais processos oceanográficos, tais como aporte costeiro e ressugência, na formação de assembleias específicas de larvas. Dentre as larvas de decápodes bentônicos, as larvas de caranguejos braquiúros apresentaram maior abundância e diversidade, especialmente próximo à costa, portanto foram selecionadas para identificar por *DNA barcoding*, em uma região costeira da plataforma continental Sudeste-Sul do Brasil. Esse trabalho constituiu o terceiro e último capítulo da tese, intitulado "*Estimating diversity of crabs (Decapoda: Brachyura) in a no-take marine protected area of the SW Atlantic coast through DNA barcoding of larvae*".

Objetivos

O objetivo principal do presente estudo é analisar a variação espacial das larvas de invertebrados, especialmente larvas de decápodes, ao longo plataforma Sudeste-Sul do Brasil (21–34 ºS), em função da ocorrência de processos oceanográficos, e contribuir para os avanços na identificação molecular de larvas de decápodes da região. Os objetivos específicos incluem:

- Investigar a distribuição do meroplâncton ao longo de um gradiente costa-oceano na plataforma Sudeste-Sul do Brasil, e associar sua ocorrência a frentes costeiras e de plataforma (Capítulo 1).

- Identificar os principais processos oceanográficos que estruturam as diferentes assembleias de larvas de decápodes pelágicos e bentônicos ao longo da plataforma Sudeste-Sul do Brasil (Capítulo 2).

- Verificar a eficiência da utilização de *DNA barcoding* para a identificação de larvas de caranguejos braquiúros, em uma região costeira do Sudeste-Sul do Brasil (Capítulo 3).

Hipóteses

- A distribuição do meroplâncton apresenta uma associação com a presença de frentes de pequena escala (Capítulo 1).

- Os processos oceanográficos de mesoescala são responsáveis por moldar as associações de larvas de decápodes (Capítulo 2).

- A identificação de larvas através de *DNA barcoding* é eficiente para acessar a diversidade de caranguejos braquiúros na plataforma rasa (Capítulo 3).

Referências

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CAPÍTULO 1

Meroplankton community structure across oceanographic fronts along the South Brazil Shelf

(Manuscrito formatado para submissão ao periódico *Hydrobiologia*)

Meroplankton community structure across oceanographic fronts along the South Brazil Shelf

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Abstract

We investigated the influence of small-scale oceanographic fronts on the meroplankton community in a large-scale area of the southwestern Atlantic shelf (21–34 ºS). Meroplankton was sampled through vertical hauls at 89 stations, distributed along 14 cross-shelf transects, during late spring 2010 and early summer 2011. High-resolution thermosalinograph data were used to detect surface frontal characteristics. In addition, salinity and temperature were obtained with a CTD/rosette system, which provided seawater for chlorophyll and nutrient concentrations. High abundances of larvae were found over frontal areas, such as in the Subtropical Shelf Front, in surface thermal and saline fronts, and to a larger extent, in the estuarine front derived from the La Plata River and Patos Lagoon estuarine complex. Moreover, meroplankton abundances were highly associated with the cross-shelf gradient and intrusions of the South Atlantic Central Water over the continental shelf. The occurrence of two cyclonic eddies also coincided with a slightly increase in the relative abundance of meroplankton. Decapod larvae were the most frequent, while gastropod larvae were the most abundant, followed by larvae of polychaetes and bivalves. In addition, distinct groups of larvae were associated with different types of fronts, besides the coastal realm, which was dominated by decapod, cirripede and bivalve larvae. Shelf fronts had a dominance of gastropod larvae, while in estuarine fronts, polychaete and echinoderm larvae prevailed.

Keywords: invertebrate larvae; horizontal distribution; large-scale variability; South Atlantic

1. Introduction

The life cycle of most benthic marine invertebrate species include microscopic, free-living dispersive stages known as meroplanktonic larvae. Larvae are morphologically and ecologically distinct from the adults, and reach the juvenile stage through a conspicuous metamorphosis (Young, 2001). The dispersal potential is considered the main advantage of going through larval stages, which is particularly relevant in sessile or sedentary organisms, like barnacles, oysters, and tube-dwelling polychaetes (Pechenik, 1999; Levin, 2006).

Analyses of long-term time series revealed that the larval abundance of echinoderms, crustaceans and mollusks responds immediately to sea surface temperature variations (Kirby et al., 2008). Despite the limited ability to move, larvae are able to control their vertical position in the water column. This behavior in conjunction with physical processes will determine whether larvae are exported, retained, or concentrated in specific locations (Cowen et al., 2000). The maintenance of adult populations is a direct consequence of larval supply, dispersal and survival (Shanks et al., 2002).

Meroplankton distribution is often marked by a decreasing in abundance and diversity from the coast towards the ocean (e.g. González-Gordillo and Rodríguez, 2003; Hidalgo et al., 2014), since many benthic species are often associated with coastal zones and estuaries (e.g. Thiébaut, 1996; Ayata et al., 2011). In neritic pelagic ecosystems meroplanktonic larvae comprise a large portion of total zooplankton community and act both as predators and prey for planktivorous fish and other zooplankton species (Beaugrand, 2005).

As a transition between the coast and the ocean, the continental shelf includes different water masses and, consequently, a series of frontal zones (Munk et al., 2003). These confluences of oceanographic processes of contrasting origin, known as fronts, can shape the spatial diversity of the planktonic community (Acha et al., 2015; Hidalgo et al., 2015). Frontal activity affects meroplanktonic larvae in a number of aspects, such as nutrient entrainment, primary/secondary production and aggregation (Munk et al., 2003; Acha et al., 2015). Furthermore, frontal zones are generally assumed to maximize diversity due to the convergence of species inhabiting different water masses (e.g. Acha et al., 2004). The role of surface fronts and mesoscale eddies on the transport of pelagic larvae has been investigated in different oceanographic settings including the Gulf Stream front (Rowell et al.,

1985), the Agulhas rings (Villar et al., 2015) and several estuarine fronts (e.g. Largier, 1993; Ayata et al., 2011).

Neritic fronts are very abundant in austral South America, caused by diverse forcing such as tides, continental run-off, currents convergence, wind and bathymetry (Acha et al., 2004). Along the South Brazil Shelf, the continental shelf is influenced by different oceanographic processes that significantly increase the availability of nutrients in the upper layers (Gaeta and Brandini, 2006), contrasting with most of the Brazilian continental margin, which is predominantly oligotrophic due to the strong influence of the Tropical Water (TW) associated with the Brazil Current (Brandini, 2006).

Studies based on the distribution of physical properties and shelf circulation have demonstrated the quasi-seasonal character of the wind-induced coastal upwelling in the inner shelf of Cape São Tomé (21 °S), Cape Frio (23 °S), and Cape Santa Marta Grande (28 °S) (e.g. Castro and Miranda, 1998; Möller et al., 2008; Campos et al., 2013), depicted by blooms of phytoplankton in satellite images (Valentin, 1984; Brandini et al., 2014; Moser et al., 2014).

The interaction between eddy-induced upwelling and wind-generated transport in summer months results in enhanced upwelling and a strong bottom intrusion of the South Atlantic Central Water (SACW) on the shelf (Lima et al., 1996). The biological activity is also enhanced by freshwater discharge of the La Plata River (35–36 °S) and Patos Lagoon (32 °S) estuarine complex, which transports nutrient-rich waters northwards (Ciotti et al., 1995; Acha et al., 2004). The Subtropical Shelf Front, a density-compensated salinity and temperature subsurface front located close to 32 °S (Piola et al., 2000), creates a region with high nutrient input, primary production, copepod and ichthyoplankton abundance (Muelbert et al., 2008).

Additionally, the outer continental shelf and upper slope waters are subjected to various mesoscale instabilities, such as frontal systems (Muelbert et al., 2008), eddies (Pereira et al., 2009) and meanders of the Brazil Current (Lorenzzetti et al., 2009), which bring about physical and chemical variations that influence the distribution patterns of nutrients and marine organisms (Brandini, 2006).

This study aims to investigate the dynamics of the meroplankton in relation to frontal zones along the continental shelf and slope area of the South Brazil Shelf. Our specific goals are (i) to identify specific frontal characteristics and their influence on the meroplankton abundance and composition, and (ii) to describe the cross-shelf distribution of the dominant groups of meroplanktonic larvae.

2. Materials and methods

2.1. Study area

The South Brazil Shelf (SBS) extends from 22 °S to 34 °S along the South American southeast coast (Heileman and Gasalla, 2008) (Fig. 1.1A). The width of the continental shelf varies according to latitude, being narrower in the northern than in the southern area (Fig. 1.1B). The continental slope is more pronounced in the northern region.

The SBS is often divided into three latitudinal subareas (Fig. 1.1B): (i) the Cape São Tomé-Cape Frio region (CSTF); (ii) the Southern Brazilian Bight (SBB) located between Cape Frio and Cape Santa Marta Grande (CSM); and (iii) the Southern Subtropical Shelf (SSS) between CSM and La Plata River. In the northern portion of SBB, the CSTF is mainly characterized by seasonal coastal upwelling, while SBB and SSS are dominated by the strong influence of less saline waters derived from the La Plata River and Patos Lagoon, which are stronger in winter and spring (Burrage et al., 2008; Möller et al., 2008).

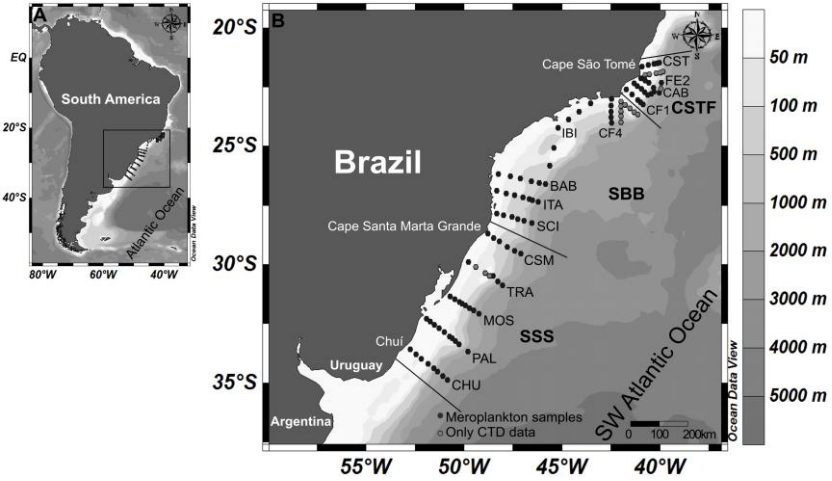

Figure 1.1. (A) Geographic location of the study area. (B) Position of the sampling stations. Transects names: CST=Cape São Tomé; FE2=Feia Lagoon 2; CAB=Campos Bight; CF1=Cape Frio 1; CF4=Cape Frio 4; IBI=Ilhabela Island; BAB=Babitonga Bay; ITA=Itajaí River; SCI=Santa Catarina Island; CSM=Cape Santa Marta Grande; TRA=Tramandaí; MOS=Mostardas; PAL=Patos Lagoon; CHU=Chuí. Subareas: CSTF=Cape São Tomé-Cape Frio; SBB=Southern Brazilian Bight; SSS=Southern Subtropical Shelf.

2.2. Sampling and laboratory procedures

Cruises were conducted between Chuí (34 °S) and Cape São Tomé (21 °S) on board of the R. V. *Cruzeiro do Sul* (owned by the Brazilian Navy). To cover the entire sampling area three consecutive cruises were conducted, with the first and second occurring in austral late spring (December 06 to 14 and 17 to 22, 2010) and the third finishing in early summer (January 04 to 11, 2011) in the CSTF region. The positions of CTD stations were strategically selected to intercept several shelf fronts that could be seen on satellite images. Prior to the cruises, high-resolution (~ 1 km) ocean colour and thermal infrared satellite images were analysed for choosing locations of CTD stations. Vertical profiles of temperature, salinity, fluorescence and dissolved oxygen were recorded at 107 stations distributed at 17 cross-shelf transects using a SeaBird CTD (conductivity, temperature and depth) profiler casts (Fig. 1.1B). During the cruises, continuous measurements of sea surface (~ 5 m) temperature and salinity were made by a well-calibrated thermosalinograph. CDT measurements were only considered at depths greater than 10 m.

In addition, water samples were collected at selected depths (3 or 5 m, maximum fluorescence depth and base of the thermocline) to determine chlorophyll and nutrient concentrations with 5-L Niskin bottles. Water was filtered on board and chlorophyll concentrations were determined by spectrophotometry using the approach detailed in Strickland and Parsons (1972). Ammonia and phosphate concentrations were determined by colorimetric analyses using a portable spectrophotometer, while nitrite, nitrate, and silicate were analyzed using Flow Injection Analysis. Nutrient analysis followed the processing recommendations in Aminot and Chaussepied (1983).

Plankton samples (89, black circles in Fig. 1.1B) were collected at 14 out of the 17 cross-shelf transects through vertical tows from the maximum fluorescence depth up to the surface in deep-water stations, from 10 m above the bottom when the water column was homogenous and from about 10 m depth at shallow stations (up to 20 m).

A conical-cylindrical net with a 0.5-m diameter mouth and 200-μm mesh equipped with a flowmeter (General Oceanics) was used for sampling planktonic organisms, through vertical tows at a speed of about 2 knots. All samples were immediately fixed and preserved in 4% buffered seawater-formaldehyde solution. The maximum fluorescence depth ranged from 7 to 125 m, and the plankton sampling depth ranged

from 12 to 130 m. The distance of sampling locations from the coast ranged from 7 to 418 km. Local depths varied from 15 to 2,800 m, thus covering coastal, shelf and slope waters.

Invertebrate larvae were counted and sorted from all 89 samples. In a few coastal stations only larvae from 1/2 or 1/4 fractions were considered due to their high densities. Larvae were identified into major taxonomic groups, under stereomicroscope, according to Smith (1977), Boltovskoy (1981) and Young (2001).

2.3. Data analysis

Larval counts were standardized to number of individuals per 100 m^3 to calculate the relative abundance (RA) of each taxon. The frequency of occurrence (FO) was also calculated.

A potential temperature–salinity (T–S) diagram was built for the studied area on Ocean Data View (Schlitzer, 2009). Water masses were determined based on thermohaline indexes in Miranda (1985), Castro and Miranda (1998), Piola et al. (2000), and Möller et al. (2008). Temperature and salinity were used as the proxies to detect fronts in the study area. Chlorophyll and nutrient data were also examined to detect responses to the fronts and some profiles are presented.

In addition to *in situ* data, we used monthly satellite images of sea surface chlorophyll concentrations for December 2010 and January 2011 with a 4 km spatial resolution from the MODIS Aqua sensor. The images were obtained from the Giovanni - Ocean Color Radiometry Online Visualization and Analysis web site.

In order to verify the distribution of the most frequent taxa in relation to environmental variables, a canonical correspondence analysis (CCA) was conducted. Biological data were log-transformed (x + 1) and environmental data were standardized. Only the most frequent taxa were considered (> 10%). CCA and additional tests were performed in R (R Foundation for Statistical Computing), with the 'vegan' and 'HH' packages (Oksanem et al., 2013; Heiberger et al., 2013).

Mean values of temperature, salinity, chlorophyll and nutrient concentrations, calculated from the surface to the plankton sampling depth, were used in the CCA. The oxygen vertical gradient was calculated using the surface oxygen value and the value at the bottom of the oxycline, as well as respective depths. These parameters were used in the CCA to characterize the environmental scenarios where meroplankton was distributed.

3. Results

3.1. Physical and biological features of the fronts

The T–S diagram until the maximum plankton sampling depth (130 m) (Fig. 1.2) showed the presence of six water masses (Table 1.1): Tropical Water (TW), Shelf Water (SW), Subtropical Shelf Water (STSW), Plata Plume Water (PPW), South Atlantic Central Water (SACW), and Subantarctic Shelf Water (SASW).

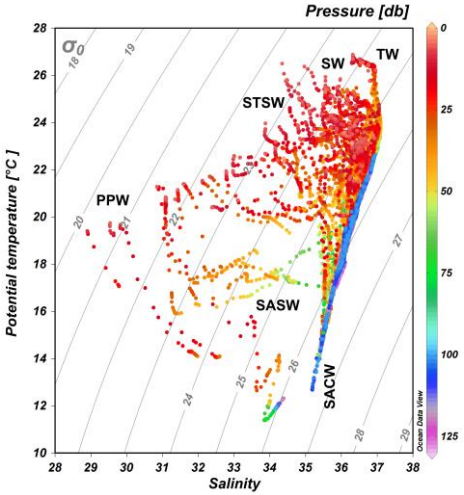

Figure 1.2. Potential temperature-salinity diagram for the first 130 m of all stations along the South Brazil Shelf during late spring 2010 and early summer 2011. TW=Tropical Water; SW=Shelf Water; STSW=Subtropical Shelf Water; PPW=Plata Plume Water; SACW=South Atlantic Central Water; SASW=Subantarctic Shelf Water.

Table 1.1. Thermohaline (TH) ranges used to characterize water masses in the region. The ranges of TW and SACW are shown separately for the latitudinal subareas (CSTF=Cape São Tomé-Cape Frio; SBB=Southern Brazilian Bight; SSS=Southern Subtropical Shelf). The TH indexes were derived from Miranda (1985), Castro and Miranda (1998), Piola et al. (2000), and Möller et al. (2008).

Water Mass	SBB and SSS	CSTF
Tropical Water (TW)	T≥18.5ºC, S≥36	T>20ºC, S>36.2
Shelf Water (SW)	-	S<36.2
Subtropical Shelf Water (STSW)	T>18.5ºC, 35.3≤S<36	-
Plata Plume Water (PPW)	T>19ºC, S≤33.5	-
South Atlantic Central Water (SACW)	T≤18.5ºC, S≥35.3	T<20ºC, S<36.2
Subantarctic Shelf Water (SASW)	T≤21ºC, 33.5<S≤34.2	-

The cross-shelf distribution of temperature and salinity are shown vertically until 200 m depth (Fig. 1.3) and through high-resolution surface data (Fig. 1.4). In both cases the distribution of meroplankton abundance (larvae/100 m^3) is displayed together.

The salty and warm Tropical Water (TW) was the dominant water mass in the surface layer over the slope area in the entire region (Fig. 1.3). Great variability was observed regarding the depth of the cool nutrient-rich South Atlantic Central Water (SACW) over the continental shelf. The thermoclines of 20°C and 18.5°C define the upper limit below which the SACW dominates the bottom layers over the shelf in the CSTF (Fig. 1.3A–F) and SBB/SSS (Fig. 1.3G–N), respectively. The strongest onshore intrusions of the SACW were observed at Feia Lagoon (FE2), Cape Frio (CF4), Santa Catarina Island (SCI) and Cape Santa Marta Grande (CSM) transects (Fig. 1.3), where it reached 15 m deep, depicting the upwelling in subsurface waters. In the Southern portion, the estuarine plume, especially represented by the low-salinity Plata Plume Water (PPW), was observed over the shelf from Chuí (CHU) to Mostardas (MOS) transects (Fig. 1.3M and N), occupying a larger area along the Patos Lagoon (PAL) and CHU transects. In the southmost transect, the Subantarctic Shelf Water (SASW) was present bellow 30 m (Fig. 1.3N).

Pelagic larvae of benthic invertebrates were present in all samples, with mean abundance of 1,350 ± 320 larvae/100 m^3. The highest abundances of meroplankton were found in the upwelling zone of Cape Santa Marta Grande (CSM) (19,250 larvae/100 m^3), in the estuarine front at Patos Lagoon transect (PAL) (13,550 larvae/100 m^3), in the Subtropical Shelf Front (STSF), located in the intermediate shelf off Chuí (CHU) (12,430 larvae/100 m^3), and in the coastal realm at Ilhabela Island transect (IBI) (10,400 larvae/100 m^3) (Fig. 1.3 and Fig. 1.4). In the area of the STSF, the plankton tow coincided with the zone of a sharp change in salinity and temperature (Fig. 1.3N and Fig. 1.4N) due to intrusion of SASW into the study area.

Several thermal and saline fronts were identified in the surface waters (Fig. 1.4) along the intermediate shelf, and their putative influence on the meroplankton abundance varied throughout the region. For instance, along the Mostardas transect (MOS) (Fig. 1.4L, green circles) the increase in meroplankton abundance (~ 2.5 times) seems to be associated with the quick increase in salinity ($\Delta S \sim 3.0$) and temperature ($\Delta T \sim 2$ºC) between stations. In addition, in Itajaí River transect (ITA) (Fig. 1.4H, green circles) the front ($\Delta S \sim 1.0$; $\Delta T \sim 1$ºC)

also coincided with an increase in meroplankton abundance (~ 2 times). And in Cape São Tomé transect (CST) (Fig. 1.4A, green circles) the increase in meroplankton relative abundance (~ 3.0 times) also coincided with the saline and thermal front ($\Delta S \sim 0.7$; $\Delta T \sim 4$ºC). On the other hand, despite the thermal surface front ($\Delta T \sim 3.0$ºC) observed in the Feia Lagoon transect (FE2) (Fig. 1.4B, green circles), meroplankton abundance was practically the same between stations.

During the cruises, the ship crossed two cyclonic vortices over the shelf zone (Ito et al., 2015). The first cyclonic eddy-like structure was found in the offshore area of CHU between the last and the third last stations, whereas the second was identified offshore Campos Bight (CAB), also between the last and the third last stations (Fig. 1.4C and N). The cyclonic eddy in CHU section was smaller and weaker than in CAB section (Ito et al., 2015). In the CAB section, the cyclonic vortex was strong enough for upwelling of the SACW from deep layers up to about 50 m deep as well as the maximum chlorophyll depth (see Fig. 1.3C and Fig. 1.5A), and for aggregating nutrients (Fig. 1.5B and C, data shown only for nitrate and phosphate). Despite plankton sampling was carried out in shallower waters (~ 30 m), a slightly increase in meroplankton relative abundance was observed (Fig. 1.4C).

In some cases, high meroplankton abundances over the oceanic waters coincided with nocturnal plankton hauls, as seen in the most offshore station of CST (Fig. 1.4A), in the second last station of IBI (Fig. 1.4F), and the second and third last stations of TRA (Fig. 1.4K).

High relative abundances of meroplankton were mainly associated with the coastal zone, seen in several transects (Fig. 1.4). In the mouth of Patos Lagoon (PAL) an estuarine front was observed (Fig. 1.4M), and high meroplankton abundance was observed until almost 200 km from the coast, where chlorophyll and nutrient concentrations were also high until 100 m deep (Fig. 1.5D–F, nutrient data shown only for nitrate and phosphate). At CHU transect, high abundances were observed in two stations, one related with and intrusion of the SASW (Fig. 1.3N) and the other associated with the occurrence of the eddy (Fig. 1.4N). In CSM and IBI transects, meroplankton highest abundances were coincident with the upwelling of the SACW (Fig. 1.3F and J). At CST, ITA and MOS thermal and saline shelf fronts seemed to be responsible for increases in larval abundances (Fig. 1.4A, H and L).

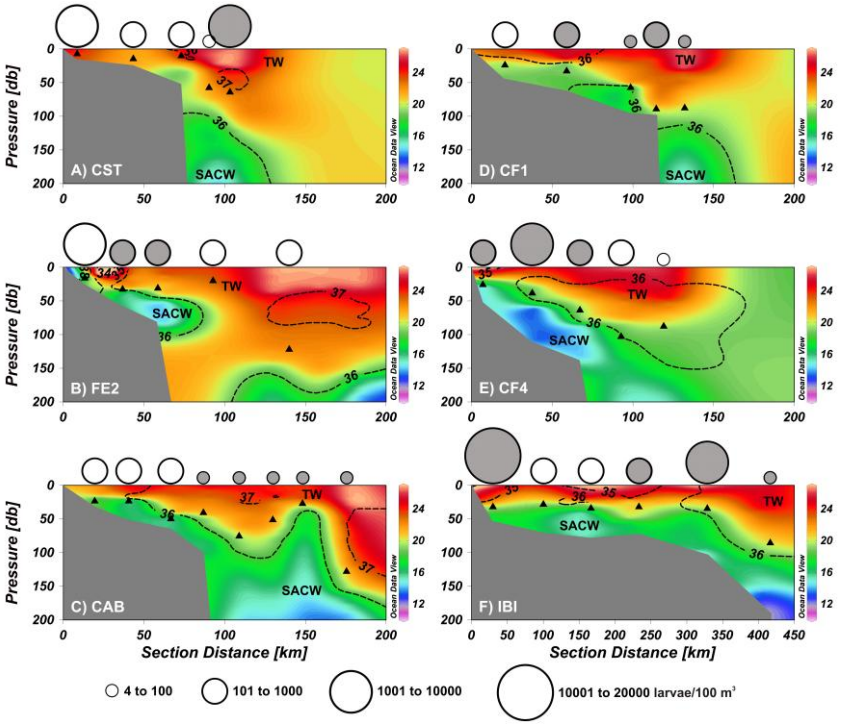

Figure 1.3. Cross-shelf distributions of temperature (°C) (colors), salinity (contour lines) and meroplankton abundance (larvae/100 m^3) (circles) for the transects along the South Brazil Shelf. Circles filled in gray represent stations conducted at night. Transects names and water masses abbreviations are according to Fig. 1.1B and Table 1.1, respectively. Black triangles indicate the plankton sampling depth, which was coincident or below (from 1 to 10 m) the maximum fluorescence depth. White triangles indicate the maximum fluorescence depth in stations where it was below plankton sampling depth (ITA and TRA).

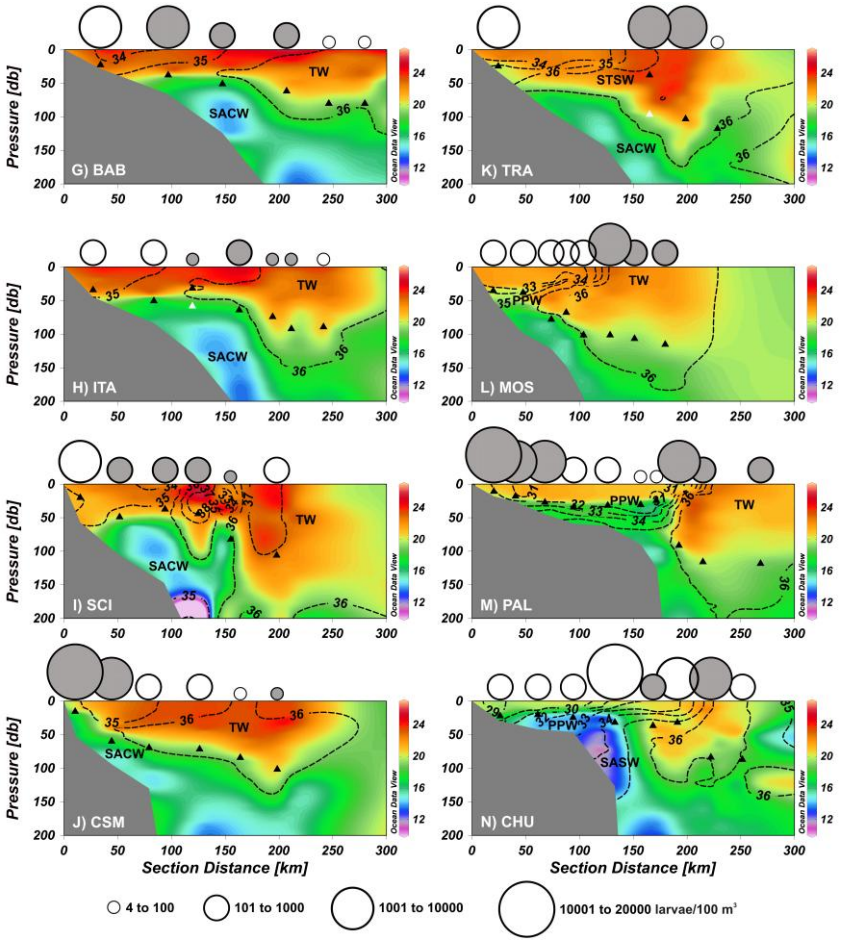

Figure 1.3. (Continued).

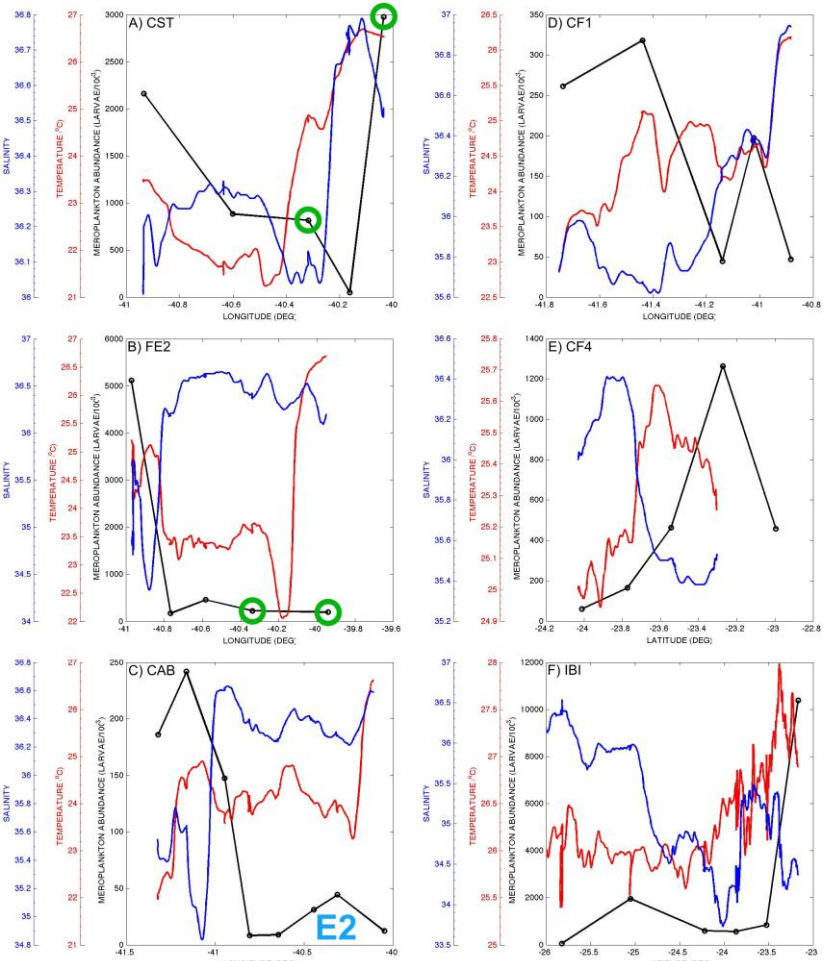

Figure 1.4. Surface salinity and temperature variability obtained from the thermosalinograph and meroplankton abundance (larvae/100 m^3) by each transect along the South Brazil Shelf. Transects name abbreviations are according to Fig. 1.1B. Note that for E and F the sections are shown by latitude, from South to North. Eddies position (E1 and E2) according to Ito et al. (2015). Green circles indicate the variation in meroplankton abundance and the corresponding limits of the shelf fronts.

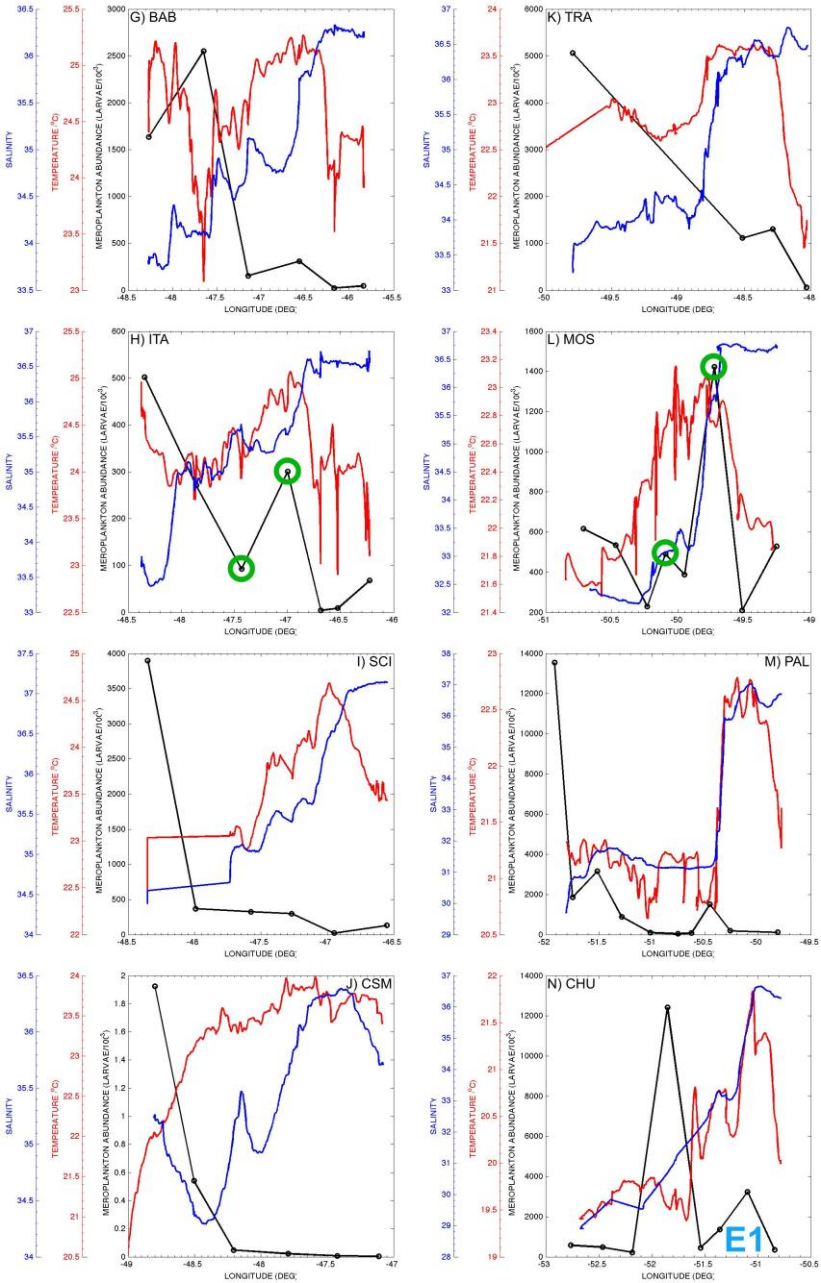

Figure 1.4. (Continued).

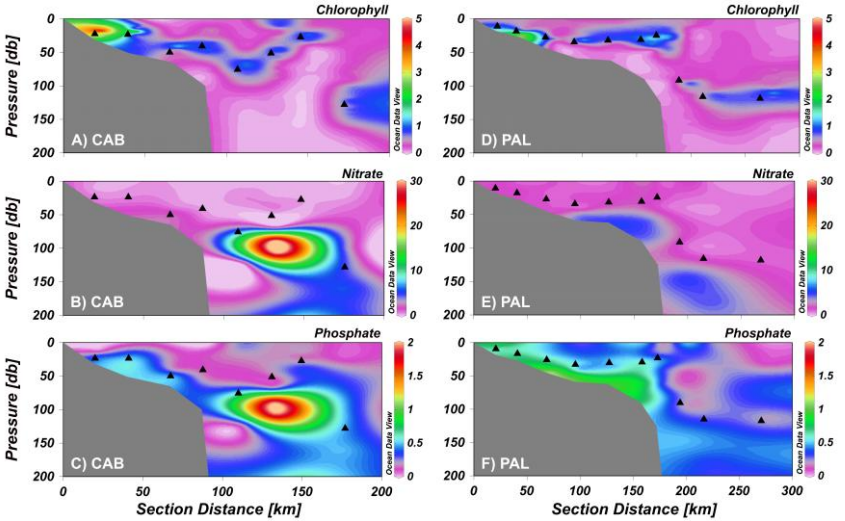

Figure 1.5. Cross-shelf distributions of chlorophyll (mg/m^3), nitrate (µM) and phosphate (µM) at Campos Bight (CAB) and Patos Lagoon (PAL) transects along the South Brazil Shelf. Black triangles indicate the plankton sampling depth, which was coincident or below (from 1 to 10 m) the maximum fluorescence depth.

3.2. Cross-shelf distribution of the meroplankton community

Overall, the surface chlorophyll concentration and the meroplankton abundance were high all along the continental shelf with maximum values in inshore waters. It was also high over the entire shelf of the three southernmost transects (Fig. 1.6), influenced by the presence of the Plata Plume Water (PPW) (Fig. 1.3L–N).

Larvae belonging to eleven phyla were found in the area. Among the groups, decapod larvae were the most frequent, while gastropod larvae were the most abundant, followed by larvae of polychaetes and bivalves, with these four groups accounting together for 80% of total larval abundance (Table 1.2). Besides these groups, cirripedes, holothurians and ophiuroids also presented relatively high mean larval abundance comparing to the others (~ 50 larvae/100 m^3).

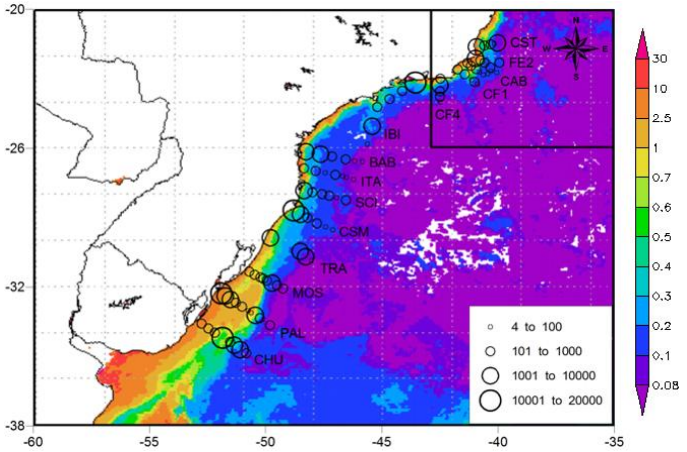

Figure 1.6. Meroplankton abundance (black circles) (larvae/100 m^3) superimposed in a chlorophyll concentration satellite image monthly composition from December 2010. Inside the square, chlorophyll concentration monthly composition image from January 2011.

Table 1.2. Mean abundance, relative abundance (RA) and frequency of occurrence (FO) of the meroplanktonic larvae sampled along the South Brazil Shelf.

Phylum/Subphylum	Taxa	Mean (larvae/100 m^3)	RA (%)	FO (%)
Porifera	Porifera	0.02	<0.01	1
Cnidaria	Cnidaria	2.26	0.17	17
Platyhelminthes	Platyhelminthes	2.90	0.21	17
Nemertea	Nemertea	4.80	0.35	8
Annelida	Polychaeta	243.68	17.99	69
Mollusca	Gastropoda	422.49	31.18	78
	Bivalvia	212.30	15.67	61
	Cephalopoda	1.92	0.14	21
Arthropoda/Crustacea	Cirripedia	59.40	4.38	36
	Stomatopoda	11.91	0.88	55
	Decapoda	197.65	14.59	96
Sipuncula	Sipuncula	3.58	0.26	19
Phoronida	Phoronida	2.02	0.15	9
Echinodermata	Ophiuroidea	49.19	3.63	16
	Asteroidea	6.77	0.50	28
	Holothuroidea	51.95	3.83	13
	Echinoidea	6.83	0.50	11
Hemichordata	Enteropneusta	31.31	2.31	11
-	Trocophores	13.61	1.00	28
-	Unidentified	30.26	2.23	48
	TOTAL	1,354.86	100.00	

Polychaete larvae presented up to 100 larvae/100 m^3 in most samples. Their abundance was very high in the coastal stations of PAL and CSM transects (~ 7,000 and 4,000 larvae/100 m^3, respectively), as well as in the intermediate shelf off CHU, near the STSF area (~ 5,000 larvae/100 m^3) (Fig. 1.7A). In turn, molluscan larvae were found in most samples with abundances of up to 1,000 larvae/100 m^3. Their highest abundances (~ 8,000 larvae/100 m^3) occurred in the nearshore stations of IBI (mostly gastropods) and CSM (mostly bivalves), and in the intermediate shelf of CHU, at the same station of polychaete larval peak (mostly gastropods) (Fig. 1.7B; Fig. 1.8). Crustacean larvae showed a clear pattern of decrease in abundance towards the ocean (Fig. 1.7C), with the highest values in the coast of FE2 and PAL transects (decapods and cirripedes) (~ 3,300 and 2,500 larvae/100 m^3, respectively) (Fig. 1.7C; Fig. 1.8). Echinoderm larvae were found in high abundances (up to 4,600 larvae/100 m^3) only in a few coastal stations located southern than 27 °S (Fig. 1.7D), among which ophiuroids and holothurians were the most representative (Fig. 1.8; Table 1.2).

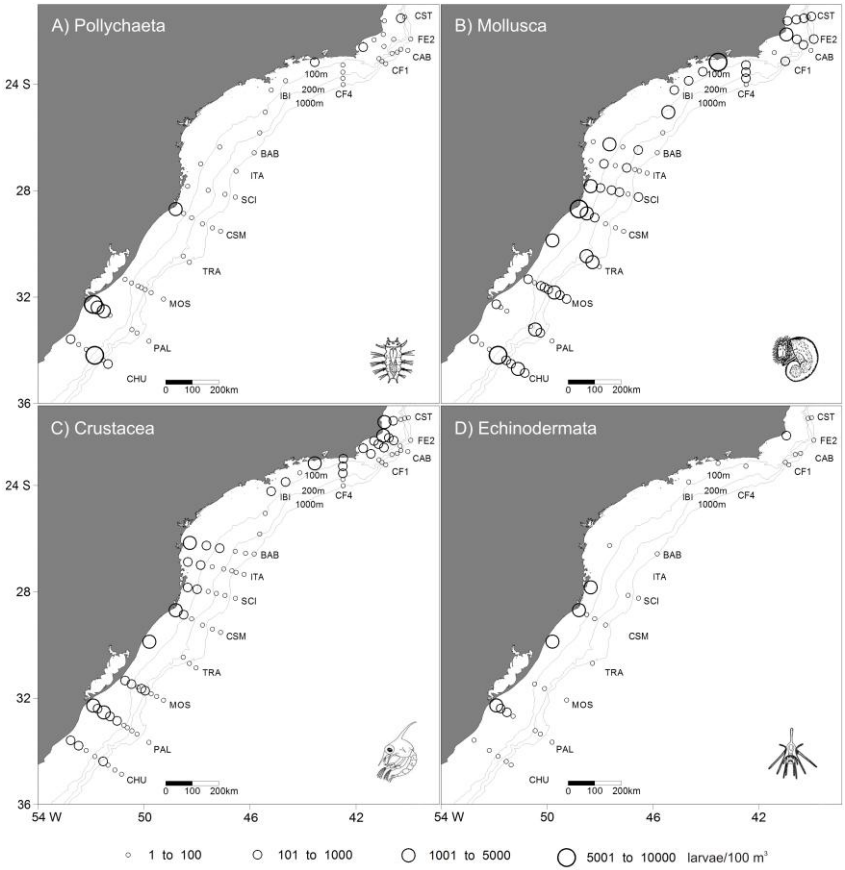

Figure 1.7. Distribution of larval abundance (larvae/100 m3) of: (A) Polychaeta, (B) Mollusca, (C) Crustacea and (D) Echinodermata in the 89 stations sampled along the South Brazil Shelf. Transects name abbreviations are according to Fig. 1.1B.

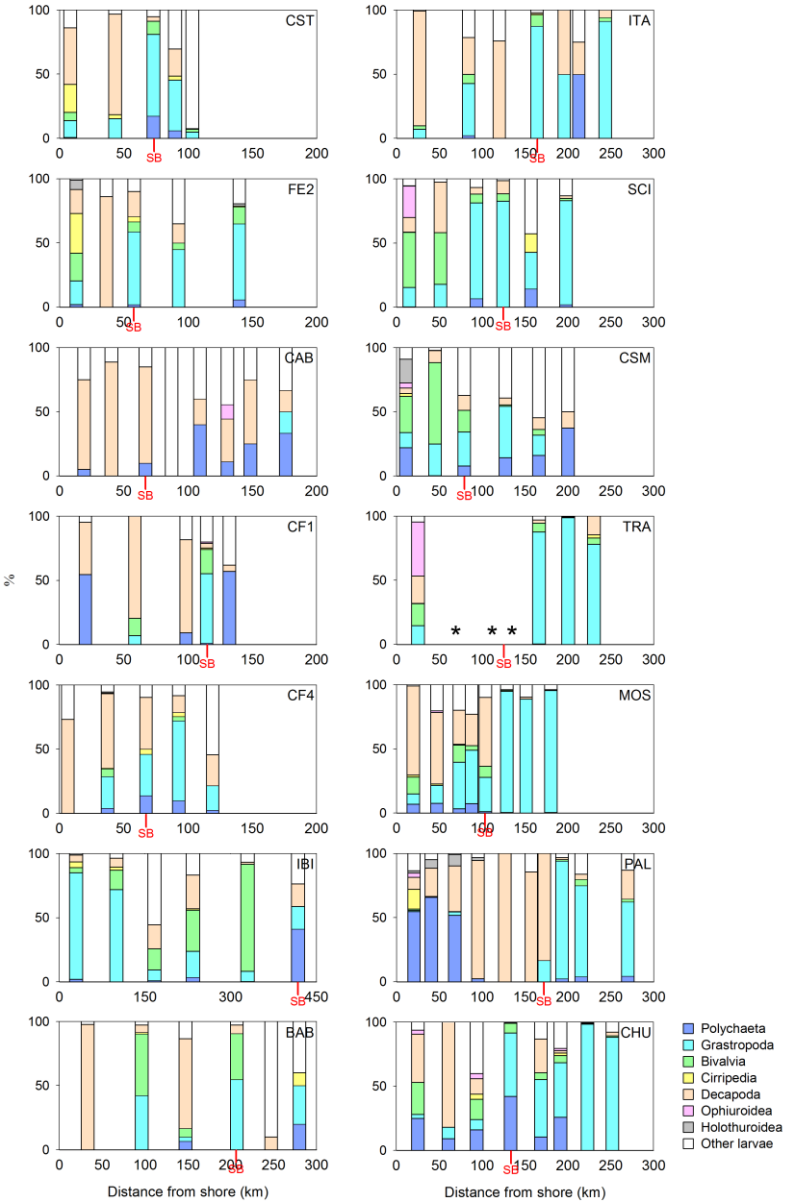

Figure 1.8. Cross-shelf relative abundance (%) of the main meroplanktonic groups of larvae by each transect along the South Brazil Shelf. Transects name abbreviations are according to Fig. 1.1B. SB indicates the approximate location of the shelf break. Asterisks indicate stations without plankton sampling.

The first and second axes of the canonical analysis ordination accounted together for 62.4% of the constrained variance (Fig. 1.8). Axis 1 represented the nutrient-rich waters, especially in silicate and phosphate, in opposition to the nutrient-poor waters, characterized by high salinity and temperature. This separation might be associated with the contrasting conditions between the PPW, which occupies the neritic waters in the south, and the Tropical Water (TW), dominant over the slope. Larvae of echinoderms and polychaetes showed an association with the estuarine plume waters, being negatively associated with axis 1 (Fig. 1.9).

Axis 2 represented the cross-shelf gradient. It was positively correlated with bathymetry, and negatively with chlorophyll concentration and oxygen stratification, distinguishing neritic from oceanic assemblages. Larvae of acorn worms (enteropneusts) and cephalopods were highly associated with offshore conditions. In contrast, crustacean larvae appeared in association with chlorophyll-rich oxygen-stratified coastal waters (Fig. 1.9).

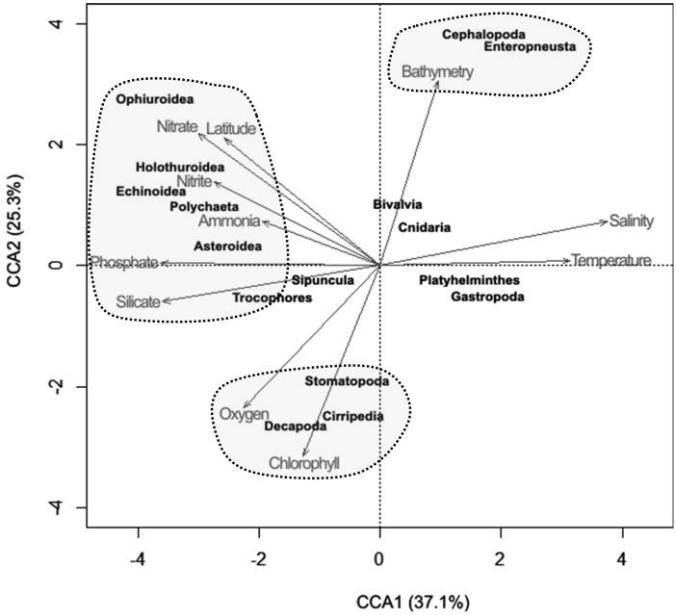

Figure 1.9. Canonical correspondence analysis plot for meroplankton composition in relation to environmental variables.

Summing up, we observed that the coastal realm was dominated by larvae of decapod, cirripede and bivalve larvae, while gastropods were the most representative larvae in shelf fronts. In turn, polychaetes and echinoderms, the latter with lower relative abundance, were the dominant larvae in the estuarine front of Patos Lagoon section.

4. Discussion

The coast-ocean gradient was the most striking feature of the meroplanktonic abundance distribution along the South Brazil Shelf during spring 2010 and summer 2011, discriminating neritic from oceanic assemblages in response to contrasting oceanographic features across the shelf. The contrast between the coastal water masses and the dominant Tropical Water (TW) over the outer shelf and slope seems to be the main driver of this pattern. This gradient was also observed in the area for holoplankon (Brandini et al., 2014; Nogueira Jr. et al., 2014) and elsewhere for meroplankton (e.g. Thatje et al., 2003; Meerhoff et al., 2014).

The oxygen-stratified coastal waters presented higher abundances of meroplankton. Previous studies in the highly productive coast of Peru have shown that production in that area is sustained by the zooplanktonic community, including larvae of benthic invertebrates in the upper oxygenated waters above the oxygen minimum layer (Criales-Hernández et al., 2008). The highest values for the oxygen vertical gradient observed in our samples corresponded to the more abrupt decrease in oxygen concentration, particularly along Cape Santa Marta Grande (CSM), which is more frequent in coastal waters that also present a shallower lowest oxygen layer.

Meroplankton abundances were higher in the nearshore stations irrespectively of the latitudinal hydroclimatic scenarios. Despite that, the highest abundances of larvae coincided with particular oceanographic conditions. Areas under the influence of coastal upwelling, with the strongest onshore intrusions of the South Atlantic Central Water (SACW), as in CSM, showed high abundance of larvae, once the enhancement of the primary production ensures the availability of food for the future larvae. This was also seen for ichthyoplankton in the area (Macedo-Soares et al., 2014). The increase in the productivity and food availability, particularly diatoms, is fueled by the intrusions of the SACW (Brandini et al., 2014).

In most coastal fronts, the meroplankton peaks, which were dominated by decapod, cirripede and bivalve larvae, were coincident

with highest surface concentrations of surface chlorophyll. In the CSTF region and from Babitonga Bay (BAB) to CSM the highest phytoplanktonic densities were observed in the coastal stations, among which, diatoms were among the dominant (Becker, 2014; Brandini et al., 2014; Moser et al., 2014). In addition, smaller size plankton fractions, including microzooplankton, were found in high abundances associated with coastal processes in BAB and CSM sections during the same cruises of the present study (Becker, 2014). These resources constitute the main prey items on the diet of bivalve veligers, cirripede nauplii and early crab zoeae (e.g. Raby et al., 1994; Turner et al., 2001; Sulkin and McKeen, 1999).

Polychaete and gastropod larval abundances were very high in the intermediate shelf off Chuí, around the shelf break area. This area corresponds to the Subtropical Shelf Front (STSF), which is subjected to a sharp change in temperature and salinity (Muelbert et al., 2008). High abundances of copepod and ichthyoplankton have been registered in the area, associated with high nutrient input and primary production (Muelbert et al., 2008).

Several other thermal and saline shelf fronts were detected during the studied period, primarily in the surface and, secondarily, in the subsurface waters. Their influence on the meroplankton abundance, dominated by gastropod larvae in these cases, was observed in some areas, such as in Mostardas (MOS) and Itajaí River transects (ITA), where higher values were found in the frontal zone, corroborating with our proposed hypothesis. On the other hand, in a few areas subjected to strong frontal influence, such as in the area off Feia Lagoon (FE2), no effect was observed in the meroplankton abundance.

A slightly increase in meroplankton relative abundance was observed in the corresponding area of the two cyclonic eddies encountered over the shelf zone of Campos Bight (CAB) and Chuí (CHU) (Ito et al., 2015). The episodic occurrence of vortices is known to enhance the primary production and, consequently, zooplanktonic community in the area (Acha et al., 2004). In addition, eddy systems may act as retention areas for neritic invertebrate larvae, as seen in the shelf of Gran Canaria in the NW Africa (Landeira et al., 2009), and in the Gulf Stream (Anderson and Robinson, 2001).

The composition of the meroplankton community observed along the South Brazil Shelf consisted mostly of larvae of gastropods, polychaetes, bivalves and decapods. These groups produce large numbers of pelagic planktotrophic larvae which spend several weeks in the water column before settling (e.g. Wilson, 1991; Raby et al., 1994).

Other studies carried out in other latitudes of the Brazilian shelf highlighted the significant occurrence of pelagic invertebrate larvae, mainly of gastropods, bivalves, polychaetes, cirripedes, decapods and bryozoans (Schwamborn et al., 2001; Koettker and Lopes, 2013). Gastropods were also the most abundant larvae found in a transect in front of Cape Frio area, with higher values at coastal stations (Yoshinaga et al., 2010).

On the other hand, larvae of cephalopods and enteropneusts showed an association with the offshore waters. In the continental shelf of southern Brazil, highest *Illex argentinus* paralarvae densities were observed offshore in spring, while in autumn and winter they were trapped in the shelf (Haimovici et al., 1995).

The separation of the assemblages of larvae in response to the contrast of the oceanic water masses and the Plata Plume Water (PPW) was clear, as indicated by the thermohaline and nutrient gradients being the most explicative variables in the CCA analysis.

The area subjected to the freshwater input of the southern estuaries presented the highest concentrations of nutrients, especially silicate and phosphate. The supply of silicate to surface waters appears to regulate new phytoplankton production, which are mostly diatoms (Turner et al., 1998). Results of the few studies concerning the relative importance of grazing by meroplankton have shown that some groups, such as larvae of polychaetes and cirripede nauplii may consume twice as many diatoms as the globally abundant nauplii of the copepod *Calanus* spp. (Martin et al., 1996; Turner et al., 2001).

The maximal larval abundances of echinoderms and polychaetes were observed in the low-salinity river plume waters, in close association with higher chlorophyll concentrations and, presumably, greater food availability for these larvae. An association between polychaete larvae and estuarine plumes has been reported in various nearshore environments (e.g. Shanks et al., 2002; Ayata et al., 2011). River plumes and associated fronts may act as physical barriers for the dispersal of pelagic larvae, retaining them in the vicinity of their spawning location, thus limiting their offshore export and allowing connectivity between neighbouring populations (Largier, 2003). Salinity may be a limiting factor in the spatial distribution of meroplankton, either by providing a density barrier against passive larval dispersal (e.g. Meerhoff et al., 2014) or by setting physiological constraints to the vertical migration of larvae (Sameoto and Metaxas, 2008). In addition to this role in larval transport, PPW has been shown to sustain high chlorophyll concentrations and consequently high phytoplankton

biomass (Ciotti et al., 1995; Möller et al., 2008). This phytoplankton biomass may thus provide planktotrophic larvae with abundant food resources. Besides, during spring, the area is subjected to onshore Ekman transport favoring even more local retention (Lima and Castello, 1995).

Summing up, our findings shed some light to the role of frontal zones in the meroplankton community along the South Brazil Shelf. Our results showed high abundances of larvae over frontal areas, such as in the STSF, in surface thermal and saline fronts, and to a larger extent, in the estuarine front derived from the La Plata River and Patos Lagoon estuarine complex. In addition, the highest concentrations of larvae coincided with the strongest upwelling event present in the studied period, reinforcing the importance of the SACW intrusions to enhance biological production in the coastal euphotic zones (Moser et al., 2014). The occurrence of two cyclonic eddies (Ito et al., 2015) also coincided with an increase in abundance of meroplankton. Different communities of larvae were observed in association with waters derived from the La Plata River and Patos Lagoon estuarine complex, the oceanic waters, and the coastal waters, indicating the influence of the oceanographic scenery in the composition and distribution of the meroplanktonic assemblages.

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CAPÍTULO 2

Large-scale spatial variability of decapod and stomatopod larvae along the South Brazil Shelf

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Large-scale spatial variability of decapod and stomatopod larvae along the South Brazil Shelf

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Abstract

The spatial distribution of a spring/summer community of combined decapod and stomatopod larvae along the southwestern Atlantic shelf, and its possible linkages with hydrographical processes and with parental habitats were investigated. Vertical plankton hauls were performed between 21 °S and 34 °S, at 107 stations, distributed along 2000 km of coastline and up to 400 km offshore, during late spring 2010 and early summer 2011. Salinity and temperature were obtained with a CTD/rosette system, which provided seawater for chlorophyll and nutrient concentrations. A coupling between chlorophyll concentration and abundance of early larvae of benthic species was observed, suggesting that the larval release could be synchronized with phytoplankton maxima. Our findings indicated that the composition and abundance of larvae are strongly influenced by distance from the coast, freshwater sources and water mass distribution. Assemblages of larvae were observed in the most relevant environmental scenarios: (1) coastal environments, dominated by intertidal, shallow water or coastal species; (2) offshore environments, with predominance of holopelagic and deepwater species; and (3) southern continental runoff, mainly represented by benthic neritic crabs and shrimps. In addition, the large-scale distribution of larvae revealed a relationship with the adult's distribution, shown mainly by the occurrence of larvae transported from the estuaries in the inner shelf and by larvae of the deepwater fauna found mainly along the outer shelf.

Keywords: crustacean larvae; distribution; oceanographic features; South Atlantic Ocean

1. Introduction

Marine larval ecology has repeatedly been emphasized as pivotal to understanding the patterns and processes influencing marine populations, communities and ecosystems. Larval supply is considered one of the major determinants of benthic population structure, stability, and distribution patterns. Most marine invertebrates produce planktonic larvae, which may spend from minutes to months in the plankton before they leave the water column and settle into their benthic habitat (Young, 2001). Decapod and stomatopod larvae often form a large portion of the coastal and oceanic temporary planktonic crustacean fauna (e.g. Schwamborn et al., 1999; González-Gordillo and Rodríguez, 2003), and their ecological importance is highly relevant due to their abundance, diversity and the different roles that they occupy in the food web (Pan et al., 2011).

Planktonic communities are often structured in assemblages with a close relationship to environmental characteristics (Munk et al., 2003). The pelagic seascape encompasses hydrographic (such as temperature, salinity or ocean currents) and biological (abundance and composition of primary and secondary producers) features of the sea (Pineda et al., 2007). A foremost question for marine ecologists is to understand to which extent larval assemblages are shaped by biological and/or physical components of the seascape under different environmental scenarios (Hidalgo et al., 2014). Studies on the distribution of crustacean larvae worldwide have shown that their presence is highly correlated with phytoplankton and sea surface temperature (SST) variations (e.g. Highfield et al., 2010). There is evidence of shifts in abundance and distribution of marine larvae that are believed to be largely driven by changes in temperature (Hsieh et al., 2009). The spawning period of several species is often timed with the phytoplankton maximum in order to diminish competition (Hoegh-Guldberg and Pearse, 1995).

In coastal environments, hydrodynamic features can tightly control larval transport (Largier, 2003) and larvae may also use the vertical migration behavior to promote their retention near suitable habitats (Queiroga et al., 2007; Morgan et al., 2009). Nearshore currents could retain larvae near their release site, causing high recruitment in the natal population and generating relatively closed populations. Alternately, larvae could be flushed from the waters nearshore (Shanks et al., 2003). Recent evidence indicates that larvae are more likely to remain near the coast and recruit closer to natal populations than was widely believed (Sponaugle et al., 2002; Levin, 2006). Larval transport

is often determined by the vertical distributions of larvae in vertically stratified currents, where larvae near the surface are carried in the opposite direction of larvae at depth (Queiroga and Blanton, 2004).

The interaction of larval behavior with the local current regime conditions can favor the larval assemblage upon the more productive water masses coming from the mainland (e.g. Morgan et al., 2009; Ayata et al., 2011). Benthic species are frequently associated to costal stations and waters derived from estuaries (e.g. Thiébaut, 1996; Shanks et al., 2002; Ayata et al., 2011).

Neritic areas usually present high diversity and abundance of decapod larvae, since larvae of many intertidal and shallow waters species complete their development in waters over the continental shelf (e.g. González-Gordillo and Rodríguez, 2003; Shanks et al., 2003). Recent studies have shown that high diversity of taxa in neritic stations is more common for meroplankton than ichthyoplankton assemblages (e.g. Hidalgo et al., 2014).

Neritic fronts are very abundant in austral South America, caused by diverse forcing such as tides, continental run-off, currents convergence, wind and bathymetry (Acha et al., 2004). Zooplankton assemblages may be strongly influenced by frontal structures (McGinty et al., 2011). Vertical movements that bring nutrient-rich waters into the well-lit surface layers are at the base of the biological production of marine fronts; phytoplankton show strong positive reaction to such nutrient enrichment (Acha et al., 2015).

Along the South Brazil Shelf, numerous mesoscale oceanographic features, including wind-and eddy-induced coastal upwelling (Valentin et al., 1987), frontal systems (Muelbert et al., 2008), river plumes (Burrage et al., 2008), and eddies (Pereira et al., 2009) have been reported. Among them, the Cape Frio upwelling (23 °S), the Cape Santa Marta Grande upwelling (28 °S), the runoff of both Patos Lagoon estuary (32 °S) and the Rio de la Plata estuary (35–36 °S) are considered the ones which mostly affect the area between 21 °S and 34 °S (Acha et al., 2004) (Fig. 2.1). These processes enhance primary production, turning the area into a highly important fishing ground, exploited by both artisanal and industrial fleet, which support important crustacean fisheries, such as pink shrimps, deep sea shrimps, and red and royal crabs (Pezzuto et al., 2006; Pereira and D'Incao, 2012).

Here, we investigate the spatial distribution of a spring/summer community of combined decapod and stomatopod larvae along the South Brazil Shelf and its possible linkages with hydrographical processes. Our main goal is to identify the components of the seascape

(environmental, spatial and biological) that structure the decapod and stomatopod larval community along the South Brazil Shelf. We tested the hypothesis that (i) the distribution of larvae discloses a relationship with the adult's distribution, (ii) the larval abundance and diversity are higher in the neritic area, and (iii) larvae of benthic species show an association with more productive waters derived from the mainland.

2. Materials and methods

2.1. Study area

According to the definition of the large marine ecosystems (LMEs), the South Brazil Shelf LME extends from 22 °S to 34 °S along the South American southeast coast (Heileman and Gasalla, 2008) (Fig. 2.1A). In this area, the average distance from the shore to the shelf-break ranges from about 50 km (Cape Frio) to more than 170 km (in front of Patos Lagoon) (Acha et al., 2004). The region is subjected to relatively intense wind-driven coastal upwelling of the South Atlantic Central Water (SACW), pumped by alongshore-northeast winds and by cyclonic vortexes originated from the Brazil Current. It is the most productive coast in the Brazil Current region with moderately high productivity (150–300 gC/m^2/year) (Heileman and Gasalla, 2008). In addition to upwelling areas, production is also sustained by several terrigenous sources such as the Patos Lagoon and La Plata River plumes (Acha et al., 2004).

The whole area is also often divided into three latitudinal subareas according to their hydrographic features: (i) the Cape São Tomé-Cape Frio region (CSTF); (ii) the Southern Brazilian Bight (SBB) located between Cape Frio and Cape Santa Marta Grande; and (iii) the Southern Subtropical Shelf (SSS) between Cape Santa Marta Grande and La Plata River. In the northern portion of SSB, the CSTF is mainly characterized by coastal upwelling events, while SBB and SSS are dominated by the strong influence of cold coastal waters derived from the La Plata River (especially SSS) and Patos Lagoon, which are stronger in winter and spring (Burrage et al., 2008; Möller et al., 2008).

The main water masses known for the whole area are the oligotrophic salty (> 36) Tropical Water (TW); the Costal Water (CW), which mixes freshwater from the continental drainage with continental shelf water; the cold (< 20 °C) nutrient-rich SACW; the Shelf Water (SW), that arises from the mix between the TW, the CW and the Subtropical Shelf Water (STSW); and the low salinity (< 30) cold (< 24

°C) Plata Plume Water (PPW) (Braga and Niencheski, 2006; Piola et al., 2008).

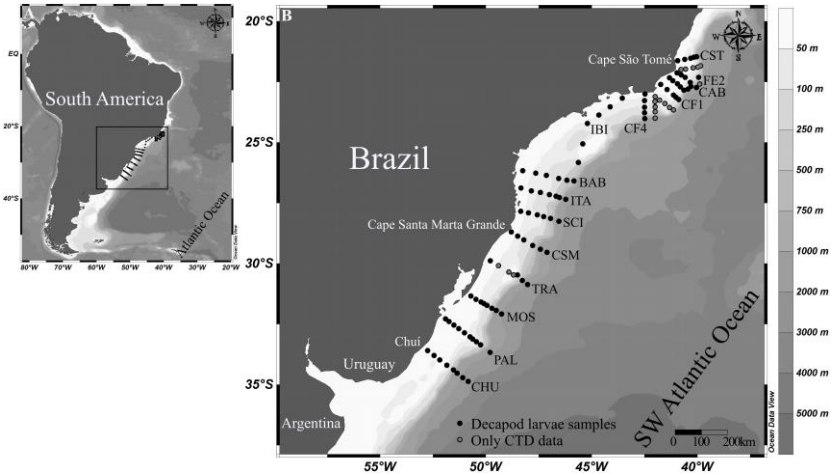

Figure 2.1. (A) Geographic location of the study area. (B) Position of the sampling stations along the South Brazil Shelf, between Cape São Tomé and Chuí. Transects names: CST=Cape São Tomé; FE2=Feia Lagoon 2; CAB=Campos Bight; CF1=Cape Frio 1; CF4=Cape Frio 4; IBI=Ilhabela Island; BAB=Babitonga Bay; ITA=Itajaí River; SCI=Santa Catarina Island; CSM=Cape Santa Marta Grande; TRA=Tramandaí; MOS=Mostardas; PAL=Patos Lagoon; CHU=Chuí.

2.2. Sampling and laboratory procedures

Cruises were conducted between Chuí (34 °S) and Cape São Tomé (21 °S). To cover the entire sampling area three consecutive campaigns were conducted, the first starting in austral late spring (December 2010) in Chuí and the third finishing in early summer (January 2011) in Cape São Tomé. Vertical profiles of temperature, salinity, fluorescence and dissolved oxygen were recorded at 107 stations distributed at 17 cross-shelf transects using a SeaBird CTD (conductivity, temperature and depth) profiler casts (Fig. 2.1B). Additionally, water samples were collected at selected depths (3 or 5 m, maximum fluorescence depth and base of the thermocline) to determine chlorophyll and nutrient (ammonia, nitrite, nitrate, phosphate and silicate) concentrations with 5-L Niskin bottles. Chlorophyll and nutrient concentrations were estimated following Welschmeyer (1994) and Grasshoff et al. (1983), respectively.

Plankton samples (89, black circles in Fig. 2.1B) were collected through vertical tows from the maximum fluorescence depth up to the surface in deep-water stations, from 10 m above the bottom when the water column was homogenous and from about 10 m depth at shallow stations (up to 20 m).

A conical-cylindrical net with a 0.5-m diameter mouth and 200-µm mesh equipped with a flowmeter (General Oceanics) was used for sampling planktonic organisms, through vertical tows at a speed of about 2 knots. All samples were immediately fixed and preserved in 4% buffered seawater-formaldehyde solution. The maximum fluorescence depth ranged from 7 to 125 m, and the plankton sampling depth ranged from 12 to 130 m. The distance of sampling locations from the coast ranged from 7 to 418 km. The mean volume of water filtered by the net was 29.4±2.3 m^3.

Decapod larvae were counted and sorted from all 89 samples. In a few coastal stations only larvae from 1/2 or 1/4 fractions were considered due to their high densities. For stomatopod larvae the entire sample was considered. Decapod and stomatopod larvae were identified to the lowest taxonomic level possible, under stereoscopic and compound microscopes. Developmental larval stages were also determined for each larva. Larval identification and stage determination followed species larval descriptions and relevant keys for each group, such as Gurney and Lebour (1940), Scelzo (1976), Calazans (1993) and Koettker et al. (2012).

2.3. Data analysis

Larval counts were standardized to number of individuals per m^3 or per 100 m^3 to calculate the relative abundance (RA) of each taxon. The frequency of occurrence (FO) was also calculated.

Shannon diversity (H') was calculated for the neritic and oceanic areas. Only taxa in species level or that represented a unique species (e.g. Hippidae sp.1, *Gennadas* sp.1) were considered in the analysis. The index was calculated in PRIMER 6 (PRIMER-E) (Clarke and Gorley, 2006).

Nonmetric multidimensional scaling ordination (NMDS) was used to verify if larval abundance and composition were correlated with the alongshore distribution (neritic and oceanic) of the samples. Multivariate analysis of variance with permutations (PERMANOVA) was conducted to test the validity of these groups formed *a priori* (Anderson, 2001). NMDS and PERMANOVA were performed in

PRIMER 6 with the PERMANOVA+ package (PRIMER-E). R^2 was obtained with the 'vegan' package (Oksanen et al., 2013) in R (R Foundation for Statistical Computing). Similarity between the samples was calculated using the Bray-Curtis similarity index (Legendre and Legendre, 2006) and taxa abundances were log-transformed (x+1) in order to reduce the weight of abundant species (Field et al., 1982). Samples collected onshore from the shelf break were classified as "neritic" (n=43), and samples collected along the shelf break and offshore were classified as "oceanic" (n=46).

Spearman rank correlations determined the relationship between combined decapod and stomatopod larval abundance and chlorophyll, temperature, salinity and distance from shore. Correlations were performed using Statistica version 7.0 (Statsoft) and larvae were separated according to the developmental period (early or late) and the adult's realm (pelagic or benthic).

Two potential temperature–salinity (T–S) diagrams were built for the studied area. The latitude of 25 °S delimited the southern and northern regions, due to differences in water mass composition. Larval abundances were plotted on the diagrams in order to verify associations between the water masses and the larval distributions. Water masses were determined based on thermohaline indexes in Möller et al., 2008.

The stratification index S, which shows the stability of the water column, was calculated by integrating the vertical density profile as the mean of the density differences along the water column, according to Fortier and Leggett (1982).

To identify the most predominant spatial patterns, principal coordinates of neighbor matrices (PCNM) was employed. This method is based on a spectral decomposition of the study area into a series of eigenvectors each representing a spatial scale and is a well-suited method to detect spatial trends across a wide range of scales (Borcard et al., 2004). A forward selection of the spatial vectors was computed (Blanchet et al., 2008). Forward selection retained 12 PCNM variables. Of these, the five first PCNM variables ($R^2 \geq 0.03$) were included in the variance partitioning procedure.

In order to evaluate the interactions among the most frequent taxa and environmental variables, a partial redundancy analysis (partial RDA) was conducted. Biological data were Hellinger-transformed to reduce the wide disparity in magnitude between taxa abundances (Legendre and Gallagher, 2001). We only considered taxa that presented FO of at least 10%. In order to avoid collinearity of explanatory variables, we applied a variance inflation factor (VIF) and removed

collinear variables before starting the correlations and the partial RDA. A cut-off VIF value of 10 was applied to get the final set of covariates (Zuur et al., 2009). Partial RDA and additional tests were performed in R (R Foundation for Statistical Computing), with the 'vegan' and 'HH' packages (Oksanen et al., 2013; Heiberger, 2013).

Variance partitioning led to split the variance of the response matrix into components explained solely by effects of environmental or spatial variables, components explained by combined effects of environmental and spatial variables, and finally unexplained components.

Package 'vegan' (Oksanen et al., 2013) was used for variation partitioning and PCNM, and 'packfor' (Dray et al., 2009) for the forward selection of PCNM variables.

Mean values of temperature, salinity, chlorophyll and nutrient concentrations, calculated from surface to plankton sampling depth, were used in the correlations and RDA. The oxygen vertical gradient was calculated using the surface oxygen value and the value at the bottom of the oxycline, as well as respective depths.

3. Results

3.1. Oceanographic scenario

The horizontal distribution of temperature, salinity and chlorophyll are shown in Fig. 2.2. The whole sampling area was characterized by the presence the TW at the slope from the surface to 100 m depth. At the shelf a latitudinal change in the distribution of the water masses was observed. South of 29°S the range of the surface salinity exceeds 7. The low-salinity waters of the PPW were found at the surface along the four southernmost transects (29–34°S) (Fig. 2.2A and 2.2D). Due to its low density, the PPW does not reach depths below 50 m, and at that depth is replaced by the Subantarctic Shelf Water (SASW) (Fig. 2.2B and 2.2E). The northernmost transects were characterized by the presence of the SW on the surface (Fig. 2.2A and 2.2D) and by the cool waters of the SACW at 50 m and deeper (Fig. 2.2B–C and 2.2E–F). On the surface, highest chlorophyll concentrations were observed in the area of the SW, STSW and PPW (Fig. 2.2G–H). At 50 m the area of the SACW corresponded to the highest chlorophyll concentrations (Fig. 2.2H). At some stations near the coast in the area between Cape São Tomé and Cape Santa Marta Grande (21–28°S), the SACW was observed closer to the surface (up to 15 m). The warm-salty

STSW was observed at 50 m depth in the transition between the PPW or the SASW and the TW (Fig. 2.2A–B and 2.2D–E). Stations located in the oceanic area, characterized by mixed waters, presented low vertical stratification ($S<0.05$), while the neritic stations of the southern transects were characterized by higher stratification index S (up to 0.13) (Fig. S2.1).

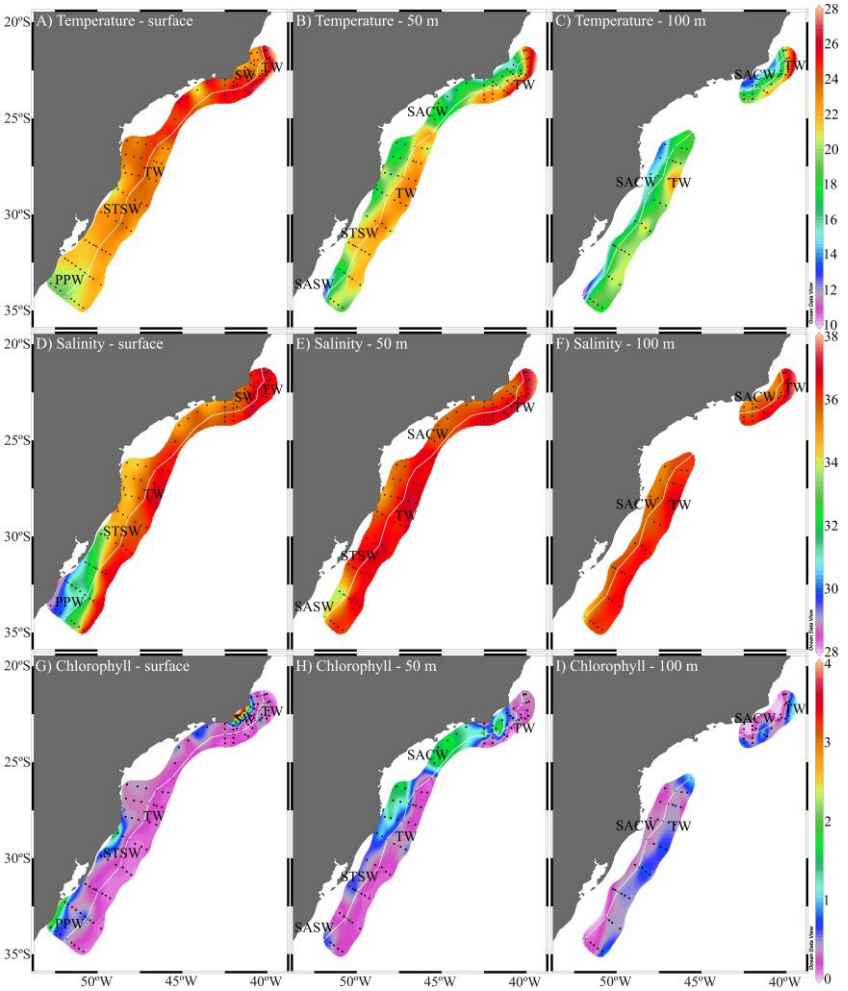

Figure 2.2. Horizontal distribution of temperature (°C) (A–C): on the surface (A), at 50 m (B) and at 100 m (C); salinity (D–F): on the surface (D), at 50 m (E) and at 100 m (F); and chlorophyll (mg/m^3) (G–I): on the surface (G), at 50 m (H) and at 100 m (I). TW=Tropical Water; SW=Shelf Water; STSW=Subtropical Shelf Water; PPW=Plata Plume Water; SACW=South Atlantic Central Water; SASW=Subantarctic Shelf Water. The white line represents the approximate position of the shelf break (~200 m deep).

3.2. Community structure

Decapod and stomatopod larvae comprised 120 taxa. Table S2.1 displays percentage of relative abundance and frequency of occurrence of all taxa included in the analyses, while Table 2.1 displays a subset of the more abundant larvae (sum of RA and FO was greater or equal than 7%). Larvae of brachyuran crabs presented the highest taxonomic richness, followed by dendrobranchiate shrimps, caridean shrimps, anomuran crabs, and stomatopods (Table S2.1). The most abundant taxa were the larvae of holopelagic shrimps, with 2 taxa accounting for 59% of total larval abundance: early larvae of *Lucifer* spp. and late larvae of *Lucifer faxoni* (Table 2.1). Among the benthic species, larvae of brachyuran crabs were dominant, especially of the swimming crabs *Achelous spinicarpus*, *A. gibbesii* and *Callinectes* spp. (Table 2.1). Larvae of neritic caridean shrimps, as Alpheidae spp. and *Leptochela* sp., and anomuran crabs, as Paguroidea sp.2 and *Dardanus insignis* (complete zoeal phase) were the most abundant taxa among these groups (Table 2.1). Larvae of thalassinidean ghost shrimps and stomatopods accounted for about 3% of total macrocrustacean larvae separately, and of these groups Callianassidae spp. and early larvae of Lysiosquilloidea were dominant (Table 2.1).

Regarding the spatial distribution of the main groups, stomatopod and caridean larvae were more abundant in the northernmost transects (Fig. 2.3A and 2.3D). Larval abundance distribution of dendrobranchiate shrimps showed spatial partitioning. Pelagic dendrobranchiates occurred in lower abundances in the southernmost transects, exactly where larvae of benthic dendrobranchiates were more abundant (Fig. 2.3B and 2.3C). It is also worth noting that larvae of pelagic species were about four times more abundant than larvae of benthic species. Larvae of anomuran and brachyuran crabs were more abundant at coastal stations, especially at the southernmost transects, including the mouth of Patos Lagoon (PAL), and also off Cape Frio (CF4) (Fig. 2.3E and 2.3F).

Table 2.1. Percentage of relative abundance (RA) and of frequency of occurrence (FO) of most common decapod and stomatopod larvae for neritic, oceanic and all samples combined (see Table S2.1 for a complete list of the species included in the study). Adult realm (pelagic or benthic) and larval stage (M=mysis; Pz=protozoea; D=decapodids; Z=zoeae; Mg=megalopae; E=erichthus; Ps=pseudozoea; An=antizoea; Al=alima) are also included.

Taxon	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Stomatopoda								
Lysiosquilloidea spp.	Benthic	Na	1.00	16	0.96	26	1.87	7
Lysiosquilloidea sp.1	Benthic	E I, II and V-VII	0.32	8	0.34	16	–	–
Squilla spp.	Benthic	Os	0.40	17	0.37	30	1.27	4
Squilla sp.1	Benthic	Al I-VI	0.20	17	0.15	21	1.36	13
Squilla sp.3	Benthic	Al III-V and VII	0.15	7	0.15	14	–	–
Dendrobranchiata								
Lucifer spp.	Pelagic	Pz I-III	31.98	35	33.00	44	8.52	26
Lucifer faxoni	Pelagic	M I and II / D	27.04	28	27.83	51	8.95	7
Lucifer typus	Pelagic	M I and II / D	0.17	15	0.01	5	3.62	24
Sergestidae spp.1	Pelagic	Pz I	0.07	11	0.02	2	1.16	20
Sergestidae spp.2	Pelagic	D	0.10	16	–	–	2.38	30
Peisos petrunkevitchi	Pelagic	Pz II and III / M II	0.24	7	0.25	14	–	–
Sergestes spp.1	Pelagic	Pz II and III	0.10	9	0.01	2	2.13	15
Sergestes spp.2	Pelagic	Pz II and III	0.09	10	0.02	5	1.68	15
Penaeoidea spp.	Benthic	Pz I-III	0.56	20	0.41	12	4.05	28
Gennadas sp.1	Pelagic	Pz III / M I-IV	0.11	11	0.01	2	2.49	20
Artemesia longinaris	Benthic	Pz II and III / M I, II and IV / D	0.36	7	0.37	12	0.19	2
Pleoticus muelleri	Benthic	Pz I-III / M I and II	0.45	12	0.38	16	1.99	9
Caridea								
Alpheidae spp.	Benthic	Z I-III	2.23	26	2.13	49	4.46	4
Alpheus sp.1	Benthic	Z II-IV	0.18	10	0.18	19	0.21	2
Alpheus sp.2	Benthic	Z III-V	0.10	8	0.10	14	0.04	2

Table 2.1. (Continued).

Taxon	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Alpheus sp.3	Benthic	Z II-IV	0.32	10	0.30	16	0.93	4
Lysmata sp.	Benthic	Z II-V	0.18	7	0.18	12	0.17	2
Palaemonidae sp.2	Benthic	Z I, III, V and VI	0.38	13	0.37	21	0.54	7
Pandalidae spp.	Pelagic/Benthic	Z I-IV	1.01	19	1.01	26	0.96	13
Leptochela sp.	Pelagic	Z I-VI / Mg	2.04	16	2.13	33	–	–
Thalassinidea								
Callianassidae spp.	Benthic	Z I-IV	3.11	12	3.23	19	0.24	7
Anomura								
Munida spp.	Benthic	Z I, III and IV	0.68	11	0.49	12	4.92	11
Pachycheles haigae	Benthic	Z I and II	0.23	8	0.20	14	0.72	2
Hippidae sp.1	Benthic	Z I-V	0.32	11	0.32	21	0.21	2
Paguroidea spp.	Benthic	Z IV / Mg	0.35	7	0.31	12	1.15	2
Paguroidea sp.2	Benthic	Z I-IV	2.04	17	2.13	35	–	–
Dardanus insignis	Benthic	Z I-VIII	0.75	33	0.50	37	6.38	28
Brachyura								
Brachyura spp.	Benthic	Mg	0.42	21	0.43	37	0.39	7
Brachyura sp.4	Benthic	Z I-IV	0.22	10	0.18	16	1.00	4
Raninidae sp.2	Benthic	Z I, IV and V	0.13	8	0.13	14	0.19	2
Persephona sp.	Benthic	Z I, II and IV	0.62	17	0.63	33	0.46	2
Parthenopidae sp.1	Benthic	Z I, III, IV and VI	0.18	7	0.16	9	0.62	4
Portunidae spp.	Benthic	Z I / Mg	0.34	21	0.32	33	0.86	11
Achelous sp.	Benthic	Z IV-VI and VIII	0.18	7	0.05	12	3.06	2
Achelous gibbesii	Benthic	Z I-III	1.61	22	1.38	30	6.74	15
Achelous spinicarpus	Benthic	Z I-VII	5.14	28	5.16	53	4.76	4
Callinectes spp.	Benthic	Z I-VII	2.26	26	2.29	40	1.57	13
Cronius tumidulus	Benthic	Z I-III	1.05	11	1.02	14	1.83	9

Table 2.1. (Continued).

Taxon	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Xanthoidea spp.	Benthic	Z III, IV / Mg	0.36	13	0.37	26	0.14	2
Hexapanopeus paulensis	Benthic	Z I-IV	0.67	13	0.70	28	–	–
Micropanope sculptipes	Benthic	Z I-ZIV	1.88	21	1.93	40	0.74	4
Pinnotheridae sp.	Benthic	Z I, III and IV	0.32	8	0.32	12	0.33	4
Pinnixa chaetopterana	Benthic	Z I and II	0.73	7	0.76	14	–	–

Figure 2.3. Distribution of larval abundance (larvae/100 m^3) of: (A) stomatopods, (B) pelagic dendrobranchiates, (C) benthic dendrobranchiates, (D) carideans, (E) anomurans and (F) brachyurans along the South Brazil Shelf.

3.3. Larvae distribution in relation to hydrological structure

Combined decapod and stomatopod larvae mean abundance was 523±131 larvae/100 m^3. Considering the different regions, the neritic area presented much higher mean abundance of larvae (1014±41 larvae/100 m^3) than the oceanic area (174±7 larvae/100 m^3). NMDS ordination also showed the separation between the neritic and oceanic communities [PERMANOVA: Pseudo-F=8.375, R^2=0.089, p(perm)=0.001; Fig. 2.4].

Diversity was higher in the neritic samples (H'=3.21) than in the oceanic ones (H'=2.35). From the 120 taxa analyzed, 46 were exclusive from neritic stations, 18 were exclusive from oceanic stations, and 56 occurred in both (Table S2.1). These evidences support the hypothesis that larval diversity is higher in the neritic area in relation to the oceanic zone. However, it also shows that the majority of the larvae inhabit both regions.

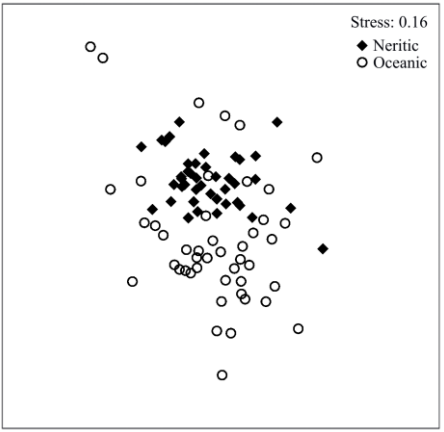

Figure 2.4. Nonmetric multidimensional scaling ordination of samples collected in spring/summer along the South Brazil Shelf, based on the abundance of 120 identified taxa. Samples were classified according to habitat (neritic or oceanic).

Larval abundance, for all stages and adult realms, presented positive and significant correlations (at 95% confidence level) with chlorophyll (Table 2.2). On the other hand, larval numbers were negatively correlated with temperature (except for late pelagic larvae, which was not significant) (Table 2.2). Salinity was only significantly correlated with early benthic larvae, with which presented a negative

correlation (Table 2.2). Distance from shore was significantly negatively correlated with early and late benthic larvae (Table 2.2).

Higher values from both chlorophyll and larval abundance were found in most coastal stations of each transect, supporting the significant positive correlations observed (Fig. 2.2G–H, Fig. 2.5 and Table 2.2). The coastal stations of Campos Bight (CAB) and Cape Frio (CF1) presented the highest chlorophyll concentration values. However, the abundance of larvae was not high in any of these samples (Fig. 2.5). The coupling between larval abundance and chlorophyll concentration was only observed for larvae of benthic species, especially seen for early larvae (Fig. 2.5B and 2.5D). In respect of proportion of larvae from early and late stages, larvae of benthic species presented a higher loss than larvae of pelagic species (Fig. 2.5).

Table 2.2. Result of Spearman rank correlations for decapod/stomatopod larval abundance (larvae/m^3) sampled along the South Brazil Shelf. Highlighted in bold are the significant p-values (< 0.05).

		N	***r***	***p***
Chlorophyll (mg/m^3)	Early pelagic	89	0.2558	**0.016**
	Early benthic	89	0.6189	**0.000**
	Late pelagic	89	0.2203	**0.038**
	Late benthic	89	0.4544	**0.000**
Temperature (ºC)	Early pelagic	89	-0.3453	**0.000**
	Early benthic	89	-0.7453	**0.000**
	Late pelagic	89	-0.1216	0.257
	Late benthic	89	-0.5282	**0.000**
Salinity	Early pelagic	89	0.0401	0.071
	Early benthic	89	-0.3144	**0.003**
	Late pelagic	89	0.0045	0.966
	Late benthic	89	-0.0974	0.364
Distance from shore (km)	Early pelagic	89	-0.0999	0.352
	Early benthic	89	-0.6701	**0.000**
	Late pelagic	89	-0.0807	0.452
	Late benthic	89	-0.5283	**0.000**

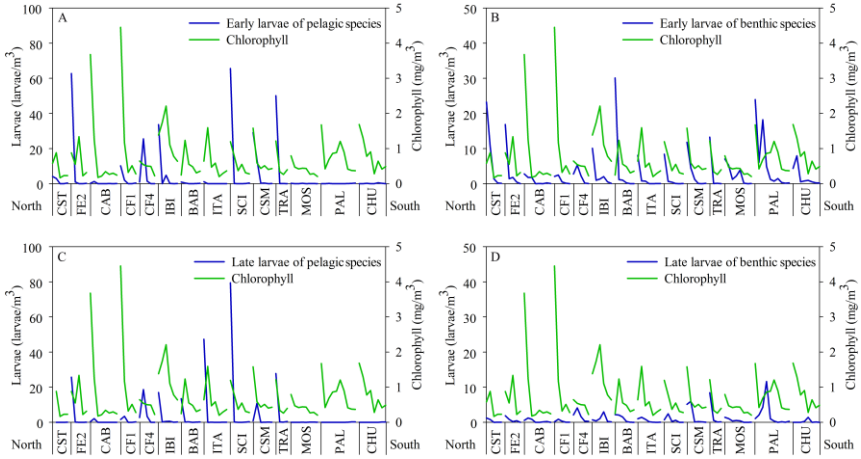

Figure 2.5. Spatial variations in early pelagic (A), early benthic (B), late pelagic (C) and late benthic (D) decapod/stomatopod larvae abundance (larvae/m^3) and depth-averaged chlorophyll concentration (mg/m^3). Samples are ordered from North to South and from coast to ocean for each transect. Note the difference between scales on the axis of abundance.

Larval abundance depicted a close relationship with the distribution of the water masses, for both northern and southern stations (Fig. 2.6). Larval abundance presented the lowest values at all stations characterized by the TW (Fig. 2.6). Regarding the stations dominated by water masses that occur in the neritic area, differences in larval abundances were observed between the northern and southern stations. In the northern area, the highest larval abundances were observed where the SW was dominant (Fig. 2.6A), while in the southern area high abundance values were found in association with the STSW and, secondarily, with the PPW (Fig. 2.6B).

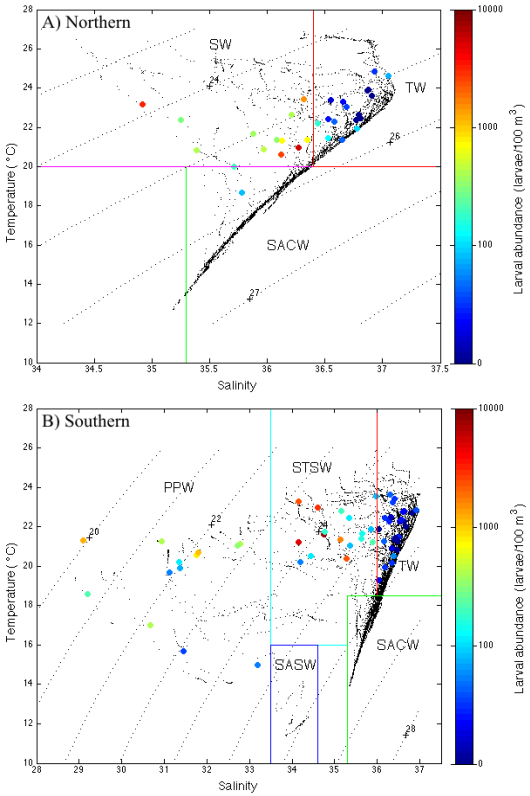

Figure 2.6. Combined decapod and stomatopod larval abundance plotted on the potential temperature-salinity diagrams from the northern (A) and southern (B) stations along the South Brazil Shelf for late austral spring and summer. TW=Tropical Water; SW=Shelf Water; STSW=Subtropical Shelf Water; PPW=Plata Plume Water; SACW=South Atlantic Central Water; SASW=Subantarctic Shelf Water. Potential salinity and temperature were from the upper 150 m.

Among the set of spatial axis produced by the PCNM procedure, five were selected by R^2 (≥ 0.03). Two spatial patterns were illustrated with the selected PCNM variables: (1) the discrimination between the samples in the northern stations (until CF4) from other samples (Fig. S2.2A), and (2) the main coast-ocean gradient, separating neritic from oceanic samples (Fig. S2.2B–D).

The partitioning of the variance estimated by the partial RDA showed that 8% of the variance was explained by the conditioned (spatial) variables, while 17% was explained by the constrained

(environmental) variables. Of the constrained variables, the first two axes explained together 54% of the variance in the most frequent taxa in relation to environmental variables. Axis 1 was mainly influenced by distance from the coast in opposition to chlorophyll, and thus described the gradient between neritic assemblages (species negatively correlated with Axis 1) and oceanic assemblages (species positively correlated with Axis 1) (Fig. 2.7). The axis 2 mainly described the thermohaline and latitudinal gradients, with highest salinity and temperature, typical of the TW, opposed to highest nutrient concentration, with major contribution of phosphate and silicate, typical of the PPW (Fig. 2.7).

Some taxa were highly associated with the oceanic stations, as *Gennadas* sp.1, Sergestidae spp.1, Sergestidae spp.2, *Lucifer typus* and *Sergestes* spp.2, which are holopelagic and presented a negligible occurrence in the neritic zone (taxa 18 to 22 of Fig. 2.7). In addition, larvae of other holopelagic species occurred exclusively in the oceanic stations, as larvae of dendrobranchiate shrimps (*Sergestes atlanticus*, *S. edwardsii*, *S. henseni*, *S. pectinatus* and *Gennadas* sp.2) and of deepwater caridean shrimps (Oplophoridae spp. and *Acanthephyra* sp.1) (Table S2.1). Their abundance and frequency of occurrence were lower than of the neritic species, though (Table S2.1).

Another group of larvae showed higher correlation with the coastal stations, wherein the chlorophyll concentration was higher. Associated with this condition we observed larvae that belong to neritic species, mainly larvae of intertidal benthic, as Paguroidea sp.2, Callianassidae spp. and Hippidae sp.1, shallow water, as *Leptochela* sp., and coastal pelagic species, as *Lucifer* spp. and *Lucifer faxoni* (taxa 27 to 34 of Fig. 2.7). This pattern is in accordance with the positive association observed between larvae and chlorophyll (Table 2.2 and Fig. 2.7) and the negative correlation between benthic larvae and distance from shore (Table 2.2).

Taxa that were observed in association with the vectors that correspond to the area influenced by the runoff of the southern estuaries, characterized by higher nutrient concentrations, and lower temperatures and salinities were mainly of early brachyuran crabs and benthic shrimps (*Achelous spinicarpus*, *Micropanope sculptipes*, *Hexapanopeus paulensis* and *Pleoticus muelleri*) (taxa 1 to 9 of Fig. 2.7). This scenario reinforces the significant negative correlation observed between salinity and early benthic larvae (Table 2.2) and also the coupling with chlorophyll, which was constant in the southern transects, from Babitonga Bay (BAB) to Chuí (CHU) (Fig. 2.5B).

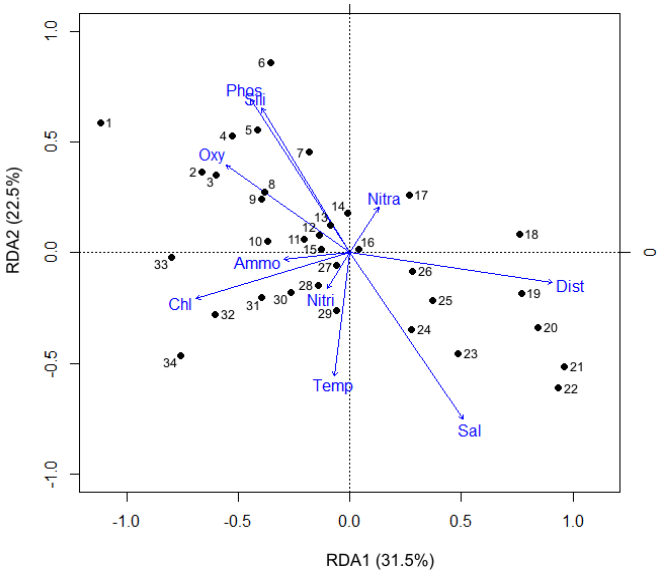

Figure 2.7. Partial RDA plot for decapod and stomatopod larvae composition in relation to environmental variables. Numbers represent taxa names: 1. *Callinectes* spp.; 2. *Pleoticus muelleri*; 3. *Achelous spinicarpus*; 4. *Cronius tumidulus*; 5. *Micropanope sculptipes*; 6. Xanthoidea spp.; 7. Brachyura spp.; 8. *Hexapanopeus paulensis*; 9. Portunidae spp.; 10. *Achelous gibbesii*; 11. *Munida* spp.; 12. Pandalidae spp.; 13. *Persephona* sp.; 14. Lysiosquilloidea spp.; 15. Palaemonidae sp.2; 16. *Alpheus* sp.3; 17. *Squilla* spp; 18. *Gennadas* sp.1; 19. Sergestidae spp.1; 20. Sergestidae spp.2; 21. *Lucifer typus*; 22. *Sergestes* spp.2; 23. *Squilla* sp.1; 24. Brachyura sp.4; 25. Penaeoidea spp.; 26. *Dardanus insignis*; 27. Alpheidae spp.; 28. *Alpheus* sp.1; 29. Paguroidea sp.2; 30. Callianassidae spp.; 31. *Leptochela* sp.; 32. *Lucifer* spp.; 33. *Lucifer faxoni*; 34. Hippidae sp.1.

Summing up, the distribution of larvae disclosed a relationship with the adult's distribution, as seen for larvae of oceanic pelagic species, which occurred in the offshore area. In addition, larvae of neritic species occurred mainly in two situations over the neritic zone: (1) larvae of intertidal benthic, shallow water, or coastal pelagic species in the nearshore stations, associated with higher chlorophyll, and (2) larvae of crabs and benthic shrimps in the area influenced by the runoff of the southern estuaries. The major area is featured by the oligotrophic TW with high salinity and temperature. In opposition to that, some areas present colder, less saline water, rich in silicate and phosphate, in

accordance with the horizontal distributions of temperature and salinity (Fig. 2.2 of Section 3.1) and the T–S diagrams (Fig. 2.6).

Variance partitioning indicated that most of the variation in decapod larval distributions is due to unexplained or stochastic variance. Of the explained portion, variation was mainly due to the combined influence of the environmental and spatial structure of the hydrological environment, which accounted for 12% of the total variation. Environmental variables alone explained 7%, while spatial variation retained 6% (Fig. S2.3).

4. Discussion

Abundance of decapod larvae were comparable to the continental shelf of other locations, such as along NE Brazilian shelf (3–9 °S) (Schwamborn et al., 1999), NW Atlantic (40–41 °N) (dos Santos et al., 2008) and NE Pacific (37 °S) (Yannicelli et al., 2006). The community of larvae was mainly dominated by early and late larvae of holopelagic shrimps, especially over the continental shelf. Typically, the high abundance of early larval stages such as protozoeae provides good evidence of any recent hatching and also presence of adult stock (e.g. Criales and Mcgowan, 1993; Rivera and Mujica, 2004). Of the two species of the genus *Lucifer* that occur in the Atlantic Ocean, *L. faxoni* is found in neritic waters, collected even in the estuaries, while *L. typus* is oceanic in distribution. Their range of distribution is from 43 °N to 23 °S and 40 °S, respectively. *L. faxoni* occurs from depths of 6 to 55 m, but is usually found in coastal waters (Bowman and McCain, 1967). We report very high densities of *Lucifer* spp. (mainly *L. faxoni*) in the study area. Other studies reported *L. faxoni* as the most frequent decapod in planktonic collections in North (Criales and Mcgowan, 1993; Webber et al., 1996) and South (Schwamborn et al., 1999; Teodoro et al., 2012) Atlantic Ocean. In addition, this species is part of the diet of coastal fish (Sedberry and Cuellar, 1993; Martins et al., 2005) and large filter feeders (Motta et al., 2010).

Brachyura was the most diverse, and their zoeae were the most abundant larvae of benthic species. Zoeae of portunids and xanthids were found up to more than 300 km offshore. These larvae have a high salinity requirement to complete larval development, being exported to oceanic waters to find optimal conditions and to avoid high predation pressure of estuaries (Morgan and Christy, 1997).

As an overall distributional pattern of the major groups, larvae occurred predominantly over the neritic zone and in lower abundances at

oceanic stations. It included stomatopods, dendrobranchiates, caridean and thalassinidean shrimps, anomuran and brachyuran crabs, in addition to rare occurrences of stenopodidean shrimps and palinuran lobster larvae (Table S2.1). A similar composition was observed on Abrolhos Bank and adjacent areas, located further north along the Brazilian shelf (Koettker and Lopes, 2013), in the Balearic front system (Western Mediterranean) (Hidalgo et al., 2014), and on the SW Iberian Peninsula (González-Gordillo and Rodríguez, 2003). The spatial differences in the distribution of benthic and pelagic dendrobranchiate larvae could be an indicative of partition in space to reduce or avoid potential competition between species. Spatial partitioning of ecosystem by shrimp species has been reported at tropical coral reefs (Purser et al., 2013) and in different zones of the Mid Atlantic Ridge (Cardoso et al., 2014). In the North Sea, the recent dominance of the larvae of benthic echinoderms in the plankton in summer (Lindley and Batten, 2002) represents a major change in the balance between the meroplankton and the holoplankton and is indicative of a shift in resource partitioning between the benthic and pelagic species (Kirby et al., 2007).

Two major features shape the structure of the spring/summer larval community along the South Brazil Shelf: (1) the cross-shelf gradient, which contrasts the water masses and chlorophyll distributions between neritic and oceanic zones and (2) the thermohaline front triggered by the continental runoff of the southern estuaries. Hence, we described three main assemblages associated with these environmental scenarios: (1) coastal environments, with higher chlorophyll concentrations, dominated by intertidal benthic, shallow water or coastal pelagic species; (2) offshore environments, dominated by the TW, with predominance of oceanic pelagic species; and (3) intrusion of fresh and nutrient-rich waters from the southern estuaries, mainly represented by larvae of crabs and benthic shrimps (Table 2.3).

Table 2.3. Assemblages of decapod and stomatopod larvae observed in the most relevant environmental scenarios along the South Brazil Shelf during the spring/summer of 2010/2011.

Environmental scenario	Dominant water mass	Dominant taxa
Coastal environments	SW – STSW	*Leptochela* sp., Paguroidea sp.2, Hippidae sp.1, Callianassidae spp. and *Lucifer faxoni*
Offshore environments	TW	Sergestidae spp.1, Sergestidae spp.2, *Gennadas* sp.1, *Lucifer typus* and *Sergestes* spp.2
Southern continental runoff	PPW	*Achelous spinicarpus*, *Micropanope sculptipes*, *Hexapanopeus paulensis* and *Pleoticus muelleri*

A general pattern of higher abundance of larvae in the coastal environments throughout the whole latitudinal gradient surveyed was observed. Larvae of many intertidal and shallow waters species go through development in waters over the continental shelf (González-Gordillo and Rodríguez, 2003; Shanks et al., 2003). Newly hatched larvae migrate to the surface, where the tidal currents carry them into the coastal waters (Tilburg et al., 2009). The corresponding species of most larvae found exclusively in the neritic area inhabit shallow waters, which were associated with highest chlorophyll concentrations and highest stratified oxygen stations, suitable habitats for the larvae as pointed out in previous research (Thorson, 1950; Criales-Hernández et al., 2008). Early larvae of Callianassidae were well represented in terms of relative abundance in neritic samples. Adult thalassinid shrimps inhabit sandy beaches, and their reproduction presents peaks during spring and summer (Hernáez et al., 2012). Even taxa that we only found late larval stages, as for *Squilla* sp.3, Lysiosquilloidea sp.1 and unidentified megalopae of Brachyura, abundance was higher in the neritic zone. Recent advances in sampling technology have provided new insights into the extent of marine larval dispersal, indicating more local retention than once appreciated (Jones et al., 2007).

The oceanic area was characterized by lower larval abundances and lower diversity, which supports the proposed hypothesis. Some taxa, mainly represented by holopelagic dendrobranchiate and caridean shrimps, were associated with the offshore stations, in which the saline and warm TW was dominant. Interestingly, larvae of holopelagic dendrobranchiate shrimps and of oceanic deep-sea caridean shrimps were dominant among the taxa that occurred exclusively at the shelf break and offshore. Other studies that took place in similar wide continental shelves did not find this high contribution of larvae of pelagic species (González-Gordillo and Rodríguez, 2003; dos Santos et al., 2008). On the other hand, our results were more similar to the ones obtained around isolated oceanic islands, with significant contribution of holopelagic shrimps, as in Saint Paul's Rocks in the Equatorial Atlantic (Brandão et al., 2013) and Gran Canaria in the NW Africa (Landeira et al., 2013), which present a much shorter shelf and a greater influence of the open ocean waters.

The contrast between these two larval assemblages (coastal and offshore environments) is associated with the cross-shelf variability of salty ocean waters and coastal productive waters (Lopes et al., 2006). It also supports the hypothesis that the neritic area harbors greater larval abundance and higher diversity than the oceanic waters. A high diversity

of taxa in neritic stations increase was observed in a meroplankton compared with an ichthyoplankton context in a frontal system of the Western Mediterranean (Hidalgo et al., 2014). A similar pattern was observed in the South Brazil Shelf, as decapod/stomatopod larvae presented higher diversity in the neritic area, while ichthyoplankton presented nearly the same number of species in both habitats (Macedo-Soares et al., 2014). Differences in larval abundance and composition were found between neritic and oceanic samples. Consistent with other studies, the shallower assemblage resulted from occurrence of common coastal species and other species with adult distributions at deeper habitats along the shelf-break and upper slope (e.g. Yoshinaga et al., 2010; Landeira et al., 2013). A pattern of cross-shelf distribution of holoplanktonic crustacean assemblages was also observed in the region corresponding to the transect off Itajaí (ITA), at which the distance from the coast was the most explicative variable (Brandini et al., 2014). Ekau (2009) also observed a separation of the zooplankton community on the eastern Brazilian coast into two major clusters, neritic and oceanic communities, and decapod larvae played an important role in such differentiation.

In our study, the maximal larval abundances of all coastal benthic species were mostly observed associated to the river plume waters, thus confirming their important role in concentrating and transporting coastal invertebrate larvae as reported in various nearshore environments (e.g. Thiébaut, 1996; Shanks et al., 2002; Ayata et al., 2011). The southernmost transects (29–34 °S) were subjected to the surface influence of PPW and presented a particularly different larval community, which might be exported from the estuaries and/or benefits from the nutrient-rich waters of their plumes. These stations were dominated by brachyuran and anomuran crab larvae and benthic dendrobranchiate shrimps. Previous studies have shown that this area is a reproductive zone for penaeids (Dumont and D'Incao, 2011) and that brachyuran zoeae are continuously exported from Patos Lagoon to the adjacent ocean (Araújo et al., 2012).

In addition to the input of larvae from benthic species, this area is subjected to high silicate and phosphate concentrations and also to sharp changes in temperature and salinity (Acha et al., 2004). A sharp thermohaline frontal system exists between STSW and SASW around 33 °S, named the Subtropical Shelf Front (STSF) (Piola et al., 2008), which increases local nutrient availability in the euphotic zone, primary production and zooplankton abundance (Muelbert et al., 2008). Frontal systems are habitats characterized by high productivity and

accumulation of planktonic organisms associated with convergence processes and may also act as retention areas (e.g. Le Fèvre, 1986). Because of the exceptional physical processes connected to frontal zones, the frontal activity affects plankton organisms in a number of aspects, among these are nutrient entrainment, primary/secondary production and plankton distribution/aggregation (Munk et al., 2003). The particular environmental conditions seem to favor the larval assemblage upon the more productive water masses coming from the mainland. This could result from the interaction of larval behavior with the local current regime (e.g. Morgan et al., 2009; Ayata et al., 2011). It is worth mentioning that historical hydrographic data suggests that in the austral winter the Plata plume reaches Cape Santa Marta Grande (28 °S) (e.g. Piola et al., 2008; Braga et al., 2008; Möller et al., 2008). This scenario could probably lead to a consequent occurrence of species associated with the PPW further north, in association with the nutrient-rich waters.

These three environmental scenarios and the assemblages of decapod and stomatopod larvae associated to them also displayed a connection between larval distribution and adults' habitat, with larvae of oceanic pelagic species dominating the offshore stations, while larvae of neritic species inhabiting the nearshore stations and the plume of the southern estuaries. In the waters associated to the plumes in the South Brazil Shelf, ichthyoplankton composition was also dominated by coastal- and estuarine-related species, showing the importance of these environments as nursery areas (Macedo-Soares et al., 2014).

The larval abundance of benthic species showed a spatial linkage with the primary production, which was more evident for early larvae. Increased concentrations of phytoplankton (here represented as chlorophyll) often coincide spatially with high larval densities (e.g. Metaxas and Young, 1998; Menge et al., 2004). This coupling is known to minimize lethal starvation periods, thus enhancing larval chances of survival (Gimenez and Anger, 2005). In contrast, the two stations with the highest chlorophyll concentrations presented low larval abundances. Whereas decapod larvae are generally able to eat and convert phytoplankton, mainly small species (Criales and Anger, 1986), for decapod larvae assemblages a higher availability of small herbivorous zooplankters is more useful (e.g. Copepoda) developed after phytoplankton blooms (González-Gordillo and Rodríguez, 2003). Availability of other potential food sources, as microzooplankton, could shed some light on this issue, but were outside the scope of our study.

The result of the variation partitioning method showed that the combined spatial and environmental variation accounted for the largest part of the explained portion. The selected PCNM variables indicate that the latitudinal and coast-ocean are the most striking spatial patterns along the studied area. The latitudinal gradient discriminates the nutrient-rich waters influenced by the southern estuaries from the northern areas. While the coast-ocean gradient, which is strong in the studied area, likewise in other regions (e.g. Pan et al., 2011) differentiates the waters rich in chlorophyll from the poor waters of slope and oceanic zones.

5. Conclusions

Our findings provide information on decapod and stomatopod larvae distribution and its relationship with the oceanographic environment within a wide latitudinal range in the South Brazil Shelf. A high degree of spatial structure in the larval assemblages was observed, strongly influenced by distance from the coast, freshwater sources and water mass distribution. The high abundance of larvae from benthic shrimps, brachyuran and anomuran crabs was indicative of the PPW; the high abundance of larvae of pelagic coastal shrimps indicated neritic waters in high and low latitudes; while the presence of larvae of oceanic or deepwater species was evincive of the oceanic zone. The large-scale distribution of larvae revealed a close relationship with the adult's distribution, shown mainly by the occurrence of larvae transported from the estuaries and by larvae of the deep bottom fauna found mainly along the outer shelf.

The high larval abundances of the genus Lucifer reflected the predominance of pelagic larvae in the studied area. On the other hand, the great input of brachyuran larvae was related to coupling with the benthic realm. The high abundance of these decapod larvae represents abundant food supply for several species that feed upon macrozooplankton (Schwamborn et al., 1999; Fanelli and Cartes, 2004). The distribution of larval abundance shown in this study suggests that macrozooplankton feeders in the pelagic chains might be also supported by the sergestid shrimps, and not only by copepods. In addition, the brachyuran larvae input should improve the coupling of benthic–pelagic chains.

The spatial distribution of decapod/stomatopod larvae (present study) and ichthyoplankton (Macedo-Soares et al., 2014) add to the accumulating evidence of a set of mechanisms that shape the plankton

ecosystems in continental shelf areas. It showed in common the association with the water masses distribution, and the relevance of the environmental circumstances in determining their assemblages. This is an essential step in advancing our understanding of processes that regulate marine planktonic communities, while it certainly calls for further research to assess the role of microzooplankton in the control of decapod and stomatopod larvae abundance.

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Supporting Information

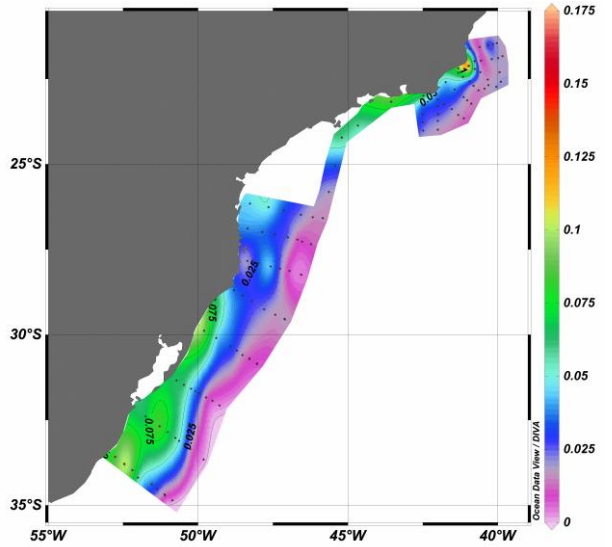

Figure S2.1. Stratification index S of the water column for the 107 stations along the South Brazil Shelf for late austral spring and early summer.

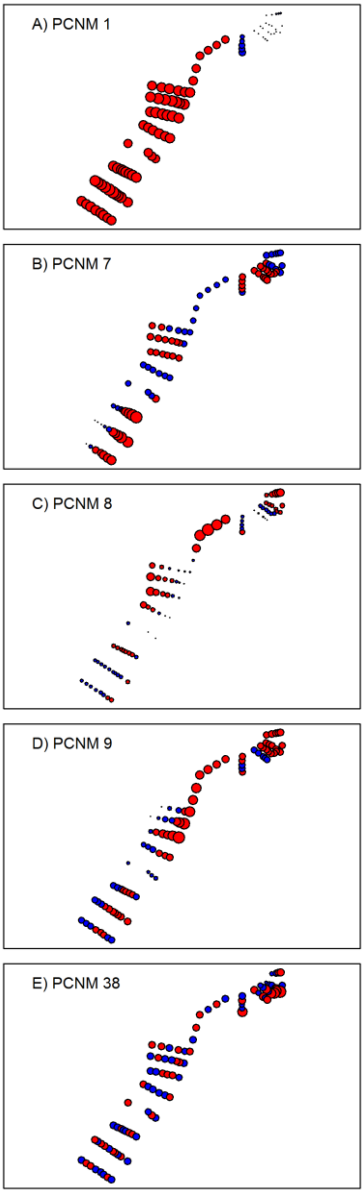

Figure S2.2. Maps of PCNM variables (A) 1, (B) 7, (C) 8, (D) 9 and (E) 38 which, together, form the large-scale spatial variation of the sampling sites. Red bubbles: positive values; blue bubbles: negative values.

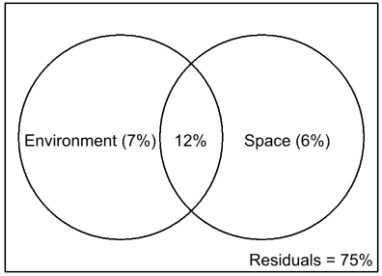

Figure S2.3. Venn diagram showing the results of the variation partitioning procedure. The variance partitioning was carried out on the selected environmental and spatial (PCNM) variables.

Table S2.1. Percentage of relative abundance (RA) and frequency of occurrence (FO) of decapod and stomatopod larvae for neritic, oceanic and all samples combined. Adult realm (pelagic or benthic) and larval stage (M=mysis; Pz=protozoea; D=decapodids; Z=zoeae; Mg=megalopae; E=erichthus; Ps=pseudozoea; An=antizoea; Al=alima) are also included.

Taxa	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Stomatopoda								
Gonodactyloidea sp.1	Benthic	E I, II and VII	0.03	4	0.01	2	0.29	7
Gonodactyloidea sp.2	Benthic	Ps II and III	0.01	2	–	–	0.18	4
Gonodactylus sp.1	Benthic	E I, III and V	0.07	4	0.04	5	0.68	4
Gonodactylus sp.2	Benthic	E I-III	0.05	3	0.05	7	–	–
Pseudosquilla sp.1	Benthic	E	0.02	3	0.02	7	–	–
Lysiosquilloidea spp.	Benthic	An	1.00	16	0.96	26	1.87	7
Lysiosquilloidea sp.1	Benthic	E I, II and V-VII	0.32	8	0.34	16	–	–
Squilla spp.	Benthic	Ps	0.40	17	0.37	30	1.27	4
Squilla sp.1	Benthic	Al I-VI	0.20	17	0.15	21	1.36	13
Squilla sp.2	Benthic	Al I-III	0.03	3	0.01	2	0.32	4
Squilla sp.3	Benthic	Al III-V and VII	0.15	7	0.15	14	–	–

Table S2.1. (Continued).

Taxa	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Dendrobranchiata								
Lucifer spp.	Pelagic	Pz I-III	31.98	35	33.00	44	8.52	26
Lucifer faxoni	Pelagic	M I and II / D	27.04	28	27.83	51	8.95	7
Lucifer typus	Pelagic	M I and II / D	0.17	15	0.01	5	3.62	24
Sergestidae spp.1	Pelagic	Pz I	0.07	11	0.02	2	1.16	20
Sergestidae spp.2	Pelagic	D	0.10	16	–	–	2.38	30
Acetes americanus	Pelagic	M II / D	0.06	4	0.07	9	–	–
Peisos petrunkevitchi	Pelagic	Pz II and III / M II	0.24	7	0.25	14	–	–
Sergestes spp.1	Pelagic	Pz II and III	0.10	9	0.01	2	2.13	15
Sergestes spp.2	Pelagic	Pz II and III	0.09	10	0.02	5	1.68	15
Sergestes atlanticus	Pelagic	M I and II	0.03	6	–	–	0.67	11
Sergestes edwardsii	Pelagic	M II	0.01	1	–	–	0.16	2
Sergestes henseni	Pelagic	M I and II	0.09	4	–	–	2.21	9
Sergestes pectinatus	Pelagic	M II	0.01	2	–	–	0.16	4
Penaeoidea spp.	Benthic	Pz I-III	0.56	20	0.41	12	4.05	28
Penaeoidea sp.1	Benthic	Pz III	0.01	2	–	–	0.34	4
Penaeoidea sp.2	Benthic	Pz II	0.01	1	–	–	0.16	2
Gennadas sp.1	Pelagic	Pz III / M I-IV	0.11	11	0.01	2	2.49	20
Gennadas sp.2	Pelagic	Pz III	0.00	1	–	–	0.08	2
Artemesia longinaris	Benthic	Pz II and III / M I, II and IV / D	0.36	7	0.37	12	0.19	2
Farfantepenaeus paulensis	Benthic	Pz III	0.04	1	0.04	2	–	–
Parapenaeus sp.	Benthic	M II-IV	0.06	4	0.04	7	0.43	2
Xiphopenaeus spp.	Benthic	Pz III	0.04	1	0.04	2	–	–
Sicyonia spp.	Benthic	Pz III / M I	0.04	2	0.02	2	0.38	2
Solenoceridae sp.1	Benthic	M I	0.00	1	0.00	2	–	–
Pleoticus muelleri	Benthic	Pz I-III / M I and II	0.45	12	0.38	16	1.99	9

Table S2.1. (Continued).

Taxa	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Caridea								
Alpheidae spp.	Benthic	Z I-III	2.23	26	2.13	49	4.46	4
Alpheidae sp.1	Benthic	Z V and VII	0.11	3	0.11	7	–	–
Alpheus sp.1	Benthic	Z II-IV	0.18	10	0.18	19	0.21	2
Alpheus sp.2	Benthic	Z III-V	0.10	8	0.10	14	0.04	2
Alpheus sp.3	Benthic	Z II-IV	0.32	10	0.30	16	0.93	4
Hippolytidae sp.	Pelagic/Benthic	Z I	0.08	3	0.08	7	–	–
Lysmata sp.	Benthic	Z II-V	0.18	7	0.18	12	0.17	2
Cangronidae sp.	Benthic	Z IV	0.02	1	0.02	2	–	–
Oplophoridae spp.	Pelagic	Z I	0.01	1	–	–	0.18	2
Acanthephyra sp.1	Pelagic	Z I-III and V	0.02	3	–	–	0.57	7
Palaemonidae sp.1	Benthic	Z I	0.01	1	0.01	2	–	–
Palaemonidae sp.2	Benthic	Z I, III, V and VI	0.38	13	0.37	21	0.54	7
Palaemonidae sp.3	Benthic	Z III and IV	0.03	2	0.01	2	0.41	2
Palaemon sp.	Benthic	Mg	0.02	1	0.02	2	–	–
Periclimenes sp.	Benthic	Z IV-VI	0.08	2	0.03	2	1.15	2
Pandalidae spp.	Pelagic/Benthic	Z I-IV	1.01	19	1.01	26	0.96	13
Pandalidae sp.1	Pelagic/Benthic	Z IV and VI	0.05	6	0.04	7	0.21	4
Leptochela sp.	Pelagic	Z I-VI / Mg	2.04	16	2.13	33	–	–
Thalassinidea								
Callianassidae spp.	Benthic	Z I-IV	3.11	12	3.23	19	0.24	7
Callianassa sp.	Benthic	Z I, II and IV	0.09	4	0.07	7	0.64	2
Stenopodidea								
Stenopus scutellatus	Benthic	Z III	0.01	1	–	–	0.14	2
Palinura								
Scyllarus americanus	Benthic	Ph II	0.01	1	0.01	2	–	–

Table S2.1. (Continued).

Taxa	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Anomura								
Munida spp.	Benthic	Z I, III and IV	0.68	11	0.49	12	4.92	11
Pachycheles haigae	Benthic	Z I and II	0.23	8	0.20	14	0.72	2
Blephoripoda doelloi	Benthic	Z I	0.01	1	0.02	2	–	–
Hippidae sp.1	Benthic	Z I-V	0.32	11	0.32	21	0.21	2
Emerita brasiliensis	Benthic	Z I, III and IV	0.22	6	0.23	12	–	–
Paguroidea spp.	Benthic	Z IV / Mg	0.35	7	0.31	12	1.15	2
Paguroidea sp.1	Benthic	Z I-III	0.29	4	0.30	9	–	–
Paguroidea sp.2	Benthic	Z I-IV	2.04	17	2.13	35	–	–
Paguroidea sp.3	Benthic	Z I and IV	0.12	2	0.13	5	–	–
Diogenidae sp.1	Benthic	Z I, II and IV	0.74	3	0.77	7	–	–
Diogenidae sp.2	Benthic	Z I	0.12	2	0.12	5	–	–
Dardanus insignis	Benthic	Z I-VIII	0.75	33	0.50	37	6.38	28
Brachyura								
Brachyura spp.	Benthic	Mg	0.42	21	0.43	37	0.39	7
Brachyura sp.1	Benthic	Z I and II	0.01	2	0.01	2	0.15	2
Brachyura sp.2	Benthic	Z II	0.00	1	–	–	0.11	2
Brachyura sp.3	Benthic	Mg	0.00	1	–	–	0.11	2
Brachyura sp.4	Benthic	Z I-IV	0.22	10	0.18	16	1.00	4
Brachyura sp.5	Benthic	Z II	0.01	1	0.01	2	–	–
Brachyura sp.6	Benthic	Z II and III	0.08	2	0.09	5	–	–
Moreiradromia antillensis	Benthic	Z I	0.01	1	0.01	2	–	–
Dromia sp.	Benthic	Z I and II	0.52	6	0.54	12	–	–
Homola barbata	Benthic	Z IV	0.00	1	–	–	0.11	2
Raninidae sp.1	Benthic	Z V	0.03	2	0.01	2	0.57	2
Raninidae sp.2	Benthic	Z I, IV and V	0.13	8	0.13	14	0.19	2
Heterotremata sp.	Benthic	Z II and III	0.08	4	0.05	5	0.75	4

Table S2.1. (Continued).

Taxa	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Dorippidae sp.1	Benthic	Z I and III	0.04	3	0.04	7	–	–
Dorippidae sp.2	Benthic	Z III	0.05	1	0.05	2	–	–
Leucosiidae sp.	Benthic	Z IV	0.15	2	0.15	5	–	–
Persephona sp.	Benthic	Z I, II and IV	0.62	17	0.63	33	0.46	2
Pyromaia tuberculata	Benthic	Z I and II	0.09	4	0.06	7	0.65	2
Libinia sp.	Benthic	Z I	0.04	3	0.04	7	–	–
Notolopas brasiliensis	Benthic	Z I and II	0.06	3	0.06	7	–	–
Pitho lheminieri	Benthic	Z I	0.02	1	0.02	2	–	–
Parthenopidae sp.1	Benthic	Z I, III, IV and VI	0.18	7	0.16	9	0.62	4
Parthenopidae sp.2	Benthic	Z VI	0.02	3	0.03	7	–	–
Parthenopidae sp.3	Benthic	Z II, III and VI	0.16	3	0.15	5	0.19	2
Platylambrus serrata	Benthic	Z I-III / Mg	0.23	3	0.24	7	–	–
Portunidae spp.	Benthic	Z I / Mg	0.34	21	0.32	33	0.86	11
Achelous sp.	Benthic	Z IV-VI and VIII	0.18	7	0.05	12	3.06	2
Achelous gibbesii	Benthic	Z I-III	1.61	22	1.38	30	6.74	15
Achelous spinicarpus	Benthic	Z I-VII	5.14	28	5.16	53	4.76	4
Arenaeus cribarius	Benthic	Z III	0.02	1	–	–	0.38	2
Callinectes spp.	Benthic	Z I-VII	2.26	26	2.29	40	1.57	13
Cronius tumidulus	Benthic	Z I-III	1.05	11	1.02	14	1.83	9
Ovalipes sp.	Benthic	Z I, II and V	0.10	3	0.05	5	1.30	2
Xanthoidea spp.	Benthic	Z III, IV / Mg	0.36	13	0.37	26	0.14	2
Eriphia gonagra	Benthic	Z I	0.12	3	0.13	7	–	–
Menippe nodifrons	Benthic	Z I	0.05	1	0.05	2	–	–
Hexapanopeus caribbaeus	Benthic	Z IV	0.02	1	0.02	2	–	–
Hexapanopeus paulensis	Benthic	Z I-IV	0.67	13	0.70	28	–	–
Panopeus americanus	Benthic	Z IV	0.01	1	–	–	0.19	2
Xanthidae sp.	Benthic	Z II-IV	1.48	2	1.55	5	–	–
Micropanope sculptipes	Benthic	Z I-ZIV	1.88	21	1.93	40	0.74	4
Pinnotheridae sp.	Benthic	Z I, III and IV	0.32	8	0.32	12	0.33	4

Table S2.1. (Continued).

Taxa	Adult realm	Larval stages	All samples		Neritic		Oceanic	
			RA	FO	RA	FO	RA	FO
Dissodactylus crinitichelis	Benthic	Z II	0.05	1	0.05	2	–	–
Pinnixa spp.	Benthic	Z II-ZIV	0.30	4	0.31	9	–	–
Pinnixa chaetopterana	Benthic	Z I and II	0.73	7	0.76	14	–	–
Tumidotheres maculatus	Benthic	Z I	0.03	2	0.03	5	–	–
Grapsidae spp.	Benthic	Z I and IV	0.09	3	0.07	5	0.58	2
Pachygrapsus sp.	Benthic	Z III and IV	0.01	3	–	–	0.25	7
Planes spp.	Benthic	Z I	0.14	2	0.14	5	–	–
Plagusia depressa	Benthic	Z II	0.01	1	0.01	2	–	–
TOTAL			100.00		100.00		100.00	

Table S2.2. Result of the PCNM analysis, showing the 12 forward selected axes.

Variables	R^2	*F*	*pval*
PCNM38	0.04996738	4.523208	0.001
PCNM9	0.04086449	3.820506	0.001
PCNM1	0.03636840	3.500168	0.001
PCNM8	0.03522038	3.490167	0.003
PCNM7	0.03264499	3.325600	0.003
PCNM4	0.02508455	2.605436	0.004
PCNM5	0.02317989	2.450727	0.005
PCNM10	0.02287316	2.462507	0.006
PCNM2	0.01988091	2.172120	0.007
PCNM3	0.01747965	1.932600	0.020
PCNM30	0.01672120	1.869624	0.035
PCNM41	0.01665302	1.883650	0.048

CAPÍTULO 3

Estimating diversity of crabs (Decapoda: Brachyura) in a no-take marine protected area of the SW Atlantic coast through DNA barcoding of larvae

(Manuscrito em revisão no periódico *Systematics and Biodiversity*)

Estimating diversity of crabs (Decapoda: Brachyura) in a no-take marine protected area of the SW Atlantic coast through DNA barcoding of larvae

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Abstract

DNA barcoding was used to identify crab larvae from the Marine Biological Reserve of Arvoredo, encompassing a coastal archipelago off the SW Atlantic coast (27 °S, 48 °W). Partial mitochondrial COI or 16S rRNA gene sequences were obtained for 488 larvae, leading to the identification of 20 species. The COI sequences generated 13 barcode index numbers (BINs) within Barcode of Life Data Systems (BOLD), among which 11 were concordant with single species. DNA from ~ 6% of the larvae did not amplify using the primers tested; based on external morphological characteristics, these larvae represented four possible additional operational taxonomic units (OTUs) at the family level. Intraspecific variation for the COI and 16S rRNA genes was found to be < 2.6% and < 2.1% respectively (Kimura 2-parameter distance), whereas interspecific divergence ranged from 7.9% to 21.5% and 6.4% to 14.5%, respectively. These results imply that both genes are suitable for use in species identification of brachyuran crabs of this area. Molecular identification of this group successfully enabled the diagnosis of larvae of closely related species, including congeners in *Mithrax*, *Achelous* and *Callinectes*. In addition, 8 out of 20 species recognized represent new records for the reserve suggesting that the brachyuran fauna in the area has been underestimated based on traditional biodiversity measures. The availability of primers suited to the targeted species, and the development of a taxonomically-comprehensive DNA barcoding database are the major recommendations to improve the accuracy and feasibility of using DNA barcoding for species identification of SW Atlantic brachyuran crabs.

Keywords: 16S, Brachyura, Brazil, COI, Decapoda, DNA barcoding, larvae, meroplankton, Southwestern Atlantic Ocean

1. Introduction

Biodiversity has long intrigued biologists and remains a central theme in ecological research (Wheeler et al., 2012; Wilson, 2003). In the marine environment, plankton sampling offers the advantage of collecting larvae of pelagic and benthic species from both shallow and deep waters. Given the difficulty of sampling many marine habitats, zooplankton sampling can be an efficient method for collecting rare and cryptic-habitat species (e.g. Harada et al., 2015).

In marine environments, decapod crustaceans are recognized for their astonishing anatomical, ecological, and behavioural diversity. Crabs from the infraorder Brachyura comprise nearly half of the estimated 15,000 extant decapod species (Martin & Davis, 2001). Powerful approaches to studying diversity include sampling different phases of a life cycle (Carassou et al., 2009). Brachyuran crabs, like a large percentage of marine species, have a bipartite lifecycle where dispersal is achieved largely by the planktonic larval period, which provides an alternative life phase to study biodiversity.

Reliable identification of crustacean larvae has traditionally required the cultivation of larvae obtained from ovigerous females of known species. Rearing of planktonic larvae through their complete developmental series is extremely demanding, particularly for groups with multiple larval instars, such as brachyuran crabs (Anger, 2006). In addition, larvae have been described from plankton samples, and in those cases their species identity often cannot be ascertained (Martin, 2014). For the majority of brachyuran species recorded so far along the Brazilian coast, larval development is either only partially or completely unknown (Koettker et al., 2012). Phenotypic plasticity has been reported in crab larvae (e.g. Charmantier et al., 2002; Howard & Hentschel, 2005) and many larvae are easily damaged during collection, adding additional complications. Both the spectacular ontogenetic changes and high levels of morphological plasticity common among marine species present challenges that can readily be addressed at the molecular level (Burton, 1996). In the past decade, the widespread application of DNA barcoding combined with the decreasing costs of DNA sequencing have contributed to the growth of molecular identification of marine invertebrate larvae; the success of this approach relies on the availability of a growing database of adult reference sequences (Hebert et al., 2003).

Considerable effort has been focused on the development and use of genetic approaches to identifying and discriminating marine species in the past ~ 20 years (reviewed by Bucklin et al., 2011). In this context,

crustaceans are an interesting target for DNA barcoding because they represent one of the most diverse metazoan groups from a morphological and ecological point of view (Radulovici et al., 2009). DNA barcoding has probably been the most widely applied molecular method for identifying plankton, including crustacean larvae (Costa et al., 2007). Fragments of the mitochondrial genes cytochrome *c* oxidase subunit I (COI) and 16S ribosomal rRNA (16S rRNA) have been used successfully to study crustacean phylogeny and biogeography (e.g. Baeza et al., 2010; Sotelo et al., 2009), as well as to distinguish between closely related species of crustaceans (e.g. Marco-Herrero et al., 2013; Palumbi & Benzie, 1991; Pardo et al., 2009).

So far, most studies using molecular tools to identify crab larvae have been successful in the detection and relative quantification of a single species (e.g. Pan et al., 2008; Ströher et al., 2011). However, studies using this approach to identify the community of crab larvae from a particular geographic area are still scarce (Harvey et al., 2009). In contrast, it has been applied to fish eggs (Gleason & Burton, 2012; Harada et al., 2015), stomatopod larvae (Tang et al., 2010), and some communities of small holoplanktonic crustaceans, such as euphausiids (Bucklin et al., 2007) and copepods (Blanco-Bercial et al., 2014).

The Marine Biological Reserve of Arvoredo [Reserva Biológica Marinha do Arvoredo (Rebio Arvoredo) (Santa Catarina)] is one of only two no-take marine protected areas (MPA) present along the nearly 8,000 km of the Brazilian coastline. It is recognized as a transitional area between tropical and temperate bioregions (Floeter et al., 2008). The sessile benthic community forms a rocky ecosystem with great heterogeneity of micro-habitats and, therefore, high biological richness (Bouzon et al., 2012). Data for many taxonomic groups suggest that the biodiversity of this region is underestimated (e.g. Padula et al., 2011; Teschima et al., 2012).

Brachyuran larvae are most abundant and taxon richness is highest around the Rebio Arvoredo in the austral fall (Koettker & Freire, 2006). Until now, 20 morphotypes of brachyuran zoeae were found in the area (Koettker & Freire, 2006). Of those, only two could be identified the species level (Koettker & Freire, 2006). So far, 33 adult crab species are known to occur in the Rebio Arvoredo region (Boos et al., 2012; Bouzon & Freire, 2007; Teschima et al., 2012). Here, the reference DNA sequences of COI and 16S rRNA genes were applied as the barcodes to identify sequences obtained from larvae sampled in the plankton. Our specific goals were: (i) to assess the diversity of crabs at Rebio Arvoredo through DNA barcoding of the larvae, (ii) to determine

if molecular identification with COI and 16S rRNA genes allows the diagnosis of closely allied brachyuran species in this region, and (iii) to increase the number of crab species from Brazil represented in GenBank and Barcode of Life Database (BOLD) (Ratnasingham & Hebert, 2007) databases.

2. Materials and methods

2.1. Study area

The Rebio Arvoredo (27° 11' to 27° 16' S and 48° 19' to 48° 24' W) occupies a polygon of 17,600 ha, comprising three islands, a few submerged rocky reefs and emerged rocks, located 11 km off the coast (Fig. 3.1).

The islands' shallow shelves shows a mosaic of rocks, boulders, caves, crevices and coarse sand arranged in a gentle slope towards the fine sandy bottom to a depth of 20 m. Around some of the islands, there are calcarious algae rodolith and *Madracis decactis* (Lyman, 1859) corallith beds (Capel et al., 2012). Fine sand and mud plains spread among the surrounding waters reaching up to a depth of 40 m. In the area, the South Atlantic Central Water (SACW) lies at > 20 m depth and the Tropical Water (TW) dominates the surface. The fauna experiences a wide range of temperatures through the seasons (15 to 27 °C) (Stramma & England, 1999).

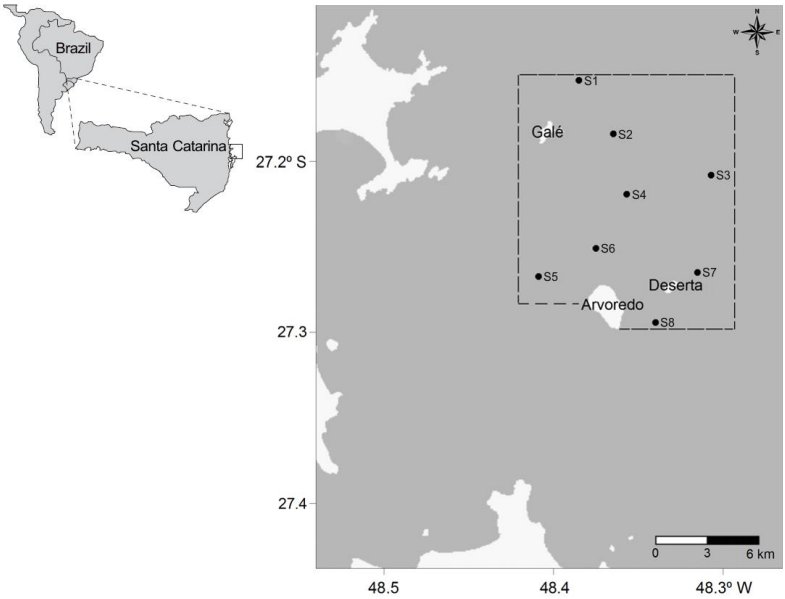

Figure 3.1. Location of the Marine Biological Reserve of Arvoredo showing the sampling sites (S1-S8) and the three main islands that constitute the Archipelago. The area of the reserve is delimited by the dashed line.

2.2. Sampling

Plankton samples were obtained using surface horizontal hauls at eight different sites around the Rebio Arvoredo during austral fall (April/May 2013) (Fig. 3.1). Four of the sites (S1-S4) were sampled in April 24, while the remaining four (S5-S8) were sampled in May 9. The hauls lasted approximately 10 min and were conducted during the morning. A conical-cylindrical net 1.8 m long, with a mouth diameter of 50 cm and 500 μm mesh size, coupled with a flowmeter, was used.

Samples were immediately fixed and preserved in 95% ethanol, which was replaced by fresh ethanol every 24 hours for the first three days. All brachyuran zoeal larvae were counted and sorted from the zooplankton samples using a stereomicroscope. Prior to DNA extraction, all larvae were examined morphologically and sorted into morphotypes according to the external characters of number and format of the carapace spines, shape of telson and abdomen. Exemplars of distinct morphotypes were photographed for future reference (Figs.

S3.1-9, supplemental material). Developmental stage was also determined for each larva.

Adult specimens of brachyuran crab species registered for the Rebio Arvoredo and for which no reference sequences were available in GenBank or BOLD databases, were collected by scuba diving. Alternatively, tissue was used from voucher specimens from the carcinological collection of the Zoology Museum of the University of São Paulo (MZUSP). Information on the collection localities can be found in GenBank, with the accession numbers provided in Table S3.1, supplemental material.

2.3. DNA extraction

Each zoea larva was individually transferred to 0.2 mL tubes and digested in 30 µL of lysis buffer/Proteinase K solution [10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.05% Tween 20, 0.2 µL of Proteinase K (20 mg/mL; Qiagen)]. Samples were incubated in a thermal cycler at 65 °C for 60 min followed by 95 °C for 10 min and then stored at –20 °C; 1 µL of the sample (approximately 10 ng of DNA) was utilized for each PCR. DNA was extracted from muscle tissue of the pereiopods of the adult crabs using the Qiagen DNeasy Blood & Tissue kit (Qiagen, Valencia, CA), following manufacturer's protocol.

2.4. Gene amplification

The COI gene was amplified using universal primers, either LCO1490 and HCO2198 (Folmer et al., 1994), or jgLCO1490 and jgHCO2198 (Geller et al., 2013), yielding an amplicon of 710 bp. If these universal primers failed, we tried the following group-specific primers to amplify COI: forward primers Brachyura1 (5´-CAACCAGGAACATTTATTG-3´; this study) or Brachyura2 (5´-CCCTTTAATACTGGGTGCTCCTGAC-3´; this study) and reverse primer DecapodaR (Geller et al., 2013), amplifying 620 and 510 bp, respectively.

If none of the COI primer sets were successful, universal 16S rRNA primers forward 16Sar and reverse 16Sbr (Palumbi, 1996) were used to produce a 570 bp amplicon. Alternatively to the 16Sar primer, the forward primer Brachyura3 (5´-TATTTTGACCGTGCAAAGGTAG-3´; this study) was used, amplifying 510 bp.

All reactions had a final volume of 20 µL and contained 1 µL of the DNA template, 1 µL of the forward primer and 1 µL of the reverse primer (final concentrations of 0.5 µM), 10 µL of Promega GoTaq Green Master Mix. PCR conditions for both COI and 16S rRNA were initial 95 °C denaturation for 2 min; then 40 cycles of 95 °C denaturation for 30 s, 47 °C annealing for 1 min, and 72 °C extension for 1 min, and a final extension at 72 °C for 5 min.

The size and quality of PCR products were visualized on 1.5% agarose gels. The products were purified using Sephadex™ G-50 spin columns, and the DNA concentration of the purified samples was measured using a NanoDrop spectrophotometer. The samples were sequenced by Retrogen, Inc. (San Diego, CA). The amplicons were Sanger sequenced in one direction, using the forward primers LCO1490, jgLCO1490, Brachyura1 or Brachyura2 for COI, and 16Sar or Brachyura3 for 16S rRNA.

2.5. Data analysis

Forward sequences from the adults and the larvae were proofread and compiled in Geneious 7.0.6 (Biomatters, Auckland, New Zealand). Larval sequences were compared to publicly available sequences using GenBank BLASTn search (Altschul et al., 1990) and BOLD Identification System tool (BOLD-IDS) (Ratnasingham & Hebert, 2007). For species diagnosis, only sequences presenting a minimum of 98% identity with an adult reference sequence were considered accurately identified.

Sequences were uploaded to BOLD, where the barcode gap analysis was used to show the distribution of the distances within each species and the distance to the nearest neighbour of each species. In addition, each specimen with a COI sequence longer than 500 bp automatically gained a barcode index number (BIN) assignment (Ratnasingham & Hebert, 2013). The BIN system clusters barcode sequences to create OTUs that closely reflect species groupings. It should be noted that BIN assignments are updated as new records are added to BOLD.

Nucleotide divergences were estimated using the Kimura 2-parameter distance model (K2P) (Kimura, 1980), implemented in the software Molecular Evolutionary Genetic Analysis Version 6 (MEGA6) (Tamura et al., 2013), to test for the efficiency of species differentiation for the two molecular markers. The mean and maximum levels of K2P distance were estimated within each taxonomic group; and the minimum

K2P distances were estimated between each taxonomic group and its nearest group.

The neighbor-joining (NJ) algorithm in Geneious 7.0.6 was used to build a phylogram for each gene in which unknown larval samples were grouped with sequences of known taxonomic identity. For the two most abundant species, which had more than 40 larvae sequenced each, only sequences representing unique haplotypes were included.

All sequences were checked on BOLD to confirm their lack of stop codons or frameshift mutations that would indicate their likely derivation from nuclear copies of mitochondrial DNA (NUMTs).

Larval counts were standardized to number of individuals per 100 m^3 to calculate the relative abundance (RA) of each taxon. The frequency of occurrence (FO) was also calculated.

3. Results

3.1. Sequencing of brachyuran adults

Ten sequences, obtained from adult specimens of six species belonging to five families, i.e., the sponge crab *Hypoconcha parasitica* (Linnaeus, 1763), the spider crabs *Epialtus bituberculatus* H. Milne Edwards, 1834, *Libinia spinosa* H. Milne Edwards, 1834 and *Stenorhynchus seticornis* (Herbst, 1788), the box crab *Hepatus pudibundus* (Herbst, 1785), and the clown crab *Platypodiella spectabilis* (Herbst, 1794), were deposited in GenBank and BOLD. Sequences had an average length of 539 ± 22 bp for COI, and 462 ± 16 bp for 16S rRNA (Table S3.1, supplemental material).

3.2. Species identification of brachyuran larvae

Overall, 851 larvae were recovered from the eight sites, of which 488 were sequenced. Combining the information obtained with COI and 16S rRNA, 482 larvae were successfully identified, representing a total of 20 species distributed across 15 genera (Table 3.1). After consistently associating sequencing results with larval morphotype, two specific morphotypes no longer required DNA analyses; in this way, sequencing 482 larvae led to the identification of 814 larvae. This large number of larvae identified by morphology was a consequence of the dominance of larvae of *Hepatus pudibundus* and *Stenorhynchus seticornis*, which together made up 78.9% of the identified larvae.

Thirty-one larvae that could represent additional species were not sequenced due to amplification failure. Seven of these were identified to family level, based on external characteristics prior to DNA extraction, leading to four distinct OTUs: Grapsidae sp. 1 and Pinnotheridae sp. 1, sp. 2 and sp. 3 (Table 3.1 and Figs. S3.7-9, supplemental material). None of the larvae of Pinnotheridae (six larvae in total) were successfully amplified for either marker locus despite repeated attempts using different primers, PCR conditions and chemical concentrations.

Table 3.1. List of taxa collected. Zoea stages are indicated in brackets. First records are shown for the MPA and Santa Catarina (SC). N = number of larvae; RA = relative abundance; FO = frequency of occurrence; RRC = rocky/reef/calcareous substrate; SM = sandy/muddy substrate; RS = rocky shores; SB = sandy beaches.

Taxa (larval stages)	First record	N	RA (%)	FO (%)	Adult's habitat
Epialtidae					
Acanthonyx petiverii (ZI-II)	MPA/SC	5	0.4	12.5	RRC
Pitho lherminieri (ZII)	-	1	0.1	12.5	RRC
Inachidae					
Stenorhynchus seticornis (ZI-II)	-	146	18.3	100.0	RRC
Majidae					
Mithraculus forceps (ZI)	-	1	0.2	12.5	RRC
Mithrax hispidus (ZI-II)	-	3	0.3	12.5	RRC
Mithrax tortugae (ZI)	-	1	0.1	12.5	RRC
Aethridae					
Hepatus pudibundus (ZI-V)	-	516	60.6	100.0	SM
Portunidae					
Achelous spinicarpus (ZI)	-	1	0.1	12.5	SM
Achelous spinimanus (ZI, III, V and VII)	-	23	3.4	62.5	SM
Arenaeus cribrarius (ZI-III and V)	MPA	26	2.5	37.5	SM
Callinectes bocourti (ZII)	MPA	2	0.2	25.0	SM
Callinectes danae (ZIII)	MPA	11	1.1	25.0	SM
Callinectes ornatus (ZI-II)	-	10	0.9	37.5	SM
Callinectes sapidus (ZI)	MPA	1	0.1	12.5	SM
Cronius ruber (ZI-IV)	-	41	4.1	75.0	SM
Menippidae					
Menippe nodifrons (ZII)	-	3	0.3	12.5	RS
Panopeidae					
Panopeus occidentalis (ZI)	MPA	1	0.1	12.5	RS
Eriphiidae					
Eriphia gonagra (ZI-II)	MPA	18	2.1	50.0	RS
Pinnotheridae					
Pinnotheridae sp. 1 (ZI)	-	3	0.5	12.5	
Pinnotheridae sp. 2 (ZIV)	-	1	0.2	12.5	
Pinnotheridae sp. 3 (ZV)	-	2	0.2	12.5	
Ocypodidae					
Ocypode quadrata (ZI and III)	MPA	3	0.5	25.0	SB
Grapsidae					
Geograpsus lividus (ZI)	MPA	1	0.1	12.5	RS
Grapsidae sp. 1 (ZI)	-	1	0.1	12.5	
Brachyura haplogroup 1 (ZI)	-	3	0.3	25.0	
Brachyura haplotype 1 (ZI)	-	1	0.1	12.5	
Brachyura haplotype 2 (ZI)	-	1	0.1	12.5	
Brachyura haplotype 3 (ZI)	-	1	0.1	12.5	
Amplification failure (ZI, III and V)	-	24	3.0	87.5	

3.2.1. Cytochrome *c* oxidase subunit I (COI)

A total of 342 COI sequences were obtained from brachyuran larvae, with an average length of 591 ± 33 bp (range: 467 – 673 bp); ~ 65% of the sequences were > 580 bp. Most of these sequences could be matched to adult reference sequences on GenBank or BOLD databases with more than 98% identity; six larvae could not be matched. The vast majority of the successfully identified larvae belong to only two species: the box crab *Hepatus pudibundus* and the spider crab *Stenorhynchus seticornis*, which together represented ~ 90% of the COI sequences obtained.

Overall, 11 species were identified using the COI data. These belonged to seven families: Epialtidae (2 species), Majidae (3 species), Portunidae (2 species), Menippidae (1 species), Inachidae (1 species), Aethridae (1 species) and Eriphiidae (1 species).

All unique haplotypes obtained for the two most abundant species and all the remaining sequences obtained for COI, together with reference sequences from the 11 corresponding brachyuran adults, were used to build a NJ phenogram, shown in Fig. 3.2. The sequences of the larvae grouped into 12 distinct clusters, of which 11 grouped with a reference adult species in monophyletic clusters (Fig. 3.2).

A total of 268 COI sequences were obtained from larvae of *Hepatus pudibundus*, showing 21 different haplotypes, which presented a maximum intraspecific variation of 2.6% K2P distance including its adult reference sequence (Table 3.2). The seven most frequent haplotypes comprised about 80% of the larvae sequenced. For *Stenorhynchus seticornis*, sequences where obtained from 42 larvae, which presented eight different haplotypes and showed a maximum of 2.2% of intraspecific variation including its corresponding adult reference sequence (Table 3.2). For this species, the three most common haplotypes included about 70% of the larvae.

Two species of the genus *Mithrax* Latreille, 1816 were identified: *Mithrax hispidus* (Herbst, 1790) and *Mithrax tortugae* Rathbun, 1920. Maximum intraspecific divergences (0.5% and 0.0%) were much lower than those between closest sister species (minimum of 7.9%) (Table 3.2).

Maximum intraspecific variations of the COI gene did not exceed 2.6% K2P distance, while variations between closest sister species ranged from 7.9% to 21.5% (Table 3.2).

Our records were divided into 13 BINs (Table 3.2). Concordance, which means species represented by a single BIN cluster, was found for

11 of them. Specimens of *Stenorhynchus seticornis* were assigned to two BINs (BOLD:AAJ5290 and BOLD:ACD1578). Brachyura haplogroup 1, which could not be matched to any reference sequences with more than 98%, shared the same BIN (BOLD:ACH6715) as *Cronius ruber*. Three sequences neither clustered with any adult reference species nor assembled within a BIN cluster (Brachyura haplotypes 1, 2 and 3), and presented a minimum divergence of 15.9% with their closest sister species (Table 3.2).

Table 3.2. Pairwise nucleotide divergences of COI and 16S rRNA sequences within species and among closest sister species for brachyuran adults and larvae, using Kimura 2-parameter (K2P) distances (%). N = number of haplotypes. Barcode index numbers (BINs) associated with COI sequences are shown.

		COI				16S rRNA			
			Within		Among		Within		Among
Taxa	**BINs**	**N**	**Mean**	**Max.**	**Min.**	**N**	**Mean**	**Max.**	**Min.**
Acanthonyx petiverii	BOLD:ACG7625	4	0.0	0.0	15.5	-	-	-	-
Pitho lherminieri	BOLD:AAY3789	2	0.5	0.5	19.3	-	-	-	-
Stenorhynchus seticornis	BOLD:AAJ5290 BOLD:ACD1578	9	0.8	2.2	13.5	-	-	-	-
Mithraculus forceps	BOLD:AAC9888	2	0.5	0.5	17.2	-	-	-	-
Mithrax hispidus	BOLD:AAF7158	2	0.5	0.5	8.5	-	-	-	-
Mithrax tortugae	BOLD:ACB6128	2	0.0	0.0	7.9	-	-	-	-
Hepatus pudibundus	BOLD:ACD9071	22	1.1	2.6	14.9	12	0.9	2.1	8.3
Achelous spinicarpus	-	-	-	-	-	2	0.3	0.3	7.1
Achelous spinimanus	BOLD:AAG1825	6	0.3	0.5	13.5	10	0.4	1.5	12.8
Arenaeus cribrarius	BOLD:AAM8140	2	0.0	0.0	14.2	6	0.6	0.9	14.5
Callinectes bocourti	-	-	-	-	-	3	0.2	0.3	6.4
Callinectes danae	-	-	-	-	-	10	0.3	0.9	9.6
Callinectes ornatus	-	-	-	-	-	11	0.6	1.8	12.2
Callinectes sapidus	-	-	-	-	-	2	0.0	0.0	7.1
Cronius ruber	-	-	-	-	-	16	0.3	1.2	11.7
Menippe nodifrons	BOLD:AAX4629	2	0.5	0.5	11.9	-	-	-	-
Panopeus occidentalis	-	-	-	-	-	2	0.3	0.3	9.2
Eriphia gonagra	BOLD:ACG8098	13	0.5	1.6	13.5	-	-	-	-
Ocypode quadrata	-	-	-	-	-	4	0.3	0.6	9.2
Geograpsus lividus	-	-	-	-	-	2	0.0	0.0	8.9
Genus *Mithrax*	-	4	11.7	17.8		-	-	-	-
Genus *Achelous*	-	-	-	-	-	12	4.2	14.0	-
Genus *Callinectes*	-	-	-	-	-	26	14.2	23.8	-
Brachyura haplogroup 1	BOLD:ACH6715	3	0.4	0.5	21.5	-	-	-	-
Brachyura haplotype 1	-	1	-	-	16.7	-	-	-	-

Table 3.2. (Continued).

		COI				16S rRNA			
			Within		Among		Within		Among
Taxa	**BINs**	**N**	**Mean**	**Max.**	**Min.**	**N**	**Mean**	**Max.**	**Min.**
Brachyura haplotype 2	-	1	-	-	18.9	-	-	-	-
Brachyura haplotype 3	-	1	-	-	15.9	-	-	-	-

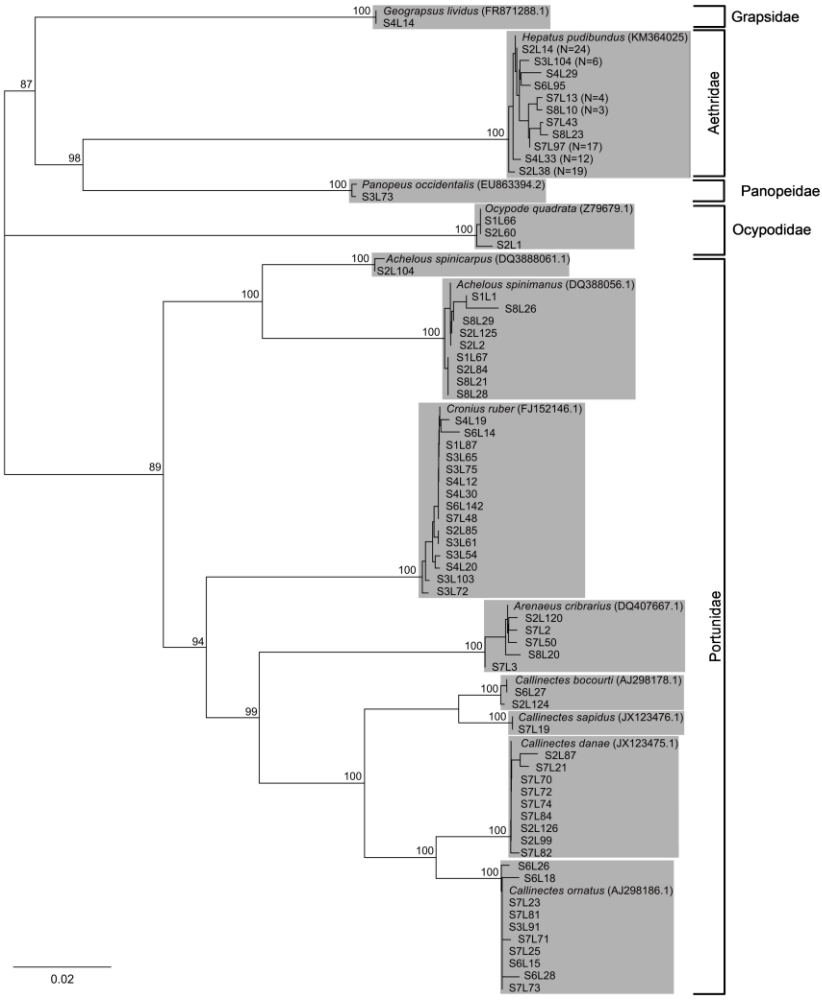

Figure 3.2. Neighbor-joining (NJ) phenogram of COI sequences, showing the clustering of 61 larvae into 11 species, plus 1 haplogroup and 3 haplotypes. Bootstrap values for 10,000 replicates are shown near the respective branches. Data from GenBank are shown with accession numbers in brackets. Each larval individual is represented by its sample (S) and number of the larva (L). For *Hepatus pudibundus* and *Stenorhynchus seticornis* only sequences representing unique haplotypes are shown; N represents the number of larvae with the same haplotype.

3.2.2. 16S ribosomal rRNA (16S rRNA)

A total of 146 16S rRNA sequences were obtained from larvae, with an average length of 502 ± 24 bp (range: 436 – 541 bp); ~ 70% of the sequences were > 500 bp. All the sequences matched an adult reference sequence from GenBank or BOLD databases with more than 98% of identity. As with COI, the majority (60%) of the larvae identified belong to *Hepatus pudibundus*.

Overall, 12 species were identified using the 16S rRNA data. These belong to five families: Portunidae (8 species), Ocypodidae (1 species), Grapsidae (1 species), Panopeidae (1 species) and Aethridae (1 species).

A NJ phenogram was built using the unique haplotypes obtained for *Hepatus pudibundus* and all the remaining sequences obtained for 16S rRNA, together with reference sequences from the 12 corresponding brachyuran adults (Fig. 3.3). The sequences of the larvae grouped into 12 distinct clusters, each including a discrete adult reference sequence (Fig. 3.3).

Eighty-nine larvae of *Hepatus pudibundus* where sequenced using this marker and 11 different haplotypes where obtained, presenting maximum intraspecific variation of 2.1% K2P distance together with its adult reference sequence, which was lower than the variation observed with COI (Table 3.2). The four most frequent haplotypes represent more than 80% of the total haplotypes.

The 16S rRNA gene enabled the discrimination of closely related species of swimming crabs, including the longspine crabs (*Achelous spinicarpus* Stimpson, 1871 and *Achelous spinimanus*) and the blue crabs (*Callinectes bocourti* A. Milne-Edwards, 1879, *Callinectes sapidus* Rathbun, 1896, *Callinectes danae* Smith, 1869 and *Callinectes ornatus* Ordway, 1863). In addition, larvae of two other species of swimming crabs were also identified [*Arenaeus cribrarius* (Lamarck, 1818) and *Cronius ruber* (Lamarck, 1818)]. The maximum intraspecific genetic divergence of these species ranged from 0.0% to 1.8%, while the interspecific divergence always exceeded 6.0% (Table 3.2). Maximum intraspecific divergences for the species of the genera *Achelous* De Haan, 1833 and *Callinectes* Stimpson, 1860 were lower than 1.5% and 1.8%, respectively (Table 3.2), while divergence between congeners was of at least 7.1% and 6.4%, respectively (Table 3.2).

Intraspecific variation with this gene did not exceed 2.1% K2P distance, while divergence between each species and the corresponding closest sister species ranged from 6.4% to 14.5% (Table 3.2).

Detailed barcode gap analysis within BOLD revealed that there was no overlap between mean intraspecific and the nearest neighbour distances considering all available sequences on BOLD, with none of the molecular markers. In general, however, the thresholds were higher with COI than with 16S rRNA (Figs. 3.4-5).

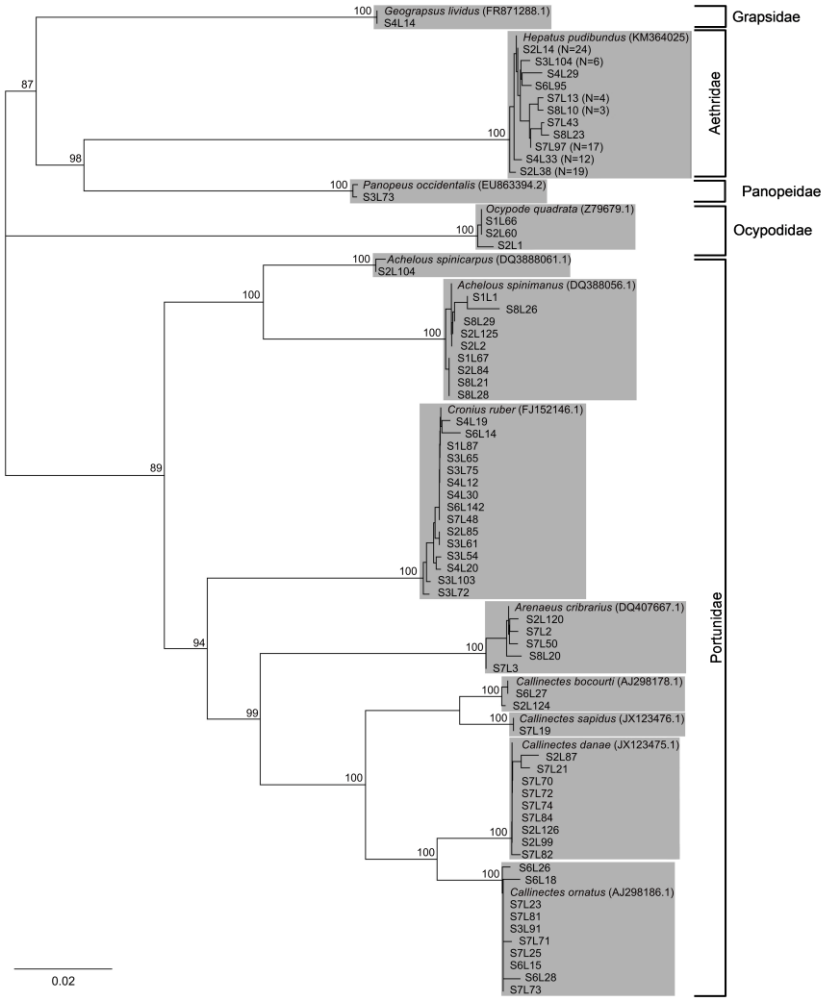

Figure 3.3. Neighbor Neighbor-joining (NJ) phenogram of 16S rRNA sequences, showing the clustering of 68 larvae into 12 species. Bootstrap values for 10,000 replicates are shown near the respective branches. Data from GenBank are shown with accession numbers in brackets. Each larval individual is represented by its sample (S) and number of the larva (L). For *Hepatus pudibundus* only sequences representing unique haplotypes are shown; N represents the number of larvae with the same haplotype.

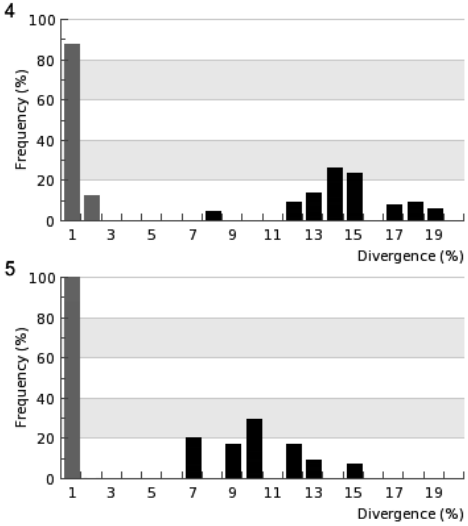

Figure 3.4-5. Distribution of the mean intraspecific distances (gray) and of the nearest neighbour distances (black) for each species within BOLD database for COI (4) and 16S rRNA (5) genes.

3.3. Biodiversity and ecological aspects

Eight of the identified species represent first records for this MPA (Table 3.1): the spider crab *Acanthonyx petiverii* H. Milne Edwards, 1834, the swimming crabs *Arenaeus cribrarius*, *Callinectes bocourti*, *Callinectes danae* and *Callinectes sapidus*, and the shore crabs *Eriphia gonagra* (Fabricius, 1781), *Panopeus occidentalis* Saussure, 1857 and *Geograpsus lividus* (H. Milne Edwards, 1837). Based on the habitat of these species, it is reasonable that they occur inside the MPA as adults, although only the larvae have been registered so far. Larvae of the ghost crab *Ocypode quadrata* (Fabricius, 1787) were also observed for the first time in the reserve, however it cannot be associated with the presence of the adult, since this species inhabits exclusively sandy beaches, which are absent on the islands of the Rebio Arvoredo.

For some species (*Hepatus pudibundus*, *Acanthonyx petiverii*, *Stenorhynchus seticornis* and *Mithrax hispidus*), the complete series of zoeal larval developmental stages were found inside the MPA. The development of the latter three conforms to the general pattern found in majoids by having only two zoeal stages before metamorphosing into

the phase of pelagic-benthic transition, in contrast to the remaining brachyurans, which have between five to twelve zoeal stages (Martin & Davis, 2001; Martin, 2014). In these cases, the second zoea already presents developed pleopods (Pohle et al., 1999) (Pohle et al., 1999) (Fig. S3.2, supplemental material). Zoeae I of *Stenorhynchus seticornis* occurred in all sites, but their last zoeal stage (ZII) was observed only northeast of Galé Island (S1) as well as in the vicinities of Deserta Island (S7). Larvae of *Hepatus pudibundus* were present in all sites, with dominance of ZI (87%). Their advanced larvae (ZIV and V) were found in five out of the eight sites, sampled in two different dates, with the highest values found east of Galé Island (S2), where abundance reached 22.0 larvae/100 m^3. It is also noteworthy that larvae (ZI and ZII) of *Acanthonyx petiverii* occurred only in one site (S6), north of Arvoredo Island, with abundance of 3.0 larvae/100 m^3. In addition, ZI and ZII of *Mithrax hispidus* were found exclusively in S3, between Galé and Arvoredo, with abundance of 2.5 larvae/100 m^3. In addition to these species, the first, last and a couple of intermediate larval stages of *Achelous spinimanus* were also found in the area (Table 3.1).

4. Discussion

4.1. Effectiveness and concerns of DNA barcoding in brachyuran species identification

In the present study, an unknown larva was determined to have been successfully identified if it: (1) matched a reference sequence available in GenBank or BOLD databases with identity > 98%, (2) was part of a cluster that included a known reference adult sample, and (3) K2P sequence distance within this cluster did not exceed 3%. For COI sequences, the BINs were also applied as molecular proxies for species boundaries (Ratnasingham & Hebert, 2013). These criteria were similarly used for species identification of other groups, such as copepods (Blanco-Bercial et al., 2014), euphausiids (Bucklin et al., 2007) and stomatopod larvae (Tang et al., 2010). Considering these parameters, we have successfully identified ~ 93% of the analyzed larvae from Rebio Arvoredo, including 20 species from 15 genera. The use of the two genetic markers was decisive in the identification of these species, because in some cases one gene amplified better than the other. Larvae of portunid crabs, for instance, presented higher levels of amplification success with 16S rRNA than with COI. The concomitant

use of these markers as barcodes for species identification was previously used for crustaceans (e.g. Tang et al., 2010).

Despite disagreements on how to account for intraspecific divergences in mitochondrial genes (Moritz & Cicero, 2004), typical intraspecific divergences are rarely greater than 2% and most are less than 1% (Avise, 2000). Within-species divergences were below 1% for 71% of the comparisons in our samples. The equivalent value in decapods in general was 86%, in amphipods of the genus *Gammarus* was 75%, while in cladocerans of the genus *Daphnia* it was 59% (Costa et al., 2007). The sequence differences among conspecific individuals for COI and 16S rRNA genes did not exceed 2.6%, whereas the interspecific divergences were always higher than 6.4%. Thus, there was no overlap between intra- and interspecific divergences which ensured a barcode gap for these samples (Meyer & Paulay, 2005). Furthermore, it is unlikely that misidentifications occurred or that cryptic species were present in the samples (Collins & Cruickshank, 2013). Based on the observed K2P distances, species identification of brachyuran crab larvae in Rebio Arvoredo waters can be confirmed with a relatively high degree of confidence. The barcode gap thresholds were higher with COI than with 16S rRNA, as also observed for other brachyuran families (Ocampo et al., 2013) and other metazoan groups (e.g. Tang et al., 2010; Vences et al., 2005). The fact that the 16S rRNA gene evolves more slowly than COI in crustaceans (Lefébure et al., 2006) could explain the difference between the barcode gap thresholds.

The highest intraspecific genetic divergences were observed for the two most abundant species for both genes, suggesting that a higher sampling effort could result in the increase of such divergence (e.g. Matzen da Silva et al., 2011; Puillandre et al., 2011). Because our work was geographically focused, we do not know if a broader scale of sampling (e.g., over the entire geographic range of the taxa) would result in an increase in intraspecific divergence and potentially obscure species designations (DeSalle et al., 2005); the substantial barcoding gap in the existing data do not hint that this would be a problem.

The phenogram-based method also supported the distinction of unidentified larvae into species. The NJ algorithm generated distinct clusters and indicated taxonomic affinities of larvae with adult reference sequences. Given the depth of genetic divergence within and among clusters, these identifications are unlikely to be affected by phylogenetic reconstruction method.

Distinguishing between larvae of closely related taxa is a bottleneck in marine ecology, even in cases where adult morphology

allows unambiguous species identification (Burton, 1996). The morphological identification of the larvae of many brachyuran species requires adequate dissection and subsequent observation of details of appendages (i.e. mandible, maxillule and maxilla). This method is time consuming and relies on the availability of comprehensive larval descriptions. Despite the extensive efforts to date, descriptions of the larval development of brachyuran species remain incomplete and much work remains to be done on brachyuran crabs worldwide.

Eggs and larvae of even common species are frequently difficult or impossible to identify on the basis of morphology (Burton, 2009). For instance, despite the wide geographical ranges and co-occurrence of the blue crabs *Callinectes danae* and *Callinectes sapidus*, morphological diagnosis of larvae of these species is not yet feasible (Koettker et al., 2012). Here, DNA barcoding successfully allowed the diagnosis of several groups of congeneric larvae, including *Mithrax*, *Achelous* and *Callinectes* species, indicating that COI and 16S rRNA are effective markers in separating closely related species of Brachyura. The diagnosis to species level is especially important in cases where species co-occur and there is overlap of niches, as for *Achelous spinicarpus* and *Achelous spinimanus* (Lima et al., 2014).

The BIN approach, applied to the COI data, confirmed the species identification through BLAST and BOLD-IDS. Eleven of the species identified with this gene were assigned to a unique BIN, while discordance was found for *Stenorhynchus seticornis*, with the assignment of two BINs. Discordant BINs may occur for several reasons, and may have a biological or operational origin (Ratnasingham & Hebert, 2013). The main biological reasons include the occurrence of several relatively distant lineages within a species, or sharing of DNA barcode haplotypes among species.

The assignement of a BIN (BOLD:ACH6715) to the sequences clustered as Brachyura haplogroup 1 suggests that these sequences are likely from *Cronius ruber* larvae. In addition, this species was also identified using the 16S rRNA data. On the other hand, sequences obtained for three larvae could not be identified (Brachyura haplotypes 1, 2 and 3), due to lack of a BIN clustering or match greater than 90% identity in GenBank or BOLD databases. Brachyura haplotype 2 showed maximum identity with *Callinectes arcuatus* (AY465911.1) and was positioned closer to portunids in the COI gene phenogram, suggesting that it belong to this family. Brachyura haplotypes 1 and 3 displayed the highest identities in GenBank or BOLD databases with species of Xanthoidea, although their placement in the NJ phenogram

does not support this. Based on the COI data, some genera were not grouped into clusters according to their recognized brachyuran families (Epialtidae, Majidae and Portunidae). However these results were not surprising, because evidence for the paraphyly or polyphyly of these nominal families has also been found in phylogenetic analyses of larvae and adults (Hultgren & Stachowicz, 2008; Mahon & Neigel, 2008). Gross morphological observation prior to DNA extraction in these cases did not allow identification to family level; dissection and analysis of the mouth appendages (i.e. maxilla and maxillule) would be required to distinguish larvae of these families due to limited morphological differentiation (Pohle et al., 1999) (Figs. S3.5 and S3.6, supplemental material). On the other hand, larvae of Pinnotheridae, for instance, were recognized based on the absence of antennal exopod and the fact that the rostral spine is approximately three times longer than the antenna (Pohle et al., 1999) (Figs. S3.7 and S3.8, supplemental material).

The lack of a barcode match for these sequences suggests that reference sequences for these species are not yet in the databases. They could possibly even correspond to species not described so far, though this is less probable. Although ascribing a species name to a sequence may not always be possible, knowledge of the sequences still permits accurate assessment of biodiversity. For example, although observed sequences could not be matched to known barcodes in the GenBank database, they could be used to count numbers of species (see Plaisance et al., 2009; Tang et al., 2010).

General consensus shows that molecular and morphological identifications should work together (e.g. Lefébure et al., 2006; Meyer et al., 2013; Pardo et al., 2009). Here, in cases of DNA amplification failure, morphological observations aided the identification to the family level. Regarding these larvae, one species of Grapsidae and three putative species of Pinnotheridae were found. Based on the morphologically described species for the reserve and surrounding waters, *Pachygrapsus transversus* (Gibbes, 1850) and *Tumidotheres maculatus* (Say, 1818) are the only Grapsidae and Pinnotheridae (respectively) registered so far (Bouzon & Freire, 2007). Although molecular data are available in GenBank for both species, we were not able to confirm the identity of these larvae due to amplification failure. Despite this, such information suggests that the known diversity of pinnotherid crabs may be underestimated.

For five species, all of which are commonly found along the Brazilian coast, including the two most abundant in this survey, the unique COI and/or 16S rRNA barcodes generated here represent the first

contributions to GenBank. COI (> 10,000 GenBank entries) and 16S rRNA (> 7,000 GenBank entries) are among the most frequently sequenced genes for ecological and evolutionary studies of Decapoda. Augmenting these records will enhance the comparative value of such standardized approaches (Matzen da Silva et al., 2011).

4.2. Biodiversity and ecological implications

This study not only describes the use of DNA barcoding for the identification of crab larvae, but also provides insights into the ecology and biodiversity of Brachyura on the SW Atlantic coast. With the addition of six new reference sequences provided by this study, sequences are now available for 27 of 33 (82%) crab species known to occur in the Rebio Arvoredo region (Boos et al., 2012; Bouzon & Freire, 2007; Teschima et al., 2012). Of the six remaining species, only two [*Neopilumnoplax americana* (Rathbun, 1898) and *Pelia rotunda* A. Milne-Edwards, 1875] belong to genera that lack any COI or 16S rRNA sequences in either the GenBank or BOLD databases. Of the 20 species identified, eight are reported for the first time in the study area. The high level of first-time records might be associated with the fact that previous studies were carried out as visual censuses, focusing on crabs that inhabit the shallow shelf of the rocky shores, rhodolith nodules and reef beds (Bouzon & Freire, 2007; Teschima et al., 2012). In contrast, the present estimate of diversity is based on plankton samples, and collected larvae of crabs that potentially live in other habitats of the reserve, including sandy or muddy bottom, and in the intertidal zone of the rocky shores. Moreover, the taxonomic resolution with which the larvae were identified through DNA barcoding was higher than in previous surveys using only morphology, which also recorded the presence of larval morphotypes of Grapsidae, Ocypodidae, Portunidae and Panopeidae, but only to the family level (Koettker & Freire, 2006).

The relative abundances of the sampled larvae only partially align with what might be expected based on adult visual census data (Gaeta et al., 2011). For example, although the high abundance observed for *Stenorhynchus seticornis* larvae is in accord with visual census data, *Mithraculus forceps* (A. Milne-Edwards, 1875) larvae were rare despite high adult abundance. The abundance of *Hepatus pudibundus* and portunid crab larvae is generally consistent with adult abundances observed through bottom trawling in an adjacent bay characterized by sandy-mud bottom (Freitas et al., 2010). A complication in making such larval/adult abundance comparisons is that the larval sampling in the

present study was restricted to April and May which may not correspond to the breeding season of all of the local crab species.

Our results, combined with the presence of suitable habitats, suggest that most of these species recorded for the first time at Rebio Arvoredo are likely to occur in the area as adults. In contrast, at least one species of brachyuran larvae, the ghost crab (*Ocypode quadrata*), was found to occur in the plankton community but does not settle or recruit inside the MPA. Larvae of this species might have been spawned on the coast (~ 11 km away) and dispersed to the MPA, as has been observed in other locations for Ocypodidae larvae (Epifanio et al., 1988). Thus, this species was not considered as a new record for the reserve.

Of the first time records, only the spider crab *Acanthonyx petiverii* has not been previously recorded south of 22 °S in the Atlantic. This species is a small crab that inhabits shallow waters, on hard substrates or associated with macroalgae. Its distribution in the western Atlantic ranges from Florida (24 °N), USA, to Rio de Janeiro (22 °S), Brazil (Retamal, 1981). The fact that all zoeal stages of this species were found in the area indicates that the adult inhabits the Rebio Arvoredo, extending its occurrence to Santa Catarina (27 °S) in the Western Atlantic. The remaining species had already been registered in the Santa Catarina state (Boos et al., 2012), but not at Rebio Arvoredo.

Our combined morphological and molecular approach brought interesting insights concerning brachyuran diversity and ecology at Rebio Arvoredo. Species that had not been recorded in the area were found, increasing the known diversity of brachyuran crabs in the area from 33 to potentially more than 45 species, when taking into account the different haplotypes and OTUs.

In addition, information regarding developmental stages indicates that *Hepatus pudibundus* develops inside the MPA, since their larvae were distributed homogeneously throughout the sites, and advanced zoeae were observed in relatively great abundance. *Stenorhynchus seticorni*s also presented the complete zoeal development inside the reserve, but for this species late zoeae were observed near the islands, what is expected since it depends on the rocky coast nearby to recruit. Results for both species suggest self-recruitment and possibly local retention (Levin, 2006). These results are particularly relevant to an MPA, indicating that it may harbor self-sustaining populations (Berumen et al., 2012). The presence of the complete zoeal development of *Acanthonyx petiverii* and *Mithrax hispidus* in specific sites (S6 and S3, respectively) suggests that these species might also recruit inside the

reserve. For these last three species as well as other species which recruit and inhabit rocky or reef substrate, greater sampling effort nearby the islands could elucidate better regarding their development and distribution.

Molecular data have an indisputable role in the analysis of biodiversity. Here we demonstrate the validity of DNA barcoding using COI and 16S rRNA genes for brachyuran crab larvae identification on a regional scale within the SW Atlantic coast. Using primers described in previous studies, successful amplification and sequencing was achieved for > 94% of the larvae sampled and all but six of those sequenced could be identified to the species level. It is not clear whether failed amplifications are due to mismatched primers or poor sample DNA quality. Some of the benefits of applying this method for identifying crab larvae are the discovery of cryptic species (Collins & Cruickshank, 2013), monitoring of invasive species (Harvey et al., 2009) and description of diagnostic morphological characters for species identification (Pardo et al., 2009). Our results suggest that biodiversity of brachyuran crabs at Rebio Arvoredo may be underestimated by a minimum of 36%. Continued improvement of this method for identification of brachyuran crabs will require: (1) optimization of PCR and sequencing primers suited to the targeted species, and (2) availability of a taxonomically-comprehensive barcode reference database. The lack of a complete DNA barcode library is currently the most limiting factor for accurate and reliable discrimination and identification of species of Brachyura.

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Supplemental online material

Table S3.1. Species identities, GenBank accession numbers and lengths of newly generated brachyuran crab sequences.

Family	Species	GenBank accession	Length (bp)
Dromiidae	*Hypoconcha parasitica*	COI: KM36402.6	545
		16S: KM36402.7	496
Epialtidae	*Epialtus bituberculatus*	COI: KM18846.6	460
	Libinia spinosa	COI: KM36402.8	513
Inachidae	*Stenorhynchus seticornis*	COI: KM18846.7	532
		16S: KM18846.8	429
Aethridae	*Hepatus pudibundus*	COI: KJ801941.1	627
		16S: KM36402.5	438
Xanthidae	*Platypodiella spectabilis*	COI: KM36402.9	557
		16S: KM36403.0	483

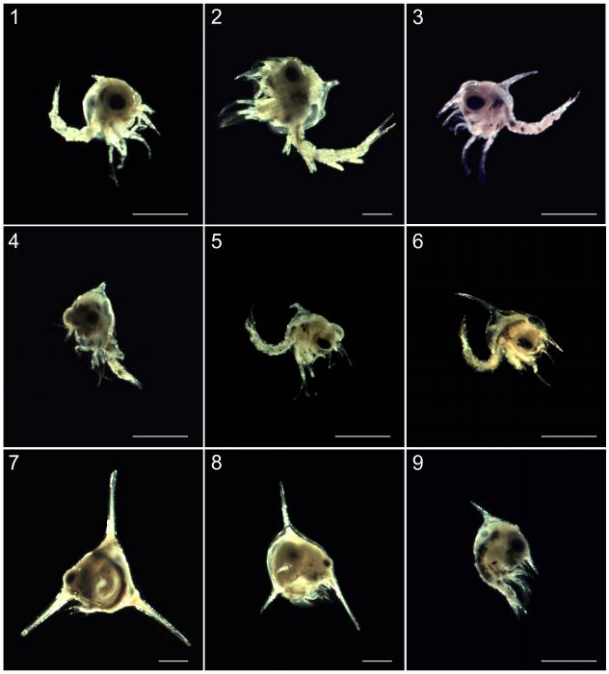

Figure S3.1-9. Larvae of *Acanthonyx petiverii* (1), *Mithrax hispidus* (2), *Eriphia gonagra* (3), *Ocypode quadrata* (4), Brachyura haplogroup 1 (5), Brachyura haplotype 2 (6), Pinnotheridae sp.2 (7), Pinnotheridae sp.3 (8) and Grapsidae sp.1 (9). Scale bar corresponds to 500 μm.

CONCLUSÃO GERAL

Os resultados possibilitaram uma melhor compreensão a respeito da estrutura da comunidade do meroplâncton e, em especial, de larvas de decápodes, ao longo de uma escala de 13º de latitude (equivalente a aproximadamente 2.000 km) e de até 400 km da costa, englobando a costa, a plataforma continental e o talude no Sudeste-Sul do Brasil.

De maneira geral, os resultados mostraram que as larvas de invertebrados respondem à presença das frentes e dos processos oceanográficos de mesoescala que ocorrem ao longo do intervalo latitudinal estudado. Mais especificamente, grupos distintos de larvas do meroplâncton foram identificados nas diferentes categorias de frentes oceanográficas encontradas. Assembleias específicas de larvas de decápodes foram reconhecidas em associação com os principais cenários observados: ambiente costeiro, oceânico e influenciado por aportes estuarinos. Outros fatores, como a dinâmica das massas de água e a distribuição dos adultos, demonstraram exercer um papel importante na estruturação da comunidade de larvas de decápodes na região.

O principal padrão observado para o meroplâncton, e principalmente para as larvas de decápodes, foi a diminuição da abundância ao longo do gradiente costa-oceano, associada principalmente com o contraste entre as massas de água mais produtivas, onde as concentrações de nutrientes e clorofila foram maiores, e a predominância da Água Tropical na região do talude. Na costa, as larvas de cirripédios, bivalves e de espécies de decápodes de águas rasas foram dominantes. Em contraste, a região oceânica mostrou predominância de larvas de cefalópodes, enteropneustos e decápodes holoplanctônicos.

Além da alta abundância associada à região costeira, o meroplâncton mostrou um acoplamento com o principal evento de ressurgência da Água Central do Atlântico Sul detectado durante o período estudado, que ocorreu no Cabo de Santa Marta Grande. Valores elevados de abundância larval também foram observados nas estações sob influência da frente estuarina na região da Lagoa dos Patos, caracterizada pela presença da Água da Pluma do Prata na plataforma continental. Nessa região o meroplâncton foi dominado por larvas de equinodermos, poliquetos, caranguejos braquiúros e camarões peneídeos. Muitas dessas larvas podem ter sido produzidas nos estuários do Rio da Prata e da Lagoa dos Patos. A pluma estuarina e o transporte de Ekman, que atua no sentido da costa, podem contribuir para a retenção nessa região.

Larvas em estágio de desenvolvimento avançado do camarão de águas rasas *Lucifer faxoni* foram as dominantes nas águas costeiras ao longo de toda a região, sugerindo que esse recurso alimentar está disponível, assim como os copépodes, para os organismos que se alimentam de macrozooplâncton. Dentre as larvas de espécies bentônicas, os caranguejos braquiúros representaram o grupo com maior diversidade e abundância, especialmente nas estações costeiras. Dos 50 táxons de braquiúros identificados na região da plataforma Sudeste-Sul pela morfologia, 21 foram identificados em nível de família ou em um nível menos preciso. Apesar da diferença no tipo de arrasto realizado, uma comparação entre as amostras coletadas no Arquipélago do Arvoredo e nas duas estações mais costeiras dos transectos de Itajaí e da Ilha de Santa Catarina, permitiu observar consistência na dominância de alguns táxons, como *Achelous* sp., *Callinectes* spp. e Xanthoidea spp.. Por outro lado, larvas das duas espécies mais abundantes no Arvoredo não foram encontradas nas estações acima mencionadas, ou foram incluídas em morfotipos de um nível taxonômico de menor resolução.

O uso de *DNA barcoding* para a estimativa da diversidade das larvas de braquiúros se mostrou um método eficiente. A partir dessa metodologia foi possível verificar, por exemplo, a ocorrência de *Acanthonyx petiverii* em Santa Catarina, espécie cuja distribuição era conhecida somente até São Paulo. Os resultados sugerem que esse método pode ser utilizado como uma alternativa mais rápida e precisa para identificar larvas de decápodes, sem necessidade de conhecimento detalhado da taxonomia do grupo. Além de estudos para estimar a diversidade, *DNA barcoding* é uma ferramenta que pode ser usada em estudos realizados em meso e larga escala.

A tese sustenta a hipótese de que a distribuição em grande escala do meroplâncton na plataforma Sudeste-Sul do Brasil é diretamente associada à presença de frentes. Os resultados ainda confirmam a hipótese de que variáveis ambientais, juntamente com variáveis espaciais, exercem um papel importante na determinação das associações de larvas de decápodes e estomatópodes. A respeito da identificação das larvas de braquiúros através do método de *DNA barcoding*, mais de 90% das larvas de um arquipélago costeiro da região foram identificadas em nível específico. Esse resultado confirma a hipótese proposta e mostra o potencial da referida metodologia em contribuir para a precisão na identificação em nível específico, que é de suma importância para compreender o papel dos processos oceanográficos nas comunidades de larvas de invertebrados marinhos.

ANEXOS

Tabela A1. Informações sobre as amostras de zooplâncton coletadas na Plataforma Sudeste-Sul do Brasil, analisadas nos capítulos 1 e 2. MF=fluorescência máxima.

Transecto	Estação	Data	Horário	Profundidade (m)			Volume filtrado (m^3)	Distância da costa (km)
				Local	FM	Arrasto		
CHU	1	06/12/2010	08:40	29	20	23	5,5	25
CHU	2	06/12/2010	11:50	42	20	22	2,3	61
CHU	3	06/12/2010	15:15	46	23	25	11,3	94
CHU	4	06/12/2010	18:40	127	30	30	7,9	133
CHU	5	06/12/2010	21:40	1052	38	40	8,4	169
CHU	6	07/12/2010	00:15	1780	30	30	3,9	191
CHU	7	07/12/2010	04:40	2252	85	85	63,3	223
CHU	8	07/12/2010	09:01	2747	87	87	29,3	252
PAL	9	07/12/2010	20:40	2404	115	120	80,2	270
PAL	10	08/12/2010	02:44	820	115	115	50,9	216
PAL	11	08/12/2010	06:20	434	95	95	38,2	193
PAL	12	08/12/2010	09:20	125	25	25	13,7	172
PAL	13	08/12/2010	11:31	90	28	30	38,8	157
PAL	14	08/12/2010	14:30	62	29	31	17,8	126
PAL	15	08/12/2010	16:30	59	30	32	14,3	95
PAL	16	08/12/2010	19:40	45	29	30	29,7	68
PAL	17	08/12/2010	22:20	27	-	17	14,5	41
PAL	18	08/12/2010	00:15	18	-	13	7,4	20
MOS	19	09/12/2010	08:55	50	34	39	20,8	20
MOS	20	09/12/2010	11:30	98	38	40	14,7	47
MOS	21	09/12/2010	14:30	121	75	80	77,3	73
MOS	22	09/12/2010	16:30	148	60	70	35,6	88
MOS	23	09/12/2010	18:45	195	90	100	23,9	104
MOS	24	09/12/2010	21:40	1535	98	100	33,0	128
MOS	25	10/12/2010	01:05	2039	93	105	29,8	151
MOS	26	10/12/2010	04:15	2331	112	110	52,2	180
TRA	27	10/12/2010	17:30	2574	110	120	65,3	230
TRA	28	10/12/2010	22:00	1452	105	105	79,1	200
TRA	29	11/12/2010	02:00	721	105	40	35,8	166
TRA	34	11/12/2010	16:50	34	25	25	9,5	25
CSM	35	12/12/2010	04:15	53	7	14	5,1	11
CSM	36	12/12/2010	07:00	95	55	58	37,6	45
CSM	37	12/12/2010	10:00	130	66	68	37,2	79
CSM	38	12/12/2010	13:45	589	67	70	42,1	127
CSM	39	12/12/2010	16:45	1375	85	85	62,0	166
CSM	40	12/12/2010	19:30	1999	95	100	34,8	200
SCI	45	17/12/2010	17:45	57	20	22	8,4	15
SCI	46	17/12/2010	21:33	105	48	50	21,4	51
SCI	47	18/12/2010	01:05	147	40	38	18,0	93
SCI	48	18/12/2010	03:34	250	40	45	46,7	124
SCI	49	18/12/2010	06:20	491	83	82	30,2	157
SCI	50	18/12/2010	09:45	1734	105	107	40,4	198
ITA	51	18/12/2010	17:21	1800	80	89	51,2	243
ITA	52	18/12/2010	21:15	1188	90	92	42,6	212

Tabela A1. (Continuação).

Transecto	Estação	Data	Horário	Profundidade (m)			Volume filtrado (m^3)	Distância da costa (km)
				Local	FM	Arrasto		
ITA	53	19/12/2010	23:17	734	76	75	42,6	195
ITA	54	19/12/2010	01:30	215	55	61	34,6	164
ITA	55	19/12/2010	04:37	129	54	30	27,0	120
ITA	56	19/12/2010	09:40	84	45	48	15,5	84
ITA	57	19/12/2010	12:40	45	41	31	36,2	27
BAB	58	19/12/2010	17:56	28	24	25	10,8	33
BAB	59	20/12/2010	22:13	66	38	37	23,9	96
BAB	60	20/12/2010	01:51	124	48	49	19,7	147
BAB	61	20/12/2010	05:50	242	67	66	24,2	207
BAB	62	20/12/2010	09:20	525	83	85	43,1	247
BAB	63	20/12/2010	12:17	995	84	85	21,6	280
IBI	64	20/12/2010	19:16	190	84	86	30,1	29
IBI	65	21/12/2010	00:40	103	40	39	23,6	100
IBI	66	21/12/2010	07:00	72	36	36	14,0	166
IBI	67	21/12/2010	11:04	78	38	37	15,0	234
IBI	68	21/12/2010	15:20	70	28	30	10,1	330
IBI	69	21/12/2010	23:02	53	32	32	11,3	418
CST	70	05/01/2011	10:25	15	-	12	6,4	8
CST	71	05/01/2011	13:00	24	22	22	7,4	43
CST	72	05/01/2011	16:00	52	13	13	7,1	73
CST	73	05/01/2011	18:35	685	60	60	66,3	90
CST	74	05/01/2011	21:50	1230	66	66	52,1	103
FE2	80	06/01/2011	14:30	25	14	14	4,3	13
FE2	81	07/01/2011	00:40	57	31	32	16,6	36
FE2	82	07/01/2011	04:20	81	28	29	11,1	58
FE2	83	07/01/2011	07:43	552	21	21	8,8	93
FE2	84	07/01/2011	12:00	1055	115	121	61,5	140
CAB	86	07/01/2011	19:20	1875	125	130	48,7	176
CAB	87	07/01/2011	23:30	976	22	25	9,0	148
CAB	88	08/01/2011	01:45	1364	48	50	28,8	130
CAB	89	08/01/2011	04:00	619	73	75	55,3	109
CAB	90	08/01/2011	06:35	100	36	39	23,8	87
CAB	91	08/01/2011	08:50	65	45	46	13,5	67
CAB	92	08/01/2011	11:50	51	18	22	11,2	40
CAB	93	08/01/2011	14:00	31	17	22	10,8	19
CF1	94	08/01/2011	17:00	44	10	20	25,2	20
CF1	95	08/01/2011	20:15	62	31	35	13,8	59
CF1	96	09/01/2011	00:15	95	53	55	24,6	98
CF1	97	09/01/2011	02:10	98	88	90	43,1	115
CF1	98	09/01/2011	04:02	1122	87	89	44,4	132
CF4	108	10/01/2011	13:00	862	71	78	130,6	119
CF4	109	10/01/2011	18:30	475	80	98	37,1	93
CF4	110	10/01/2011	21:05	138	55	60	26,8	67
CF4	111	11/01/2011	00:30	110	35	40	22,3	37
CF4	112	11/01/2011	04:00	52	17	22	6,6	7

Tabela A2. Informações sobre as amostras de zooplâncton coletadas na Reserva Biológica Marinha do Arvoredo, analisadas no capítulo 3.

Estação	Data	Horário	Volume filtrado (m^3)
1	24/04/2013	09:15	48,5
2	24/04/2013	09:54	108,0
3	24/04/2013	10:26	122,1
4	24/04/2013	10:50	174,1
5	09/05/2013	10:15	111,7
6	09/05/2013	10:45	168,3
7	09/05/2013	11:15	138,6
8	09/05/2013	09:35	78,6